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Effects of autologous conditioned serum on the physiology of aged skin.

Establishment of feasible conditions and evaluation of efficiency and compatibility of autologous conditioned serum with in vivo and in vitro measurement methods.

DISSERTATION

with the aim of obtaining the doctoral degree

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Submitted by Dominique Hertz-Kleptow

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This doctoral project was carried out between October 2016 and March 2023 at the Faculty of Mathematics, Informatics and Natural Sciences, Institute of Biochemistry and Molecular Biology, AG Cosmetic Sciences at the University of Hamburg by Dominique Hertz-Kleptow and under the direction of Prof. Dr . Martina Kerscher.

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Second reviewer: Prof. Dr. Volkmar Vill

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„In nature nothing exist alone”
Rachel Carson

List of publications

Hertz-Kleptow D, Hanschmann A, Hofmann M, Reuther T, Kerscher M. Facial skin revitalization with CPM®-HA20G: an effective and safe early intervention treatment. Clin Cosmet Investig Dermatol. 2019; 12: 563-572.

Hausmann C, Hertz-Kleptow D, Zoschke C, Wanjiku B, Wentzien-Odenthal A, Kerscher M, Schäfer-Korting M. Reconstructed Human Epidermis Predicts Barrier-Improving Effects of Lactococcus lactis Emulsion in Humans. Skin Pharmacol Physiol. 2019; 32(2): 72-80.

Kerscher M, Hertz-Kleptow D, Drabik A, Kaptan T, Reinecke J. Cell-Free Blood Cell Secretome (BCS) Counteracts Skin Aging and Restores Firmness and Elasticity. J Drugs Dermatol. 2021; 20(6): 682-688.

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List of abbreviations

ACS ... *autologous conditioned serum*
ANOVA ... *analysis of variance*
AP-1 ... *activator protein 1*
BDDE ... *butanediol diglycidyl ether*
BSA ... *bovine serum albumin*
COL1A ... *collagen 1A*
CTGF ... *connective tissue growth factor*
DMEM ... *Dulbecco's Modified Eagle's Medium*
ECM ... *extracellular matrix*
EEMCO .. *European Group for Efficacy Measurements on Cosmetics and Other Topical Products*
EGF ... *epidermal growth factor*
EIA ... *enzyme immunoassay*
ELISA ... *enzyme-linked immunosorbent assays*
EPCs ... *emergent perceptual categories*
ERK ... *extracellular-signal-regulated kinase*
FCS ... *fetal calf serum*
FGF-2 ... *fibroblast growth factor-2*
HAS ... *hyaluronic acid synthases*
IFN-α ... *interferon-a*
IGF1 ... *insuline-like growth factor-1*
IL-1 ... *interleukin-1*
IL-1ra ... *interleukin-1 receptor antagonist*
JNK ... *c-Jun amino-terminal kinase*
MAPK ... *mitogen-activated protein kinas*
MMP ... *matrix metalloproteases*
MTT ... *methyl-thiazolyl-tetrazolium*
NF-κB ... *nuclear factor 'kappa-light-chain-enhancer' of activated B-cells*
PBS ... Phosphate Buffered Saline
PDAF ... *platelet-derived angiogenesis factor*
PDGF ... *platelet-derived growth factor, platelet-derived growth factor*
PIP ... *procollagen type I carboxy-terminal peptide*
PRIMOS ... *Phase shift rapid in-vivo measurement of the skin*
PRP ... platelet rich plasma
ROS ... *reactive oxygen species*
SASP ... *Senescence-Associated Secretory Phenotype*
SD ... *standard deviation*
SLEB ... *subepidermal low echogenic band*
TBST ... *Tris-buffered saline with Tween*
TGF-ß1 ... *transforming growth factor-ß1*
TIMPs ... *tissue inhibitor of metalloproteinase*
TNF ... *tumor necrosis factor*
UV ... *ultraviolet*
VEGF ... *vascular endothelial growth factor*

Abstract

Background: Regenerative cosmetic products are a major segment of the cosmetic industry. The intervention of oxidative damaged cellular mechanism with antioxidative substances are well known and studied, but the interest in new products is still omnipresent. Around 2010, a new approach developed with the use of injectable autologous growth factors and cytokines. They have anti-inflammatory and regenerative effects to the skin's cellular structures. Autologous conditioned serum (ACS) is part of this new approach and known for its healing potential in musculoskeletal disorders. As skin aging is also correlated with oxidative damage and inflammatory processes, the use of ACS could help cellular structures to regenerate and rejuvenate.

Aim: The aim was to evaluate the efficiency and compatibility of injected ACS in three clinical studies on female facial skin and the in vitro effects on human dermal fibroblasts.

Material and Methods: For the in vitro effects, human dermal fibroblast were incubated with ACS and a combination of ACS and hyaluronic acid (HA) from six patients, generating eight ACS and two ACS + HA samples. An methyl-thiazolyl-tetrazolium (MTT) assay for cell viability, western blot for collagen I A (COL1A) and TGF-ß1, and an enzyme immunoassay for procollagen type I production were conducted. For the different assays, fetal calf serum (FCS) of the culture medium was replaced by ACS and ACS + HA samples, respectively. Medium with FCS was used as a control. Cell viability and intra and extracellular procollagen type I concentrations were examined after two, six and 24 hours of incubation. Collagen I and TGF-ß1 were analyzed after 24 hours of incubation.

The clinical part consisted of three clinical studies with a total of 66 patients between the ages of 30 to 64. They received four injections of ACS or ACS + HA in the left and right side of the face with at least two weeks intervals. Efficacy was assessed by biophysical measurements over a time period of up to 48 weeks. Skin hydration was measured with the Corneometer®, skin firmness, recovery and elasticity with the Cutometer®, density and thickness via sonography, and skin topography via an optical digital 3D device (PRIMOS). Means and standard deviations (SD) were calculated. The in vivo trials were additionally statistically evaluated using the analysis of variance.

Results: Cell viability of human dermal fibroblasts increased with increasing incubation time for all incubated samples. Procollagen type I concentrations were measured in all samples without a noticeable increase over incubation time. TGF-ß1 was detected in all ACS and ACS + HA incubated cells but not in the control; collagen type I could not be detected in any of the probes.
Data from 57 (nine dropouts) female patients were evaluated in total of the three clinical studies. All three studies showed steady to slightly increasing skin hydration values, all within the range of normal hydrated skin. The first ACS in vivo study showed a significant increase in skin firmness and skin recovery until the end of the study in week 24. Also, skin fatigue parameters decreased significantly. The parameters that describe the elasticity of the skin showed the highest values in week 8. The second ACS in vivo study showed significant increase in all skin firmness, recovery and elasticity parameters until week 12; the final values in week 24 were comparable to the initial values at the beginning of the study. The second study also evaluated the treatment with ACS + HA, but this combination showed no significant advantage over the single treatment with ACS only. In the third ACS in vivo trial, skin firmness decreased until the end of the study in week 48 but significantly only for the right side. Skin fatigue increased, and the parameters that describe the elasticity of the skin showed, in parts, a significant decrease. Skin thickness and density were evaluated in the first two ACS in vivo studies only and showed no significant changes between screening and the end of the studies in week 24.

Conclusion: A positive influence of ACS to cell viability of human dermal fibroblast was confirmed, as was the detection of TGF-ß1 and procollagen type I production, which are crucial proteins for the synthesis of collagen and the extracellular matrix. The first two clinical ACS studies showed improvements of skin mechanical properties until week 12 and 24, respectively. The third study did not show consistent improvements in the mechanical properties of the skin. Compared to the longevity of HA injections, ACS could be an alternative as it has no volume increasing potential, unlike HA. Additionally, as an autologous product, ACS has no allergic potential, which could be a benefit for sensitive skin. More detailed studies concerning the composition of ACS and skin biopsies after injection would be a good future approach to further assess the influence of ACS on collagen synthesis in vivo.

Zusammenfassung

Hintergrund: Regenerative Kosmetikprodukte sind ein bedeutendes Segment der Kosmetikindustrie. Die Intervention von oxidativ geschädigten zellulären Mechanismen mit antioxidativen Substanzen wurde bereits vielseitig untersucht. Das Interesse an neuen und innovativen Produkten ist weiter steigend. Ein neuer Ansatz ist die Verwendung von injizierbaren autologen Wachstumsfaktoren und Zytokinen, die entzündungshemmende und regenerative Wirkungen auf die Zellstrukturen der Haut haben. Autolog konditioniertes Serum (ACS) ist Teil dieser neuen Ansätze und bekannt für sein regeneratives Potenzial bei Erkrankungen des Bewegungsapparates. Da die Hautalterung auch mit oxidativen Schäden und entzündlichen Prozessen korreliert, könnte die Verwendung von ACS der Haut und deren Zellstruktur helfen, sich zu regenerieren und zu verjüngen.

Ziel: Ziel war die Bewertung der Wirksamkeit und Verträglichkeit von injiziertem ACS durch drei klinische Studien an weiblicher Gesichtshaut und die in vitro Wirkung auf menschliche dermale Fibroblasten.

Material und Methoden: Für die in vitro Versuche wurden humane dermale Fibroblasten mit ACS und einer Kombination aus ACS und Hyaluronsäure (HA) von sechs Probandinnen inkubiert. Insgesamt wurden acht ACS und zwei ACS + HA Proben erstellt und ausgewertet. Es wurden ein MTT-Assay für die Zellvitalität, ein Western Blot für Kollagen I A und TGF-ß1 und ein Enzymimmunoassay für die Produktion von Prokollagen Typ I durchgeführt. Für die verschiedenen Analysen wurde fötales Kälberserum (FCS) des Kulturmediums durch die ACS- bzw. ACS + HA-Proben ersetzt. Als Kontrolle wurde Medium mit FCS verwendet. Die Zellvitalität und die intra- und extrazellulären Prokollagen Typ I Konzentrationen wurden nach zwei, sechs und 24 Stunden Inkubation untersucht. Kollagen Typ I und TGF-ß1 wurden nach 24-stündiger Inkubation analysiert.

Für den klinischen Teil wurden drei Studien mit insgesamt 66 Patientinnen im Alter von 30 bis 64 Jahren durchgeführt. Diese bekamen vier Injektionen ACS bzw. ACS + HA in die linke und rechte Gesichtshälfte in zweiwöchigem Abstand. Die Hautfeuchtigkeit wurde mittels Corneometer® gemessen, mechanische Eigenschaften der Haut mit dem Cutometer®, Hautdichte und Hautdicke mittels Sonografie und Hauttopografie mit einem optisch-digitalen 3D-Gerät

(PRIMOS). Mittelwerte und Standardabweichungen wurden berechnet. Die in vivo Studien wurden zusätzlich statistisch mittels Varianzanalyse ausgewertet.

Ergebnisse: Die Zellvitalität menschlicher dermaler Fibroblasten nahm mit zunehmender Inkubationszeit für alle Proben zu. Die Prokollagenkonzentrationen blieben in allen Proben ohne merklichen Anstieg. TGF-ß1 wurde in den mit ACS und ACS + HA inkubierten Zellen nachgewiesen, aber nicht in der Kontrolle. Kollagen Typ I konnte in keiner der Proben sicher nachgewiesen werden.
In den drei klinischen Studien wurden die Daten von insgesamt 57 (neun Dropouts) Patientinnen ausgewertet. Alle drei Studien zeigten konstante bis leicht ansteigende Hautfeuchtigkeitswerte, die alle im Bereich normal hydratisierter Haut lagen. Die erste ACS in vivo Studie zeigte eine signifikante Steigerung der Hautfestigkeit und Rückbildungsfähigkeit bis zum Ende der Studie nach 24 Wochen. Auch Hautermüdungsparameter wurden signifikant besser. Die Parameter, die die Elastizität der Haut beschreiben, zeigten in Woche 8 die höchsten Werte. Die zweite ACS in vivo Studie zeigte signifikante Verbesserungen aller mechanischen Parameter bis Woche 12. Die Werte der Abschlussvisite nach 24 Wochen waren vergleichbar mit den Werten zu Beginn der Studie. Die zweite Studie bewertete zusätzlich die Behandlung mit ACS + HA. Diese Kombination zeigte allerdings keinen signifikanten Vorteil gegenüber der Einzelbehandlung mit ACS. In der dritten ACS in vivo Studie nahm die Hautfestigkeit bis zum Ende der Studie in Woche 48 ab, jedoch nur signifikant für die rechte Seite. Die Hautermüdung nahm zu und die Parameter, die die Elastizität der Haut beschreiben, zeigten teilweise eine Abnahme. Hautdicke und -dichte Parameter wurden in den ersten beiden ACS Studien bewertet und zeigten keine signifikanten Veränderungen zwischen dem Screening und dem Ende der Studien in Woche 24.

Schlussfolgerung: Ein positiver Einfluss von ACS auf die Zellvitalität humaner dermaler Fibroblasten konnte bestätigt werden. Auch die Produktion von TGF-ß1 und Prokollagen Typ I konnte nachgewiesen werden. Beide sind wichtige Proteine für die Synthese von Kollagen und der extrazellulären Matrix. Die ersten beiden klinischen ACS Studien zeigten Verbesserungen der mechanischen Eigenschaften der Haut bis Woche 12 bzw. 24. Die dritte Studie zeigte keine konsistenten Verbesserungen der mechanischen Eigenschaften der Haut. Im Vergleich zur Langlebigkeit von HA-Injektionen könnte ACS eine Alternative sein, da es im Gegensatz zu HA kein volumensteigerndes Potenzial hat. Außerdem hat ACS als autologes Produkt kein allergisches Potential. Dies könnte besonders für empfindliche Haut von Vorteil sein. Genauere Untersuchungen zur Zusammensetzung von ACS sowie Hautbiopsien nach Injektion wären ein guter zukünftiger Ansatz, um den Einfluss von ACS auf die Kollagensynthese in vivo weiter beurteilen zu können.

1. Introduction

The aging process is a complex cumulation of environmental, endogenous and genetic factors. Yaar et al. state that "Aging, a process that results in cellular attrition and senescence eventually terminating in decreased viability and death, is effected by genetic program as well as by cumulative environmental and endogenous insult that take place throughout the organism's live-span" [1]. This highlights the essence of the aging process and indicates possible interventions. To postpone, slow or even reverse aging, the molecular and cellular understanding of aging is crucial. Technological advancements have led to the growing knowledge of specific genes involved in the aging process in yeast, nematodes, fruit flies, and mice, which are proposed to be similar in humans [2]. The reduced potential of cell doubling with age or the "inflamm-aging" process, where pro-inflammatory stressors induce aging, are some findings [3, 4]. The whole process of aging is also based on a combination of intrinsic (chronological and genetic) and extrinsic (ultraviolet exposure and noxious environmental factors) factors [5].
Skin aging is one part of the research focus of senescence, especially for the cosmetic market, where numerous antioxidative substances like Vitamin C, flavonoids, resveratrol, retinoids or treatments with hyaluronic acid, botulinum toxin or microneedling have been studied extensively for their skin anti-aging effects. Despite the variety of anti-aging products available [6-10], the rising demand for such interventions continues to drive the search for new innovations. This demand is reflected in the American society of Plastic Surgeons annual statistics report, which lists various minimally-invasive procedures like botulinum toxin, chemical peel or soft tissue fillers [11]. The number of minimally-invasive procedures showed an average annual increase of 1.3 % between 2015 and 2019 and is approximately 15 million procedures per year (*Figure 1a*).

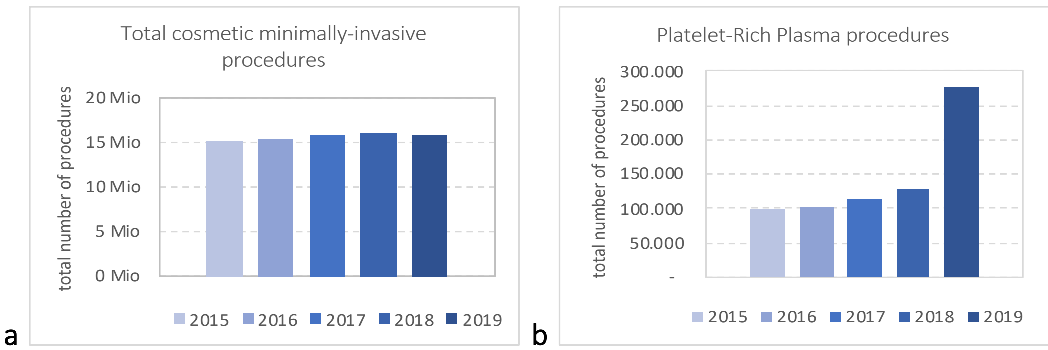


Figure 1: Development of cosmetic procedures, data from the American Society of Plastic Surgeons (ASPS) between 2015 and 2019 [11-13].

Part of the minimally-invasive procedures is platelet-rich plasma (PRP), which has been listed since 2015 as a new approach for minimally-invasive anti-aging procedures as part of the soft tissue fillers. Since then, its request has experienced a strong growth with an annual increase above 10 % between 2016 and 2018 and a doubling from 2018 to 2019 to above 250.000 procedures, as shown in *Figure 1b* [12, 13].

PRP is a substance expected to have anti-inflammatory, wound healing, and juvenescent effects due to specifically enriched proteins within it [14]. This treatment has been observed to have a cell proliferation stimulating effect by collagen-I synthesis in dermal fibroblasts [15]. PRP is an autohemotherapy and is known under a variety of different names with different protein compositions and possible additives. The idea behind PRP treatment is the use of an autologous serum obtained from the whole blood and individualized for the same individual to whom it should be injected [16]. For this reason, autologous blood-derived products are known for low to no unwanted side effects and a low allergic potential [17].

PRP has been used for wound healing and healing stimulation as a component of fibrin glues since the 1970s, especially in the maxillofacial and oral surgery [18-20].

Nowadays, platelet concentrates are successfully used in aesthetic dermatology and are described in the literature in several reviews for indications like scar healing, vitiligo, hair growth, alopecia, wrinkles or skin rejuvenation [21-23]. Autologous conditioned serum (ACS) is part of this regenerative approach and is known from the area of orthopedic diseases in animals and humans [24-26]. ACS is obtained from whole blood after incubation and centrifugation. For this reason, it obtains an increased number of anti-inflammatory cytokines, including interleukin-1 receptor antagonist (IL-1ra) and several growth factors. These growth factors include insulin-like growth factor-1 (IGF1), platelet-derived growth factor (PDGF) and transforming growth factor-β1 (TGF-ß1) [24]. Such components are of importance for cell growth and regeneration, and interfere in the inflammatory process – all factors relevant for skin aging processes [27].

1.1 Background and current state of knowledge

The characteristics of the skin and its aging processes result from changes of the skin tissue components, such as keratinocytes, fibroblasts, and collagen. Biochemical changes can also play a role. Anti-aging treatments aim to influence these changes, and HA and autologous blood products are two important injectables for this purpose [23, 28]. In the following chapters, the influence of these two products, the anatomy of the skin and its cells, biochemical pathways for the synthesis of important substances like collagen, elastin, and hyaluronic acid, the skin aging processes, and the compositions and processing of autologous blood products will be described.

1.1.1 Anatomy and physiology of the skin

The skin is the biggest organ of the human, covering 1.5 – 2 square meters and weighing 3.5 – 10 kg. It serves as the outer barrier between the environment and the inside of the body, consisting of three major layers, the epidermis, dermis and subcutis. The thickness of the epidermis and dermis together ranges from 1.5 to 4 mm, while the epidermis alone measures only about 0.03 to 0.3 mm, depending on body location, gender or age [29-31]. *Figure 2* shows the microscopic structure of the skin with all its layers, schematically and as histological section and is closer described in the following chapter.

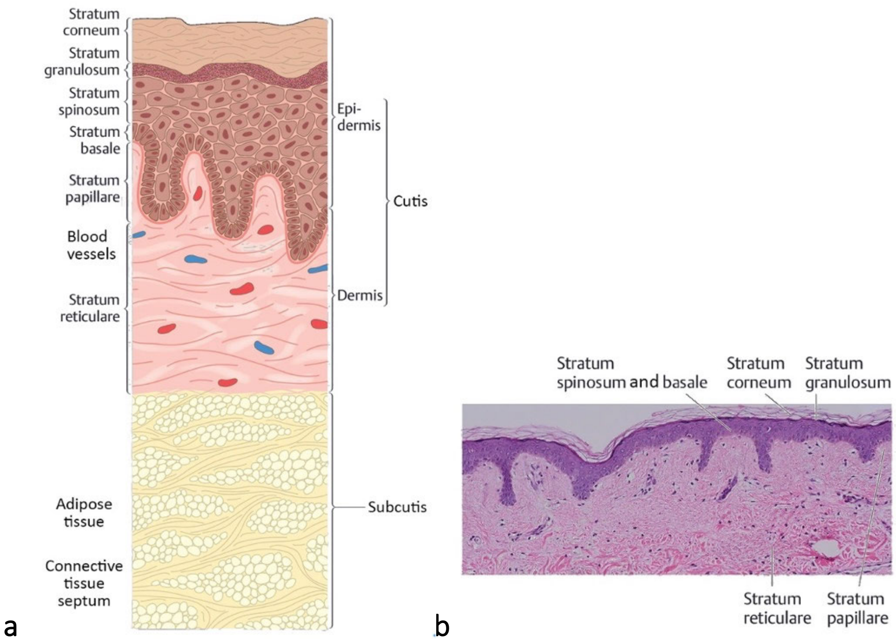


*Figure 2: Schematic (**a**) and histologic (**b**) structure of the skin, adapted from Moll, I.[31].*

1.1.1.1 Epidermis

The epidermis is the outer most layer of the skin, consisting of a multi-layered squamous epithelium that is constantly renewed. The epidermis is divided into four different layers of cells, or strata: the stratum basale, stratum spinosum, stratum granulosum and stratum corneum, which is the outer most layer (*Figure 2)* [31].

The main cell population in the epidermis are the keratinocytes, which undergo different degrees of differentiation as they move through the layers. They change their morphological and functional characteristics and synthesize and express different structural proteins and lipids within the different strata. Their main function is the limitation of passive water loss from the body, protect the skin and body from environmental threats and provide mechanical strength [31-34].

In the single layered stratum basale, mitotically active stem cells generate new daughter cells. These germinative active keratinocytes have a cylindrical shape and a specialized fibrous network of keratin and actin filaments with mechanical and structural properties. As they move into the stratum spinosum, they increase in cell volume and reorientate into a horizontal cell axis. The stratum spinosum has about 2 – 5 cell layers and is also called the prickle layer due to numerous cell-cell-interactions, desmosomes and hemidesmosomes that create a prickle-like appearance. Here, they are important for the intercellular adhesion and insertion of filaments. Above the stratum spinosum is the stratum granulosum with 1 – 3 cell layers. The keratinocytes of this layer have basophilic keratohyalin granules, which form the precursor of the keratin matrix. The upper stratum granulosum is where the last differentiation processes occur, leading to flattening of the cells, disappearance of the cell nuclei and cell organelles, dehydration and formation of a cementitious lipid substance [35].

The stratum corneum is formed by about 10 – 20 cell layers of solid, coherent, platelet-like, hexagonal corneocytes, with a thickness of around 8 – 13 µm [31, 35]. The cells become rigid, immobile and form a strictly geometric arrangement surrounded by the cementitious lipids. This arrangement is also called ‘‘Bricks and Mortar’’ model, where the mortar is formed by glucosyl ceramides, cholesterol, cholesterol esters and long-chain fatty acids which were synthesized and excreted by the keratinocytes beforehand [35].

The time it takes for a keratinocyte to be newly generated and its transit through the epidermis is called the “epidermal turnover time” or “epidermal renewal time”. It can be divided into three segments: the new generation, the transit through the viable cell layers of the stratum spinosum and granulosum, and the final termination of the cell fragments as corneocytes in the stratum corneum [36]. Depending on the different methods and calculations used in literature, different time spans are found, varying between 27 to 45 days of total epidermal turnover time [36-39].

From age 50 onwards, there is a prolonged turnover time for the stratum corneum, taking around 20 to 30 days [40]. Also, the regeneration time of the epidermis after injury is prolonged with age. This can lead to a decreased barrier integrity and increased vulnerability to exogenous insults, which can accelerate the aging process [41].

Two other important cell types in the epidermis are the melanocytes and Langerhans cells. Melanocytes are responsible for producing pigment in the skin. They transfer pigment granules, known as melanosomes, to epidermal keratinocytes, providing protection to the cell nucleus from ultraviolet (UV) light and give the skin its color.

Langerhans cells are dendritic immune cells that play a vital role in the immune barrier. They are essential in contact allergy because to their surface antigen-presenting cells [33].

1.1.1.2 Dermis and subcutis

The dermis is a tear-resistant connective tissue that lies beneath the epidermis and extends down to the subcutis. The main cell type in the dermis are fibroblasts, which are surrounded by a mesh-like structure of collagen and elastin tissue fibers. Other cell types in the dermis include histiocytes (active form: macrophage), mast cells, melanocytes, Langerhans cells and lymphocytes. The cells and tissue fibers are embedded in a gel-like substance of glycosaminoglycans and proteoglycans, which also contains mast cells, lymphocytes, leukocytes and macrophages. The gel-like basic substance together with the tissue fibers is called extracellular matrix (ECM). The ECM is responsible for maintaining the water and electrolyte balance, tissue turgor, fulfills important tasks in cell migration, differentiation, and in wound healing. Due to the ability of glycosaminoglycans and proteoglycans to bind large amounts of water, they maintain viscoelastic properties and are responsible for the attractive complexion of the skin [31, 35]. One well known glycosaminoglycan is HA, which is also a major component of the ECM. Up to 50 % of HA is located in the dermis of an adult human [42].

The thickness of the dermis is between 1 – 4 mm and, like the epidermis, is very variable depending on the location, age and gender [31, 43, 44]. It can be divided into the dermo-epidermal junction zone with a basement membrane, stratum papillare and the stratum reticulare [35]. The dermo-epidermal junction zone connects both layers and is formed by the “rete ridges” of the epidermis, a villous like cytoplasmatic formation of basal cells reaching into the dermis [45]. This basement membrane consist of three major parts: the cell membrane of the basal keratinocytes with hemidesmosomes and a gab area called lamina lucida, the lamina densa consisting of collagen IV and laminin I, and the sublaminar room with manly anchoring fibrils. Its main function is the mechanical connection between epidermis and dermis and is therefore a highly strained area with an ingenious reticulate system [35].

Fibroblasts synthesize all fibers and the ECM. The fibers are mainly of the structural protein collagen. Collagen fibers are responsible for the mechanical stability and distensibility of the dermis. Collagen type I is the most common structural protein type in the skin. More filigree collagen type III fibrils surround the basal membrane and the cutaneous appendages. Anchoring fibrils of collagen type VII traverse from the lamina densa to the upper most dermis and cohere epidermis and dermis together [31].

The last and deepest layer of the skin is the subcutis. It consists of lobular fatty tissue, divided by connective tissue. These septa carry the vascular and nerve supply and build the tight structure around the fat lobules. It acts as thermal isolation, mechanical protection and energy storage. It is also important for the flexibility of the skin [35]. The reduction and reorganization of subcutaneous fatty tissue with age also triggers the characteristic appearance of old people with wrinkles and less skin elasticity [29].

1.1.2 Skin aging and its extrinsic and intrinsic influence

The processes of skin aging are complex and involve multiple layers of the skin, with the dermis playing a crucial role. Both extrinsic and intrinsic factors contribute to skin aging, but the most significant extrinsic factor for premature skin aging is exposure to UV radiation, which generates free radicals and reactive oxygen species (ROS) in the skin [5, 46]. Flament et al. calculated that UV exposure was responsible for 80 % of visible facial aging signs in Caucasian women [47].

ROS generation initiates a cascade of different inflammatory biochemical processes that can deplete cellular antioxidant enzymes such as superoxide-dismutase and catalase, cause DNA damage and activate the neuroendocrine system, leading to immunosuppression and the release of pro-inflammatory mediators. These processes increase the permeability of capillaries and lead to infiltration and activation of neutrophils and other phagocytic cells into the skin. Additionally, they stimulate the generation of further free radicals, elastases, other proteases such as cathepsin G, and activates various matrix metalloproteases (MMP) [48].

One major structural change in aged skin is the decrease in skin thickness compared to young skin [45, 49, 50]. There are also alterations in the biochemical processes of cell regeneration and collagen synthesis [51].

In detail, Lavker conducted a study where he took skin biopsies from the dorsal forearm (UV exposed skin) and upper inner arm (UV unexposed skin) from two age cohorts of 20 to 25 and 68 to 84- year-old Caucasian male volunteers. The specimens were evaluated using light and electron microscopes. Lavker found an almost flat dermal-epidermal junction zone and a surplus amount of lamina densa with attached anchoring fibrils in UV unexposed and exposed senile skin. He theorized that the increased lamina densa-anchoring fibril complex was an attempt to compensate for the flattening and form a better bond between dermis and epidermis. He also found differences in fibroblast structure and constitution of microfibrils in the papillary dermis [45].

Similar modifications in intrinsically aged skin were found by Mine et al. They took mammary skin samples from human adult women aged 19 – 74 and investigated the age-related alterations of fibroblasts obtained from the papillary dermis and reticular dermis, respectively. Like Lavker, they found morphological changes, especially of the rete-ridges structure of the dermal-epidermal junction zone, which was clearly flattened, illustrated in *Figure 3a*. The analysis of MMPs and tissue inhibitor of metalloproteinases (TIMPs) in papillary fibroblasts revealed a progressive increase with age, which possibly triggers the degradation of collagenous structures of the ECM. In general, the papillary fibroblasts showed the most alterations with decreased growth potential, change in protein secretion and functionality, whereas the reticular fibroblasts of old donors showed less overall changes [49].

Varani et al. found that sun-protected chronologically aged skin, taken as biopsies of the hip in individuals of 80+ years (old cohort), has a declined number of fibroblasts, collagen and procollagen type I synthesis compared to young skin (18 – 29 years) of up to 75 %. Due to their findings, they propose cellular fibroblast aging and a lower level of mechanical stimulation resulting from decreased intact collagen fibers as two different mechanisms for reduced collagen synthesis in chronologically aged skin, shown in *Figure 3b* [51].

Changes in the epidermis include fewer melanocytes and Langerhans cells and a prolonged cell regeneration time. But the epidermal skin structure and integrity of the stratum corneum, as long as it’s not injured, remains mostly unchanged in senile skin [1, 40, 52].

These data suggest a profound degradation of dermal connective tissue, with loss of collagen and elastic fibers and a disorganization and loss of cells. The clinical manifestation of chronological aged skin is xerosis, laxity and wrinkles, as well as benign changes such as seborrheic keratoses or cherry angiomas [1, 53].

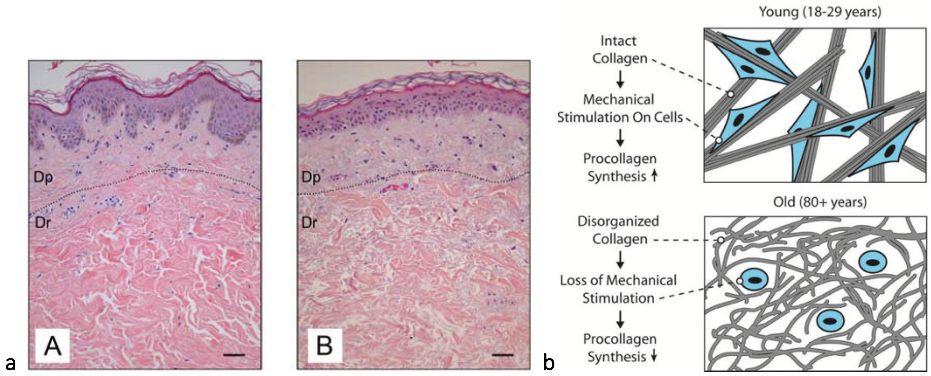


*Figure 3: Histologic (**a**) and schematic (**b**) illustration of skin aging.*
*(**a**) Histological biopsies of mammary skin obtained from a 19 year old (**A**) and a 74 year old donor (**B**), Scale bars = 50 µm. Dp = papillary dermis. Dr = reticular dermis; (**b**) Reduced collagen synthesis in aged skin due to a declined number of fibroblasts and mechanical tension [49, 51].*

1.1.3 Inflammation as part of skin aging

Wound healing is associated with an inflammation, new tissue formation and tissue remodeling phases [54]. Considering this knowledge, parallels can be drawn between the molecular processes of wound healing and those involved in skin photoaging. These parallels can effectively be used for regenerative approaches [55].

Franceschi et al. coined the term “inflamm-aging” as a “global reduction in the capability to cope with a variety of stressors and a concomitant progressive increase in the pro-inflammatory status” as a major characteristic of the aging process. Depending on the level of inflammation processes over the years and genetic precondition, an organism can handle aging differently. If a threshold of pro-inflammatory status is reached, age related diseases occur [4].

External stressors, such as UV rays from sun, cigarette smoke, and other environmental pollutants, as well as the natural process of aging contribute to the generation of ROS that stimulate the inflammatory process in the skin, as described in the chapter above [48]. In particular, senescent fibroblasts show an altered pattern of gene expression that leads to secretion of a wide range of soluble and insoluble factors, referred to as Senescence-Associated Secretory Phenotype (SASP). SASP includes pro-inflammatory cytokines / chemokines (IL-1, IL-6, and IL-8), growth factors, MMPs, and other soluble factors like tumor necrosis factor (TNF) [56, 57].

It has been shown that IL-1 is a mediator for paracrine senescence through activation of the inflammasome complex, which is responsible for the inflammatory response [58]. This inflammasome complex induces degradation of membrane receptors, signaling pathways, proteins, and other components of the ECM. It changes the functions of the stem cells, disrupts autophagy processes and activates the transcription factor NF-κB (nuclear factor 'kappa-light-chain-enhancer' of activated B-cells), promoting inflammation in tissues [56, 59]. This inflammation is followed by high levels of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, that disrupt the transmission of anabolic signals and lead to dermal matrix breakdown [60, 61]. Additionally, UV radiation or inflammation can activate AP-1 (activator protein 1), a transcription factor composed of Jun and Fos proteins, which interferes with collagen degradation [61]. Therefore, the term inflamm-aging or inflammatory aging comes from the close connection between aging and inflammatory processes in fibroblast [4, 56]. Several publications visualize the biochemical signal cascades with the inflammatory process in the skin induced by UV light and environmental factors [4, 48, 61, 62]. An overview by Kim et al. is shown in *Figure 4*, which includes the activation of mitogen-activated protein kinase (MAPK) due to reactive oxygen species, followed by activation of AP-1 and NF-κB [62]. AP-1 is an effective inhibitor of TGF-ß, a ubiquitous, multifunctional cytokine important for regulating procollagen synthesis in fibroblast [63]. TGF-β initiates cellular effects by binding to specific cell surface receptor complexes, TGF-β type I (TβRI) and TGF-β type II (TβRII) receptors. Binding of TGF-β to the receptor activates a biochemical cascade resulting in collagen synthesis. This pathway is shown in *Figure 7* and further describes in section *1.1.6 Collagen*. The downregulation and degradation of collagen and elastin in the skin result in wrinkles and sagging [62].

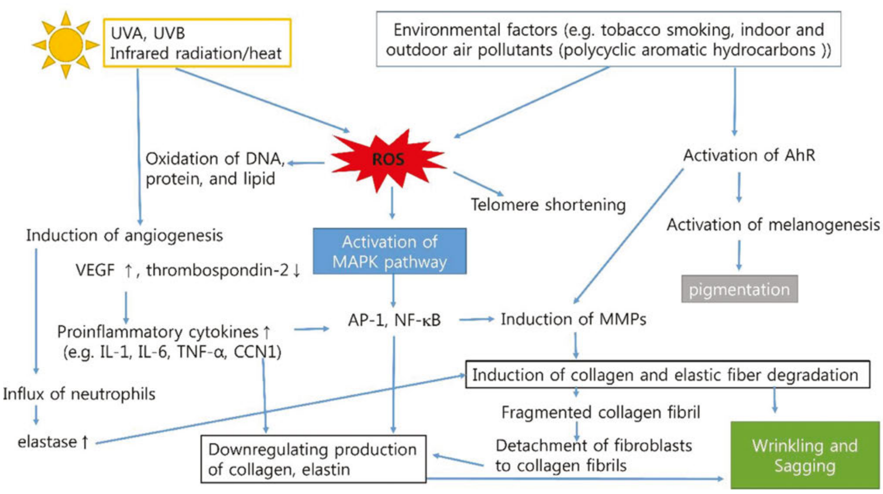


Figure 4: Biochemical signal cascade of extrinsic influenced skin cells by Kim et al. [62].
ROS: reactive oxygen species, AhR: arylhydrocarbon receptor, NF-kB: nuclear factor kappa-B, IL-1: interleukin-1, TNF-α: tumor necrosis factor, CCN1: cysteine-rich protein 61, MAPK: mitogen-activated protein kinase, AP-1: activator protein 1, and MMPs: matrix metalloproteinases.

1.1.4 Wound healing

Wound healing can be divided in to three (four) overlapping phases, as shown in *Figure 5*. The inflammation accompanied with hemostasis, tissue formation and final tissue remodeling [64]. Hemostasis is associated with clotting cascades and thrombocytes activation, leading to the formation of a fibrin cloth to seal the wound and prevent blood loss. The inflammatory phase includes the cleaning of the wound and preparation for new tissue formation via the infiltration of neutrophils, macrophages and lymphocytes. This phase is often associated with edema, erythema, and pain [64, 65]. Neutrophils release various antimicrobial substances such as reactive oxygen species, proteases like elastase, and mediators like TNF-α, IL-1ß and IL-6, which amplify the inflammatory response and stimulate vascular endothelial growth factor (VEGF) and IL-8 [54]. Macrophages play an essential role in the secretion of growth factors (PDGF, VEGF), chemokines, and cytokines (TGF-ß) to eliminate apoptotic cells and stimulate cell proliferation and tissue recovery. This phase lasts about 2 – 5 days [64].

The proliferation phase is dominated by re-epithelialization, initiated by epidermal growth factor (EGF), and angiogenesis, stimulated by VEGF [65]. Keratinocytes and fibroblasts are stimulated, and the later synthesizes new ECM and collagen fibers, proteoglycans and fibronectin [66]. This phase lasts about 6 – 21 days and is the most important [64]. The last remodeling phase can last several months to years and is associated with the skins ability to regain elasticity and tensile strength through continuous collagen synthesis (collagen III to collagen I replacement) [64, 65]. Senescence slows all these processes and leads to a possibly prolonged and less perfect healing process with an increasing inflammatory status [62].

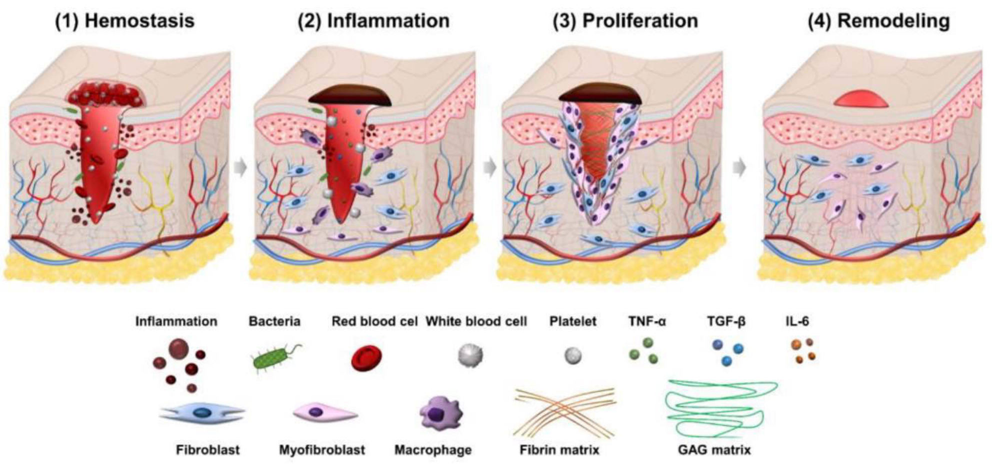


Figure 5: Wound healing process by Trinh et al. [64].

1.1.5 Fibroblasts – function and important signal transduction

Dermal fibroblasts and their mature form, fibrocytes, are the predominant cell types in the human dermis [31]. Fibroblasts have a variety of functions, making them a major subject of research [67-72]. They perform both physiological and reparative mechanisms in the dermis, such as the repair and recovery of wound healing in injured skin [73, 74]. They are involved in the regulation of angiogenesis and induce the migration and differentiation of endothelial cells [75]. They are also the primary cells for the synthesis and remodeling of the ECM through growth factors and cytokines, such as platelet-derived growth factor (PDGF), EGFs or TGF-β1 [69, 76, 77].

In addition to collagen fibers, glycosaminoglycans and glycoproteins are important proteins of the ECM. They are synthesized in the endoplasmic reticulum and Golgi apparatus of the dermal fibroblasts by specific enzymes called HA synthases (HAS). In the skin, HA plays a vital role because, in addition to its strong water binding potential, it is also important for cell growth and adhesion as well as antioxidative processes [78-80].

In aged or stressed skin, biochemical signal transductions changes occur, including alterations to the signal cascades pathways of cell proliferation and differentiation. One important pathway is the MAPK pathway. This pathway is mediated by a phosphorylation process which occurs, for example, under the influence of reactive oxygen species, particularly under UV irradiation. Three families of the MAP kinases exist: extracellular-signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK) and p38 MAP kinase. The ERK pathway primarily activates cellular response to growth factors, while JNK and p38 mediate cellular response to cytokines and physical stress [81]. JNK is activated through phosphorylation of different MMPs [82]. MMPs also lead to dermal matrix breakdown and further inflammation. This pathway is also seen in keratinocytes of the epidermis (*Figure 6*) [61].

In the course of aging, a reduction in the components of the ECM and the dermal fibers are observed [51, 83]. The decrease in collagen concentration in the dermis is considered to be a major feature of the functional weakness of aged dermal fibroblasts [51].

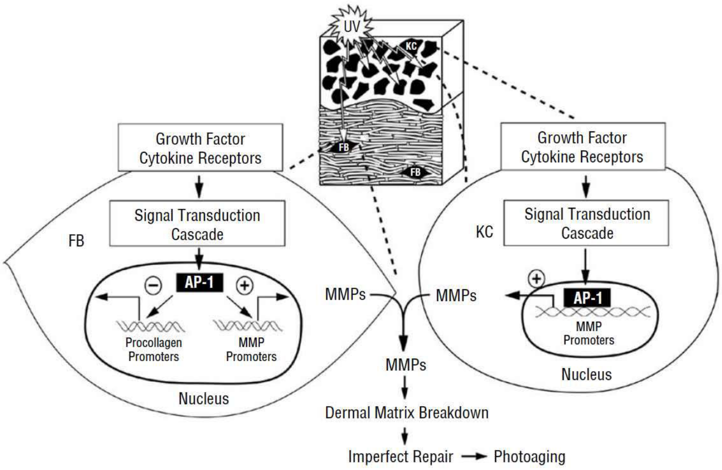


Figure 6: Model illustrating biochemical pathways on fibroblast (FB) and keratinocytes (KC) due to UV irradiation, by Fisher et al. AP-1: acute phase protein 1, MMP: matrix metalloproteinase [61].

1.1.6 Collagen and elastin

Collagen is a ubiquitous fiber found in the human body and is composed of 28 different collagen types that can be divided into subfamilies based on to their supramolecular assembly [84]. In the skin, the primary type of collagen is type I [31], which is a heterotrimer consisting of two α1(I) chains and one α2(I) chain. It has a typical triple helical structure due to the tandemly repeated Gly-X-Y triplets, with the X and Y positions often occupied by proline and hydroxyproline, respectively [85].

Collagen is synthesized in the endoplasmatic reticulum as procollagen, which consist of a central triple helical domain and two non-collagenous propeptide domains at both their N- and C-terminal ends. The procollagen molecules pass through several modifications and folding and are then transported to the Golgi apparatus. In the Golgi vesicle, the procollagen molecules are further aggregated and secreted into the extracellular space. The N- and C-terminal ends are enzymatically cleaved off, generating the mature fibrillar supramolecules [80].

Collagen type I represents about 90 % of the composition of dermal collagen fibrils and is therefore essential for the organization of the dermal ECM and tensile strength of the skin. It also provides a scaffold for anchoring other proteins, which enhances the stability of the ECM [84].

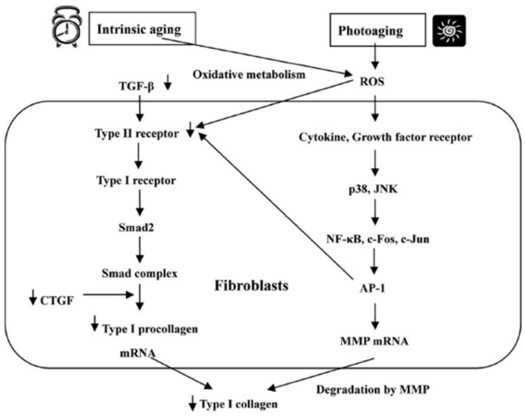


Figure 7: TGF-β/Smad/CTGF/procollagen axis by Hwang et al. [67].

A decreased synthesis of type I collage is observed in intrinsic aging due to the down-regulation of TGF-β and connective tissue growth factor (CTGF) in the TGF-β/Smad/CTGF/procollagen axis (*Figure 7*). In photoaging, growth factors and cytokine receptors on fibroblasts are activated via UV induced ROS. They stimulate p38 and JNK (members of the MAPK signaling cascade), which further activate AP-1, stimulating MMP transcription. Increased MMP transcription accelerates the degradation of collagen, inducing dermal matrix alterations. ROS can also be generated from oxidative metabolism and accumulate during the intrinsic aging process [67]. A depressed collagen synthesis leads to a reduced interaction of fibroblast with intact collagen, followed by a loss in mechanical tension and further depletion in collagen synthesis [86].

Elastin is another key protein of the ECM. Even though elastic fibers represent only about 2 – 4 % of the total dry weight of the dermis, they provide resilience and elasticity to tissue and are roughly 1000 times more flexible than collagens [87, 88].

The elastogenesis in dermal skin is facilitated by fibroblasts. During this process, the precursor protein tropoelastin is synthesized on the rough endoplasmic reticulum of the cells, undergoes intracellular modification, and is then released to the ECM through the Golgi apparatus [89]. This polypeptide chain forms the basis of the elastin molecule with a hydrophobic domain rich glycine, valine and proline, and a hydrophilic domain rich in lysine and alanine, which also participate in the crosslinking of the fibers (desmosines) [87, 90]. In contrast to collagen fibers, the metabolic turnover of elastin is slow and has a half-time comparable to a human life span. Pathological conditions such as inflammation or sun damage can therefore lead to a permanent loss of elastin fibers in the skin [87]. This accumulation of dystrophic elastotic material in the dermis is also known as solar elastosis. One of the mechanisms responsible for increased protein degradation, just like in collagen degradation, results from the activation of MMPs [27, 89].

1.1.7 Hyaluronic acid (HA)

HA is a non-sulfated glycosaminoglycan with a basic disaccharide structure consisting of N-acetyl-D glucosamine and D-glucuronic acid, linked by a glycosidic β(1→3) bond. Each disaccharide unit is further linked by a ß(1→4) glycosidic bond forming a stable helical acid polysaccharide [42, 91]. HA is found in prokaryotic to eukaryotic cells, with up to 50 % of the total body HA is found in the skin [78]. In the ECM, HA is an essential stabilizing component that contributes to the construction of a mechanically resilient, three-dimensional network between cells and collagen fibers [78, 92].

HA is highly hydrophilic and polyanionic due to its free carboxyl groups (COO^-) of the glucuronic acid. The multiple hydroxyl groups (-OH) of the saccharides also contribute to the pronounced water-binding capacity (of up to 1000 times) [93, 94]. Continuous formation and elimination of HA are required to ensure its diverse functions in tissue [42]. The half-life ($t_{½}$) of the skin HA is less than a day [78, 95]. Dermal fibroblasts and epidermal keratinocytes synthesize a large amount of HA on the inside of the cell membrane by the catalytically active enzymes HAS 1, 2 and 3, linking the substrates UDP-glucuronic acid and UDPN-acetylglucosamine to the HA polymer [92-94].

HA synthesis is regulated by the cell density in the skin, with low cell density correlating with high synthesis rate and high mobility and proliferation rates of the cells. High cell density correlates with low levels of HA synthesis. Different growth factors (EGF, PDGF, TGF-ß2, IGF-I, FSH) and cytokines (TGF-ß1, IL-1, interferon gamma) activate the biosynthesis of HA via phosphorylation of the HAS enzymes [78, 94].

Besides synthesis, HA is metabolized relatively quickly into monosaccharides and then further metabolized. Hyaluronidases or a free-radical mechanism degrade HA [78]. Two important receptors, CD44 (cluster of differentiation 44) and RHAMM (receptor for hyaluronan-mediated motility) are crucial for the cell-cell and cell-matrix communication with HA. These receptor induce the transduction of intracellular signals. TGF-β1 stimulates the synthesis and expression of RHAMM and HA, initiating cell mobility, growth and maturation [78, 94]. Proper functioning of these receptors and signal cascades is important as they are also related to tumor genesis [28, 96].

Besides the physiological properties of HA, such as the high water-binding capacity, it exhibits outstanding rheological behavior with pronounced viscoelastic properties. These properties include hydration of the skin, lubrication of joints, space filling capacity, and building a framework through which cells migrate or receive protection [78, 93, 96]. These properties are closely related to the current use of HA in a wide variety of medical, pharmaceutical and cosmetic applications [28, 93, 97, 98].

HA is not only used in aesthetic medicine for wrinkle reduction and volumizing effects, but also for its biochemical functions. The intradermal application of stabilized, cross-linked, non-animal HA restores the hydrodynamic balance of the ECM, thereby reducing the clinical effects of skin aging [99]. In addition, it stimulates the proliferation of fibroblasts, indirectly enhancing collagen production and its metabolism through biomechanical stretching or triggering the cytokine TGF-ß1 [98, 100].

1.1.8 Blood and autologous blood-derived products

Autologous blood-derived products have been used for wound healing and healing stimulation since 1970s, particularly in maxillofacial and oral surgery as fibrin glue [18-20]. These products are components of blood enriched with wound healing and anti-inflammatory proteins [14]. Nowadays, different kinds of platelet concentrates are successfully used in aesthetic dermatology for indications such as scar healing, vitiligo, hair growth, alopecia, wrinkles, and skin rejuvenation [22, 23]. They are commonly referred to as platelet rich plasma (PRP), but the production methods is not consistent, resulting in different "kinds" of PRP. However, all methods involve the centrifugation of the patient's own blood, and the autologous plasma solution should contain 4 – 7 times the baseline concentration of human platelets [23].

1.1.8.1 Blood, chemical composition and characteristics

In the human body, like in all mammals, blood is used as a transport system, supplying the organism with necessary substances and as an excretion system of metabolic substances. Blood serves as a carrier-, storage-, and communication system. It is part of the immune system and prevents blood loss after injuries with its blood coagulation system. Blood makes up about 8 % of the human body weight and consist of cells and fragments of cells in an aqueous solution, the blood plasma [101].

Blood is composed of cellular elements and aqueous blood plasma. It contains soluble substances like the respiratory gases, oxygen and carbon dioxide, electrolytes, nutrients, metabolites, proteins, vitamins, trace elements and signal substances. Blood cells include erythrocytes (red blood cells), leukocytes, lymphocytes (white blood cells) and thrombocytes (platelets). The total blood volume of an adult human is about 4 liters, the volume fraction of the blood cells is approximately 45 % of the total volume, referred to as hematocrit [101, 102].

Blood plasma without coagulation factors is called blood serum. It differs from blood plasma only with the absence of fibrinogen and coagulation proteins and is slight yellowish. Besides electrolytes, like Na^{+}, Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+} or phosphate ions, it contains proteins, which can be divided into five big fractions, the albumin and α_1-, α_2 -, β- and γ-globulins [101, 103].

These proteins have a concentration between 60 – 80 g / liter. Albumin is the main protein with 60 %, followed by the globulins. Their tasks include the transport of hydrophobic substances, regulation of water balance, hemostasis and defense against pathogens. Albumin is particularly responsible for the maintenance of the colloid osmotic pressure and for the transport of uni-polar substances [101]. The soluble antibodies (immunoglobulins) are part of the γ-globulins and form the humoral branch of the adaptive immune response. The β-globulins include acute phase proteins. In case of tissue damaged or infections, macrophages and other inflammatory cells release cytokines. IL-6 and IL-1 cause further increased production and secretion of the acute phase proteins in the hepatocytes, including the C-reactive protein [103].

Thrombocytes, or platelets, are the smallest anucleate blood cells, measuring approximately 1 – 4 µm in diameter. The normal number of platelets in blood is 150,000 – 350,000/µl [104]. Their functions include homeostasis and the initiation of wound healing, as described in section *1.1.4 Wound healing* [105].

1.1.8.2 Classification and characterization of platelet rich plasma (PRP)

As already briefly described in the introduction, there are different ways of producing PRP, and different names are used in literature. Ehrenfest et al. and Alves et al. both describe how PRP is processed and which different fractions and systems are used [16, 106]. First, whole venous blood is taken form the patients in syringes with anticoagulants and immediately processed by centrifugation. A two-step centrifugation, shown in *Figure 8*, results in two different PRP product: pure PRP and leucocyte-rich PRP. Ehrenfest et al. classifies PRP into four different categories: pure platelet-rich plasma PRP (P-PRP), leucocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leucocyte- and platelet-rich fibrin (L-PRF). This classification is depending on pharmacological and material characteristics [16]. A concentration of at least 1,000,000 platelets/µl in 5-ml or 4 – 7 times the baseline concentration is assumed to be necessary for the desired effects [23, 107]. Also, the platelets need to be activated, with, for example, calcium chloride, to achieve a release of various proteins [16]. These are cytokines, chemokines or growth factors such as PDGF, VEGF, PDAF (platelet-derived angiogenesis factor), EGF, IGF, FGF-2 (fibroblast growth factor-2) and TGF-β1 [106, 108].

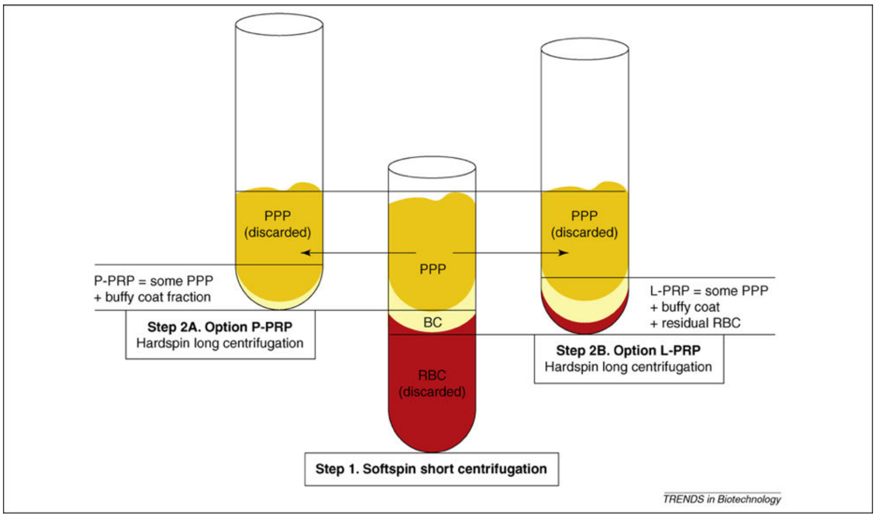


Figure 8: Platelet-rich plasma (PRP) preparation protocol using a two-step centrifugation procedure.
Step 1: Whole blood with anticoagulants is centrifuged with low forces (softspin). Three layers are obtained: red blood cells (RBCs), 'buffy coat' (BC) layer and platelet-poor plasma (PPP). Step 2A: For pure PRP (P-PRP): PPP and superficial BC are transferred to another tube. After centrifugation with high force (hardspin), most of the PPP layer is discarded for final P-PRP concentrate. Step 2B: For leucocyte-rich PRP (L-PRP): PPP, the entire BC layer and some residual RBCs are transferred to another tube. After centrifugation with high force (hardspin) PPP is discarded. The final L-PRP consists of the entire BC and residual RBCs [16].

The activated platelets in PRP stimulate cell growth by promoting protein synthesis and stimulating the cell cycle. In addition to regulating cell growth, some of the released factors also regulate cell migration, differentiation, and cell survival [106, 109, 110].

TGF-β1, FGF-2 and IGF are known to stimulate proteoglycan synthesis and ECM formation in mesenchymal stem- and progenitor cells, including dermal fibroblasts. TGF-ß1 is an important mediator in delaying the aging process in the skin. In vitro and in vivo studies have shown that TGF-ß1 activates the synthesis of collagen type I [75, 111, 112].

PRP is also a good source of anti-inflammatory cytokines such as IL-4, IL-13 and interferon-α (IFN-α) [113]. These properties of PRP are successfully used in clinical therapy [109].

1.1.8.2.1 Platelet rich plasma for skin rejuvenation

There are several clinical trials investigating the use of PRP or similar called autologous blood products for the treatment of facial skin aging signs. However, the preparation technique, amount of injected material, injection technique, frequency and localization of injections varied for each study. Additionally, the evaluation methods, treatment and follow-up visits differed. Despite these variations, the outcomes are generally beneficial and the conclusions positive [114-126].

For example, Cameli et al. investigated the effect of pure PRP on four locations of the face in twelve patients. Three sessions were conducted at one-month intervals with 4 ml PRP, of which 1 ml was injected into the forehead and crow's feet area, 2 ml into the cheeks (1 ml per side) and 1 ml into the nasolabial folds. Transepidermal water loss, skin hydration, skin elasticity and smoothness were measured at baseline and one month after the last treatment. Flow cytometry for the PRP count was also conducted. All measurements showed improvements. However, patients evaluation showed mixed opinions ranging from good to insufficient improvement [115].

In contrast, Abuaf et al. only injected approximately 2 ml of PRP once into the forehead and infra-auricular area for biopsies. Twenty patients were treated, and punch biopsies were taken of a PRP-treated infra-auricular and a saline- treated infra-auricular area at baseline and after 28 day. Collagen fibers in the dermis increased as well as elastic fibers for both treatments. No further measurements were conducted [114].

The available data is therefore rather variable and the outcomes partly questionable. Nevertheless, there is a general positive impression, which can be confirmed from other aesthetic indications, like androgenetic alopecia or acne scars [23, 127, 128].

1.1.8.3 Classification and characterization of autologous conditioned serum (ACS)

ACS is a serum without cells, clotting factors, or additives that has healing and anti-inflammatory potential similar to that of PRP [17]. However, ACS differs in its preparation and composition, which will be described below.

ACS was first initially developed in the 1990s for orthopedic diseases such as osteoarthritis, rheumatoid arthritis and spinal disorders [17, 129]. Its focus was on the generation of IL-1ra, an inhibitor of interleukin-1 (IL-1), which is known as pro-inflammatory cytokine for various inflammatory and autoimmune diseases [54, 130]. In the Orthokine® therapy, the patient's venous blood is drawn into syringes with medical-grade glass beads and incubated at 37 °C for several hours. The blood is then centrifuged, and the serum is extracted for direct injection into the affected region or can be stored for later use [24]. The glass beads have a purely physical effect on the blood cells, similar to a catalyst. The main difference between ACS and PRP is that the whole blood with all its cells is incubated and preserved during incubation. This exploits the ability of leukocytes to produce growth factors and cytokines induced by the specially prepared surfaces of the glass beads. After incubation, the blood is centrifuged, and the serum is aspirated for further use. This technique produces a cell-free serum that has elevated levels of signaling proteins such as IL-1ra and numerous growth factors [24, 25, 131].

The concentrations of proteins and growth factors in ACS probes compared to whole blood were determined using enzyme-linked immunosorbent assays (ELISA). The concentrations of anti-inflammatory cytokines IL-1Ra, IL-10 and growth factor TGF-ß1 were significantly increased in the ACS probes compared to whole blood [24, 132]. A significant increase of the pro-inflammatory cytokines IL-1ß, IL-6 and TNF-α can also be observed in the ACS probes [132]. In order to efficiently block IL-1-mediated reactions, IL-1Ra must be present at least in a ratio of 1:10 excess over IL-1 [24, 129, 133]. In addition, IL-10 and TGF-ß1 as well as FGF act synergistically to IL-1ra. Thus, the excess of anti-inflammatory cytokines and growth factors present in the ACS can antagonize the effect of IL-1, IL-6 or TNF-α, present in inflamed tissue and interrupt the inflammatory process [134, 135]. A ACS composition profile by Wehling et al. is shown in *Table 1* [24].

Table 1: Content of cytokines and growth factors in human ACS by Wehling et al. [24].
ACS is obtained using 10 mL whole blood using the Orthokine® system.

	Cytokine										
	IL-1ra	IL-1β	IL-6	TNFα	IL-10	FGFb	VEGF	HGF	IGF1	PDGF AB	TGFβ
No. of patients	224	224	200	92	92	92	92	92	92	92	80
Concentration (pg/mL) prior to incubation (0 hours)											
Basal[b]	236	<3.9	<12.5	<15.6	<7.8	14.6	61	431	86 000	205	1 165
Concentration (pg/mL) after incubation (6 hours)											
Mean	2 014.8	7.9	28.7	10.1	33.4	26.6	508.6	1 339.3	117 208.8	39 025.6	97 939.0
SD	4 381.1	8.7	48.1	9.6	18.9	20.8	307.7	928.7	51 644.4	10 515.8	113 418.3
Minimum	390.3	1.4	0.9	3.0	4.1	2.8	114.1	691.4	37 430.0	19 601.0	13 067.0
Maximum	31 057.0	48.9	250.2	69.7	105.0	104.5	1 694.0	6 473.0	440 000.0	66 208.0	823 000.0

a All measurements were performed using enzyme-linked immunoabsorbant assay kits (ELISA; R&D Systems, Minneapolis, MN, USA). Basal values are normal values of healthy donor as measured by the kit manufacturer. Serum retrieved from 10mL of whole blood.

b All basal levels given with a '<' are lower limits of the kit's sensitivity. The accuracy of readings lower than these values are suboptimal.

FGFb = fibroblast growth factor-b; **HGF** = hepatocyte growth factor; **IGF1** = insulin-like growth factor-1; **IL** = interleukin; **PDGF** = platelet-derived growth factor; **TGF**β = transforming growth factor-β; **TNF**α = tumor necrosis factor-α; **VEGF** = vascular endothelial growth factor.

The anti-inflammatory and TGF-ß1 rich serum derived from autologous conditioned blood shows promising effects in the treatment of osteoarthritis, and it can be assumed that it may have positive effects on the inflammatory processes during skin aging as well. The parallels between these conditions suggest that the positive cytokine and growth factor profile of ACS can counteract the aging process. A study by Pinto et al. found an increase in IL-1Ra, EGF, TGF-ß1, IGF-1 and PDGF-AB levels and positive developments of skin hydration, firmness and visco-elasticity, suggesting potential aesthetic skin improvement [136].

1.2 Aim of the thesis

Skin regeneration and achieving a more youthful appearance are common aims in the cosmetic market [13, 137]. HA therapy is a well-established method for correcting fine lines, reducing nasolabial folds and marionette lines, and augmenting lips and cheeks [28, 138-140]. PRP is a newer product that is still under research [23, 106]. ACS, on the other hand, is mainly known for its anti-inflammatory and healing properties for orthopedic applications [17, 24, 131]. The question of whether ACS can be used for skin enhancing and anti-aging indications arises from the idea of its composition.

Therefore, this research project aims to investigate the therapeutic efficacy and safety of ACS on human skin. The potential of ACS for skin regeneration and rejuvenation will be examined in vitro and in vivo.

Appropriate conditions for preparing and administering ACS will be established, along with objective measurement methods to evaluate its effects.

In vivo, the clinical effects of ACS micro injections in the cheeks of healthy female volunteers were assessed. Three clinical studies will be conducted, and the effects will be evaluated using biophysical measurements. Skin hydration, elasticity, roughness, thickness and depth of the skin will be the main criteria for evaluating the therapeutic effect.

In addition to the vivo measurements, in vitro assays will be conducted to assess the biochemical effects of ACS on human dermal fibroblast cultures and gain further insights on human skin cells.

2 Material and Methods

In the following chapters the used materials and methods for the in vitro assays and in vivo studies, and the measurement devices will be described. Starting with the explanation of the processing of ACS itself. Then, the in vitro material and methods will be explained. Following, the study designs of the three ACS in vivo studies will be described, the biophysical measurements, and the statistical analyzation.

2.1 Autologous test product

The processing of ACS as test product for the in vitro assays and in vivo studies follows a strict protocol. The required steps will be described in the following.

2.1.1 Material for blood withdrawal and ACS processing

One way gloves, hand disinfection, swab, butterfly needle with adapter for the EOT®II syringes, blood collection syringes with a special filtration system (EOT®II syringes), tourniquet, stand for the EOT®II syringes, cannula disposal container, laboratory book, and labels for the syringes.

Centrifuge, incubator, sterile cloth, disposal container for needles, surface disinfectant, Luer-Lock syringes for reinjection (1 ml syringe), needles (20G), needles (30G), membrane filters for venting, membrane filters (Pall PharmAssure® 25mm Syringe Filter with 0.2 µm Super® membrane), caps, labels with patient names for the injection syringes, storage packaging, laboratory book.

2.1.2 Processing of ACS

Date, time, amount of blood withdrawal, and processing of the patient's serum is recorded in a laboratory book. The labels and the storage packaging are provided with first names, surnames, dates of birth and a batch number.

The ACS processing starts with withdrawal of the patient’s blood. For a sufficient amount of ACS for the whole treatment regime, 4 syringes (ACS in vivo I study), 8 syringes (ACS in vivo II study), and 9 syringes (ACS in vivo II study) a 10 ml blood were withdrawn from each patient. They were labeled with first name, surname and date of birth. The syringes were incubated for 3 hours (ACS in vivo II study) or 6 hours (ACS in vivo I and III study) in an 37°C preheated incubator, removed from the incubator and centrifuged for 10 minutes at 3000 g (5000 rpm). ACS was then aliquoted via bacterial filter to 1 ml syringes and stored at -18 °C for the single treatment sessions. For the ACS+HA injection group of the second ACS in vivo study, a 5 ml syringe was filled with ACS.

Belotero Soft® was used in the second ACS in vivo study.
Belotero Soft® (Merz Pharmaceuticals, Frankfurt am Main, German) is a monophasic HA-filler with cohesive polydensified matrix (CPM®) with HA concentrations of 20 mg/ml.
The CMP® technology is based on a first crosslinking process with butanediol diglycidyl ether (BDDE) and a further process where the monophasic gel is linked with non-crosslinked hyaluronic acid and BDDE. This process results in a monophasic HA gel with different density zones and lower viscoelasticity, compared to other HA filler products [141].

2.2 Material and Methods in vitro

Two in vitro experiments to evaluate the effect of ACS on human dermal fibroblasts were carried out in October 2017 and June 2019 in cooperation with the Institute of biochemistry, University of Hamburg, parallel to the clinical trials.

2.2.1 Study protocol of the in vitro I and in vitro II tests

To examine the influence of ACS and ACS + HA on human dermal fibroblasts for anti-aging properties, assays for cell viability, procollagen I, collagen I and TFG-ß1 production after incubation were performed. For that purpose human dermal fibroblasts were cultivated in culture medium with 10 % FCS at 37˚C and 5% CO_2 to 70 – 80% confluency and passage numbers 3 to 5 were used for the assays. In the first test series (in vitro I) ACS and ACS + HA of two female volunteers was examined. In the second test series (in vitro II) only ACS of six different aged volunteers was used. For the different assays FCS of the culture medium was replaced by the ACS and ACS + HA samples, respectively. Medium with FCS was used as control. Cell viability and intra and extracellular procollagen concentration was tested after 2, 6 and 24 hours incubation. TGF-ß1 and collagen was analyzed via western blot assay after 24 hours incubation. *Table 2* gives an overview of the in vitro tests.

Table 2: In vitro test overview

Cell viability	Procollagen I	Collagen I and TGF-ß1
MTT assay	PIP EIAssay	Western Blot
Whole cells	Lysate and supernatant	Lysate and supernatant
Incubation 2, 6 and 24 h	Incubation 2, 6 and 24 h	Incubation 24 h
Measurement with spectrophotometer at λ=550 nm	Measurement with spectrophotometer at λ=450 nm	Visual image on blotting gel, collagen I at 130-140 kDa, TGF-ß1 at 25 kDa

2.2.2 Materials in vitro

In the first experiment ACS samples of two female subjects, aged 36 and 61, were used. Additionally, a combination of ACS and hyaluronic acid was used in a portion of 4:1. In the second experiment ACS of six female subjects between 36 and 61 was used, two of the ACS samples were from the first experiment. The specifications of the used ACS samples are listed in *Table 3.*

Vital human dermal fibroblasts (HFIB-D, provitro AG, Berlin) were used as cell cultures. Cell culture medium was Dulbecco's Modified Eagle's Medium (DMEM; PAN Biotech). For the determination of cell viability an MTT assay was conducted. For the procollagen type I concentration a PIP EIA Assay Kit (Takara Bio Inc.) was use and absorbance was measured with a microplate reader (Tecan Infinite® 200Pro / Sparkcontrol). Collagen type I and TGF-ß1 were detected via anti-Collagen I and anti-TGF-ß1 antibodies (Santa Cruz Biotechnology) with western blot.

Table 3: ACS test products

Study	Date of birth	Dare of blood extraction	Age at time of blood extraction	Labeling	ACS appearance
ACS in vivo study III	1980	24.10.18	38	yACS#1	Clear, dark yellow
ACS in vivo study III	1978	29.10.18	40	yACS2#	Turbid, light yellow
ACS in vivo study II	1980	15.11.16	36	yACS; yACS#3	Slightly turbid, light yellow
ACS in vivo study III	1965	22.10.18	53	aACS#1	Clear, light yellow
ACS in vivo study III	1965	03.12.18	53	aACS#2	Clear, light yellow
ACS in vivo study II	1955	08.11.16	61	aACS; aACS#3	turbid, light yellow

2.2.2.1 Chemicals and reagents in vitro

- Humane fibroblasts, dermis (HFIB-D), cryo — provitro AG Berlin
- COL1A mouse monoclonal antibody — Santa Cruz Biotechnology®
- TGF-ß1 mouse monoclonal antibody — Santa Cruz Biotechnology®
- Procollagen Type I C-Peptide (PIP) EIA Kit — Takara Bio Inc. Europe
- FCS Premium Fetal Calf Serum — PAN Biotech
- Phosphate Buffered Saline (PBS) pH 7.4 (10x) — gibco® by Life Technologies
- Pen Strep Penicillin Streptomycin — gibco® by Life Technologies
- L- Glutamine 200 mM — gibco® by Life Technologies
- Dulbecco's Modified Eagle's Medium (DMEM), sterile filtered — PAN Biotech
- 0.25% Trypsine — gibco® by Life Technologies
- 50 ml Isopropanol: 0.04 M HCl:
  50 ml Isopropanol, 167 µl conc. HCl
- MTT 5 mg / ml:
  22.9 mg MTT in 4.6 ml PBS
- 1 ml Western blot lysis buffer:
  100 µl MNT buffer, 100 µl 10% Tritow X100, 40 µl complete lysis buffer, 760 µl H_2O
- 3 ml PIP lysis buffer:
  300 µl PBS, 60 µl 50 mM EDTA, 30 µl mM PMSF, 150 µl 10 % Tritow X100, 2,460 µl H_2O
- Tris-buffered saline with Tween (TBST):
  Tris-buffered saline with Tween 20; 10 mM TrisHCl; pH 7.5; 150 mM NaCl; 0.1 % Tween20

2.2.2.2 Devices in vitro

- Centrifuge 5804 R — Eppendorf
- Clean bench — BDK Luft- & Reinraumtechnik
- Microscope Wilovert s — Hund Wetzlar
- Incubator — Thermo Fisher scientific
- Microplate reader — Tecan Infinite® 200Pro / Sparkcontrol

2.2.3 Methods in vitro

In the following chapters the methods for the fibroblast preparation and the subsequent analyses with ACS and ACS + HA are described in detail.

2.2.3.1 Cell cultivation and preparation

The thawing for later cultivation of the cryopreserved human dermal fibroblasts was done following the manufactures instruction. In short, the cryopreserved fibroblasts vial was thawed in a warm water bath at 37°C and added to 10 ml 37°C preheated DMEM with 10 % fetal calf serum (FCS), 100 U/ml penicillin, 100 mg/ml streptomycin and then centrifuged at 250 x g for 5 min. The liquid cell-free supernatant was discharged. The cell pellet was maintained in a petri dish with 10 ml DMEM, 10 % fetal calf serum supplemented with 2 mM L-glutamine (culture medium) at 37°C in a humidified atmosphere containing 5 % (V/V) CO_2 in an incubator. The culture medium was changed after 24 hours. The cells were grown adherently in monolayer and were passaged when about 70 % – 80 % confluence was reached.
For the passaging the cells were removed from the incubator and checked for vitality under the microscope. The cells were washed with 10 ml phosphate-buffered saline solution (PBS), then 1 ml of 0.25% trypsin solution was added to start the detachment process. After four minutes of incubation at 37 °C and soft shaking, the detachment of the cells was checked again under the microscope. The detached cells were suspended in 10 ml culture medium and centrifuged at 230 x g for 5 min. The medium was aspirated and the cell pellet was dissolved in 5 ml fresh culture medium. The suspended cells were divided into three petri dishes of 2.5 ml, 1.5 ml and 1 ml each and replenished to 5ml with culture medium for further cultivation. For the experiments passage numbers 3 to 5 were used.
For the different assays 21 ml cells with 29 ml culture medium and 12 ml with 16 ml culture medium was seeded to 6 well plates with 100,000 cells/well. For the residual cells 14.2 ml cells with 5.8 ml culture medium was seeded to 48 well plates with 20,000 cells/well. The 48 well plates were used for the MTT assays, the 6 well plates for the procollagen type I C-peptide (PIP) enzyme immunoassays and western blot.
For the different assays the culture medium, containing 10 % FCS, was changed to DMEM with 10 % of the different ACS or ACS + HA samples supplemented with 2 mM L-glutamine. Culture medium with 10 % FCS was used as control.

2.2.3.2 Cell viability with MTT assay

The **m**ethyl-**t**hiazolyl-**t**etrazolium (MTT) assay is a colorimetric assay to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. It is a colorimetric assay system, firstly described by Mosmann et al. in 1983 [142]. It is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow salt, to purple formazan crystals by NAD(P)H-dependent oxidoreductase enzymes of metabolic active cells. The purple color solution can be quantified by measuring the absorbance at 500 – 600 nm with a spectrophotometer. The greater the number of viable, metabolic active cells, the higher the amount of reduced MTT and consequently the more intensely colored the solution [142, 143]. The chemical structure of MTT and its reduced formazan salt and the absorption curve is shown in *Figure 9*.

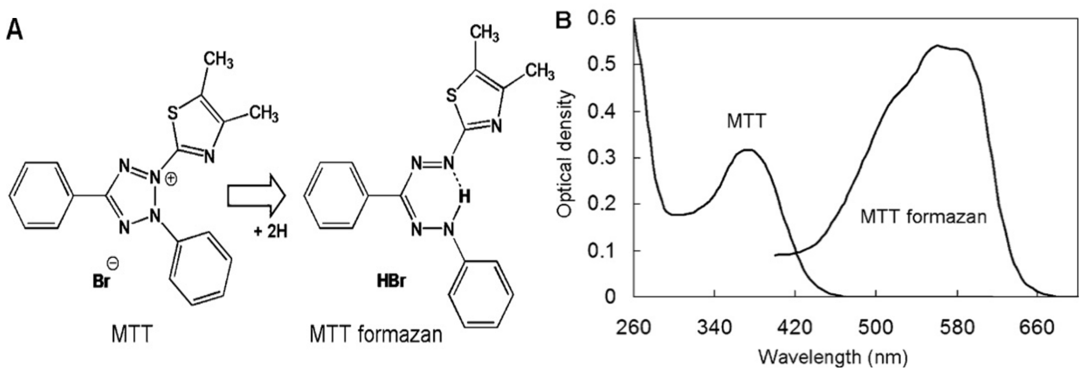


Figure 9: MTT structure and absorption curve.
*(**A**) Chemical structure of MTT and its reduced formazan product; (**B**) absorption spectra of MTT in distilled water and MTT formazan in sunflower oil, both at the same concentration (0.016 mg/ml) [144].*

For the MTT assay the fibroblasts were seeded to 48 well plates with 20,000 cells/well. The fibroblasts were then incubated for 2, 6 and 24 hours with 10 % addition of the different ACS or ACS + HA (4:1) samples instead of FCS in the culture medium. Culture medium with 10 % FCS was used as control. The medium was withdrawn immediately before the test. 225 µl of fresh medium and 25 µl 5 mg/ml MTT solution was added to each well. The wells were incubated for further 3 hours at 37°C. After each hour the formation of formazan crystals was checked under the microscope. The incubation ended by aspirating the MTT containing medium. To dissolve the crystals 250 µl isopropanol with 0.04 M HCl solution was added to each well and placed on an orbital shaker.

For measuring the MTT concentrations of the probes a Tecan Infinite® 200 Pro and a Sparkcontrol microplate multi-mode reader were used for the two tests, respectively. The absorbance wavelength was λ= 550 nm, reference wavelength was λ= 690 nm. For the in vitro I test duplicates were taken, for the in vitro II tests triplicates.
The vitality of the cells was calculated from the ratio of the amount of formazan salt formed in ACS treated cells to the amount of formazan salt in untreated cells in percent.

2.2.3.3 Procollagen Type I C- Peptide (PIP) with ELISA

Collagen is synthesized in the endoplasmic reticulum (ER) as procollagen, containing additional peptide sequences at the amino- and carboxy-terminal ends of the protein. Their function is to facilitate the winding of procollagen molecules into their typical triple-helical conformation. During its secretion from the cell these propeptides are enzymatically cleaved off the collagen triple helix and the molecule polymerizes into the extracellular collagen fibrils [80]. Thus, the amount of free propeptides reflects stoichiometrically the amount of collagen molecules in a ratio of 2:1 [145]. Quantitative detection of collagen synthesis was first reported by Taubman et al., who performed radioimmune assays for the carboxy-terminal end of the propeptide, the procollagen type I carboxy-terminal peptide (PIP), using polyclonal antibodies [146].
The assay used for this study is a solid phase in vitro procollagen Type I C-peptide enzyme immunoassay (EIA) Kit by TaKaRa Bio Inc. The principle of this kit is based on a sandwich method by a one-step procedure with two mouse monoclonal anti-PIP antibodies. During the incubation, PIP is bound to anti-PIP (solid phase) on one side and tagged by peroxidase (POD)-labelled-anti-PIP on the other. The color development results from the reaction of POD and added substrate (H_2O_2 and tetramethylbenzidine) with intensities proportional to the amount of PIP present in the samples, detectable via absorbance with an EIA plate reader .
For this assay the fibroblasts of the 6 well plates were incubated with 10 % addition of the different ACS and ACS + HA samples for 2, 6 and 24 hours. To prepare extracellular supernatant 2 ml of the samples supernatant was transferred into 15 ml flacons with 8 ml cold acetone and stored at – 20°C. For the intracellular lysate cells were washed with PBS, precipitated in acetone and centrifuged for 10 min at 15,000 x g at 4°C, the supernatant was discarded. Dry pellets were resuspended in 400 µl PIP lysis buffer (400 µl), un-resuspended material was pelleted again via centrifugation at 13,000 x g for 10 min, the resulting supernatant of the lysate was transferred into fresh tubes.

The analyses were carried out according to the supplier's instruction. In short, 100 µl of antibody-POD conjugate solution was transferred into the kit's wells. 20 µl of standard, sample supernatant and lysate was added to the wells within 5 minutes. Standard and samples were incubate for 3 hours at 37°C. The standard and sample solutions were removed and the wells washed 4 times with 400 µl PBS. 100 µl substrate solution was added to each well and incubated at room temperature for 15 minutes. 100 µl stop solution was added to all wells and mixed gently.

Standard and samples were read at 450 nm with a Tecan Infinite® 200 Pro and a Sparkcontrol microplate multi-mode reader for the in vitro I and in vitro II tests, respectively. For both tests duplicates were taken and mean and SD calculated.

2.2.3.4 TGF-ß1 and COL1A measurement with western blot

TGF-ß1 and COL1A are both important proteins for the synthesis of collagen and the extracellular matrix [147]. As the collagen synthesis decreases with age the synthesis of TGF-ß1 and COL1A is of interest for regenerative purposes [51, 67].

For the detection of both proteins the fibroblasts were incubated with the different ACS samples for 24 hours in 6 well plates (100,000 cells/ well). The supernatant was transferred to a 15 ml flask with 8 ml of ice-cold acetone and stored overnight at – 20°C for precipitation. The cells were washed with PBS. 50 µl western blot lysis buffer was added, the cells were scraped from the plates and transferred to 1.5 ml tubes. The cells were incubated on ice for 10 minutes and then centrifuged at 14,000 x g for 10 minutes at 4°C. The supernatant of the cell's lysate was transferred to fresh tubes.

For the western blot equal amounts of protein were separated electrophoretically on a 10 % SDS-PAGE, stacking at 8 mA/gel and separated at 12 mA/gel. Then blotted on a polyvinylidene difluoride (PVDF) membrane at 100 V for 1.5 hours, ice was exchanged after 30 minutes. The membranes were blocked for one hour in TBST with 1 % bovine serum albumin (BSA), then sealed in a foil bag in TBST with 0.2 % BSA and the specific antibodies (1:500 TGF-ß1, 1:1000 COL1A, 1:4000 Actin-ß, all by Santa Cruz Biotechnology®) overnight at 4°C with shaking. The membranes were washed three times with TBST and incubated for two hours at room temperature with secondary antibodies in 0.2 % BSA in TBST with shaking. After repeated washing with TBST, the membranes were incubated with chemiluminescence solutions for about one minute and the chemiluminescence of the specific band was detected. The molecular weight of TGF-ß1 monomer is 13 kDa, that of TGF-ß1 dimer 25 kDa [148]. Collagen Type I precursor has a molecular weight of 130-140 kDa, mature Collagen Type I 70-90 kDa [149].

2.3 Material and methods in vivo

Three consecutive clinical studies were conducted between 2015 and 2019. All three studies build on each other. Therefore they have modified treatment regimens and measurement methods. The designs and proceedings will be described in detail in the following chapters. All evaluated treatments took place at the Institute of cosmetic sciences at the University of Hamburg.

2.3.1 Ethical and legal aspects

The ACS producing syringe is an approved medical device product in Germany and the European Union and is therefore subject to the Medical Device Act in Germany [17].

The purpose of the medical devices act is the regulation of the trade with medical devices, to set high standards of quality and safety and ensure the protection of health for patients and users. It regulates the requirements for clinical studies (§ 19 – 22) and sets the standards according to the principles of the ICH GCP based on the "Declaration of Helsinki" (Ethical principles for medical research on humans). The ICH GCP standard serves to protect test persons and is intended to contribute to scientifically reliable results of the test. It represents an international ethical and scientific standard for the planning, implementation, documentation and reporting of clinical trials on humans [150].

Therefore the three studies will be carried out according to the requirements and specifications of the Medical Device Act valid at the time of execution, the guidelines of the Declaration of Helsinki and the International Conference of Harmonization Guidelines for Good Clinical Practice (ICH GCP). As prescribed by the Medical Devices Act, all three studies will be approved by a local ethics committee and registered with the German Clinical Trials Register.

2.3.2 Study protocol in vivo

In the following the study protocols and an overview in tabular form will be provided. The different inclusion and exclusion criteria of the three trials will be listed afterwards in an consolidated chapter.

2.3.2.1 ACS in vivo study I

The purpose of the first study was the evaluation of in vivo effects by micro puncture injections with ACS on skin elasticity. Efficacy and safety of cell-free ACS were investigated over 24 weeks in 20 patients with a loss of facial skin elasticity (age 35-55 years).

Patients underwent a series of three treatment sessions of ACS at day 0, week 2 and 4. An additional ACS administration was performed at week 12. About 1 ml cell-free ACS was injected intradermal on the lower face on each side and at each session. An exemplary treated areal is shown in *Figure 10*. Between 20 to 25 injections were performed on each side in an 4 x 4 cm areal.

For the primary efficacy evaluation the suction principle for mechanical properties of the skin was used. With a Cutometer® Dual MPA 580 (Courage & Khazaka, Cologne, Germany) viscoelastic properties of the skin were assessed over 24 weeks for 6 times (screening, weeks 2, 4, 8, 12, and 24). This investigator-initiated study was conducted at the University of Hamburg, Institute of cosmetic sciences, after the positive vote of the ethical committee of Freiburger Ethik Kommission in Freiburg, Germany and after patients signed patient's written informed consent forms. Further investigations which served as secondary efficacy parameters were standardized photography, PRIMOS, corneometry, sonography and different questionaries. In total, 24 women were enrolled in the study to compensate possible dropouts.

Figure 10: Exemplary injection areal left side, ACS in vivo I study, patient 09.

Table 4: Study chart ACS in vivo study I

	Screening Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Timepoint	day –14	day 0	week 2	week 4	week 8	week 12	week 24
Written informed consent	x						
Demographic data	x						
Verification of inclusions/ exclusion criteria	x						

Medical history	x						
Physical examination	x						
Blood withdrawal and serum processing	x						
ACS intradermal injections, 2 ml per treatment		x	x	x		(x)	
Assessments: photo documentation, PRIMOS, corneometry, cutometry, sonography, questionnaires)	x		x	x	x	x	x
Lab test and vital signs	x						
Recording of adverse events		x	x	x	x	x	x

2.3.2.2 ACS in vivo study II

The purpose of the second study was to evaluate in vivo effects of cell-free ACS and ACS + HA on skin elasticity. Based on the first results of the ACS in vivo study I, where the measurements with the cutometer for elasticity and firmness were significantly improved 12 weeks after the start of the treatments, this second study was initiated [151].

Efficacy and safety of cell-free ACS and ACS + HA were investigated in this prospective, randomized, controlled trial over 24 weeks in 20 patients (10 in each treatment group) with a high loss of facial skin elasticity (age 35- 65 years).

Patients in one study arm were treated with ACS only (n = 10), and in the other study arm with ACS + HA (n = 10). Patients underwent a series of three treatment sessions at day 0, week 2 and week 4 with manual micro needling. An additional ACS and ACS + HA administration was performed in week 12, respectively. At each treatment session 5 ml cell-free ACS was injected in a max. depth of 0.5 – 2 mm (intradermal) on the cheeks of both sides (2.5 ml for each side). In the group with ACS + HA, 4 ml ACS plus 1 ml HA was administered in the same way. An exemplary treated areal is shown in *Figure 11.*

For the primary efficacy evaluation the suction principle for mechanical properties of the skin was used. With a Cutometer (Cutometer® Dual MPA 580, Courage & Khazaka, Cologne, Germany) viscoelastic properties of the skin were assessed over 24 weeks for 3 times (screening, weeks 12 and 24). Further investigations, which served as secondary efficacy parameters, were standardized photography, PRIMOS, Corneometrie, Sonography and different questionaries.

This investigator-initiated study was conducted in at the University of Hamburg, Institute of cosmetic sciences, after the positive vote of the ethical committee of Freiburger Ethic Commission in Freiburg, Germany and after patients signed patient's written informed consent forms. In total, 22 women were enrolled, to compensate possible dropouts. This work was part of a bi-center study, where only the data of the patients treated at the University of Hamburg, Institute of cosmetic sciences, were considered.

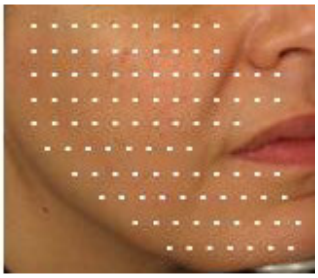


Figure 11: Exemplary injection areal right side, ACS in vivo II study, patient 01.

Table 5: Study chart ACS in vivo study II

	Screening Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Timepoint	day –14	day 0	week 2	week 4	week 8	week 12	week 24
Written informed consent	x						
Demographic data	x						
Verification of inclusions/ exclusion criteria	x						
Medical history	x						
Physical examination	x						
Blood withdrawal and serum processing	x						
ACS alone (5 ml) or ACS + HA (4 + 1 ml) intradermal injection		x	x	x		(x)	
Assessments: standardized photo documentation, PRIMOS, corneometry, cutometry, sonography, questionnaires	x					x	x
Lab test and vital signs	x						x
Recording of adverse events	x	x	x	x	x	x	x

2.3.2.3 ACS in vivo study III

The therapeutic safety and efficacy of cell-free ACS for facial skin elasticity loss has been shown in the first two ACS in vivo studies over 12 and 24 weeks, respectively [151, 152].

In this prospective, open, clinical study over 48 weeks, the efficacy of four treatment session and long-term safety of cell-free ACS was investigated in 20 patients. Female patients with a confirmed loss of facial skin elasticity (classified according to the skin firmness (R0, Uf) value by cutometry), and within an age of 30 – 65 years, were enrolled into the study.

Skin elasticity loss according to the firmness (R0, Uf) values of the ACS in vivo study II, were defined from slight elasticity loss (0.21 – 0.30 mm) up to extreme elasticity loss (> 0.41 mm), as shown in *Table 6* [152].

Table 6: Skin elasticity loss according to firmness values of ACS in vivo study II

Skin Elasticity Loss	R0 value (mm)
Grad 3: extreme Elasticity loss	> 0.41
Grad 2: strong Elasticity loss	0.31 – 0.40
Grad 1: slight Elasticity loss	0.21 – 0.30

The patients underwent a series of four treatment sessions with 4 ml cell-free ACS injections at day 0, in week 2, week 4, and week 6. ACS was applied manually with a serial puncture technique, with injections placed approximately 1 cm apart in a max. skin depth of 0.5 – 2 mm (intradermal) on both sides of the cheeks at each session (2 ml per side). An exemplary treated areal is shown in *Figure 12*.

For the primary efficacy evaluation the suction principle for mechanical properties of the skin was used with the Cutometer® Dual MPA 580 (Courage & Khazaka, Cologne, Germany). Visco-elastic properties of the skin were assessed over 48 weeks for 6 times (screening, weeks 0, 12, 24, 36 and 48). Further investigations, which served as secondary efficacy parameters, were standardized photography, corneometry, and different questionaries. This study was conducted after positive vote of the ethical review committee of Hamburger Ethic Commission in Hamburg, Germany, and after patients signed patient's written informed consent forms.

In total, 20 women were enrolled. This work was part of a multicenter study, where only the data of the patients treated at the University of Hamburg, Institute of cosmetic sciences, were considered.

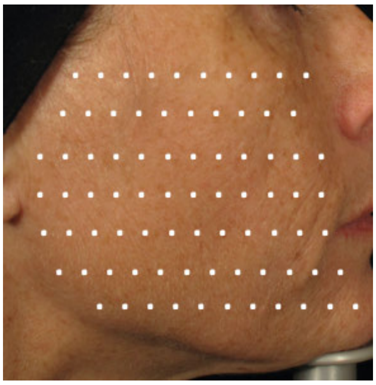


Figure 12: Exemplary injection areal right side, ACS in vivo III study, patient 03.

Table 7: Study chart ACS in vivo study III

	Screening Visit 0	Baseline Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Timepoint	day -14	day 0	week 2	week 4	week 6	week 12	week 24	week 36	week 48
Written informed consent	x								
Demographic data	x								
Verification of inclusion / exclusion criteria	x								
Medical history	x								
Physical examination	x								
Blood withdrawal and serum processing	x								
ACS intradermal injection, 4 ml per treatment		x	x	x	x				
Assessments: corneometry, cutometry, questionnaires	x	x				x	x	x	x
Lab test (facultative) and vital signs	x								x
Recording of adverse events		x	x	x	x	x	x	x	X

2.3.3 Inclusion and exclusion criteria

Inclusion and exclusion criteria for all three studies were identical with some additions for the in vivo ACS study II and III. These additional criteria are listed below the overall inclusion and exclusion criteria.

Inclusion criteria:

- Provision of signed and dated informed consent form to participate in the study
- Non-pregnant, non-breast feeding female aged 35 – 55 years
- Loss of facial skin elasticity according to the Investigator's opinion
- Intent to improve skin structure and the elasticity of the skin using cell-free ACS

Exclusion criteria:

- Severe skin diseases e.g. psoriasis, atopic eczema (neurodermatitis), acne, active herpes zoster or other autoimmune skin diseases in the face
- Skin cancer in the anamnesis
- Treatment with chemotherapy, immunosuppressive agents or immunomodulatory therapy (e.g. corticosteroids, monoclonal antibodies) within three months before study treatment
- Systemic diseases with skin involvement (SLE)
- Pre-treatment with laser, botulinum toxin or HA in the lower face (treatment areal)
- Severe diet in the last 3 months or nutritional supplementary during the study duration of 24 week
- Acute infection
- Pregnancy or woman who plan to become pregnant during the course of the study
- History of bleeding disorders or treatment with anticoagulants or inhibitors of platelet aggregation (e.g. ASS or other non-steroidal anti-inflammatory drugs [NSAIDs])
- Tendency to form keloids, hypertrophic scars or other healing disorder
- Any medical history that, in the opinion of the Investigator, would make the subject unsuitable for inclusion (e.g. chronic, relapsing or hereditary disease that may affect the outcome of the study.)

For the in vivo ACS study II additional criteria apply:

- 35-65 years old females (10 pre-menopausal and 10 post-menopausal patients)

For the in vivo ACS study III additional inclusion criteria apply:

- Confirmed skin elasticity loss: Cutometer R0 value > 0.20 (elasticity loss at least Grade I)
- Female aged 30-65 years (10 pre-menopausal and 10 post-menopausal patients)
- Patients accept not to alter their usual skincare routine during the study
- Botulinum toxin: no treatments 6 months before enrolment in the study. Patients accept not to start with treatment during the study.
- Hyaluronic acid (HA): no treatments with low-viscoelastic HA in the last 12 months, no treatments with high-viscoelastic HA in the last 24 months. Patients accept not to start with any HA treatment during the study.
- Skin booster and laser: no treatments in the past and during the study.
- Corneometer (hydration) measurements: Patients accept not to wash their face and not to treat with any products minimum 6 hours before measurements are performed on the study visit days.

2.3.4 Biophysical measurements

For evaluating the skin physiological treatment results *Table 8* lists the used biophysical measurement methods and devices.
These in vivo methods are non-invasive and cause no pain or side effects to the patients skin. But they are easily affected by internal and external factors such as sweating, outside temperature or humidity [153, 154]. To obtain reproducible readings the recommendations of the **E**uropean Group for **E**fficacy **M**easurements on **C**osmetics and **O**ther Topical Products (EEMCO) guidelines were applied in all three studies. They include, measurements in an air-conditioned room with 20 – 22°C and a relative humidity between 40 – 60 % after the patients had an acclimatization time of at least 30 minutes (20 minutes are recommended). Patients did not wash and use products on the investigation area for at least 6 hours before measurements (depending on the product 2 – 10 hours no washing and 8 – 12 hours no products are recommended) [155, 156].
The measurements were conducted in the laboratories of the Institute of cosmetic sciences at the University of Hamburg, Papendamm 21, 20146 Hamburg.
To minimize further interactions the measurements followed a strict routine. After the patient's questionnaires and physical assessments, photographs were taken succeeding with the PRIMOS, then corneometry, cutometry and at last sonography. Cutometry and sonography both

interact with the skin (suction and water) and have to be performed last. *Figure 13* shows the schematic measurement areas with the different allocations for the devices.

Table 8: Overview devices and parameters

Parameter	Device / Method
Skin topography	Optical 3D skin surface measurement device (PRIMOS®, LMI Technologies GmbH, Berlin)
Skin mechanical properties	Cutometer® (MPA 580 Dual, Courage und Khazaka, Cologne)
Skin thickness and density	20 MHz ultrasound (DUB 20, Taberna pro medicum, Lüneburg)
Skin hydration	Corneometer® (MPA 580 Dual, Courage und Khazaka, Cologne)

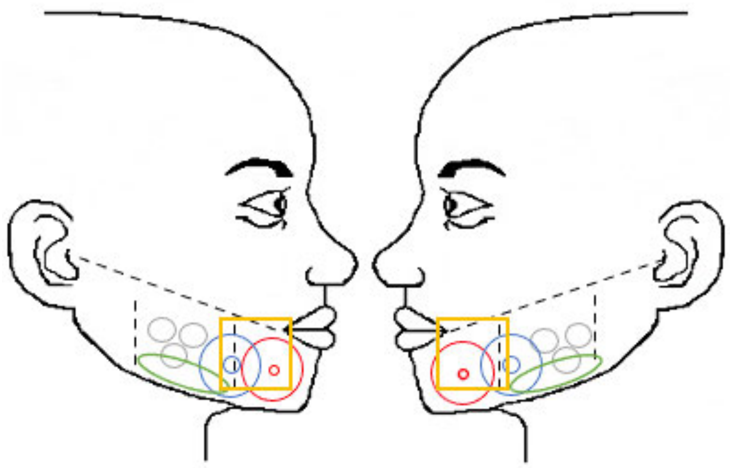


Figure 13: Schematic image of the measurement areas with measurement methods.
Orange: PRIMOS; red: cutometry 2 mm; blue: cutometry 4 mm; green: conography; grey: corneometry

2.3.4.1 Corneometry

Stratum corneum hydration was measured with the Corneometer® CM 825 (Courage & Khazaka electronic GmbH, Cologne). The technique is based on the capacitance measurement of a dielectric medium [157].

The Corneometer® was established in the 1970s to 1980s and is nowadays one of the most easily used methods for quantifying stratum corneum hydration. The Corneometer®'s probe head is a capacitor. It is built of parallel arranged gold lines (conductor tracks/ electrodes) on a ceramic tile (isolating material) sealed with a thin glass lamina to protect the skin from the flow of electricity [158]. The isolating material is called dielectric and in most cases the dielectric

constant of isolating material is around 7 [159]. When a power source is put on the capacitor an electric field is build up within the conductor tracks resulting in an alternating electron surplus (minus charge) or electron lack (positive charge), respectively. A material with a high dielectric constant will increase the capacity of the capacitor. This concept is used in the Corneometer® as the stratum corneum is built of various materials, including water which has a dielectric constant of about 81. Once the probe is put on the skin the capacity of the measuring capacitor will change according to the amount of water in the skin [157-160]. *Figure 14* shows the principle construction of the probes head [161].
The probe has a measurement depth of around 10 – 20 µm and will therefore measure the hydration of the stratum corneum [31, 157, 160].
The measurement units are arbitrary Corneometer® units and range from 0 to 130 a.u. [160].
According to Heinrich et al. the interpretation of the values can be distinguished into three skin categories: Very dry skin shows units below 30 a.u., dry skin ranges between 30 – 40 a.u. and normal skin shows units above 40 a.u. [162].

Below the technical data for the Corneometer® CM 825 is summarized:
Measurement principle: capacity
Units: arbitrary Corneometer® units
Dimensions: probe approx. 11 cm long, Weight: approx. 41 g, Measurement head: 49 mm²
Measurement time: 1 s
Frequency: 0.9-1.2 MHz
Pressure: approx. 1 N ± 10%
Accuracy: ± 3 %
Operation conditions: temperature & relative humidity: T: 5-40° C, RH: 30-70 % RH
Optimal working conditions: 20° C, 50 % RH
Storage conditions: T: 0-70° C, RH: 0-80 % RH [157]

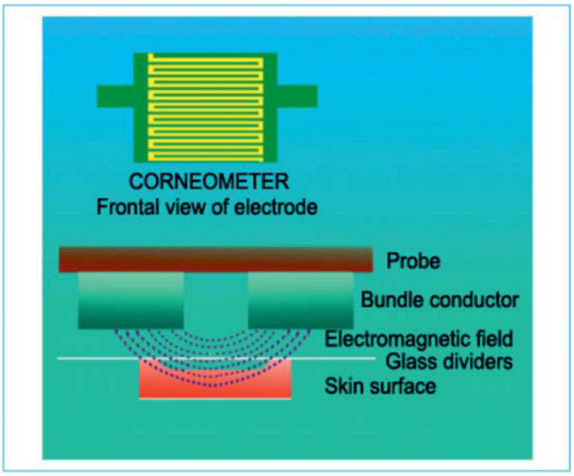


Figure 14: Principle construction of the Corneometer® probe [161].

2.3.4.1.1 Corneometer® measurement and evaluation method

For the practical measurement, the probe is placed on the skin with a constant pressure which is compensated by a spring inside the probe`s head. The result of the measurement is shown in the software program within 1 second [157]. To avoid measurement errors, standardized conditions for the patients and the room apply as described in *Section 2.3.4 Biophysical measurements*. Following the EEMCO guidelines, during each visit three consecutive measurements at slightly adjacent skin areas with 5 seconds pause in between were taken [155]. The mean values were used for further calculations.

2.3.4.2 Cutometry

For the quantification of mechanical properties of the skin, like distension (firmness), elasticity or viscoelasticity, the Cutometer® Dual MPA 580 (Courage & Khazaka electronic GmbH, Cologne) was used.
Cutometry is the measurement of the skin with mechanical force. It can provide information about the structure and composition of the skin, as the mechanical function is related to the epidermal stratum corneum, dermal collagen and elastic fibers, and the viscosity of interstitial fluid [163]. It is widely known in the cosmetic field, especially to evaluate differences in skin aging stages, anatomical regions, sex or efficacy of topical or minimal invasive cosmetic products like HA [9, 164-166].
The working mechanism is, that the skin is vertically drawn into a circular aperture of the measurement probe by constant negative air pressure over a defined time period. The handheld measuring probe consists of a 3 mm^2 suction head with an optical measuring system inside the

aperture of the probe's head. The measuring system is composed of a light source (transmitter), a light receptor and two oppositely arranged prisms. It is connected via an air tube to the measurement instrument with an integrated vacuum pump. Depending on the used probe the circular aperture has a diameter of 2, 4, 6 or 8 mm, respectively. At rest the incoming light beam is fully reflect and the intensity of the transmitted light equals the intensity of the reflected light [167]. Perpendicular deformation of the skin changes the intensity of the reflected light proportionally to the skins penetration depth when pressured is initiated (*Figure 15*). This results in an deformation (extension) in millimeter versus time in seconds curve (strain-time mode) or deformation (mm) vs vacuum (mbar) (stress-strain mode) on the computer software system. The software provides different modes with different suction and relaxation options. In this study the mode 1 was used where constant negative pressure over a specificized time was applied. Also a variety of parameters can be defined by the software, including the applied pressure in millibar (mbar), the on- and off-time (suction and relaxation interval) in seconds (s), the rate of increase or decrease of the air-negative pressure in mbar/s or the repetition of measuring cycles [167, 168].

A typical viscoelastic skin deformation curve of one cycle in mode 1 is shown in *Figure 16 a*, another one with 5 repetitions (cycles) in *Figure 16 b*. As mode 1 is widely used in research the interpretation of the resulting curve refers to this mode [165, 169, 170]. The curve can be divided into two parts, the suction phase during the phase of applied pressure at the beginning and the relaxation phase, when no pressure is applied, this resembles one cycle.

All parameters and their meaning are listed in *Table 9*. The most important will be described in the following. Courage & Khazaka electronic GmbH uses the so called R-Parameters to describe the course of the curve. The international term is labelled "U". There are either absolute or relative parameters, which are functions of the absolute parameters in % [168]. After Dobrev all parameters can be divided into three groups according to their biological informativeness [167].

1. Elastic parameters:

- Absolute parameters – Ue and Ur
- Relative parameters – Ua/Uf (R2), Ur/Ue (R5) and Ur/Uf (R7)

2. Viscoelastic parameters:

- Absolute parameters – Uv and Uf_x – Uf (R9)

- Relative parameters – Uv/Ue (R6)

3. Mixed parameters:

- Uf (R0), Uf_x (R3), Uf – Ua (R1) and Ua_x (R8)

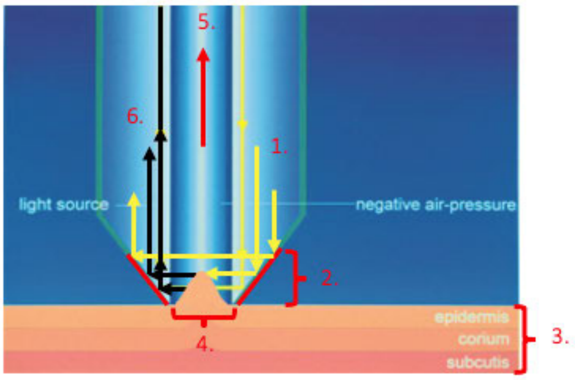
The firmness or distensibility of the skin (Uf = R0) consists of an elastic part (Ue) and an viscoelastic part (Uv) and is the maximum amplitude of the suction phase. The elastic part relates to the stretching of collagen and elastic fibers in the skin, the viscose part to the movement of interstitial fluid. Plastic properties of the stratum corneum and overall skin thickness play an additional role for the degree of skin distensibility. Here it is important to differentiate between the different size of the aperture. The 2 mm probe is appropriate to observe the mechanical properties of the epidermis, the 4 and 6 mm probes are assumed to determine the epidermal and dermal structures [163, 167].

Focusing on the development of facial skin, the immediate distention (Ue) is suggested to decreases with age, probably as a result of a decline in collagen synthesis in intrinsically aged skin or the elastosis in sun exposed areas [51, 167]. The delayed distension (Uv) shows an increase due to lower amounts of glycosaminoglycans and soluble collagen of interstitial fluid which results in a reduced viscosity [163]. But Uv can also increase after application of emollients due to a higher water content in the skin and softening of the epidermis which was also found by Dobrev [171]. The total skin firmness or distensibility (Uf = R0) is therefore influenced by a variety of different factors and an increase or decrease always depends on either Ue and Uv [163]. As a consequence the skin distensibility (Uf = R0) may decrease with age, which was found by Luebberding et al. They found a negative correlation between Uf, Ue and age in different skin localizations of different age groups, with a decrease of up to 50 %. But they conclude that the skin recovery is more affected by age than the skin firmness [165].

The ratio Uv/Ue (ratio of viscoelastic to elastic distention, R6) is a parameter to determine viscoelastic to elastic components of the skin. An simultaneous increase of Uv and decrease of Ue, as suggested in skin aging, results in an increased value of Uv/Ue (R6) [167]. The biological skin elasticity described by Ur/Uv (R7) on the other hand decreases with age [164, 172, 173].

To evaluate the skin properties and in special the intrinsic and extrinsic aging processes with the cutometer, further relative parameters should be considered. Here, gross and net elasticity (Ua/Uf = R2 and Ur/Ue = R5) are of interest. Gross elasticity is the ration of the maximum retraction to whole distensibility and includes the viscosity of the skin. Net elasticity is focused on elastic fibers only, as both parameters (Ur/Ue) represent only the immediate deformation and retraction [167]. Collagen, one of the main insoluble fibers with high tensile strength in the skin, is known to decrease during aging [29]. Therefore gross and net elasticity also decrease with less collagen fibrils in the skin [51, 167].

Ur and Ua (R8) describe the second part of the curve, the relaxation of the skin. They represent the skins ability to restore to its initial state and are dependent to the well function of elastic fibers in the skin. Hysteresis, also described as skin tiring or skin fatigue, is another viscoelastic parameter and relates to the observation that after repeated stress the skin does not immediately return to its initial position. The curve increases with each cycle and the greater the difference between the first maximal deformation (R0 = Uf) and the last maximal deformation (R3 = Uf_x) the stronger the tiring of the skin (R9). R9 is also an indicator to the water content of the skin [167].



1. Direction of the beam of light (incoming)
2. Optical lenses (prisms)
3. Skin
4. Probe opening
5. Direction of suction (when negative air-pressure is applied)
6. Direction of reflected light (transmission, only yellow arrows will cause a signal)

Figure 15: Schematic image of the working mechanism of the Cutometer®. Adapted after C&K Manual [168].

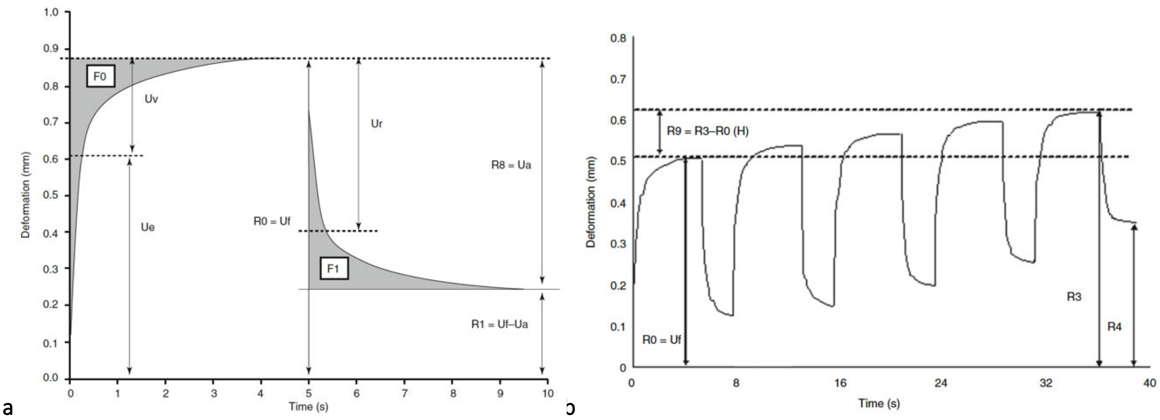


Figure 16: Cutometer curve of a viscoelastic material.
*(**a**) Skin deformation in mm / time, 5 sec. suction time, 5 sec relaxation time,1 repetition. (**b**) Exemplary skin deformation curve, 2 mm aperture, 4 sec. suction time,2 sec. relaxation time, 5 repetitions [167].*

Table 9: Cutometer parameters adapted after Dobrev et al., used parameters are highlighted in bold [167].

Cutometer-term	International term	Description of the curve	Meaning	Unit
-	Ue	Straight increase	Immediate (elastic) deformation /dis-tension	mm
-	Uv	Delayed increase	Delayed (viscoelastic) deformation / distension, viscoelasticity or plasticity	mm
-	Ur	Straight decrease	Immediate (elastic) retraction or recov-ery	mm
-	Ua	First min. amplitude at the end of the relaxation phase	Maximum retraction / relaxation	mm
-	Ua – Ur	Delayed decrease	Delayed (viscoelastic) retraction	mm
R0	**Uf (Ue + Uv)**	**First max. amplitude at the end of the suction phase**	**Final deformation or distensibility / firmness**	**mm**
R1	Uf – Ua	Residual value of first max and first min. amplitude	Residual deformation at the end of 1st measuring cycle (resilient distension or recoverability)	mm
R2	**Ua / Uf**	**Ratio of min. amplitude to max. amplitude**	**ratio of total retraction to total defor-mation – gross elasticity or overall visco-elasticity**	**%**
R3	**Uf_x**	**Last max. amplitude at the end of the suction phase**	**Deformation or distensibility after re-peated cycles / firmness after repeated stress (tiring effect)**	**mm**
R4	Ua_x	Last min. amplitude	Recoverability after repeated cycles	mm
R5	**Ur / Ue**	**Ratio of straight decrease to straight increase**	**Net elasticity, without viscous defor-mation**	**%**
R6	**Uv / Ue**	**Ratio of delayed to straight increase**	**Ratio of viscoelastic to elastic disten-tion, indicates the relative contribu-tions of the viscoelastic plus viscous and elastic distension to the total de-formation**	**%**
R7	**Ur / Uf**	**Ratio of straight decrease to first max. amplitude**	**Ratio of immediate (elastic) retraction / recovery to total distensibility (called biological elasticity)**	**%**
R8	**Ua**	**First min. amplitude**	**Total recovery**	**mm**
R9 (R3 – R0)	**Uf_x – Uf**	**Last max. amplitude minus first max. amplitude**	**Skin tiring, hysteresis effect**	**mm**

2.3.4.2.1 Cutometer® measurements and evaluation method

In all three studies mode 1 was used with 450 mbar, 2 seconds tension, 2 seconds relaxation time, 5 repetitions, so the total of one measurement was 20 seconds. The 2 and 4 mm probe was used on the left and right cheek. One measurement was performed on each side. *Figure 13* shows the location of the measurements.

Based on the literature the following important parameters will be evaluated within the three studies. They include skin firmness (R0, Uf; mm), skin gross elasticity (R2, Ua/Uf, %), skin firmness after repeated suction – in this case 5 repetitions (skin tiring, R3, Uf_5, mm), net elasticity (R5, Ur/Ue, %), ratio of viscoelastic to elastic extension (R6, Uv/Ue, %), ratio of elastic recovery to total extension (R7, Ur/Uf, %), total recovery (R8, Ua, mm) and skin tiring (R9, Uf_5 – Uf, mm) [165-167, 173-175].

2.3.4.3 Sonography

For the measurement of skin density and thickness the ultrasound DUB Skin Scanner (Tpm taberna pro medicus) device with a 22 MHz transducer was used. For the evaluation of the sonographic pictures the DUB SkinScanner 5 Software was used.

For skin diagnostics ultrasound devices with a range of 20 MHz to 100 MHz are used and referred to as high-frequency sonography. Within these frequencies cutaneous and subcutaneous structures up to a depth of 0.8 to 1 cm can be visualized [176, 177] . It is a non-invasive and side-effect-free procedure and enables the measurement of skin thickness and skin density while generating a cross-sectional image of the skin. With software-based calculations the skin structures can then be displayed in two or three dimensions [177].

Sonography is widely used for clinical investigations like the determination of skin tumors, the monitoring and treatment of psoriatic inflamed skin or the imaging of skin involvement in systemic sclerotic processes [176, 178-181]. It is also well established for the evaluation of effectiveness of cosmetic products in aesthetic clinical studies. For example to analyze the changes of skin density and thickness and as a consequence the anti-aging effect of injected HA [9, 182, 183]

Ultrasound refers to longitudinal and transversal sound waves with frequencies above the human hearing range, starting at around 16 kHz to 1 GHz [184]. The imaging of ultrasound waves relies on the echo – impulse method, where properties of reflected waves from tissue can be visualized. Based on the knowledge that different tissues reflect ultrasound waves distinctively. Therefore, differences in keratin, collagen or water content can be seen on a sonographic image [185]. Collagen and skin connective tissue have a high echogenicity, water a low echogenicity. This information can be used to draw conclusions about the mechanical properties of the skin, like elasticity and firmness [186].

The DUB® ultrasound device is built of a probe head in which a piezoelectric transducer is controlled by a motor system moving it during the measurement parallel to the skin surface over a distance of 12.8 mm [187]. The generation of ultrasound waves is based on the piezoelectric effect where, in this case, electric energy is transferred into mechanic energy. The transducer is supplied with high-frequency electrical signals from the pulse generator. This leads to an oscillating change in thickness of the piezoelectric crystals within the transducer and the generation of mechanical or acoustic energy (sound waves), respectively [188] The ultrasound signals are emitted and, after reflection of the tissue, received by the transducer. Depending on the depth of penetration and intensity of the reflected echo, one signal can be converted into an amplitude value and visualized as an peak on an oscilloscope (A-scan). As the transducer of the DUB® ultrasound device is moved along the skin surface, several A-scans are acquired and visualized as an sonogram on a monitor in a so called brightness mode (B-mode). The intensity of the echo is visualized in a pseudo color coding, according to its amplitude from 0 to 256 color values, i.e. the higher the amplitude (high echogenicity), the brighter the color. For a loss-free coupling of the transducer to the skin water is used to minimize the impedance before the ultrasound waves reach the skin surface [186, 187, 189]. A typical device with signal transduction and resulting image is shown in *Figure 17.*

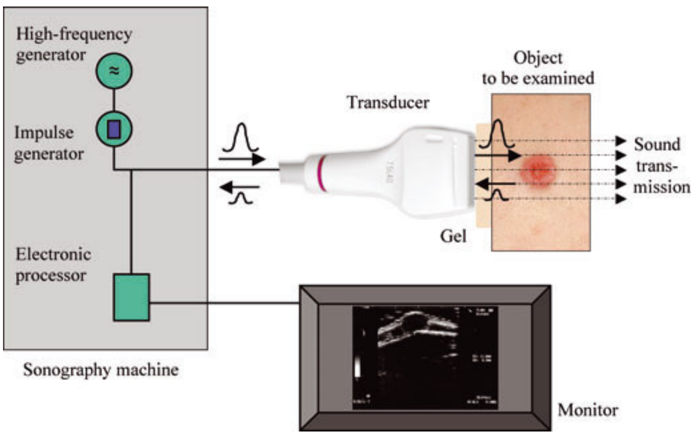


Figure 17: Ultrasound device with signal transduction and image [186].

2.3.4.3.1 Sonography measurements and evaluation method

The DUB – SkinScanner with a 22 MHz transducer, 4 mm penetration depth and 42 dB amplification was used. One measurement on each side of the lower cheek was performed according to the predefined schema shown in *Figure 13 (2.3.4 Biophysical measurements)*. For a standardized measurement the patient has to lay down on an examination table to ensure skin tension is as constant as possible [188].

The transducer is placed perpendicular on the cheeks skin in order to keep the skin tension and impulse entry angle constant. In this position, the probe head is filled with water and the measurement is started. As soon as a clear image is shown on the computer software the measurement can be stopped and the image is saved with the patients study number, visit number and examination area for later evaluation.

For the analyzation of the images the DUB SkinScanner 5.0 software was used. Automatic skin analyzation for skin density (arbitrary units) and thickness (µm) was used for a standardized evaluation of the ultrasound scans. The software uses various algorithms to determine the whole skin as well as epidermis thickness and density parameters. Firstly an automatic phase correction is carried out. The sum A-scan function is activated and the two horizontal measurement lines are positioned at a distance of 10 A-scans, which results in a measurement width of 12,100 µm for each scan [190]. The vertical lines set by the software are checked by the investigator and adjusted manually if necessary. Also the phase correction is set manually in case of interfering signals from, for example, skin peels, hair reflections or dust particles. *Figure 18* shows an evaluated image with manually set phase correction due to interfering signals, shown as bright points left to the red vertical line. Those signals are not part of the evaluation. The skin thickness and density is shown in the grey field in the right corner of the software and exported into excel for further calculations.

One measurement on each side of the lower cheek was performed following the schematic shown in *Figure 13 (2.3.4 Biophysical measurements).*

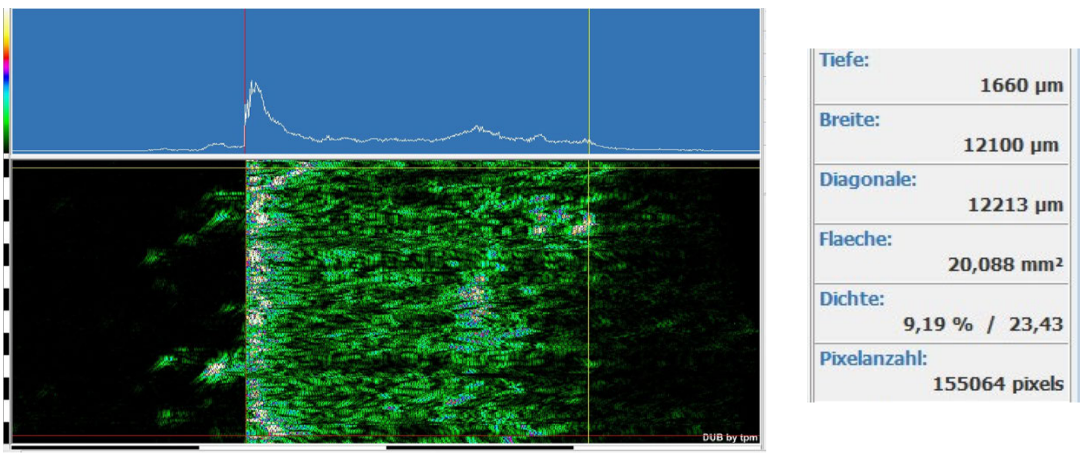


Figure 18: Ultrasound image of ACS in vivo study I, Patient 01, Screening, left side.

2.3.4.4 Phase shift rapid in-vivo measurement of the skin (PRIMOS)

PRIMOS stands for phase shift rapid in-vivo measurement of the skin and is an optical three dimensional (3D) measurement device for determining skin topography (GFMesstechnik GmbH, Teltow, Germany). The PRIMOS device was used in this study to analyze skin surface roughness parameters.

The principle of the optical profilometry is based on the triangulation method which is a geometric method for optical distance measurement. The image visualization is possible via a digital fringe (stripe) projection technique and a camera system. The fringe projector, here a digital micro mirror device (DMD^{TM} by Texas Instruments), casts patterns of parallel stripes onto the skin surface. These stripes (s) are projected to a CCD camera system (charged-coupled device camera) in a triangulation angel and appear at a coordinate system (v) depending on the distance of the object pixel (skin surface). The distance resolution ΔZ is given by a function of the projected pixel sizes of projector and camera and the triangulation angle. The PRIMOS camera is arranged perpendicular to the skin surface and the projection is done at an angle β relative to the perpendicular camera system. For this configuration, Z resolution can be calculated as $\Delta_z = \Delta_x / \tan(\beta)$ (with a lateral camera resolution Δ_x) [191]. The functional principle of the light stream is shown in *Figure 19*.

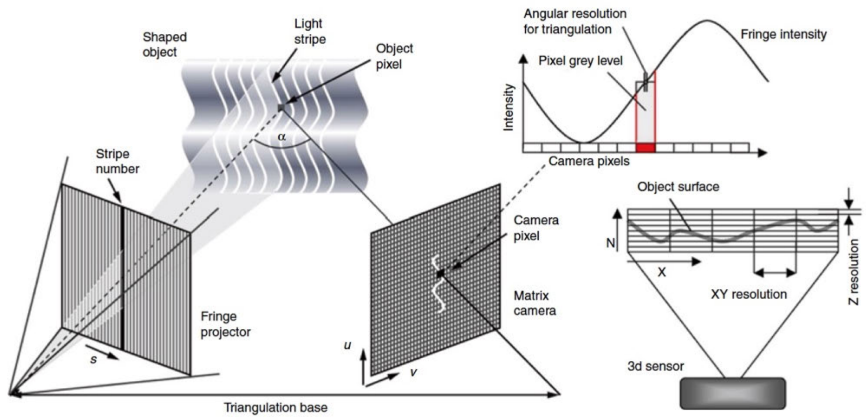


Figure 19: Functional principle of fringe (stripe) projection.
Fringe intensity and resulting pixel gray levels for fringe projection (left), derivation of exact phase values by gray levels (upper right), resulting XY and Z resolutions within the measuring volume (lower right) [191].

The finest differences on the skin's surface deflect the strictly parallel stripes in a distorted angel. This distorted deflection represents a qualitative and quantitative measure of the skin profile. With the information of the projected stripes and its phase shift after reflection, the software calculates a color coded 3D height image [192]. An exemplarily picture with evaluation lines and applied high pass filter is shown in *Figure 21*.

2.3.4.4.1 PRIMOS measurements and evaluation method

The measurement area of the PRIMOS image is shown in *Figure 13 (2.3.4 Biophysical measurements)*. The maximum measurement area is 30 x 40 mm [192]. To enable multiple recordings of the same area, the overlay function of the software was used. A semi-transparent image of the first measurement appears on the screen. The live image can then be placed over the first image. This enables the evaluation of changes of the same skin area [193]. The images were then evaluated using the software PRIMOS 5.6 (GFMesstechnik, Teltow, Germany). All images were adjusted to the first recoding using the automatic matching function to determine the maximum common image section. They were then converted with the robust high-pass filter into a color coded 3D image and the star roughness of 16 circularly arranged lines was calculated. An sample image with the star roughness lines is shown in *Figure 21*. Following Jacobi et al. and Kottner et al. the surface roughness parameters mean roughness (Ra), mean depth of roughness (Rz), maximum roughness (Rmax), height of the greatest profile peak (Rp), number of peaks (PC) and waviness (Wt) were assessed in the first and second ACS in vivo study [194, 195]. They represent DIN ISO norms and are based on calculation of five consecutive equally spaced profile sections (Z1-Z5) of a defined overall length of measurement (Lm) [196]. A schematic profile section is shown in *Figure 20.* Lm in this study was 25 mm. All parameters, but PC, are calculated in µm.

In dermatological terms, Ra is the most important and represents the overall roughness of the surface structure [197, 198]. Rz additionally represents minor furrows and anatomical lines, while Wt indicates deeper furrows, represents the skin’s wave structure and is independent of the roughness parameters [195, 196]. All three parameters decrease in amplitude in case of skin hydration. An increase is associated with skin aging [191, 199].

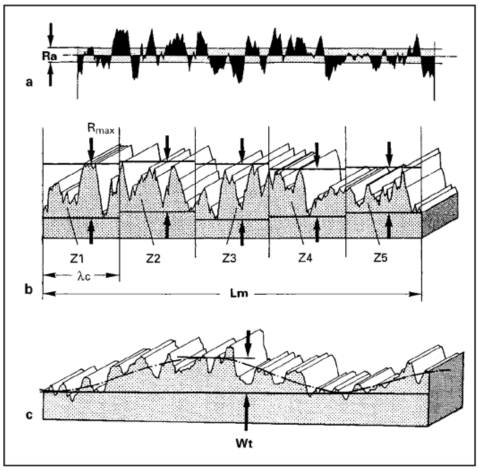


Figure 20: PRIMOS profilometric parameter.
***a** Ra = Mean arithmetic roughness value. **b** Rz = Mean depth of roughness (distance between upper and lower arrow) of five consecutive equally spaced profile sections (Z1–Z5); Rmax = maximum depth of roughness; Lm = overall length of measurement; λc = length of measurement of each section. **c** Wt = Waviness, i.e. height of maximum peak to valley tangents within the profile [196].*

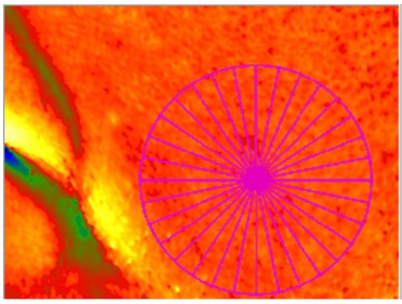


Figure 21: PRIMOS image of ACS in vivo study I, Patient 01, Week 2, left side.

2.3.5 Questionnaires

Different questionnaires were handed out to the patients to evaluate subjective changes after the treatments. Theses questionnaires will not be evaluated within this thesis but are published by Kerscher et al. [200, 201].

2.3.6 Photography

For the documentation of visible changes of the facial skin the Fotofinder system including software (FotoFinder mediscope, FotoFinder Systems GmbH, Bad Birnbach, Germany) was used. With this systems it is possible to generate standardized before/after pictures. The photographs were taken in a room specially equipped for this purpose, with constant lighting and a standardized black background. The camera settings are standardized by the software and thus ensure consistent image quality. A spacer on which the head is positioned ensures that the distance between the subject and the camera remains constant and in the same angel. An transparent image of the first recording is displayed in the recording mode of the software. Through this overlay function an exact positioning of the live image is possible. The Fotofinder System was used for documentation purposes and possible later visualization.

2.3.7 Patients files

A medical record was maintained for each patient. These records include the patient's medical history, all relevant medical events during the studies, medications before and during the studies, and documentation of the treatments and follow-up visits.

2.3.8 Statistical analyses

Data analyses were conducted using descriptive statistics and inferential statistics, by using IBM® SPSS® Statistics for Mac, Version 28.0.1.1 (14) as well as Microsoft® Excel Version 16.64. Microsoft® Excel was used for generating all graphs and diagrams within the result section based on the calculation of the means and SDs.

The in vitro measurements will be described descriptively only due to the small sample sizes. Here the means and SDs will be shown in graphs in the result section of the in vitro part.

For the in vivo results of the clinical studies means and SDs will be visualized in different graphical plots in the results section of the in vivo part. Statistically significant results are based on the differences regarding their mean values and are visualized within the graphs with asterisks. Statistical significance is based on a few elementary principles. The hypothesis testing, normal distribution and a defined p value. There are two complementary hypothesis – the null hypothesis H_0 and the alternative hypothesis H_1. The null hypothesis is true if the observed data do not differ from what would be expected on the basis of chance alone. The counterpart of the null hypothesis is called the alternative hypothesis H_1. The alternative hypothesis assumes that the examined data differ systematically [202].

Since the observed data is usually based on a subset of data in the population, there is always a uncertainty whether the hypotheses are true or not. When performing statistical tests there is always the chance for the wrong decision, meaning that the null hypothesis is rejected even though it is true (false positive – type I error) or it is not rejected even though it is false (false negative – type II error) [203].

The acceptable level of a type I error is labelled by alpha (α), while the acceptable level of a type II error is titled beta (β). Based on the type I error a typical significance level of $\alpha = 0.05$ is used. This means a tolerance of up to 5 % of type I errors, i.e. a 5 % chance of rejecting the null hypothesis even though it is true. The probability value (*p*-value) is the value of the statistic used to test the null hypothesis. If $p < \alpha$ then the null hypothesis is rejected [204].

Rejection of the null hypothesis means that the observed data is not based on chance and in the case of the treatments with ACS and ACS + HA, an systematic and therefore statistical significant effect would occur.

For the evaluation of the three trials the level of significance was set to 5 % ($p < 0.05$) [203]. Statistical overall significance was calculated using the analysis of variance (ANOVA) with repeated measurements with two within-subject factors. The two within-subject factors were the left and right side measurements of the face and the visits. This model analyses the overall effect of the visits, sides and interaction of visits and sides on the dependent variables of the respective measurement parameter.

If an overall significant effect was found within the results of the sides, further analyses were conducted. Therefore, in a next step, the measurement results of the right and left sides were considered separately. For this purpose, the analysis of variance with repeated measurements with only one within-subject factor, the visits, was used. This model examines significant differences between each visit of the respective measurement parameter. In a further post-hoc test significant changes before, during and after the treatments with ACS and ACS + HA can be shown, respectively. Following, a two-sided t-test for paired samples for the right and left side was computed for each visit. In case of the second ACS in vivo study also a t-test for independent variables, to compare the two different treatments with ACS and ACS + HA, was calculated, respectively.

One condition to be able to interpret the analyses of variance appropriately, is the examination of the normality assumption. This was done by checking all data by the normal quantile-quantile plot (Q-Q plot). Besides this graphical method, the Shapiro-Wilk test is often mentioned in the literature for sample size of less than 50 and was therefore analyzed as well [205].

But as the analysis of variance and t-tests are rather robust to violations of normal distribution for sample sizes, at least above 10, this assumption was neglected and is only mentioned for reasons of completeness [203, 206].

Mauchly's test of sphericity was checked but the Greenhouse-Geisser adjustment was used for all repeated measures ANOVA to minimize the type I error [207]. Post hoc tests after Bonferroni were used, as it controls the overall false positivity rate at a set significance level of $\alpha = 0.05$ more conservative than most others [208]. Effect size will be reported considering the classification after Cohen: small effect: 0.01, medium effect size: 0.06 – 0.14, lager effect size > 0.14 [207, 209].

Statistical significance was marked in the graphs with differently colored asterisks (*), so that significant changes between the individual test days and between the treatments can be seen from the graphs (right side: blue, left side: red; in case of combined analyzation: grey; significant difference between the right and left side: grey; significant difference between the treatments of the ACS in vivo study II: grey).

By convention, values $p \leq 0.05$ have been marked with one asterisk (*), values $p \leq 0.01$ with two asterisks (**), and values $p \leq 0.001$ with three asterisks (***) [202].

The aim of the these tests were the determination of statistically relevant differences between the measurement times (visits) and treatments (ACS in vivo study II only).

3 Results

In the following chapters the results of the two in vitro tests and of the three clinical trials will we described. The in vitro and in vivo trials were conducted parallelly between 2016 and 2020. A total of 66 perspective subjects were screened. After inclusion and exclusion criteria were checked and all questions were discussed, informed consent was signed. All 66 screened patients were included in the three studies, of which 57 completed the studies. In detail, 21 (3 dropouts) subjects completed the ACS in vivo study I, 16 (6 dropouts) the ACS in vivo study II and 20 (no dropouts) the ACS in vivo study III study.
For the in vitro measurements ACS probes of 6 subjects were used, two for the first in vitro test and six for the second.

3.1 Results in vitro

The impact of ACS on human dermal fibroblasts was examined in two separate test series in October/November 2017 and June/July 2019 together with the Institute of biochemistry, University of Hamburg.
ACS of six different volunteers was examined. Two samples were used in both tests (aACS and aACS#3 and yACS and yACS#3). The ACS samples were derived from three young and three old volunteers from the ACS in vivo study II and III. In the first series of tests ACS of a 36 and 61 year old volunteer was used, in the second tests series the mean age of the young group was 38 ± 2 (n = 3) and of the old group 55.7 ± 4.6 (n = 3).
Cell viability was examined with an MTT assay after 2, 6 and 24 hours incubation with autologous conditions serum and autologous conditioned serum in combination with hyaluronic acid (4:1). Production of procollagen of the extracellular supernatant and intracellular lysate was measured with a procollagen type 1 C-peptide enzyme immunoassay (PIP EIA) kit after 2, 6 and 24 hours of incubation via ELISA. TGF-ß1, COL1A and ACTB were evaluated via western blot after 24 hours incubation.
For all assays, except for the western blot, duplicates were taken. For the MTT assay of the second test triplicates were taken. Mean and SD was calculated, a further statistical evaluation was not performed due to the small sample sizes.

3.1.1 Results of the MTT assay

The results of the MTT assays, to analyze the fibroblasts cell viability, are shown below in *Figure 22* to *Figure 24*. *Figure 22* shows the results of the first MTT assay with fibroblasts incubated in 10 % ACS and ACS + HA (4:1) solution, respectively. *Figure 23* shows only fibroblasts incubated in 10 % ACS solution of the second in vitro test. *Figure 24* compares the results of the in vitro test I and II, as the samples were from the same patients. The fibroblasts were incubated with the different solutions for 2, 6 and 24 hours. The results are shown in percent with SDs, normalized to 10 % FCS solution as 100 % reference.

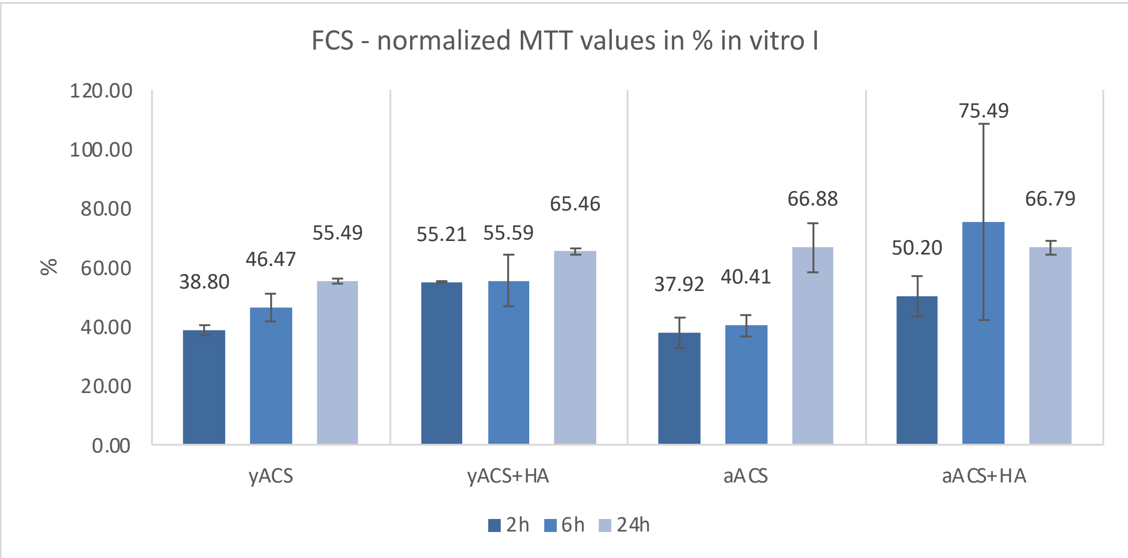


Figure 22: MTT results of the in vitro test I.
Results of fibroblasts activity in percent calculated to FCS as 100 % standard after 2, 6 and 24 hours incubation with ACS and ACS+HA solutions of a 36 and 61 year old volunteer, respectively; FCS= fetal calve serum, yACS = young autologous conditioned serum, aACS = aged autologous conditioned serum, HA = hyaluronic acid.

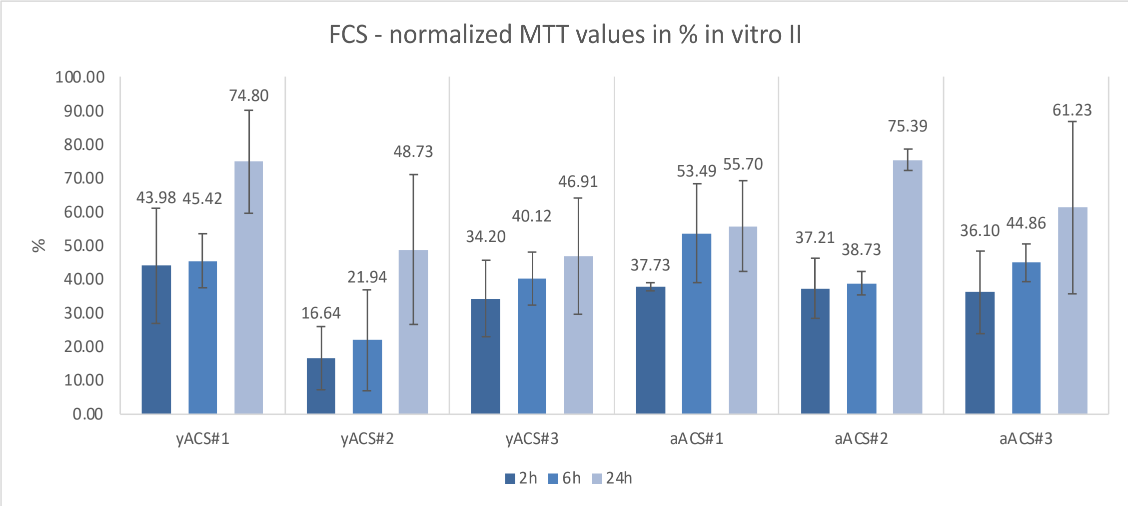


Figure 23: MTT results of the in vitro test II.
Results of fibroblasts activity in percent calculated to FCS as 100 % standard after 2, 6 and 24 hours incubation with young and aged ACS solutions of six different volunteers, mean values with SD; FCS= fetal calve serum, yACS = young autologous conditioned serum, aACS = aged autologous conditioned serum.

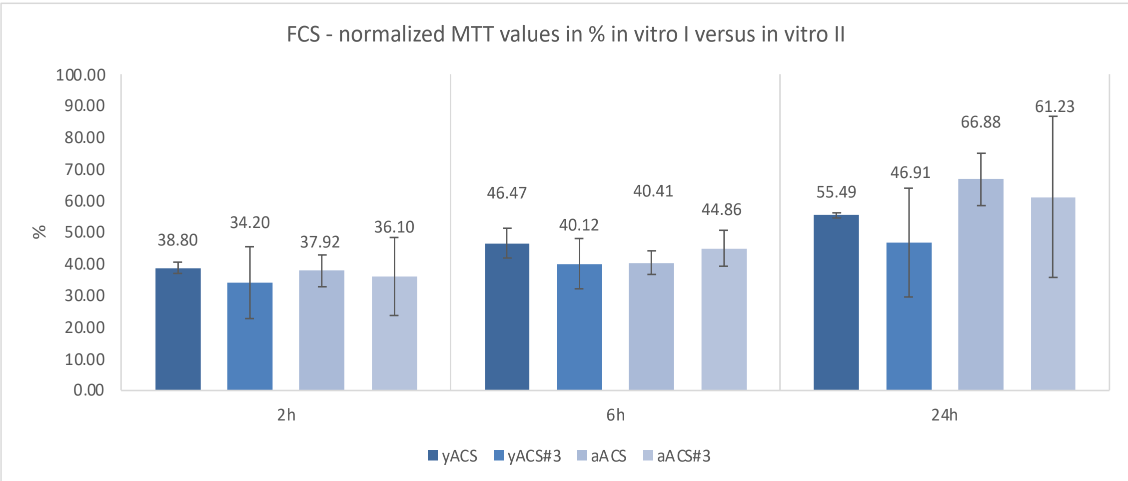


Figure 24: MTT results of the in vitro test I versus in vitro II.
Results of fibroblasts activity in percent calculated to FCS as 100 % standard after 2, 6 and 24 hours incubation with ACS. Comparison of the first (yACS, aACS) and second (yACS#3, aACS#3) test series from the same volunteers; FCS= fetal calve serum, yACS = young autologous conditioned serum, aACS = aged autologous conditioned serum.

The results of the first in vitro test (*Figure 22*) showed an steady increase, except for the sample with ACS + HA of the 61 year old patient (aACS + HA). This sample started with a viability of 50.2 ± 6.76 % and showed the highest value after 6 hours of incubation albeit with a high SD (75.49 ± 33.15 %). After 24 hours incubation with ACS + HA the value decreased to 66.79 ± 2.4 %. The other samples started with a cell viability of 38.80 ± 1.85 % (yACS), 37.92 ± 4.99 % (aACS) and 55.21 ± 0.34 % (yACS + HA), all compared to the incubation with FCS. The final cell activity after 24 hours incubation was 55.49 ± 0.88 % (yACS), 66.88 ± 8.26 % (aACS) and 65.46 ± 0.99 % (yACS + HA). The highest overall increase in cell viability with 28.96 % was seen in the sample of the 61 year old (aACS). The sample with additional HA increased by 16.60 %, discounting the high value after 6 hours incubation. The sample of the 36 year old increased by 16.68 % for just ACS and 10.26 % for ACS in combination with HA.

The second in vitro test also showed increasing cell viability with continuous incubation (*Figure 23*). Due to a high value of the aACS#1 sample after 2 hours incubation (176.40 %) the 2 hour results only consider the mean and SD of two out of the triplicates. All samples, except the 40 year old (yACS#2: 16.64 ± 9.44 %), started with values above 30 %. The greatest increase in cell viability compared to FCS was seen in the sample of the 53 year old (aACS#2), with an increase of 38.18 % from 37.21 ± 8.99 % to 75.39 ± 3.31 % cell viability. The sample of the 36 year old patient (yACS#3) showed the slightest increase with 12.70 %, starting with 34.20 ± 11.38 % cell viability after 2 hours and increasing to 46.91 ± 17.22 % after 24 hours incubation.

yACS (36 years old sample) and aACS (61 years old sample) of the first in vitro test and yACS#3 and aACS#3 of the second in vitro test were from the same patients and therefore compared in *Figure 24*. The repeated use after two years storage below - 18 °C showed similar results. As described above the sample with the young ACS started with 38.80 ± 1.85 % (yACS) and 34.20 ± 11.38 (yACS#3) cell viability after 2 hours incubation and increased to 55.49 ± 0.88 % (yACS) and 46.91 ± 17.22 % (yACS#3) metabolic activity after 24 hours incubation with ACS, respectively. The ACS sample of the old volunteer showed similar results, with 37.92 ± 4.99 % (aACS) and 36.10 ± 12.29 % (aACS#3) after 2 hours up to 66.88 ± 8.26 % (aACS) and 61.23 ± 25.64 % (aACS#3) metabolic activity after 24 hours incubation, respectively. All values were similar and showed a comparable trend, which can be determined via the mean difference of all four samples over incubation time together with just 5.24 ± 2.25 % variation.

3.1.2 Results of the procollagen type 1 C-peptide enzyme immunoassay (PIP EIA)

The concentration of procollagen C1 peptide after 2, 6 and 24 hours incubation of human dermal fibroblast with ACS or ACS + HA was measured with an PIP assay kit by Takara Bio Inc. via ELISA. The results are shown in *Figure 25* and *Figure 26.* Duplicates were taken of each sample, the values are presented in mean ng/ml ± SD of the mean.

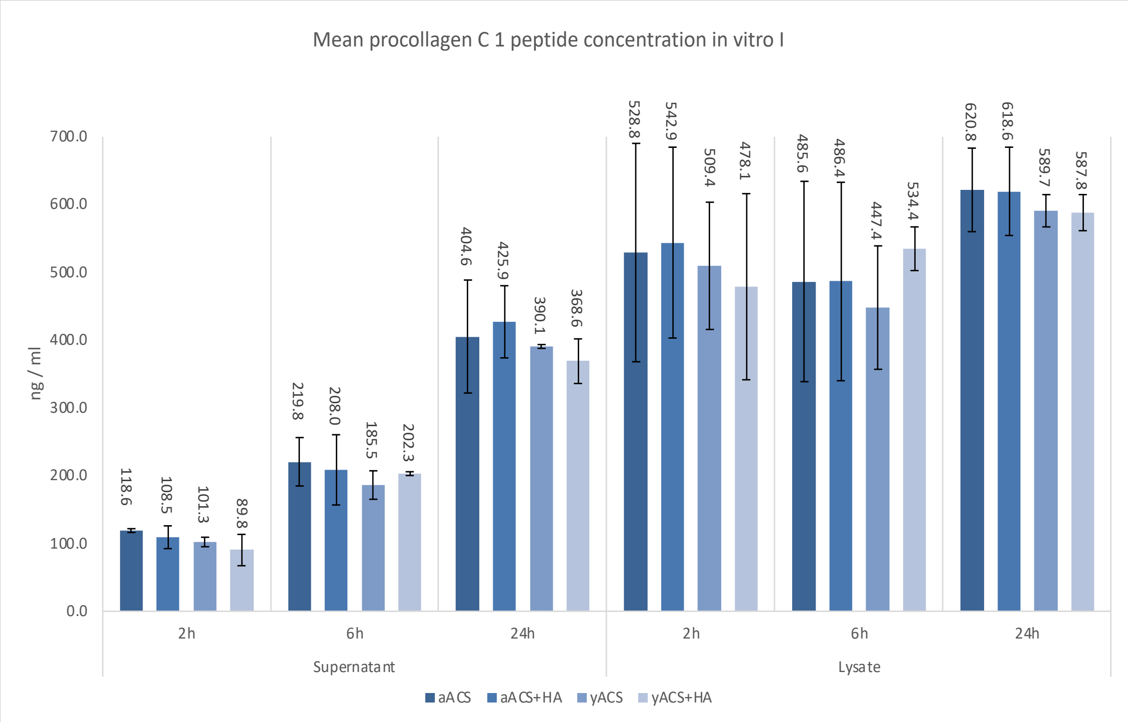


Figure 25: Procollagen C 1 Peptide concentration of the in vitro I test.
Results of fibroblasts procollagen C1 peptide production after 2, 6 and 24 hours incubation with ACS and ACS+HA solutions of a 36 and 61 year old volunteer, respectively; mean and SD of duplicates, yACS = young autologous conditioned serum, aACS = aged autologous conditioned serum, HA = hyaluronic acid.

Figure 25 illustrates the procollagen production of the incubated fibroblasts of the first in vitro test. The measured concentrations of the extracellular supernatant increased with incubation time, while the concentrations of the intracellular lysates remained consistent at around 535.8 ± 57.7 ng/ml. The extracellular medium of all samples showed approximately doubling after 6 hours and quadrupling after 24 hours of incubation. The samples of the 61 year old (aACS and aACS + HA) showed continuously higher values compared to the samples of the 36 year old (yACS and yACS + HA).

Within the extracellular medium, aACS had the highest start concentration of 118.6 ± 2.9 ng/ml after 2 hours and increased to 404.6 ± 83.5 ng/ml after 24 hours incubation, which indicates an increase of 3.4 times. aACS + HA showed a concentration of 108.5 ± 17.3 ng/ml after 2 hours and showed the highest concentration of all samples after 24 hours (425.9 ± 53.4 ng/ml, 3.9 times increase). The highest 4.1 times increase over 24 hours was found in the young women's ACS + HA sample (increase from 89.9 ± 23.3 ng/ml at 2 hours to 368.6 ± 2.7 ng/ml after 24 hours incubation).

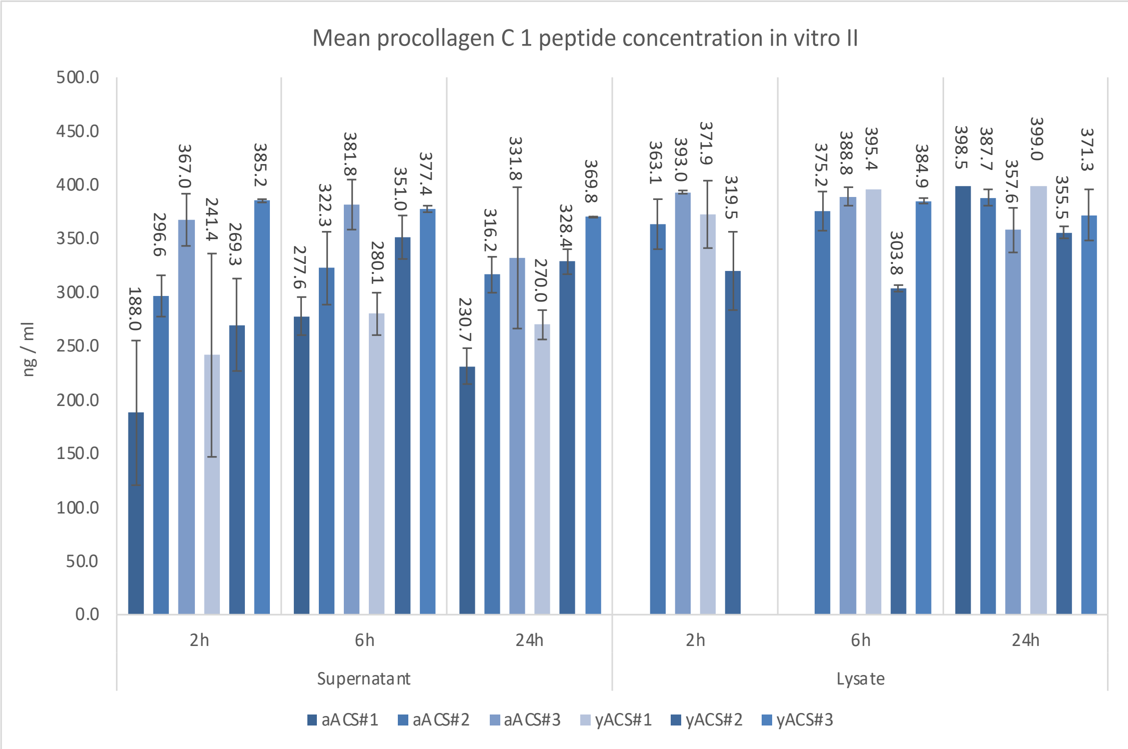


Figure 26: Procollagen C 1 Peptide concentration of the in vitro II test.
Results of fibroblasts procollagen C1 peptide production after 2, 6 and 24 hours incubation with ACS and ACS+HA solutions of three young and three old volunteers, respectively; mean and SD of duplicates, yACS = young autologous conditioned serum, aACS = aged autologous conditioned serum, HA = hyaluronic acid. Due to missing data no values for aACS#1 at 2 and 6 hours, yACS#3 at 2 hours and no SD for yACS#1 at 6 and 24 hours and aACS#1 at 24 hours.

In *Figure 26* the results of the PIP assay of the second in vitro test are shown. Both values of the intracellular lysates of the aACS#1 at 2 and 6 hours, yACS#3 at 2 hours and one value for yACS#1 at 6 and 24 hours and aACS#1 at 24 hours were outside the measurable range of the spectrophotometer and could not be evaluated.

All other values showed similar PIP concentrations, varying between the lowest 188.0 ± 67.4 ng/ml for the 53 year old (aACS#1) after 2 hours and highest 399.0 ng/ml for the 36 year old after 24 hours (yACS#3, no SD due to missing data). There was no obvious increase over incubation time or difference between the young and old ACS samples.

3.1.3 Results of the TGF-ß and COL 1A western blot measurements

The amount of TGF-ß1 and collagen type I (COL1A) in the cells (lysate) and extracellular medium (supernatant) were measured by western blot after 24 hours incubation time. The lysates were also stained against beta-actin (ACTB) as loading control. The western blot images are shown below in *Figure 27.*

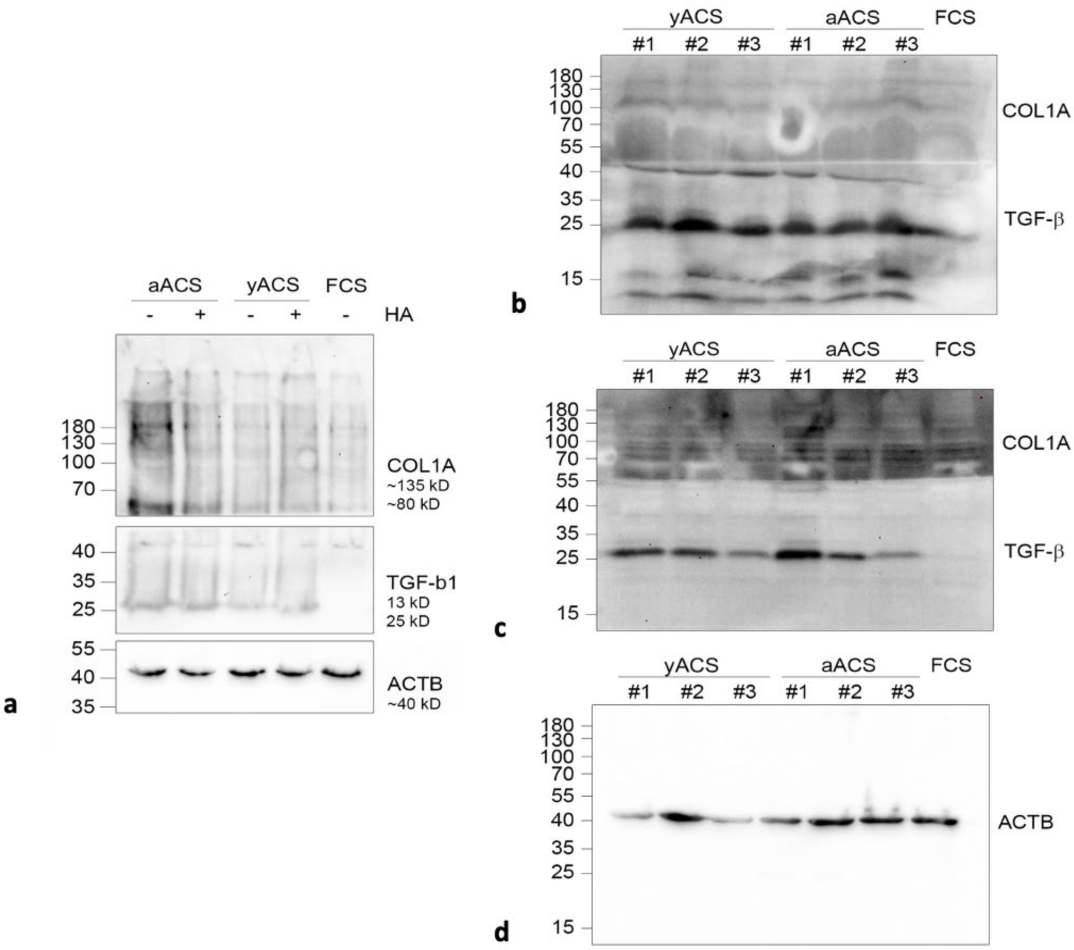


Figure 27: Western blot of the in vitro I and II tests.
In vitro I lysate only ***(a)****, in vitro II* ***(b-d)****; extracellular supernatant* ***(b)****, intracellular lysate* ***(c-d)****.*

Western blot of the first in vitro test was from the cell lysates only (*Figure 27a*). ACTB, as loading control, showed about the same intensity for all samples, indicating that the protein concentration for all samples was about the same. TGF-ß1, at 25 kDa, was detected in all samples incubated with ACS but not in the FCS incubated fibroblasts. The samples of the 61 year old (aACS and aACS + HA) showed, due to a more intensified band, a greater TGF-ß1 concentration than the samples of the 36 year old (yACS and yACS + HA). Collagen type 1 could not clearly be detected as the bandings did not fit the molecular weight of collagen type 1 precursor at 130 – 140 kDa or of mature collagen type 1 at 70-90 kDa. The combined incubation of ACS + HA showed no difference in signal intensity compared to the ACS incubated samples. Overall, the sample of the 61 year old (aACS) showed the strongest signals. Due to the generally rather weak signals no quantification was carried out.

The second western blot of the in vitro test II was carried out with cell supernatant (*Figure 27 b*) and lysate (*Figure 27 c-d)* and showed similar results to the in vitro I tests. It was found that TGF-ß was only present in the lysates and supernatant of the ACS incubated fibroblasts but not the FCS incubated fibroblast. The extracellular supernatant showed no obvious difference for TGF-ß between young and aged ACS. COL1A and ACTB were present in all samples. Again, the COL1A banding could not clearly be detected as the bandings did not fit the molecular weight of collagen.

3.2 Results in vivo

To evaluate the clinical effects of ACS on facial skin parameters, three clinical trials were conducted at the University of Hamburg, Institute of cosmetic sciences. Distinctive biophysical measurement method were used to examine the facial skin. The results will be described in separate sections, starting with the first ACS in vivo study, followed by the second and third ACS in vivo study. The first screening visit, was identical for all three studies. The patients were informed about the study process, read the patients information and signed the informed consent. Inclusion and exclusion criteria were verified, demographic data, medical history, and physical examinations were recorded.
For the statistical analysis, data was checked for normal distribution using the Shapiro-Wilk test. The data was further checked by the normal Q-Q plots. Although some (minor) violations of the assumption of normality were observed, a ANOVA with repeated measurements was conducted due to robustness of the ANOVA to violation of the normality assumption [206, 210].

3.2.1 ACS in vivo study I

In the first ACS in vivo study 24 female patients were enrolled. There were three dropouts during the study period. Those patients provided personal and time management reasons. Therefore, the data of 21 female patients was analyzed. In total, the patients attended seven visits: screening (with measurements), day one (first treatment – just treatment, no measurements), week 2 (measurements and second treatment), week 4 (measurements and third treatment), week 8 (measurements), week 12 (measurements and treatment, patient 9 and 14 declined additional treatment) and week 24 (measurements).
Skin condition, hydration, mechanical properties of the skin, skin density and thickness, and skin topography were evaluated.

Some parameters of the Cutometer® measurements, sonography, and PRIMOS measurements showed significant differences between the sides and the interaction of sides and visits in the combined analysis of variance. Hence, a separate repeated measures ANOVA for the right and left side for each parameter of those devices was calculated, followed by t-tests to evaluate differences between the sides (compare *2.3.8 Statistical analyses*).

3.2.1.1 Age and skin condition

The age ranged from 35 to 55 years with a mean age of 46.75 ± 5.87 years. Skin condition was rated as not sensitive by 67 % of the patients and as sensitive by 33 %. 14 % of the patients characterized their skin as being oily, 38 % as normal and 48 % as dry. The results are visualized in *Figure 28.*

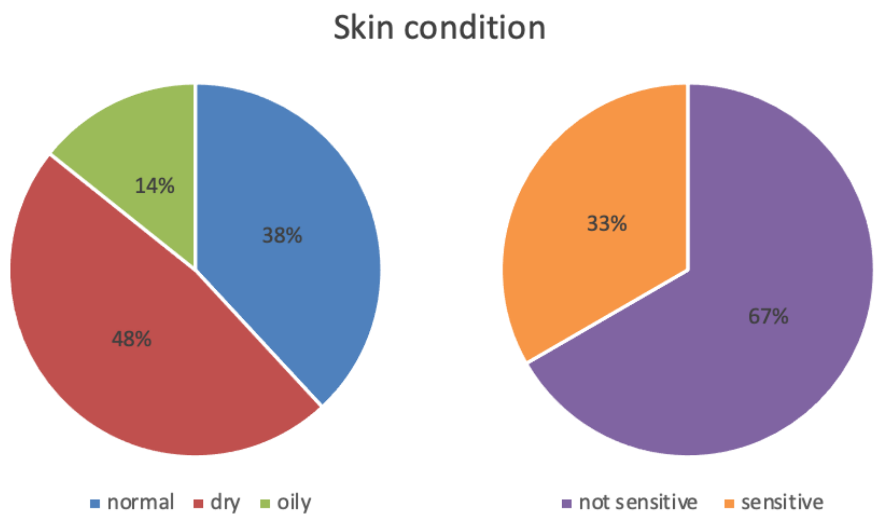


Figure 28: Skin condition ACS in vivo study I, n = 21.

3.2.1.2 Skin hydration

Skin hydration was measured at each visit on both sides three times. The mean of these measurements was calculated for each patient. Means and SDs were then calculated from all 21 patients and are shown in *Figure 29*. After Heinrich et al. skin hydration measurements with the Corneometer® CM 825 can be categorized to very dry skin (< 30 a.u.), dry skin (30 – 40 a.u.), and normal skin hydration (> 40 a.u.) [162].

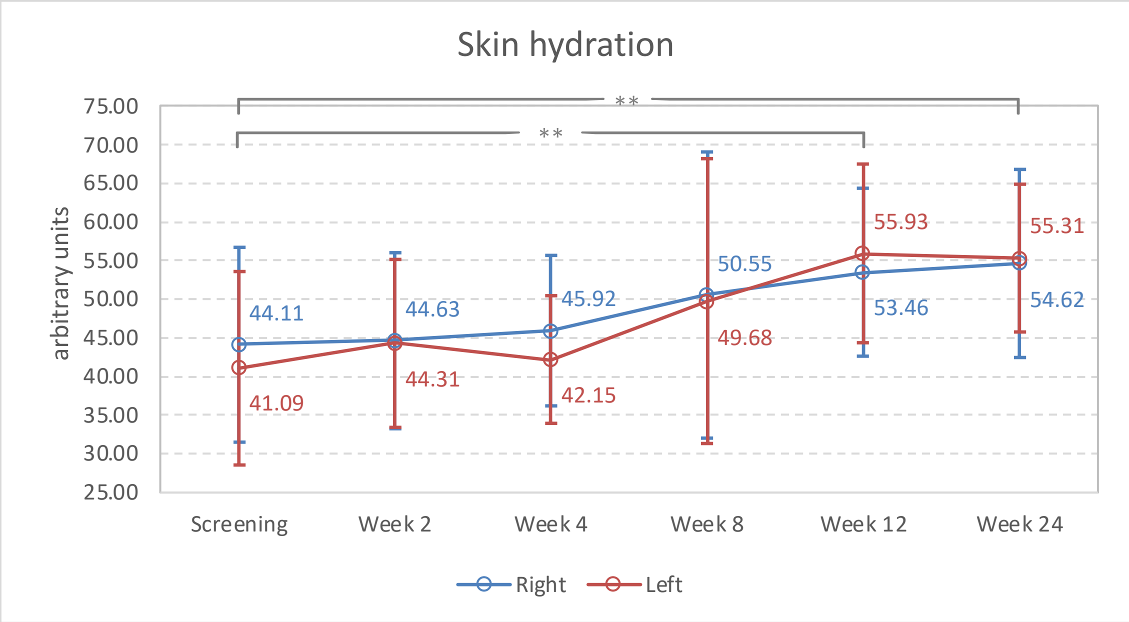


*Figure 29: Skin hydration ACS in vivo study I, n 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Skin hydration increased on both sides from below 45 a.u. to around 55 a.u. There was a statistically significant difference between the visits, F(3.374, 67.487) = 6.551, p < 0.001, partial η^2 = 0.247, but no overall difference between the right and left side, F(1.000, 20.000) = 40.507, p = 0.479, partial η^2 = 0.025. Neither was the interaction of the sides and visits statistically significant, F(3.817, 76.348) = 1.688, p = 0.164, partial η^2 = 0.078. Therefore, the Bonferroni-adjusted post-hoc results included the mean of both sides together. The mean skin hydration increase was with 28.40 % (p = 0.010) after 12 weeks and 29.04 % (p = 0.009) after 24 weeks significantly higher compared to the screening values (*Figure 29*). Also, week 2 compared to week 12 (p = 0.024, + 23.00 %), and week 4 compared to week 12 (p = 0.007, + 24.20 %) and week 24 (p = 0.02, + 24.83 %) showed statistically significant higher values (not visualized in the graph). According to Cohen the skin hydration increase showed a large effect size (partial η^2 = 0.247) [209].

3.2.1.3 Cutometry

Skin mechanical properties were measured with the 2 mm and 4 mm Cutometer® Dual MPA 580 (Courage & Khazaka electronic GmbH, Cologne) probe on each side of the face for one time in modus 1 (compare *2.3.4.2.1. Cutometer® measurements and evaluation* method).

3.2.1.3.1 Skin firmness (R0, Uf)

The maximum amplitude R0 (Uf = Ue + Uv) of the cutometer curve describes the maximum expansion of the skin during the suction phase and gives information about the skin's firmness. The lower the amplitude, the firmer or less distensible the skin [168].

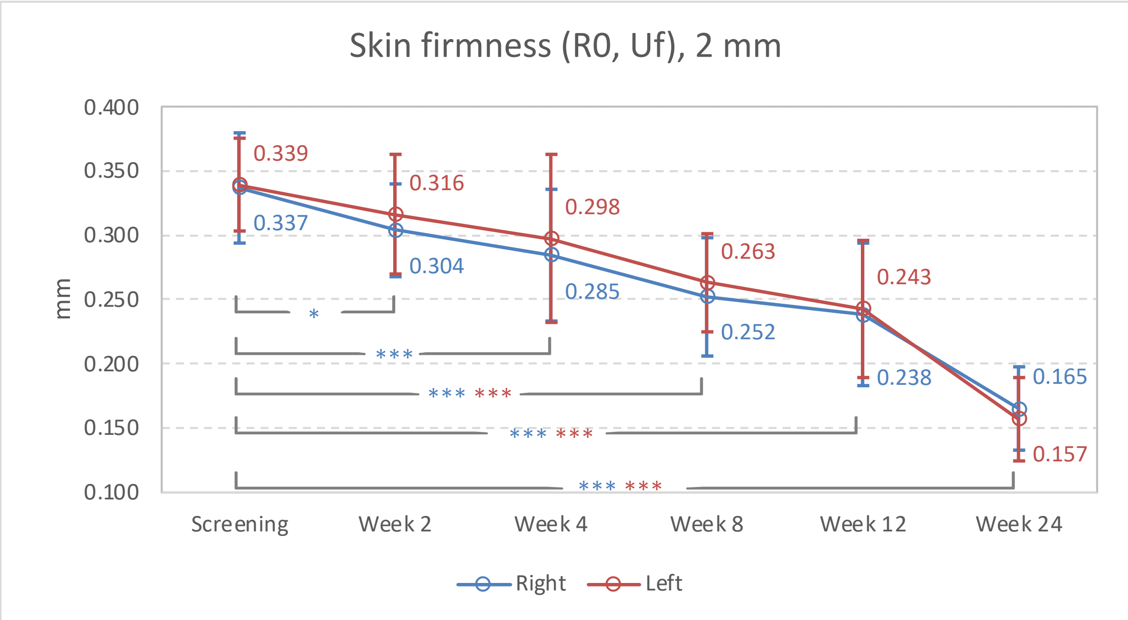


*Figure 30: Skin firmness, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

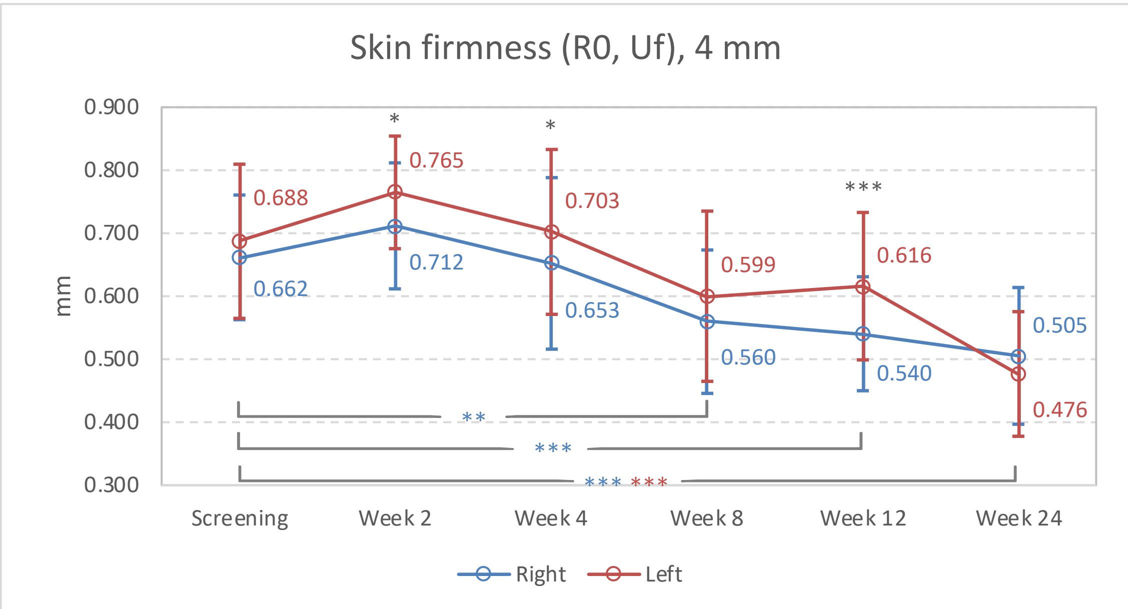


*Figure 31: Skin firmness, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

For the 2 mm probe, mean skin firmness was statistically significant different between the visits for the right side, $F(4.048, 80.959) = 55.389$, $p < 0.001$, partial $\eta^2 = 0.735$ and for the left side,

$F(3.597, 71.937) = 56.555$, $p < 0.001$, partial $\eta^2 = 0.739$. Significant results were seen comparing the screening visit with all following visits, shown in *Figure 30*. For the right side, all visits showed significantly lowers values compared to screening, with increasing difference (week 2: p = 0.019, – 9.80 %; week 4: – 15.62 %, week 8: – 25.18 %, week 12: – 29.37 %, and week 24: – 51.06 %, all showed a significance of p < 0.001). For the left side, week 2 (p = 0.812, – 6.82 %) and week 4 (p = 0.126, – 12.29 %) were insignificantly lower. Week 8, 12 and 24 were significantly different compared to screening p < 0.001 (– 22.46 %, – 28.42 % and – 53.74 %). Left and right side mean values and SDs were comparable at each visit, which was confirmed by the insignificant paired samples t-tests (results in appendix). Taken together, the skin distensibility continuously decreased from 0.338 mm to 0.161 mm (mean of both sides). In other words skin firmness increased by over 50 %, comparing the screening values with week 24 (51.06 % right side, 53.74 % left side). The effect size was large (partial $\eta^2 = 0.735$ right side, $\eta^2 = 0.739$ left side).

The results of the 4 mm probe showed a different course compared to the 2 mm probe (*Figure 31)*. There was also a statistically significant difference between the visits for the right side, $F(3.438, 68.758) = 23.803$, $p < 0.001$, partial $\eta^2 = 0.543$ and the left side, $F(3.723, 74.464) = 28.755$, $p < 0.001$, partial $\eta^2 = 0.590$. Like the measurements of the 2 mm probe, both sides showed a large effect size. But the left side measurements were significantly higher compared to the right side in week 2 (p = 0.033), week 4 (p = 0.041), and week 12 (p < 0.001). However, they had a similar trend and showed a final significant increase in skin firmness of 23.68 % for the right side (p < 0.001) and 30.75 % for the left side (p < 0.001) in week 24, compared to the baseline measurements during the screening visit. Conversely, two weeks after the first treatment (week 2), there was an insignificant 7.58 % (p = 0.889) and 11.29 % (p = 0.089) decrease of skin firmness for the right and left side, respectively. After week 2, skin firmness increased again and was significant in week 8 (p = 0.004, – 15.34 %) and 12 (p < 0.001, – 18.34 %) for the right side only, in week 24 for both sides accordingly (p < 0.001).

3.2.1.3.2 Skin firmness after repeated suction (R3, Uf_5)

The last maximum amplitude R3 (Uf_5) is the last maximum amplitude of the suction phase after 5 repetitions of one measurement cycle. It gives information about the skin firmness and skin tiring effects. The closer it is to R0, the lower the tiring effect [168].

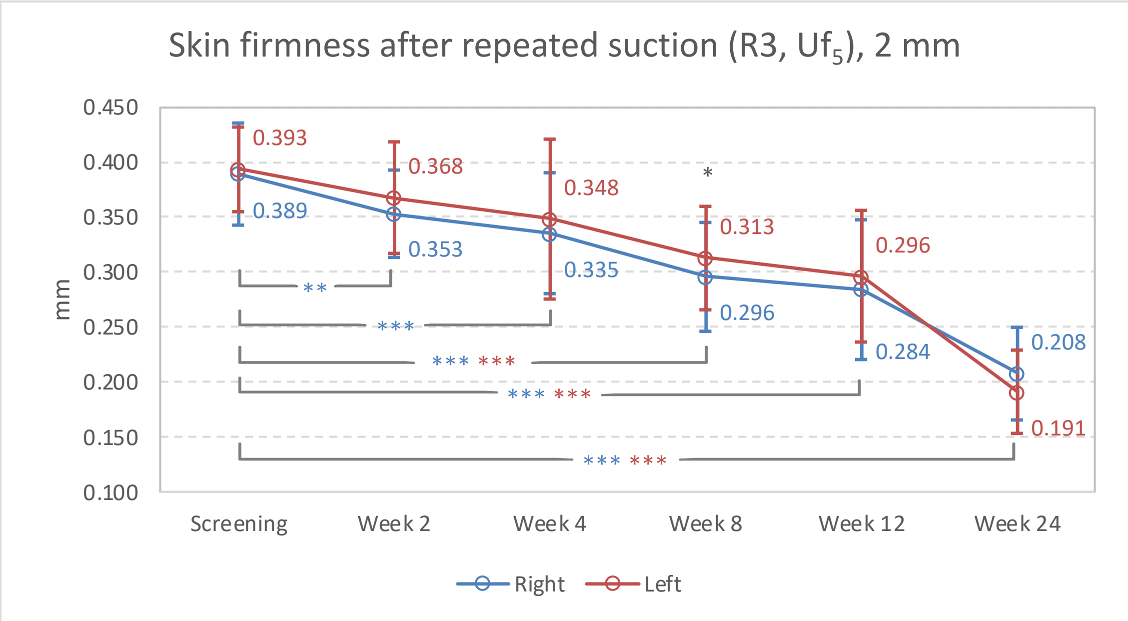


*Figure 32: Skin firmness after repeated suction, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0..05, ** p≤0.01, *** p≤0.001.*

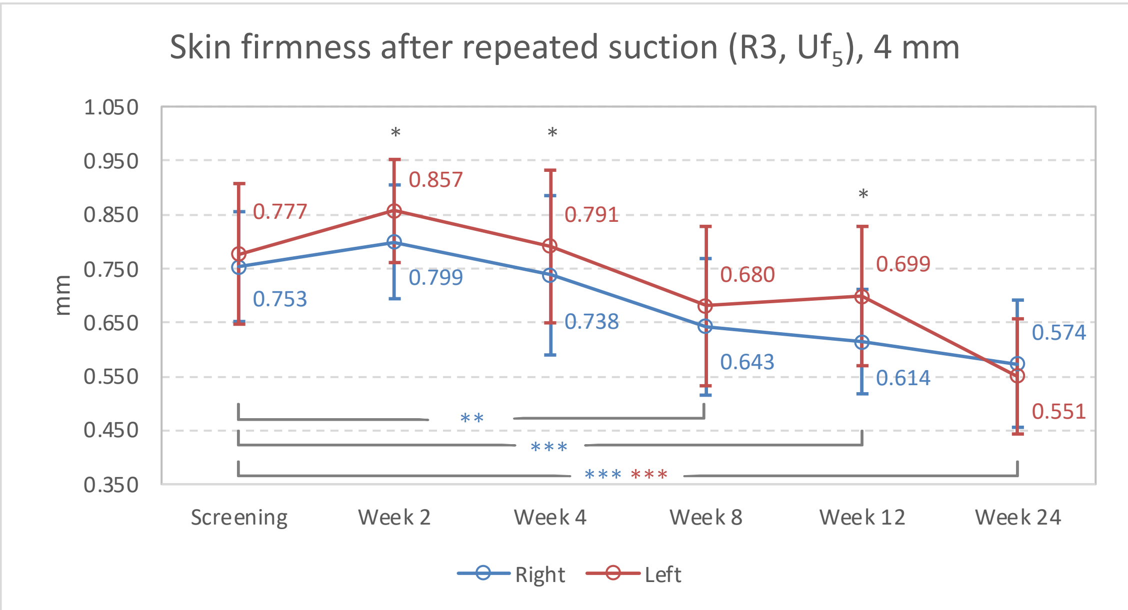


*Figure 33: Skin firmness after repeated suction, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The development of the skin firmness after repeated suction (R3, Uf_5) was for both probe sizes similar compared to skin firmness (R0, Uf), and they showed similar significant results.

For the 2 mm probe, the mean values were statistically significant different between the visits for the right side, $F(4.078, 81.556) = 55.691$, $p < 0.001$, partial $\eta^2 = 0.736$ and for the left side, $F(3.276, 65.516) = 58.727$, $p < 0.001$, partial $\eta^2 = 0.746$ (*Figure 32*).

The values decreased from 0.39 mm to 0.20 mm (mean of both sides). Therefore, skin firmness after repeated suction significantly increased by 46.58 % ($p < 0.001$) for the right side and by 51.56 % ($p < 0.001$) for the left side, comparing baseline values form the screening visit with week 24. The right side showed a significant increase for all visits compared to screening (week 2: p = 0.010, – 9.37 %; week 4, 8, 12, 24: $p < 0.001$, – 13.90 %, – 23.94 %, – 26.96 % and – 46.58 %). The left side was significantly increased in week 8, 12 and 24 ($p < 0.001$, – 20.42 %, – 24.78 % and – 51.56 %). The effect size was large and approximately the same compared to skin firmness (R0, Uf). There was a significant difference between the right and left side in week 8 (p = 0.049).

The statistical analyzation of the 4 mm probe measurements also showed statistically significant differences between the visits for the right side, $F(3.452, 69.039) = 24.785$, $p < 0.001$, partial $\eta^2 = 0.553$ and for the left side, $F(3.674, 73.486) = 26.638$, $p < 0.001$, partial $\eta^2 = 0.571$ (*Figure 33*). The left side measurements were significantly higher compared to the right side in week 2 (p = 0.031), week 4 (p = 0.035), and week 12 ($p < 0.001$). However, they had a similar trend and showed a final significant increase in skin firmness after repeated suction of 23.78 % ($p < 0.001$) for the right side and 29.07 % ($p < 0.001$) for the left side in week 24, compared to the screening visit. Like the skin firmness (R0, Uf) measurements, there was an insignificant 6.08 % (p = 1.000) and 10.27 % (p = 0.133) decrease of for the right and left side in week 2, respectively. From week 2 onwards skin firmness after repeated suction increased again and was significantly different in week 8 (p = 0.006, – 14.73 %) and 12 ($p < 0.001$, – 18.57 %) for the right side, in week 24 for both sides accordingly ($p < 0.001$). Like the measurements of the 2 mm probe and the skin firmness (R0,Uf), both sides had a large effect size.

3.2.1.3.3 Skin tiring (R9, Uf_5 – Uf)

The difference of the last maximum amplitude and the first maximum amplitude is described as skin tiring R9 (Uf_5 – Uf). The smaller the value, the smaller the tiring effect. R9 visualizes therefore the difference between R0 (Uf) and R3 (Uf_5) [168].

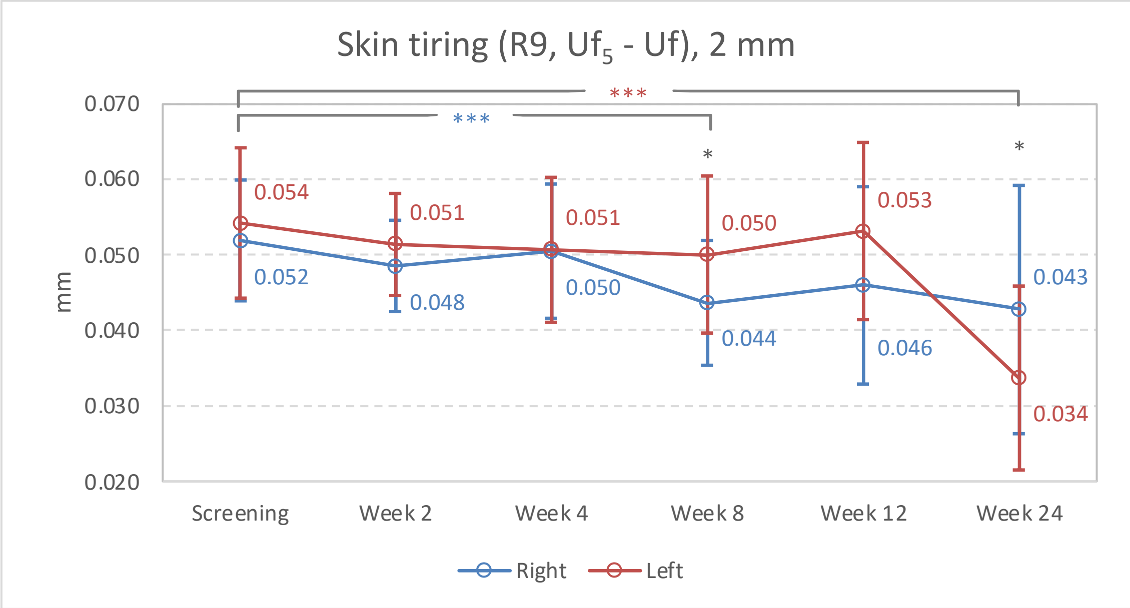


*Figure 34: Skin tiring, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

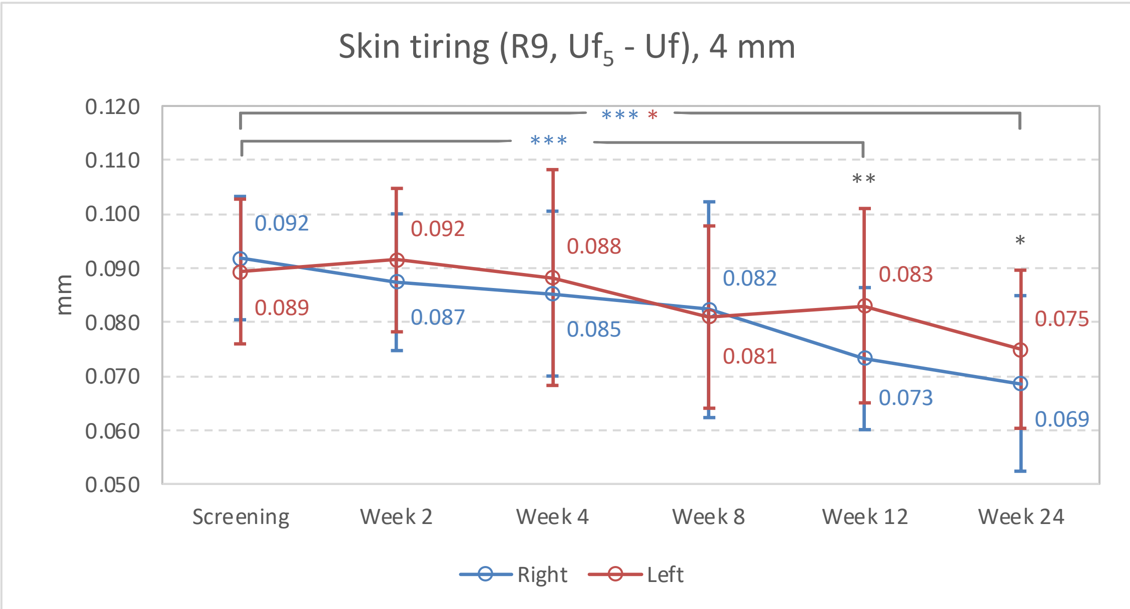


*Figure 35: Skin tiring, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Skin tiring decreased, in parts significantly, for the 2 mm probe and the 4 mm probe. For the 2 mm probe measurements, the repeated measures ANOVA determined a statistically significant difference between the visits for the right side, $F(2.837, 56.730) = 4.282$, $p = 0.010$, partial $\eta^2 = 0.176$ and for the left side, $F(3.862, 77.244) = 18.230$, $p < 0.001$, partial $\eta^2 = 0.477$. Bonferroni-adjusted post-hoc analysis revealed significantly higher values for all visits of the left side compared to week 24 ($p < 0.001$), but due to distinctness in the graph they were not visualized. Screening compared to week 24 showed the strongest decrease with – 37.91 % ($p < 0.001$).

The right side was only significantly lower in week 8 ($p < 0.001$, – 15.89 %) compared to screening, but showed the strongest decrease compared to screening in week 24, with – 17.45 % ($p = 0.202$). A significant difference between the right and left side was found in week 8 ($p = 0.008$) and week 24 ($p = 0.003$) (*Figure 34*).

Figure 35 shows the results of the 4 mm probe. Here, the statistical analysis also revealed a significant difference between the visits for the right side, $F(4.058, 81.155) = 9.980$, $p < 0.001$, partial $\eta^2 = 0.333$ and for the left side, $F(3.826, 76.525) = 4.159$, $p = 0.005$, partial $\eta^2 = 0.173$. The effect size was large. The highest significant difference was between screening and week 24 with – 25.26 % ($p < 0.001$). Week 12 showed a significant decrease of – 20.18 % ($p < 0.001$) compared to screening. The left side was only significantly lower in week 24 with – 16.14 % ($p = 0.024$) compared to screening. With 0.010 mm and 0.006 mm difference, the left side was significantly higher compared to the right side in week 12 and 24 ($p = 0.006$ and $p = 0.038$), respectively.

3.2.1.3.4 Skin recovery (R8, Ua)

The first minimum amplitude R8 (Ua) shows the maximum recovery of the skin during the relaxation phase and allows conclusions about the recovery ability of the skin. The closer the value is to 0, the higher the skin's recovery ability [168]. Skin recovery (R8, Ua) is, like the skin firmness (R0, Uf), an absolute parameter and consists of an elastic and viscoelastic part.

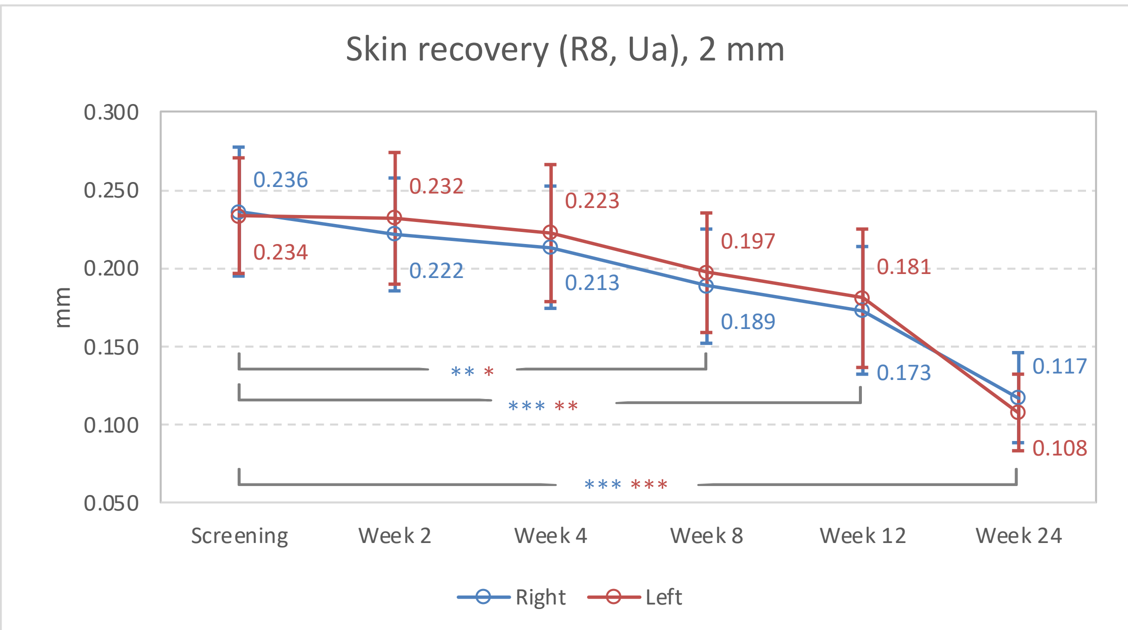


*Figure 36: Skin recovery, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

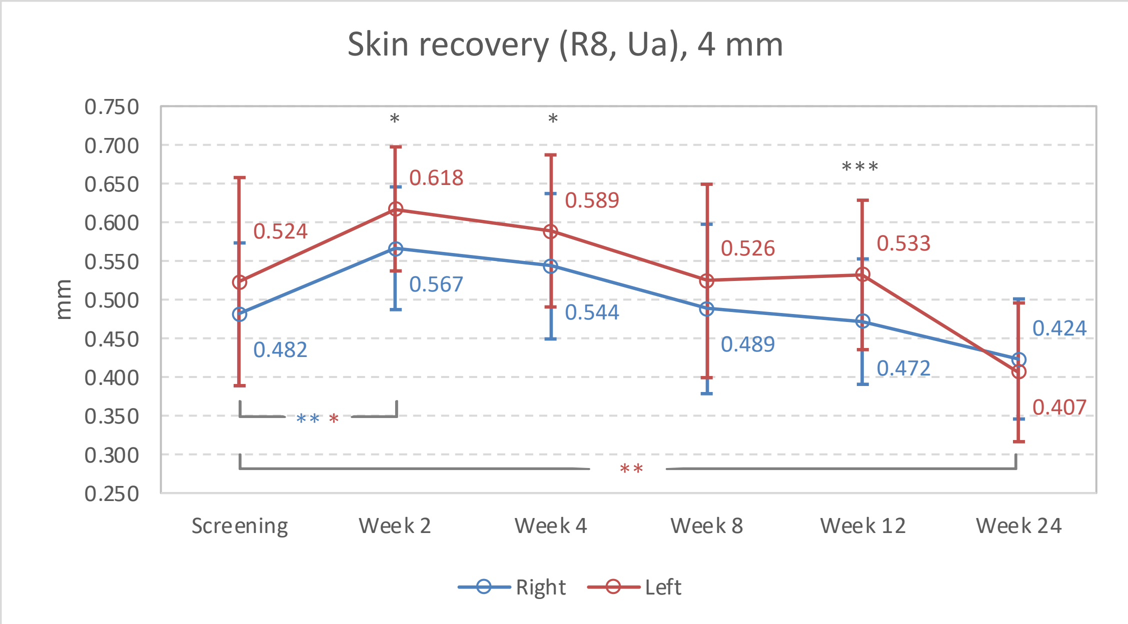


*Figure 37: Skin recovery, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0,05, ** p≤0,01, *** p≤0,001.*

Skin recovery (R8, Ua) showed the same development like the skin firmness (R0, Uf) for both probe sizes and sides. For the 2 mm probe, skin recovery values continuously decreased from 0.235 mm to 0.112 mm for both sides (average of both sides) (*Figure 36*). In other words skin recovery increased by over 50 %, comparing screening with week 24 (50.47 % right side, 53.89 % left side). The mean skin recovery was statistically significant different between the visits for the right side, $F(4.158, 83.168) = 35.626$, $p < 0.001$, partial $\eta^2 = 0.640$ and for the left side, $F(3.806, 76.121) = 36.638$, $p < 0.001$, partial $\eta^2 = 0.647$. Significant results were seen in week 8, 12 and 24 compared to the screening visit for both sides. The right side increase by 20.05 % ($p = 0.002$) in week 8, by 26.72 % ($p < 0.001$) in week 12 and by 50.47 % ($p < 0.001$) in week 24. The left side similarly increased by 15.47 % ($p= 0.014$) in week 8, 22.54 % ($p = 0.003$) in week 12 and 53.89 % ($p < 0.001$) in week 24. Left and right side mean values and SDs were comparable during each visit, which was confirmed by the insignificant paired samples t-tests (data in appendix). The effect size was large for both sides (partial $\eta^2 = 0.640$ right side, $\eta^2 = 0.647$ left side).

The results of the 4 mm probe showed, like the skin firmness (R0, Uf) data, a different course compared to the 2 mm probe (*Figure 37*). The statistical analysis revealed a significant difference between the visits for the right side, $F(3.692, 73.836) = 13.833$, $p < 0.001$, partial $\eta^2 = 0.409$ and for the left side, $F(3.685, 73.696) = 15.995$, $p < 0.001$, partial $\eta^2 = 0.444$. Like the measurements of the 2 mm probe, both sides had a large effect size. The left side measurements were significantly higher compared to the right side in week 2 (p = 0.027), week 4 (p = 0.048) and week 12 (p < 0.001). However, they had a similar trend and showed a final increase in skin recovery of 12.06 % (p = 0.004) for the right side and 22.27 % (p = 0.010) for the left side in week 24, compared to the screening visit. Conversely, 2 weeks after the first treatment (week 2), there was a significant 17.59 % (p = 0.004) and 17.92 % (p = 0.014) decrease of skin recovery for the right and left side, respectively (increase in values). After week 2 the skin recovery increased again but was significant for the left side only, as described above.

3.2.1.3.5 Skin gross elasticity (R2, Ua/Uf)

The skin gross elasticity (Ua/Uf) is a relative parameter. It is the ratio of the first minimum amplitude of the relaxation phase (Ua) divided by the first maximal amplitude of the suction phase (Uf). The closer the value is to 1 (100 % retraction) the more elastic is the skin [168]. As the amplitude also includes the viscoelastic parts of the curve, the skin gross elasticity gives additionally information about the skin viscosity.

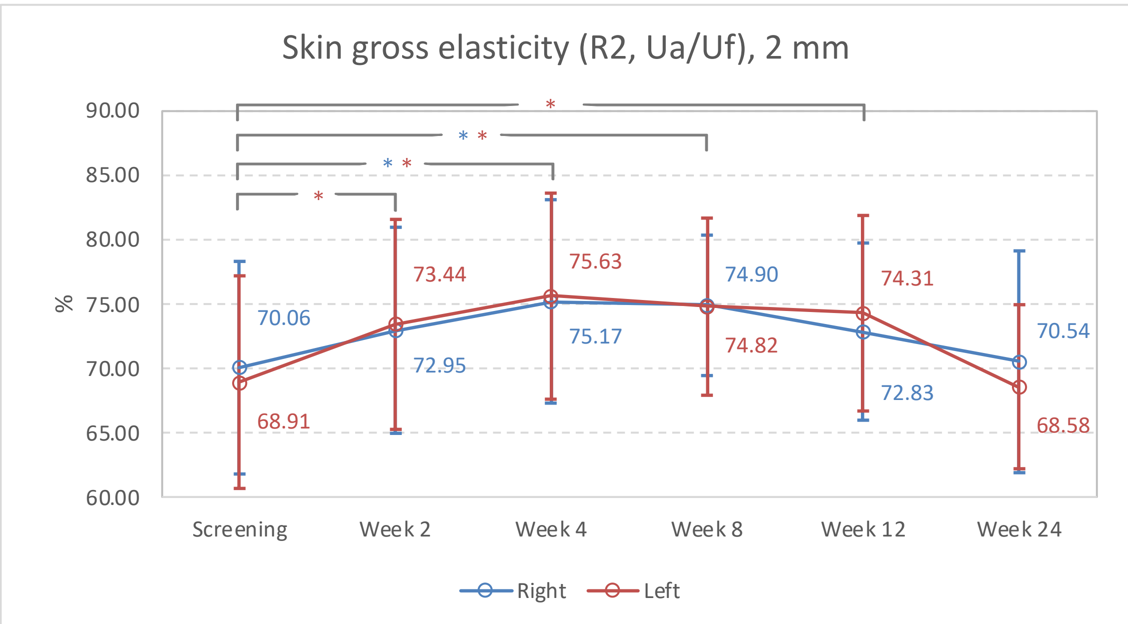


*Figure 38: Skin gross elasticity, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

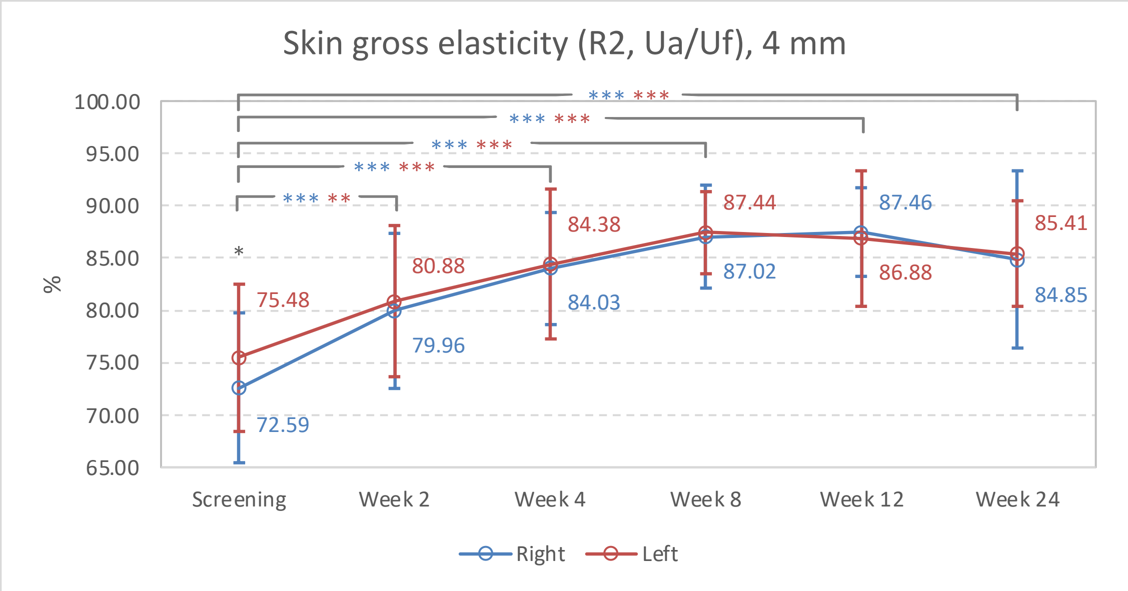


*Figure 39: Skin gross elasticity, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Skin gross elasticity of the 2 mm probe showed statistically significant differences between the visits for the right side, $F(3.327, 66.540) = 2.669$, $p = 0.049$, partial $\eta^2 = 0.118$ and for the left side, $F(3.660, 73.197) = 5.913$, $p < 0.001$, partial $\eta^2 = 0.228$ (*Figure 38*). The initial skin gross elasticity of the 2 mm probe was 70.06 % and 68.91 % for the right and left side, respectively. The left side increased significantly until week 12 up to 74.31 % ($p = 0.040$). At the end of the study period the value was slightly underneath the initial value with 68.58 % ($p = 1.000$). The right side increased significantly until week 8 to 74.90 % ($p = 0.030$) and was with 70.54 % ($p = 1.000$) slightly above the initial value at the end of the study. The highest values for both sides were in week 4 (75.17 % right side, 75.63 % left side), which was two weeks after the third treatment. Both sides were nearly identical in values and performance during the study (results of t-test in appendix). Although, skin gross elasticity was nearly the same at the end of the study compared to the first measurement, there was a medium effect size for the right side and a lager effect size for the left side ($\eta^2 = 0.118$ the right, $\eta^2 = 0.228$ left side).

In *Figure 39* the results of the skin gross elasticity of the 4 mm probe are visualized. There was a statistically significant difference between the visits for the right side, $F(3.034, 60.678) = 32.981$, $p < 0.001$, partial $\eta^2 = 0.623$ and for the left side, $F(3.421, 68.413) = 17.554$, $p < 0.001$, partial $\eta^2 = 0.467$. The initial skin gross elasticity measurements were 72.59 % and 75.48 % for the right and left side, respectively. The left side was statistically significant higher compared to the right side at screening ($p = 0.025$). All other visits showed similar values. All visits were statistically significant higher compared to screening ($p = 0.007$ week 2 left side, all others: p <

0.001). At the end of the study the right side was increased by 12.26 %, the left side by 9.93 %. The effect size was large for both sides.

3.2.1.3.6 Skin net elasticity (R5, Ur/Ue)

The parameter R5 (Ur/Ue) is the ratio of the elastic part of the suction phase (Ur) and the elastic part of the relaxation phase (Ue). It gives information about the elastic fibers in the skin. Like the gross skin elasticity (R2), the closer the value is to 1 (100 %) the more elastic is the skin [168].

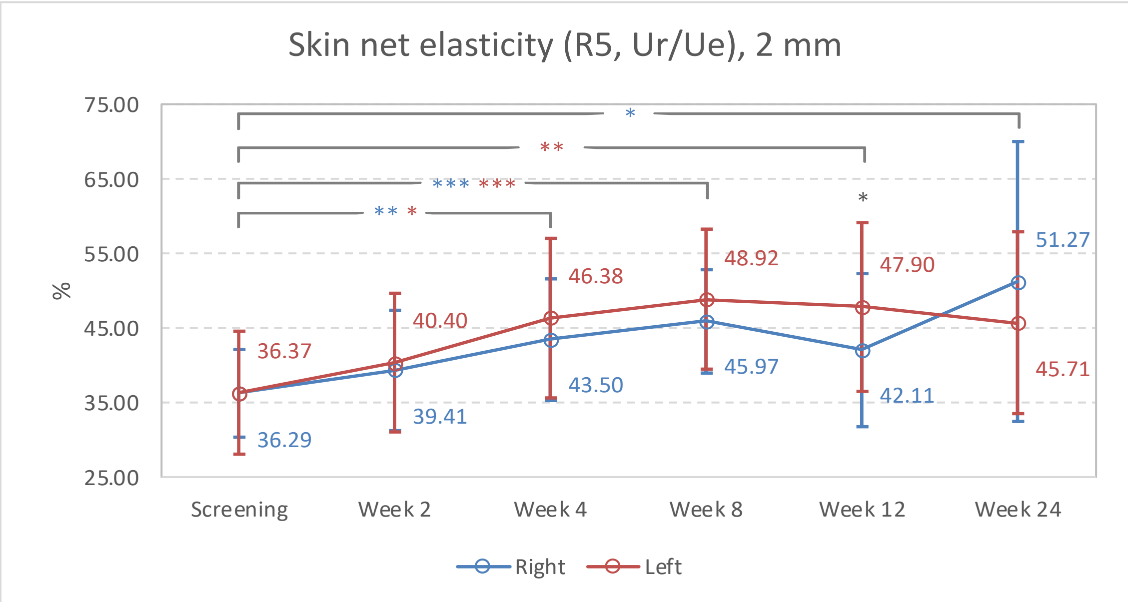


*Figure 40: Skin net elasticity, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

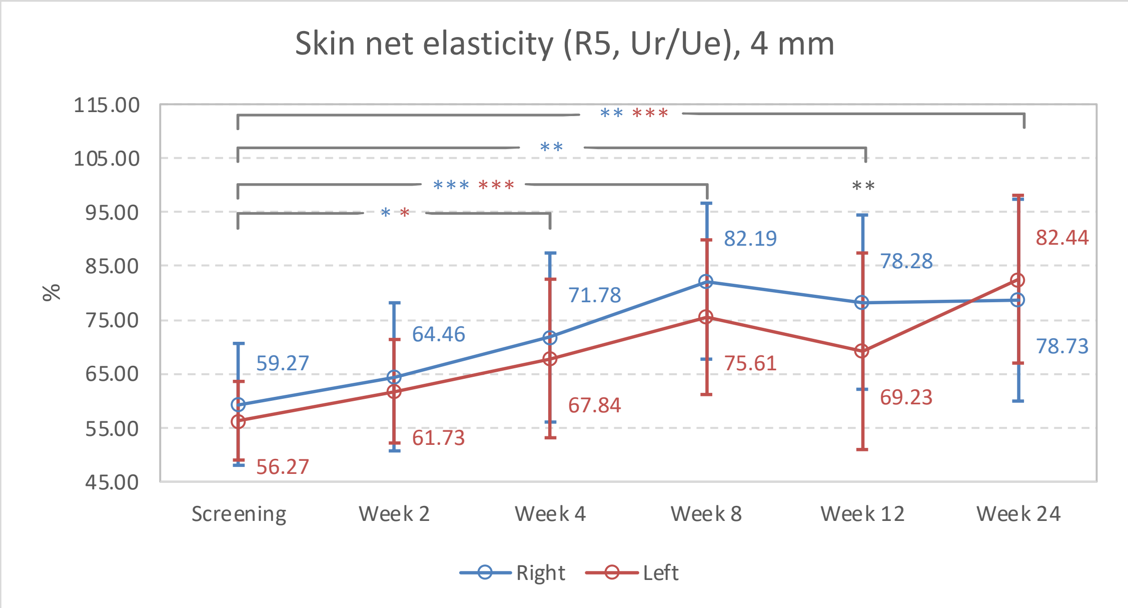


*Figure 41: Skin net elasticity, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The 2 mm probe measurements of the skin net elasticity are shown in *Figure 40*. There was a statistically significant difference between the visits for the right side, F(2.025, 40.496) = 7.387, p = 0.002, partial η^2 = 0.270 and for the left side, F(3.684, 73.686) = 6.542, p < 0.001, partial η^2 = 0.246. The effect size of this parameter was lager. Skin net elasticity started with nearly the same values, 36.29 % and 36.37 % for the right and left side, respectively. The highest and significant value for the left side was four weeks after the last treatment in week 8 with 48.92 % (p < 0.001). After week 8 the skin net elasticity decreased slightly for the left side and was 45.71 % (p = 0.139) at the end of the study. The right side significantly increased until week 8 as well to 45.97 % (p < 0.001), then dropped to 42.11 % (p = 0.112) in week 12 and increased to significantly 51.27 % (p = 0.021) in week 24 compared to screening again. In week 12 the left side was significantly higher compared to the right side (p = 0.016).

With swopped sides, skin net elasticity measurements of the 4 mm probe showed a similar trend compared to the 2 mm probe (*Figure 41*). Here, the repeated measures ANOVA determined a statistically significant difference between the visits for the right side, F(2.025, 40.496) = 7.387, p = 0.002, partial η^2 = 0.270 and for the left side, F(3.684, 73,686) = 6.542, p < 0.001, partial η^2 = 0.246. The effect size was lager for both sides.

The measurements started with values above 55 % (59.27 % right side, 56.27 % left side). The highest and significant value, now for the right side, was four weeks after the last treatment in week 8 with 82.19 % (p < 0.001). After week 8 the skin net elasticity decreased slightly for the right side, but was still significantly increased with 78.73 % (p = 0.008) at the end of the study. The left side significantly increased until week 8 as well to 75.61 % (p < 0.001), then dropped to 69.23 % (p = 0.064) in week 12 and increased to significantly 82.44 % (p < 0.001) in week 24 compared to screening again. In week 12, the right side was significantly higher compared to the left side (p = 0.004).

3.2.1.3.7 Ratio of viscoelastic to elastic extension (R6, Uv/Ue)

The relative parameter R6 (Uv/Ue) describes the viscoelastic versus the elastic ratio of the curve in the suction phase. The smaller the value the higher the elasticity and the more elastic fibers are in the skin [168].

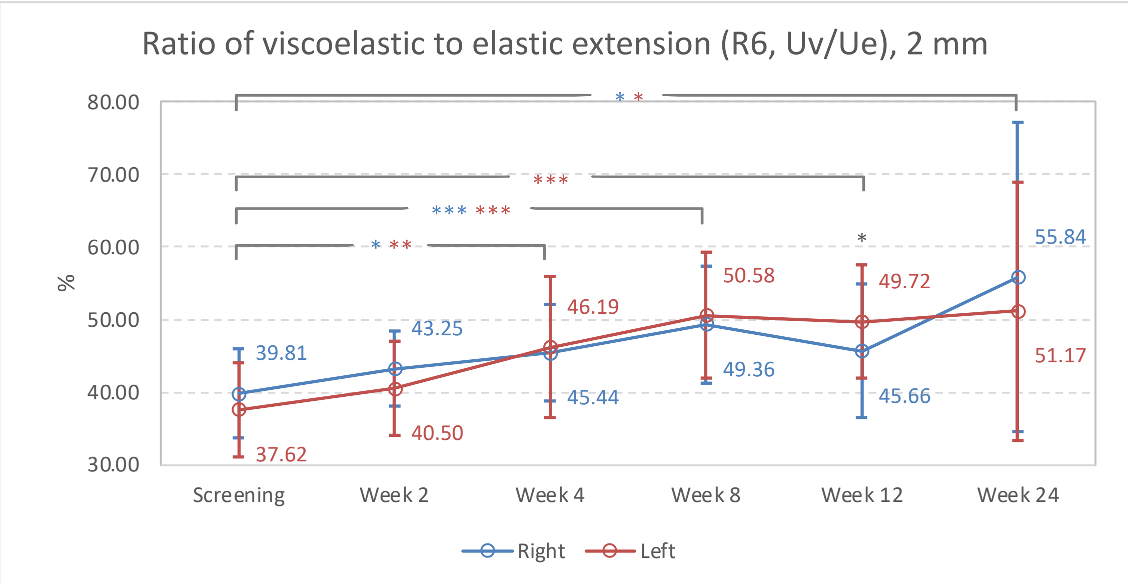


*Figure 42: Ratio of viscoelastic to elastic extension, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

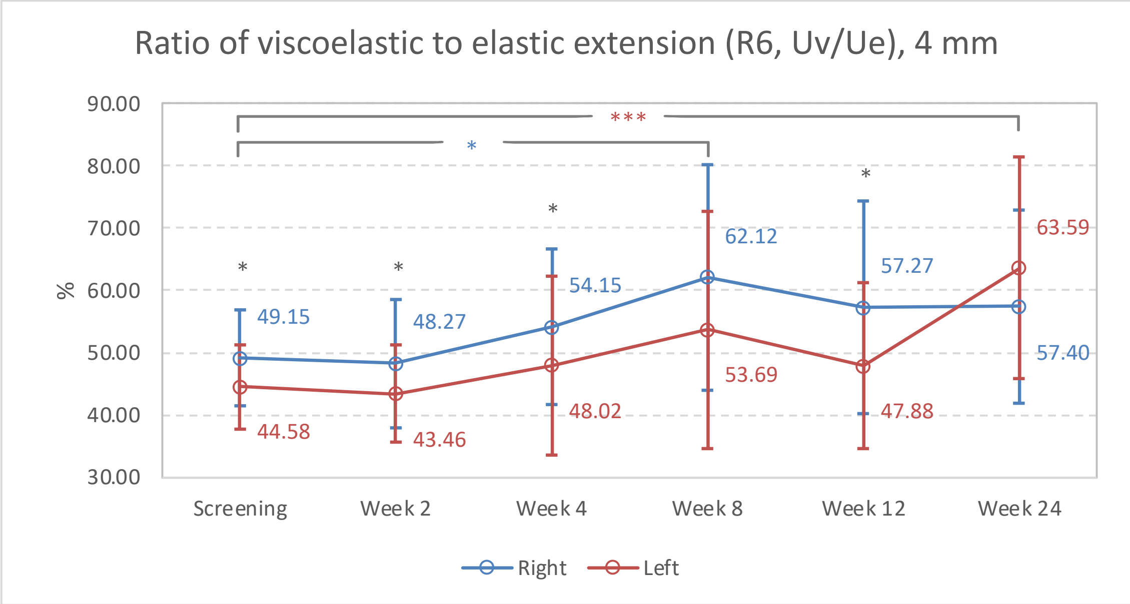


*Figure 43: Ratio of viscoelastic to elastic extension, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The measurements of the 2 mm probe of the parameter R6 (ratio of viscoelastic to elastic extension) are shown in *Figure 42*. The repeated measures ANOVA determined a statistically significant difference between the visits for the right side, $F(2.123, 42.460) = 6.107$, $p = 0.004$, partial $\eta^2 = 0.234$ and for the left side, $F(2.590, 51.796) = 7.582$, $p < 0.001$, partial $\eta^2 = 0.275$. Here as well, the effect size showed lager results. The initial ratio of viscoelastic to elastic extension was 39.81 % and 37.62 % for the right and left side, respectively. Both sides increased and were in week 24 with 55.86 % (p = 0.02) for the right side and 40.70 % (p = 0.044) for the left side significantly higher compared to screening. The right side value decreased from week 8 to week 12 to 45.66 % (p = 0.533) and was additionally significantly lower compared to the left side (p = 0.047). The right side value increased again between week 12 and week 24 to 55.84 % and was significantly higher (p = 0.02) compared to screening. Also, the right side was nearly 5 % above the left side value (51.17 % left side) in week 24.

The measurements of the 4 mm probe were again vice versa compared the 2 mm probe like the results of the skin net elasticity (*Figure 43*). The repeated measures ANOVA determined a statistically significant difference between the visits for the right side, $F(3.522, 70.447) = 4.952$, $p = 0.002$, partial $\eta^2 = 0.198$ and for the left side, $F(3.779, 75.574) = 8.073$, $p < 0.001$, partial $\eta^2 = 0.288$. The overall effect size showed large results for both sides.

The results of the right side showed significantly higher values from screening to week 4 (screening: p = 0.05, week 2: p = 0.033, week 4: p = 0.019) and in week 12 (p = 0.015) compared

to the left side. The right side was 49.15 % at screening, then increased until week 8, to significantly 62.12 % (p = 0.038) and then slightly decreased to 57.40 % (p = 0.547) in week 24.
The left side increased from 44.58 % at screening to 53.69 % (p = 0.567, compared to screening) in week 8 as well. Then decreased between week 8 and week 12 to 47.88 % (p = 1.000, compared to screening) and finally, significantly increased to 63.59 % (p < 0.001, compared to screening) in week 24.

3.2.1.3.8 Ratio of elastic recovery to total extension (R7, Ur/Uf)

The parameter R7 is another relative parameter (Ur/Uf). It is the ratio of the elastic recovery (Ur) of the relaxation phase and the maximum firmness (Uf). The closer the value is to 1 (100 %) the more elastic is the skin [168]. This parameter is discussed to decrease with age [173].

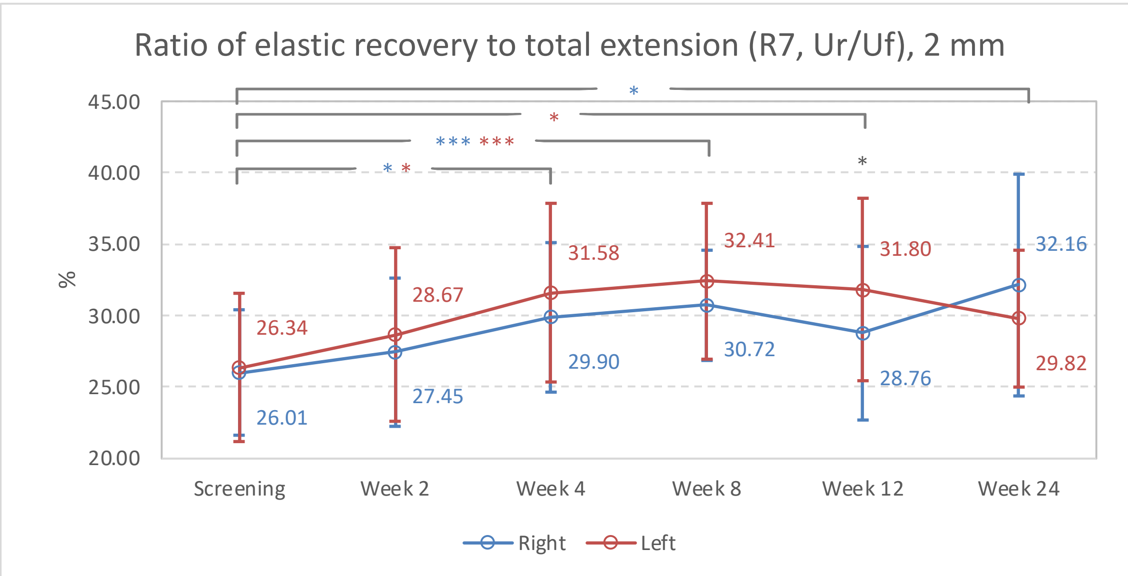


*Figure 44: Ratio of elastic recovery to total extension, ACS in vivo study I, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

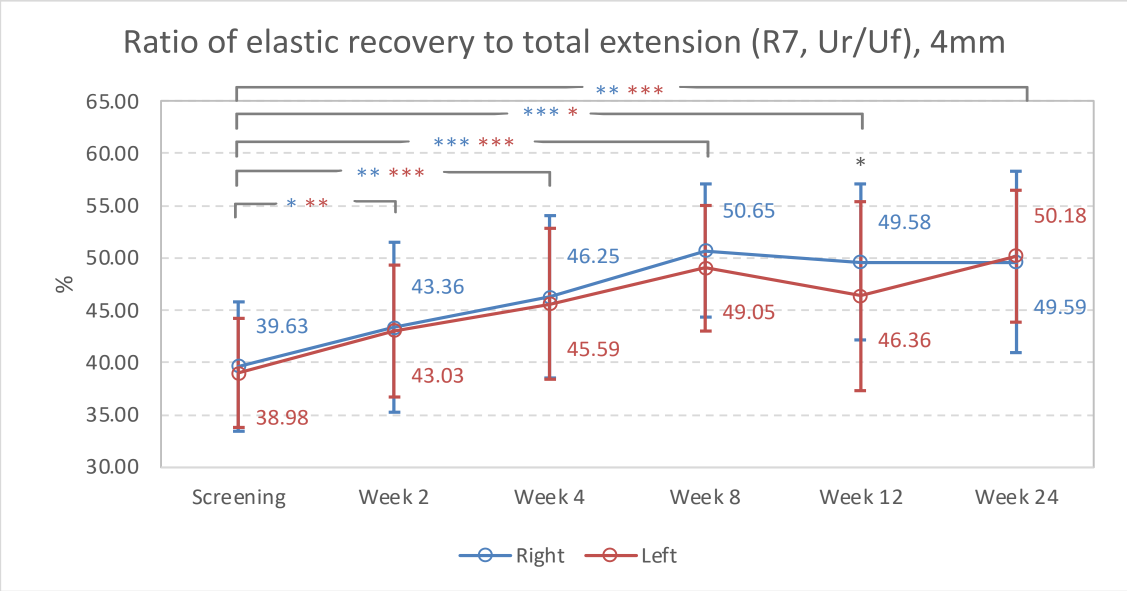


*Figure 45: Ratio of elastic recovery to total extension, ACS in vivo study I, 4 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The ratio of elastic recovery to total extension (R7, Ur/Uf) performed nearly identically to the skin net elasticity (R5, Ur/Ue) and similar to the ratio of viscoelastic to elastic distensibility (R6, Uv/Ue) for both sides and both probe sizes.

The 2 mm probe measurements showed statistically significant differences between the visits for the right side, $F(2.891, 57.827) = 5.946$, $p = 0.001$, partial $\eta^2 = 0.229$ and for the left side, $F(4.012, 80.233) = 5.355$, $p < 0.001$, partial $\eta^2 = 0.211$. The effect size of both sides were lager again (*Figure 44*). Both sides started with nearly the same values, 26.34 % and 26.01 % for the right and left side, respectively. The highest and significant value for the left side was four weeks after the last treatment in week 8 with 32.41 % ($p < 0.001$, compared to screening). After week 8 the value decreased slightly for the left side and was 29.82 % ($p = 0.520$, compared to screening) at the end of the study. The right side significantly increased until week 8 as well to 30.27 % ($p < 0,001$), then decreased to 28.76 % ($p = 0.462$) in week 12 and increased to significantly 51.27 % ($p = 0.043$) in week 24 compared to screening again. In week 12, the left side was significantly higher compared to the right side ($p = 0.041$).

Likewise with swopped sides, the measurements of the 4 mm probe showed a similar trend compared to the 2 mm probe and the skin net elasticity (R5, Ur/Ue) (*Figure 45*). The statistical analyzation showed a statistically significant difference between the visits for the right side, $F(3.232, 64.636) = 17.640$, $p < 0.001$, partial $\eta^2 = 0.469$ and for the left side, $F(3.300, 65.992) = 15.362$, $p < 0.001$, partial $\eta^2 = 0.434$. Like the 2 mm probe and the skin net elasticity (R5, Ur/Ue), the effect size showed lager results as well. Here, the measurements started with 39.63 % for the right side and 38.98 % for the left side. The highest and significant value was now found for the right side, four weeks after the last treatment, in week 8 with 50.65 % ($p < 0.001$, compared to screening). After week 8 the value decreased slightly for the right side but was still significantly higher with 49.59 % ($p = 0.002$) at the end of the study compared to screening. The left side significantly increased until week 8 as well to 49.05 % ($p < 0.001$), then decreased to 46.36 % ($p = 0.016$) in week 12 and increased to significantly 50.18 % ($p < 0.001$) in week 24 again compared to screening. In week 12, the right side was significantly higher compared to the left side ($p = 0.018$).

3.2.1.4 Sonography

The skin density and thickness was measured with the ultrasound DUB Skin Scanner (Tpm taberna pro medicus) device with a 22 MHz transducer (compare *2.3.4.3.1 Sonography measurements and evaluation method*). Each side was measured one time at each visit. The results of the skin density and thickness are listed in the graphs below with the means and SDs of all 21 patients of the right (blue) and left (red) side.

3.2.1.4.1 Skin density

A high skin density is visualized by a high echogenicity of the skin connective tissue and can be seen as a bright colored image in the sonogram in the b mode. In aged skin (> 70 years) the echogenicity decreases especially in sun damaged skin areas and is visualized with a less brighter image and lower values [177].

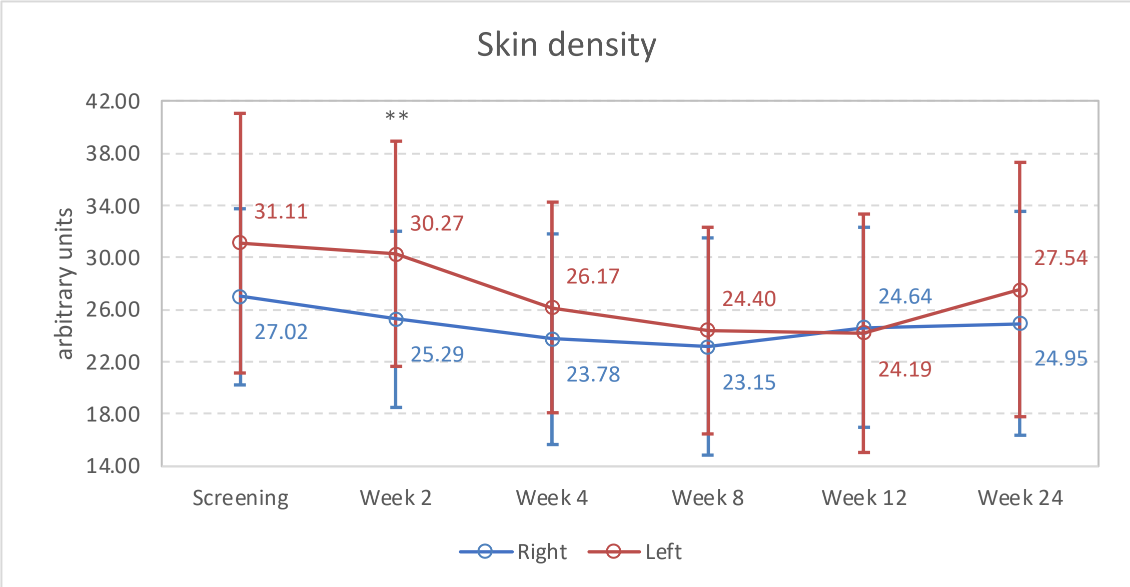


*Figure 46: Skin density, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Skin density stayed about the same, with a slight decrease throughout the whole study period, staring with 31.11 a.u. and 27.02 a.u. for the left and right side, respectively (*Figure 46*).
The statistical analysis showed no statistically significant differences between the visits for the right side, F(4.290, 85.810) = 1.314, p = 0.270, partial η^2 = 0.062 but for the left side, F(4.357, 87.137) = 3.497, p = 0.009, partial η^2 = 0.149. This overall significance for the left side (p = 0.009) was not seen in the multiple comparisons of the visits (post hoc tests), as they were conservatively Bonferroni adjusted. Generally, the left side showed higher values at screening, in week 2 and week 4. In week 2, the left side was significantly higher compared to the right side (p = 0.003). In week 12, both sides were nearly the same (24.64 a.u right side, 24.19 a.u. left side). The final skin density in week 24 was 27.54 a.u., 11.47 % below the screening value, for the left side. The right side decreased by 7.64 % and was 24.95 a.u. at the end of the study.

3.2.1.4.2 Skin thickness

Skin thickness is calculated in µm and measured from the skin entrance echo to the last echogenic area seen in the image of the B-mode. In ultrasound images skin thickness decreases with age [50].

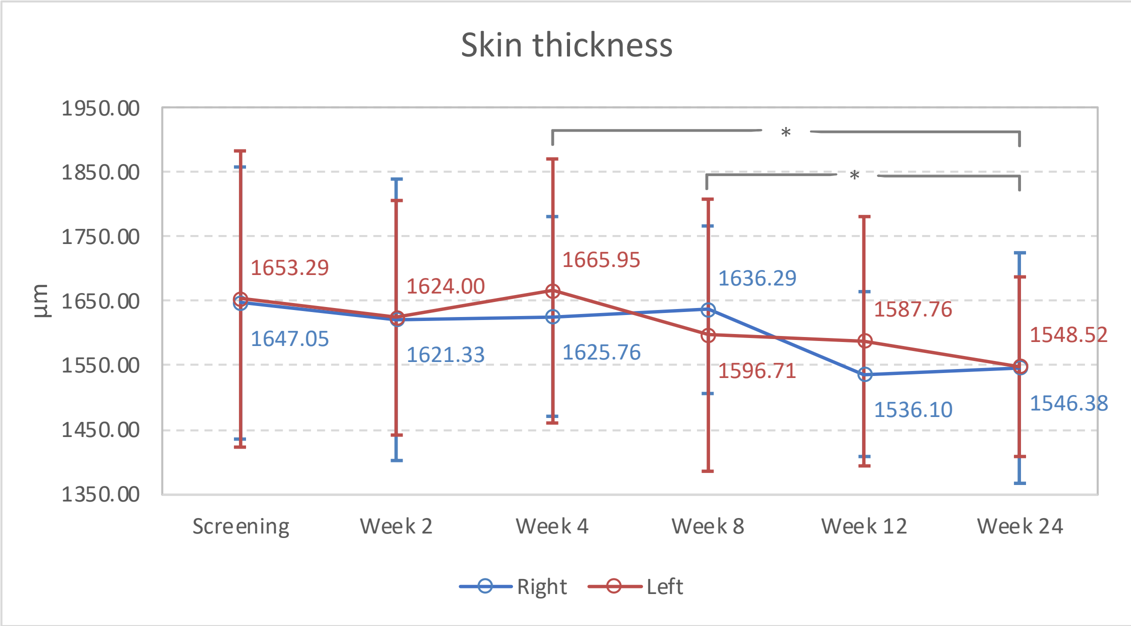


*Figure 47: Skin thickness, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Skin thickness was for both sides similar throughout the study period and showed no statistically significant difference between the sides, as shown in *Figure 47*. The statistical analyzation showed a significant difference between the visits for both sides together, $F(3.976, 79.528) = 5.442$, $p < 0.001$, partial $\eta^2 = 0.214$ but no significance for the sides $F(1.000, 20.000) = 0.535$, $p = 0.473$, partial $\eta^2 = 0.026$ or interaction visits and sides $F(3.581, 71.624) = 0.857$, $p = 0.484$, partial $\eta^2 = 0.041$. Statistically significant differences were seen between week 4 ($p = 0.042$) and 8 ($p = 0.034$) compared to week 24. During screening there was a skin thickness of 1647.05 µm for the right side and 1653.29 µm for the left side. Both sides decreased to 1546.38 µm (right side) and 1548.52 µm (left side), which is an overall decrease of – 6.22 %.

3.2.1.5 PRIMOS – skin topography

The topography of the skin surface was measured with the PRIMOS camera system (GFMesstechnik GmbH, Teltow, Germany). Each side was measured one time at each visit. The results are listed in the graphs below with the means and SDs of all 21 patients of the right (blue) and left (red) side.

3.2.1.5.1 Mean skin roughness (Ra)

The mean skin roughness (Ra) is the average roughness of the set star lines separated into five sections each. The higher the values, the rougher the skins surface and an increase is associated with skin aging [191, 199].

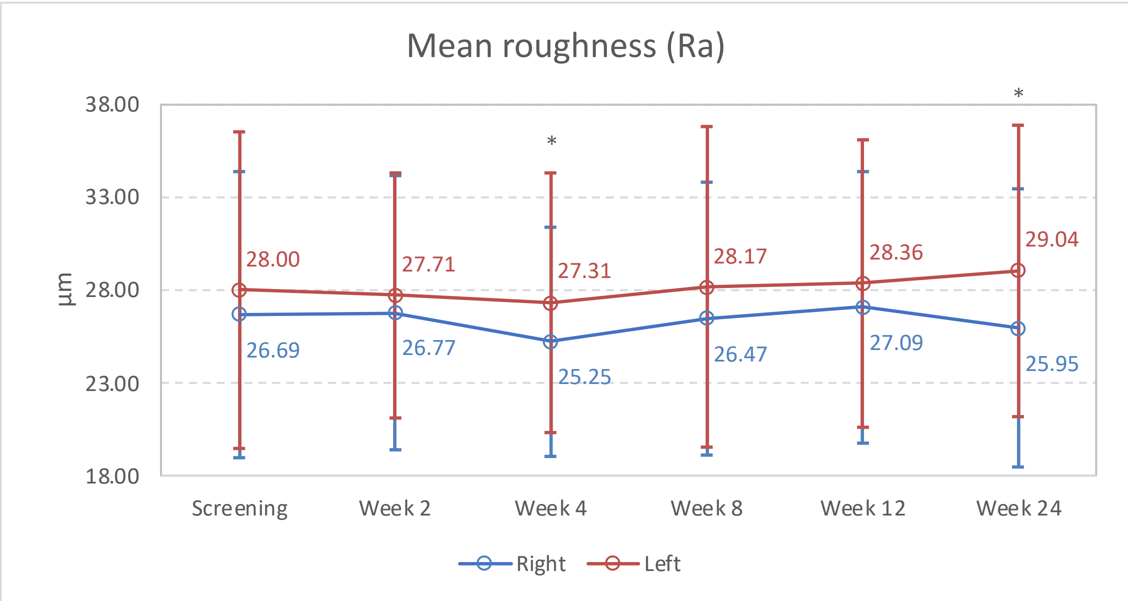


*Figure 48: Mean roughness, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The mean skin roughness was 28.00 µm for the left side and 26.69 µm for the right side at the beginning of the study. At the end of the study the values were 29.04 µm (left side) and 25.95 µm (right side) and showed no significant change (*Figure 48*). The result of the statistical analysis for the difference between the visits was for the right side, $F(4.154, 83.087) = 1.131$, $p = 0.348$, partial $\eta^2 = 0.054$ and for the left side, $F(3.862, 77.237) = 0.875$, $p = 0.480$, partial $\eta^2 = 0.042$. As the partial η^2 was below 0.06 for both sides, the effect size was small. The percentage difference between screening and week 24 was + 3.69 % and – 2.77 % for the left and right side, respectively. The left side was throughout the study higher than the right side and showed a significant difference in week 4 ($p = 0.027$, 8.19 %) and week 24 ($p = 0.025$, 11.91 %) compared to the right side. On average the left side was 6.57 % higher than the right side.

3.2.1.5.2 Maximum roughness (Rmax)

The maximum roughness (Rmax or Rm) is the greatest minimum-maximum difference of all segments of the measured profile. Rmax has also been shown to increases with age [195].

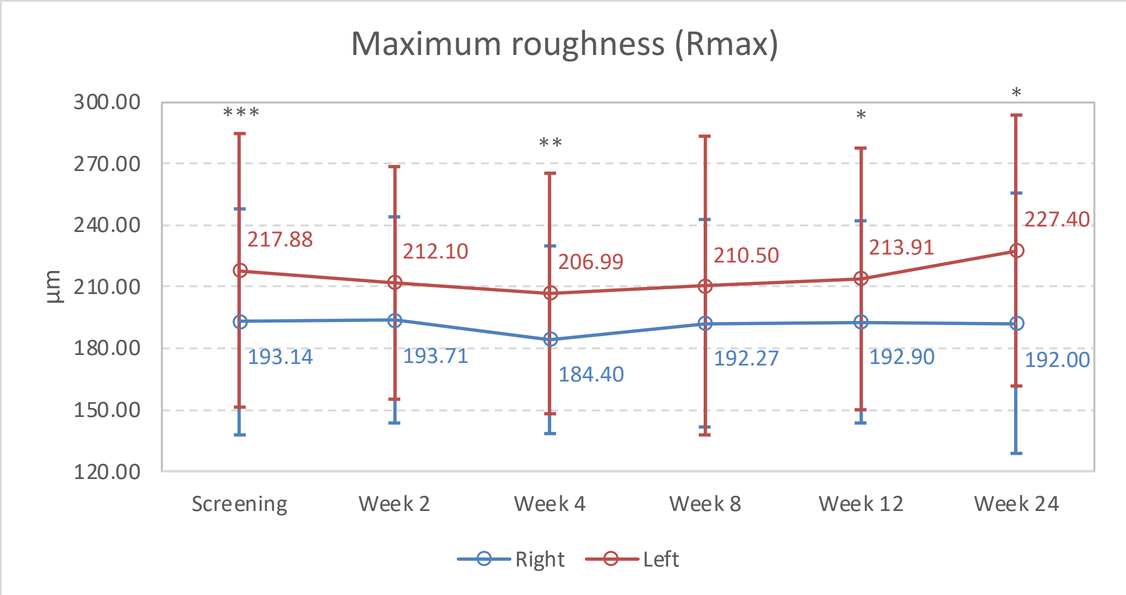


*Figure 49: Maximum roughness, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The maximum roughness was like the mean skin roughness insignificant throughout the study period (*Figure 49*). The statistical analyzation showed no significant difference between the visits for the right side, $F(3.492, 69.842) = 0.370$, $p = 0.805$, partial $\eta^2 = 0.018$ or the left side, $F(3.561, 71.219) = 1.611$, $p = 0.187$, partial $\eta^2 = 0.075$. The effect size was small for the right side and medium for the left side. The left side increase by 4.37 % from 217.88 µm to 227.40 µm ($p = 1.000$), the right side decreased by 0.59 % ($p = 1.000$) from 193.14 µm to 192.00 µm, comparing screening with week 24. The left side was again on average 12.22 % higher than the right side and significantly higher at screening ($p < 0.001$, 12.81 %), in week 4 ($p = 0.010$, 12.25 %), week 12 ($p = 0.022$, 10.89 %), and week 24 ($p = 0.011$, 18.44 %).

3.2.1.5.3 Mean depth of roughness (Rz)

The mean depth of roughness is calculated as the average from all minimum-maximum difference within each measurement section [211]. An age correlation has been found here as well, with higher values with increasing age [195].

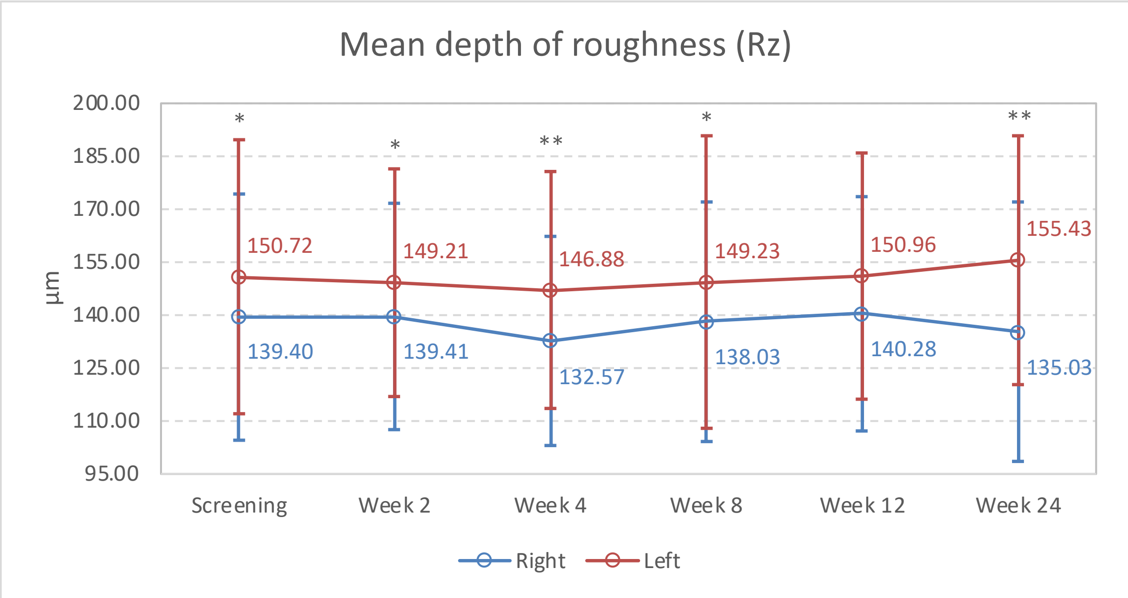


*Figure 50: Mean depth of roughness, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The mean depth of roughness is shown in *Figure 50* and like the two other roughness parameters Ra and Rmax, no statistical significant change was seen throughout the study period. But the left side was, apart from the data in week 12, significantly higher than the right side. The repeated measures ANOVA showed no statistical significant difference between the visits for the right side, $F(4.032, 80.634) = 0.827$, $p = 0.513$, partial $\eta^2 = 0.040$ or for the left side, $F(4.135, 82.709) = 0.813$, $p = 0.524$, partial $\eta^2 = 0.039$. The effect size was small for both sides.

The left side increase by 3.12 % from 150.72 µm to 155.43 µm (p = 1.000), the right side decreased by 3.14 % (p = 1.000) from 193.40 µm to 135.03 µm, comparing screening with week 24. The left side was on average 9.42 % higher than the right side and significantly higher at screening (p < 0.012, 8.12 %), in week 2 (p = 0.050, 7.03 %), week 4 (p = 0.006, 10.79 %), week 8 (p = 0.046, 8.11 %), and week 24 (p = 0.006, 15.11 %).

3.2.1.5.4 Maximum profile peak (Rp)

Rp represents the highest peak within the measurement profile and is part of the DIN roughness parameters [194].

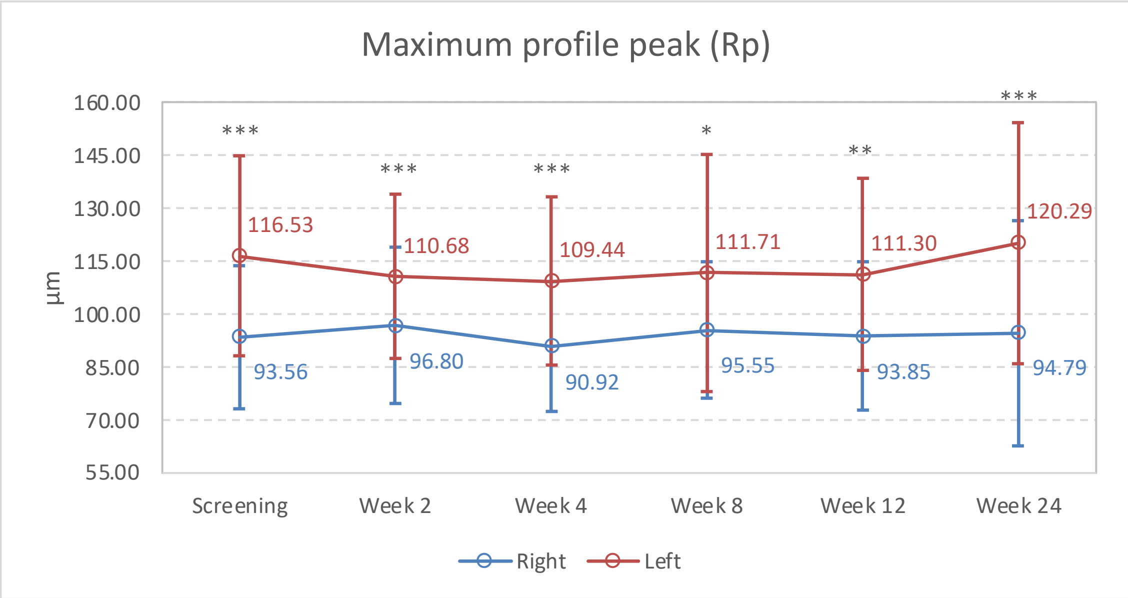


*Figure 51: Maximum profile peak, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The maximum profile peak of the skin surface measurements stayed throughout the study for both sides about the same (*Figure 51*). The overall statistical analyzation revealed no significant difference between the visits for the right side, $F(3.411, 68.226) = 0.389$, $p = 0.786$, partial $\eta^2 = 0.019$ or the left side, $F(3.743, 74.866) = 1.459$, $p = 0.226$, partial $\eta^2 = 0.068$. The effect size was small for both sides. At the end of the study, the percentage increase was 3.23 % for the left side, from 116.53 µm to 120.29 µm. The right side increased by 1.31 %, from 93.56 µm to 94.79 µm. The values of the left side were during the study significantly higher compared to the right side with an average of 20.24 % (screening: $p < 0.001$, week 2: $p = 0.001$, week 4: $p < 0.001$, week 8: $p = 0.014$, week 12: $p = 0.005$, week 24: $p < 0.001$).

3.2.1.5.5 Waviness (Wt)

The waviness of the skin is the sum of the largest profile peak and valley and is an indicator to deeper furrows and changes of the cutaneous turgor [192, 196].

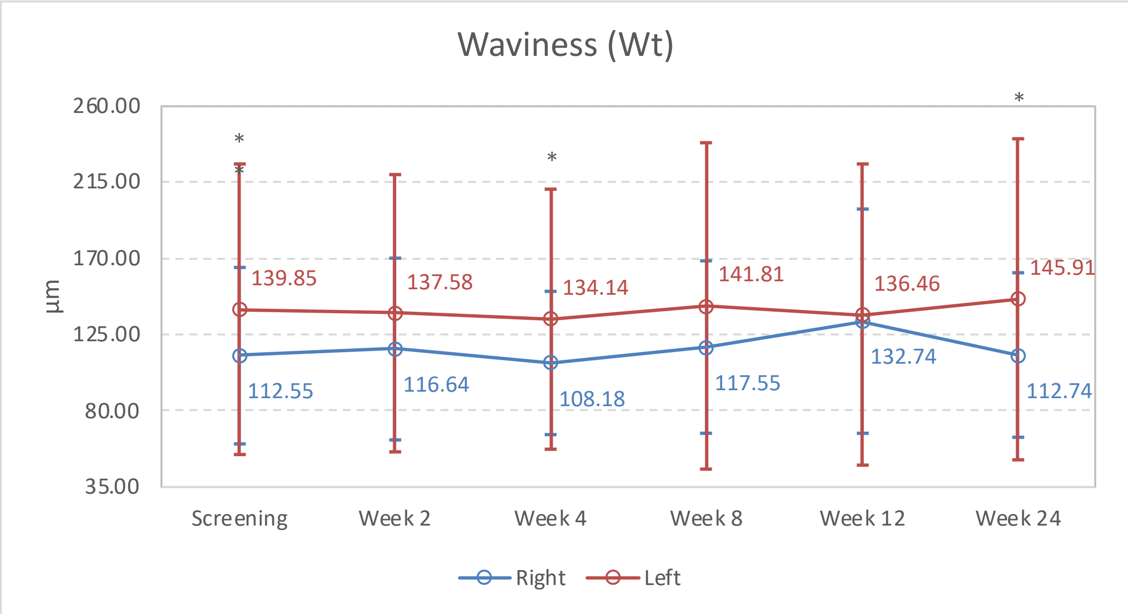


*Figure 52: Waviness, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The statistical analyzation of the skin waviness showed for the right side an overall significant difference between the sides, $F(2.679, 53.570) = 3.513$, $p = 0.025$, partial $\eta^2 = 0.149$ but not for the left side, $F(3.228, 64.566) = 0.388$, $p = 0.777$, partial $\eta^2 = 0.019$ (*Figure 52*). The overall significant difference for the right side ($p = 0.025$) was not confirmed by the Bonferroni adjusted post-hoc tests. The effect size was for the right side large, left side showed a small effect size. The waviness of the skin was 139.85 µm for the left side and 112.55 µm for the right side at the beginning of the study. The left side increased by 4.33 % to 145.91 ($p = 1.000$) in week 24. The right side had a peak in week 12 to 132.74 µm ($p = 0.485$, 17.93 %) but decreased until week 24 again by 0.17 % (comparing screening and week 24) to 112.74 ($p = 1.000$). The left side was throughout the study higher compared to the right side and showed significantly higher values at screening ($p = 0.013$, 24.26 %), in week 4 ($p = 0.027$, 24.00 %), and week 24 ($p = 0.024$, 29.43 %). On average, the left side was 19.33 % higher than the right side.

3.2.1.5.6 Number of peaks (PC)

PC is the number of peaks within the measurement profile and part of the surface roughness parameters [194].

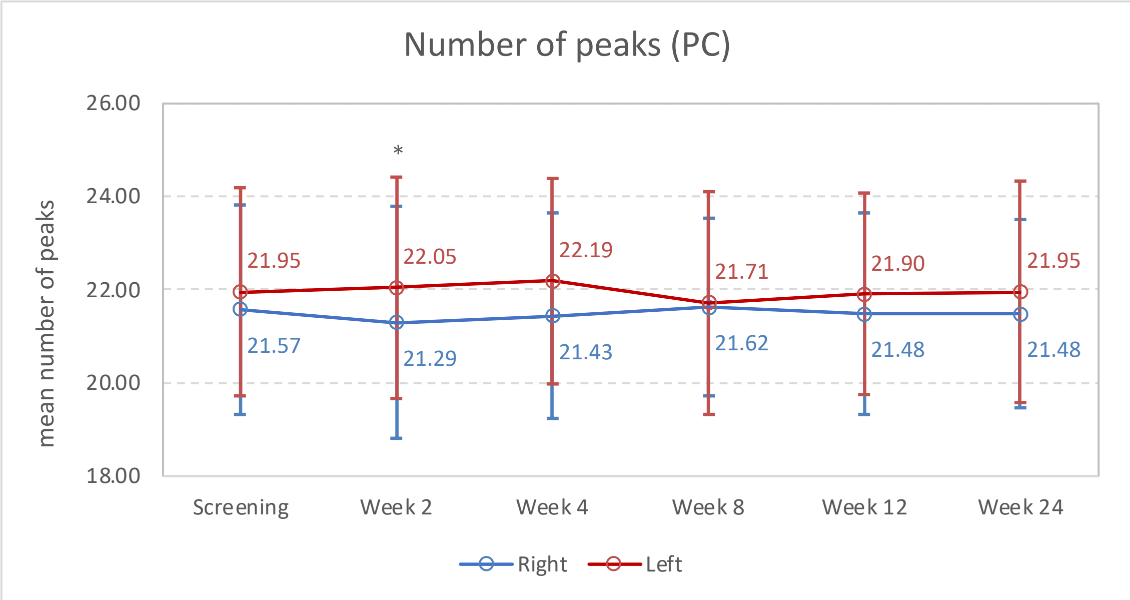


*Figure 53: Number of peaks, ACS in vivo study I, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The number of peaks of the skin surface measurements stayed throughout the study for both sides the same, as shown in *Figure 53*. At the end of the study, the percentage difference was zero for the left side and – 0.44 % for the right side. The values of the left side of the face were on average 2.25 % above the right side, in week 2 significantly higher (p = 0.032, 3.58%).

The statistical analysis showed no significant difference between the visits for the right side, $F(3.988, 79.753) = 0.313$, $p = 0.868$, partial $\eta^2 = 0.015$ nor for the left side, $F(3.794, 75.880) = 0.590$, $p < 0.662$, partial $\eta^2 = 0.029$. The effect size of both sides was small again.

3.2.2 ACS in vivo study II

For the second in vivo ACS study, 22 female patients were enrolled. This investigator-initiated study was conducted at two sites, but only the results of the patients treated at the University of Hamburg, Institute of cosmetic sciences, were evaluated. During the screening visit 10 patients were included for the ACS + HA treatment group and 12 patients for the ACS group. There were six dropouts during the study period. One in the ACS group and five in the ACS + HA group. Thus, the data of 16 female patients was analyzed (n = 6 ACS + HA, n = 10 ACS).

The patients attended seven visits: Screening, day one (first treatment), week 2 (second treatment), week 4 (third treatment), week 8, week 12 and week 24.

Measurements were taken at screening, in week 12 and 24. During the treatment sessions at day one and in week 2 and 4 there were no measurements taken.

Some parameters of the Cutometer® and PRIMOS measurements showed significant differences between the left and right face side in the combined analysis of variance. Thus, a separate repeated measures ANOVA for each side was calculated for all parameters obtained by these two devices (compare *2.3.8 Statistical analyses*).

Significant results were visualized in the graphs with whiskers, for the right side in blue and for the left side in red. A significant difference between the sides is marked in grey. All statistical results are listed in full detail in the appendix.

3.2.2.1 Age and skin condition

The age ranged from 35 to 63 years with a mean age of 48.8 ± 11.4 years for the ACS group and 53 to 64 years with a mean age of 56.0 ± 4.1 years for the ACS + HA group.

For the ACS group the skin condition was rated as dry by 80 % of the patients and as normal by 20 %. 80 % of the patients characterized their skin as being sensitive, 20 % as not sensitive.

For the ACS + HA group the skin condition was rated as dry by 75 % of the patients and as normal by 25 %. 69 % of the patients characterized their skin as being sensitive, 31 % as not sensitive. The results are visualized in *Figure 54* and *Figure 55.*

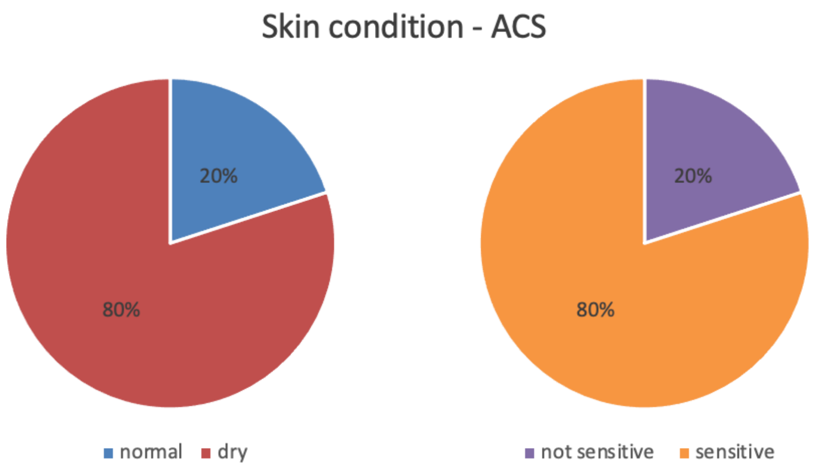


Figure 54: Skin condition, ACS in vivo study II, ACS, n = 10.

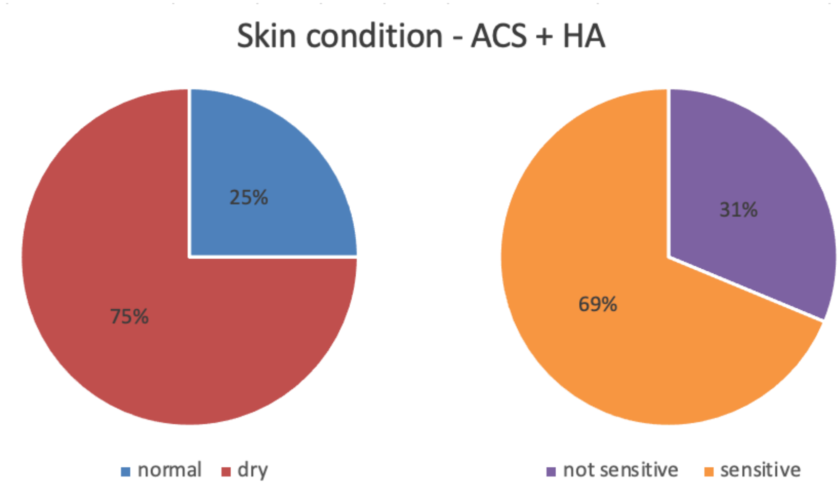


Figure 55: Skin condition, ACS in vivo study II, ACS + HA, n = 6.

3.2.2.2 Skin hydration

The skin hydration was measured at each visit on both sides three times. The mean of these three measurements was calculated for each patient. Means and SDs were then calculated from all 16 patients and are shown in *Figure 56*.

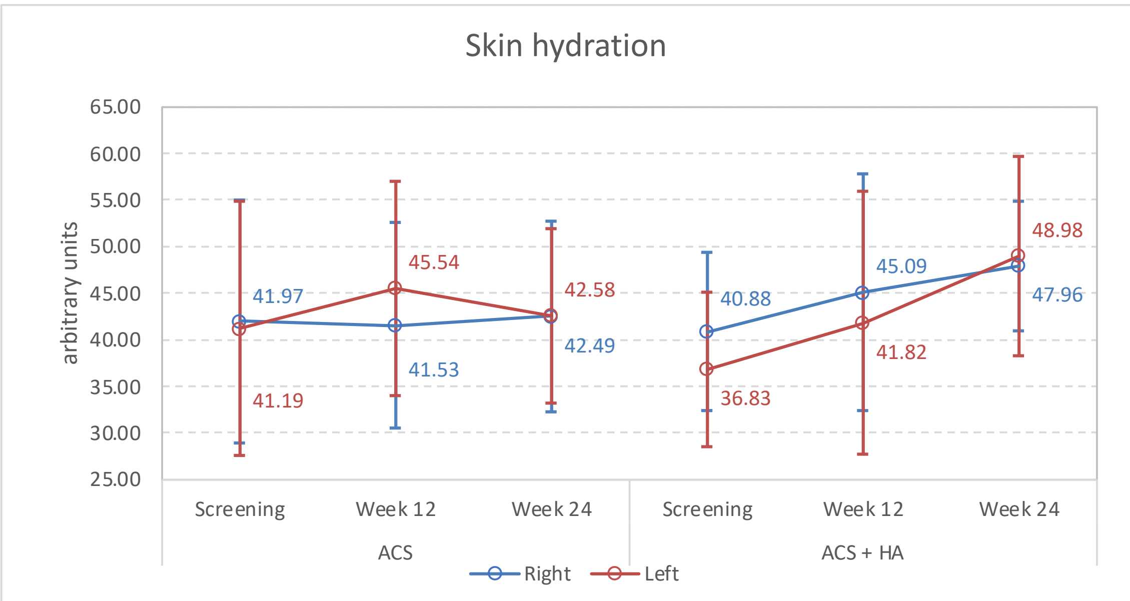


Figure 56: Skin hydration, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

For the ACS group, skin hydration stayed throughout the study and for both sides accordingly around 42.44 ± 1.56 a.u. The highest value was 45.54 a.u. on the left side in week 12. The total increase from screening to week 24 was 2.29 %. There was no statistically significant difference between the visits, $F(1.381, 12.431) = 0.141$, $p = 0.793$, partial $\eta^2 = 0.015$ and no overall difference between the left and right side, $F(1.000, 9.000) = 0.593$, $p = 0.416$, partial $\eta^2 = 0.062$.

Neither was the interaction of the sides and visits statistically significant, $F(1.962, 17.656) = 2.757$, $p = 0.092$, partial $\eta^2 = 0.235$.

Skin hydration of the ACS + HA group showed an continuous increase of 17.33 % for the right side and 32.97 % for the left side. However, the statistical analyzation showed no statistically significant increase. There was no statistically significant difference for the visits, $F(1.116, 5.580) = 1.770$, $p = 0.239$, partial $\eta^2 = 0.261$ or for the sides, $F(1.000, 5.000) = 2.004$, $p = 0.216$, partial $\eta^2 = 0.286$. Neither was the interaction of the sides and visits statistically significant, $F(1.352, 6.759) = 0.732$, $p = 0.463$, partial $\eta^2 = 0.128$.

The comparison of the two treatment groups included the mean of both sides together and showed no significance, either (screening: $p = 0.602$, week 12: $p = 0.991$, week 24: $p = 0.202$).

Except for the left side screening value (36.83 a.u.) of the ACS + HA group, all values were at all visits above 40 a.u. and hence in a normal hydration rate [162].

3.2.2.3 Cutometry

The skin mechanical properties were measured with the 2 mm and 4 mm Cutometer® Dual MPA 580 (Courage & Khazaka electronic GmbH, Cologne) probe on each side of the face for one time in modus 1 (compare *2.3.4.2.1. Cutometer® measurements and evaluation* method).

Due to technical problems with the Cutometer probes, the screening measurements had to be repeated. This was done in week 2 during the second treatment session. These measurements will still be labelled as screening data. Also, there was an incorrect measurement at screening for patient 2 concerning the 2 mm probe, right side data. This measurement was repeated in week 4 during the third treatment session and is labelled as screening data, too.

3.2.2.3.1 Skin firmness (R0, Uf)

The maximum amplitude R0 (Uf = Ue + Uv) of the cutometer curve describes the maximum expansion of the skin during the suction phase and gives information about the skin's firmness. The lower the amplitude, the firmer or less distensible the skin [168].

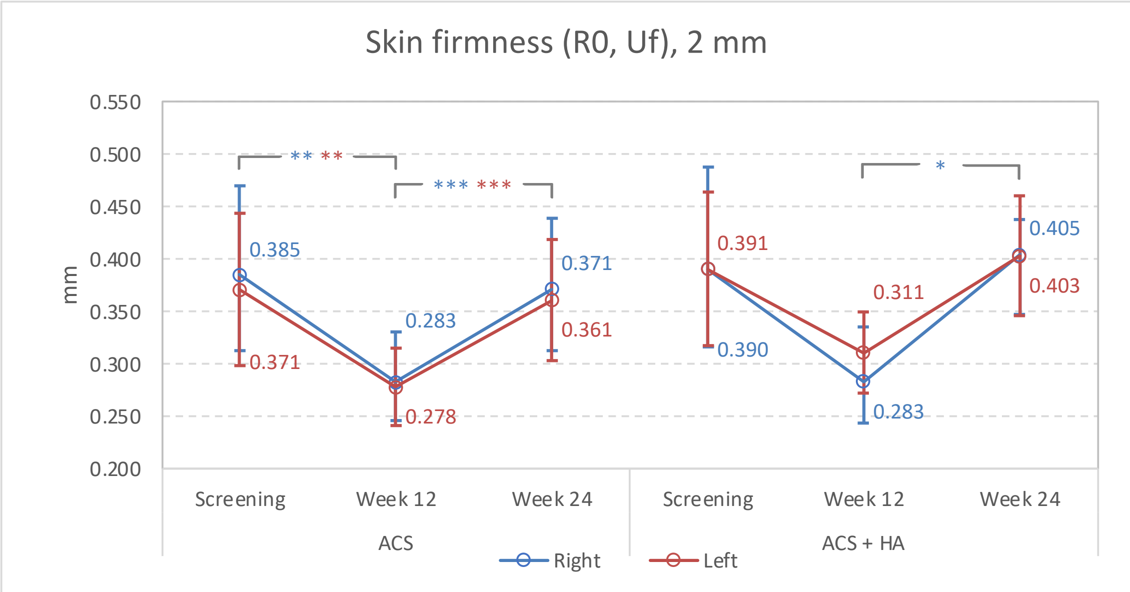


*Figure 57: Skin firmness, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

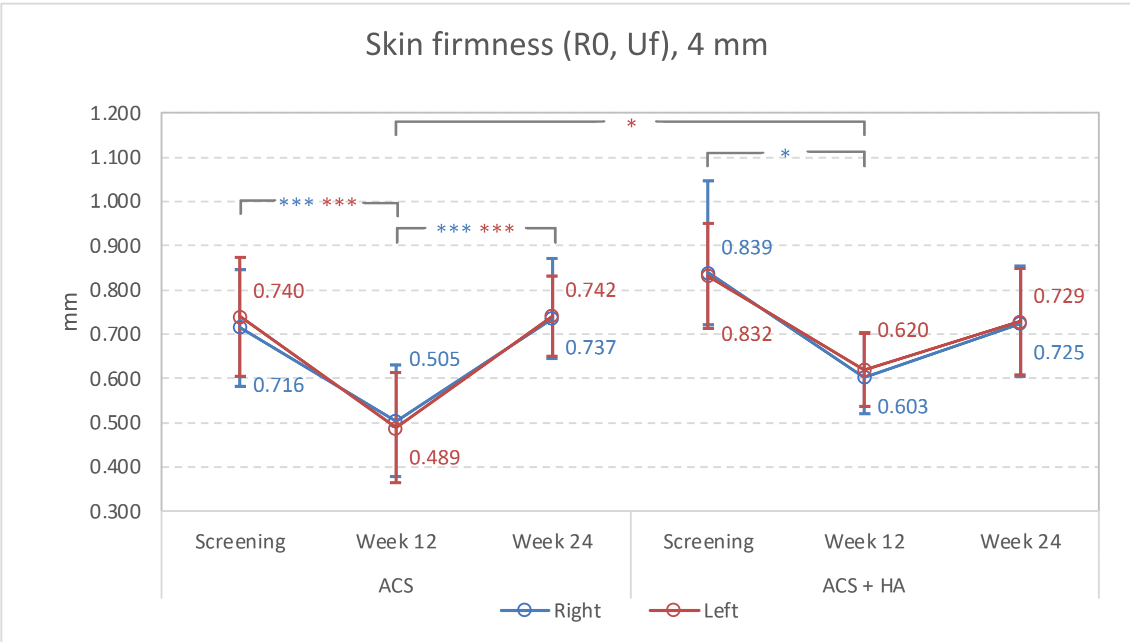


*Figure 58: Skin firmness, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

For both probe sizes and treatment groups skin firmness increased until week 12, then decreased again until week 24 to a similar level compared to screening.

For the 2 mm probe ACS group skin firmness significantly increased until week 12 by 26.50 % (p = 0.006) and 25.03 % (p = 0.003) for the right and left side, respectively. After week 12 the skin firmness significantly decreased again by 31.25 % (p = 0.001) and 29.76 % (p < 0.001) for the right and left side, respectively. However, the final difference between screening and week

24 was not significant, with – 3.53 % ($p = 1.000$) for the right side and – 2.72 % ($p = 1.000$) for the left side. The ANOVA for the difference between the visits was for the right side, $F(1.701, 15.305) = 15.332$, $p < 0.001$, partial $\eta^2 = 0.630$ and for the left side, $F(1.730, 15.573) = 18.444$, $p < 0.001$, partial $\eta^2 = 0.672$.

Skin firmness increased for the 2 mm probe ACS + HA group until week 12 by 27.46 % ($p = 0.089$) and 20.51 % ($p = 0.237$) for the right and left side, respectively. After week 12 the skin firmness significantly decreased for the right side by 42.85 % ($p = 0.013$) and 29.83 % ($p = 0.053$) for the left side again. However, the final difference between screening and week 24 was not significant, with 3.63 % ($p = 1.000$) for the right side and 3.20 % ($p = 1.000$) for the left side. The ANOVA for the difference between the visits was for the right side, $F(1.655, 8.276) = 8.052$, $p = 0.014$, partial $\eta^2 = 0.617$ and $F(1.759, 8.795) = 4.483$, $p = 0.049$, partial $\eta^2 = 0.473$ for the left side.

Left and right side mean values and SDs were comparable during each visit and for both treatment groups, which was confirmed by the insignificant paired samples t-tests (data in appendix). As the significant values of the individual visits already suggested, the effect size was large for both treatment groups. There was no statistically significant difference between the ACS and ACS + HA treatment group. But the ACS + HA group showed higher values during screening with 3.33 %, in week 12 with 5.83 %, and in week 24 with 10.31 % (average of both sides).

The 4 mm probe measurements developed similarly for both treatment groups compared to the 2 mm probe measurements. The ANOVA of the ACS group showed a statistically significant difference between the visits for the right side, $F(1.329, 11.957) = 28.916$, $p < 0.001$, partial $\eta^2 = 0.763$ and $F(1.686, 15.170) = 37.278$, $p < 0.001$, partial $\eta^2 = 0.806$ for the left side. There was a significant increase in skin firmness for the ACS group until week 12 by 29.53 % ($p < 0.001$) and 33.88 % ($p < 0.001$) for the right and left side, respectively. After week 12 skin firmness significantly decreased again by 45.90 % ($p = 0.002$) and 51.65 % ($p < 0.001$) for the right and left side, respectively. However, the final difference between screening and week 24 was not significant, with 2.82 % ($p = 1.000$) for the right side and 0.27 % ($p = 1.000$) for the left side.

For the ACS + HA group, the ANOVA for the difference between the visits was for the right side, $F(1.720, 8.602) = 9.907$, $p = 0.007$, partial $\eta^2 = 0.665$ and for the left side, $F(1.706, 8.528) = 5.908$, $p = 0.028$, partial $\eta^2 = 0.542$. Skin firmness significantly increased until week 12 for the right side by 28.15 % (p = 0.014). The left side increased by 25.50 % (p = 0.084). After week 12 the skin firmness decreased for the right side by 20.21 % (p = 0.137) and by 17.59 % (p = 0.484) for the left side again. The final values were 13.63 % (p = 0.384) and 12.40 % (p = 0.244) below the screening values.

Left and right side mean values and SDs were comparable during each visit and for both treatment groups, which was confirmed by the insignificant paired samples t-tests.

The values of the ACS + HA group were during screening 14.77 % higher compared to the ACS group and showed a statistically significant difference in week 12 (p = 0.025, 26.73 %), but only for the left side. The average difference was in week 12 23.02 %. In week 24 the ACS group was 1.67 % above the ACS + HA group.

3.2.2.3.2 Skin firmness after repeated suction (R3, Uf_5)

The last maximum amplitude R3 (Uf_5) is the last maximum amplitude of the suction phase after 5 repetitions of one measurement cycle. It gives information about the skin tiring effects. The closer it is to R0 (Uf), the lower the tiring effect [168].

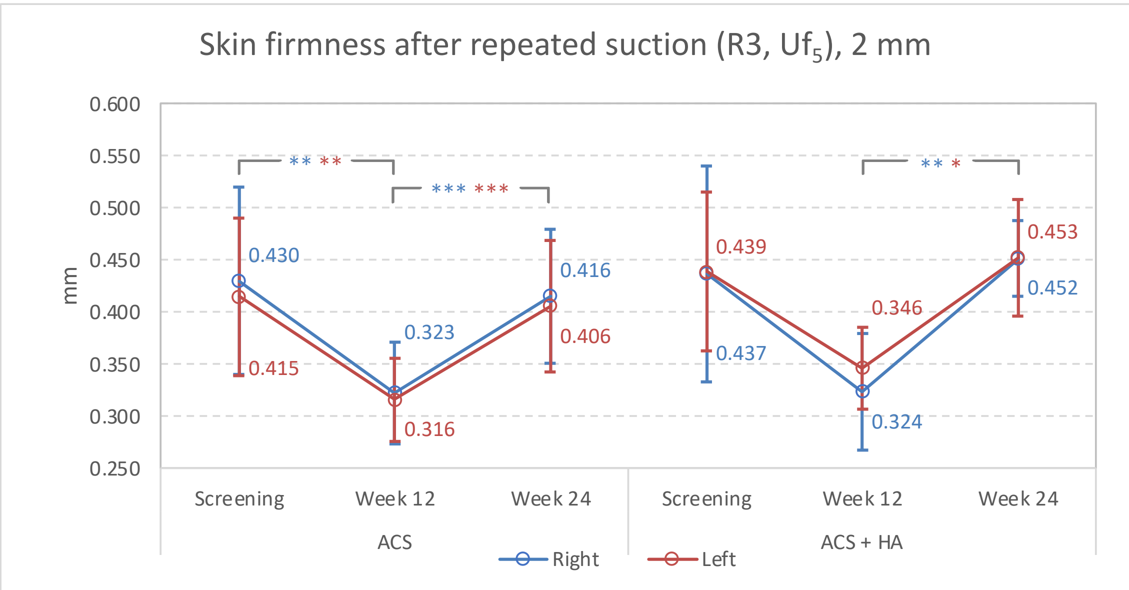


*Figure 59: Skin firmness after repeated suction, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

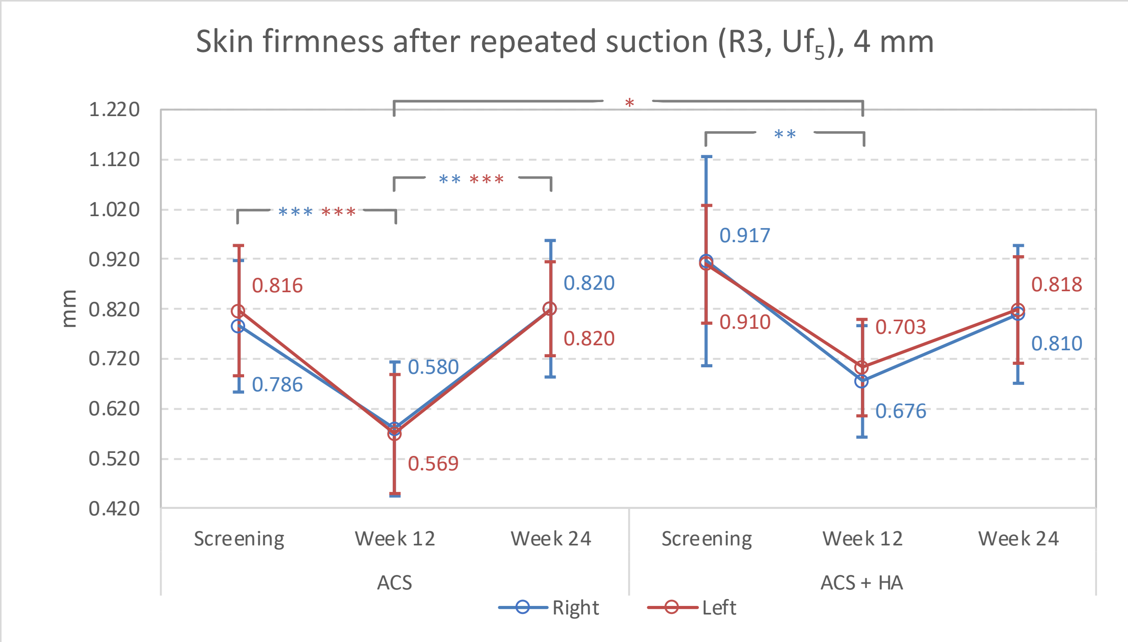


*Figure 60: Skin firmness after repeated suction, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The development of the skin firmness after repeated suction (R3, Uf_5) showed for both treatment groups and probe sizes similar developments to those of the R0 (Uf) measurements.

For the ACS group 2 mm probe, the mean values were statistically significant different between the visits for the right side, $F(1.566, 14.096) = 17.123$, $p < 0.001$, partial $\eta^2 = 0.655$ and for the left side, $F(1.805, 16.258) = 17.968$, $p < 0.001$, partial $\eta^2 = 0.666$. The comparison between both sides was not significant, as the matching values already suggested (data in appendix).

Skin firmness after repeated suction decreased from 0.422 mm during screening to 0.319 mm in week 12, then increased to a final value of 0.411 mm in week 24, on average of both sides. Hence, skin firmness after repeated suction significantly increased on average by 24.41 % ($p = 0.005$ right side, $p = 0.003$ left side) from screening to week 12. The decrease between week 12 and 24 was for both sides on average 28.62 % ($p < 0.001$ right side, $p = 0.001$ left side). The final skin firmness after repeated suction was 2.77 % below screening ($p = 1.000$ for both sides).

The results of the ACS + HA group 2 mm probe were nearly the same as the ACS group. The mean values were statistically significant different between the visits for the right side, $F(1.566, 7.830) = 9.087$, $p = 0.011$, partial $\eta^2 = 0.645$ and for the left side, $F(1.784, 8.920) = 5.830$, $p = 0.021$, partial $\eta^2 = 0.538$. The right and left side values were nearly the same and hence, the comparison between the sides insignificant.

Skin firmness after repeated suction decreased from 0.438 mm during screening to 0.335 mm in week 12, then increased to a final value of 0.452 mm for both sides identically. Therefore, skin firmness after repeated suction increased by 23.47 % ($p = 0.078$ right side, $p = 0.167$ left side) from screening to week 12. The decrease between week 12 and 24 was for both sides on average significantly 34.95 % ($p = 0.007$ right side, $p = 0.035$ left side). The final skin firmness after repeated suction was 3.27 % higher than screening ($p = 1.000$ for both sides).
Both treatment groups developed similar, showed a lager effect size and no significant difference between the sides (data in appendix).

The 4 mm probe results are visualized in *Figure 60*. The results of the ANOVA for the difference between the visits was for the right side, $F(1.225, 11.026) = 24.904$, $p < 0.001$, partial $\eta^2 = 0.735$ and for the left side, $F(1.686, 15.170) = 37.278$, $p < 0.001$, partial $\eta^2 = 0.806$. There was a significant decrease in skin firmness after repeated suction for the ACS group in week 12 by 26.17 % ($p < 0.001$) and 30.24 % ($p = 0.010$) for the right and left side, respectively. After week 12 the values significantly increased again by 41.47 % ($p = 0.002$) and 44.05 % ($p < 0.001$) for the right and left side, respectively. However, the final difference between screening and week 24 was not significant, with 4.44 % ($p = 0.529$) for the right side and 0.49 % ($p = 1.000$) for the left side above screening.
For the ACS + HA group, the statistical analyzation for the difference between the visits showed significant results for the right side, $F(1.622, 8.108) = 10.318$, $p = 0.007$, partial $\eta^2 = 0.674$ and for the left side, $F(1.696, 8.478) = 4.912$, $p = 0.042$, partial $\eta^2 = 0.496$. Skin firmness after repeated suction significantly decreased by 26.30 % ($p = 0.010$) from screening to week 12 for the right side. The left side decreased by 22.77 % ($p = 0.130$). After week 12 skin firmness after repeated suction increased for the right side by 19.81 % ($p = 0.103$) and by 16.43 % ($p = 0.438$) for the left side again. The final values were 11.71 % ($p = 0.475$) and 10.07 % ($p = 0.418$) below the screening values.
Left and right side mean values and SDs were comparable during each visit in both treatment groups, which was confirmed by the insignificant paired samples t-tests (data in appendix).
As the significant values of the individual visits already suggested, the effect size was large for both treatment groups.

The values of the ACS + HA group were during screening and in week 12 higher compared to the ACS group and showed a statistically significant difference in week 12 (p = 0.030, 23.46 %), but like the skin firmness measurements (R0, Uf), only for the left side.

3.2.2.3.3 Skin tiring (R9, Uf_5 – Uf)

The difference of the last maximum amplitude and the first maximum amplitude is described as skin tiring R9 (Uf_5 – Uf). The smaller the value, the smaller the tiring effect. R9 visualizes therefore the difference between R0 (Uf) and R3 (Uf_5) [168].

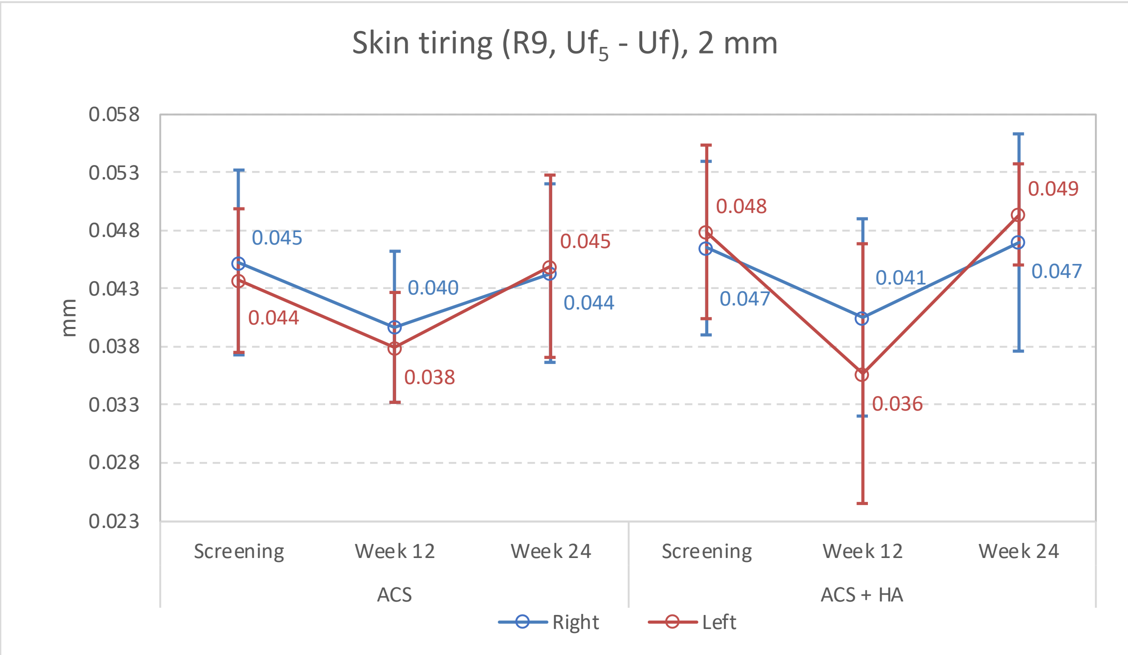


Figure 61: Skin tiring, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

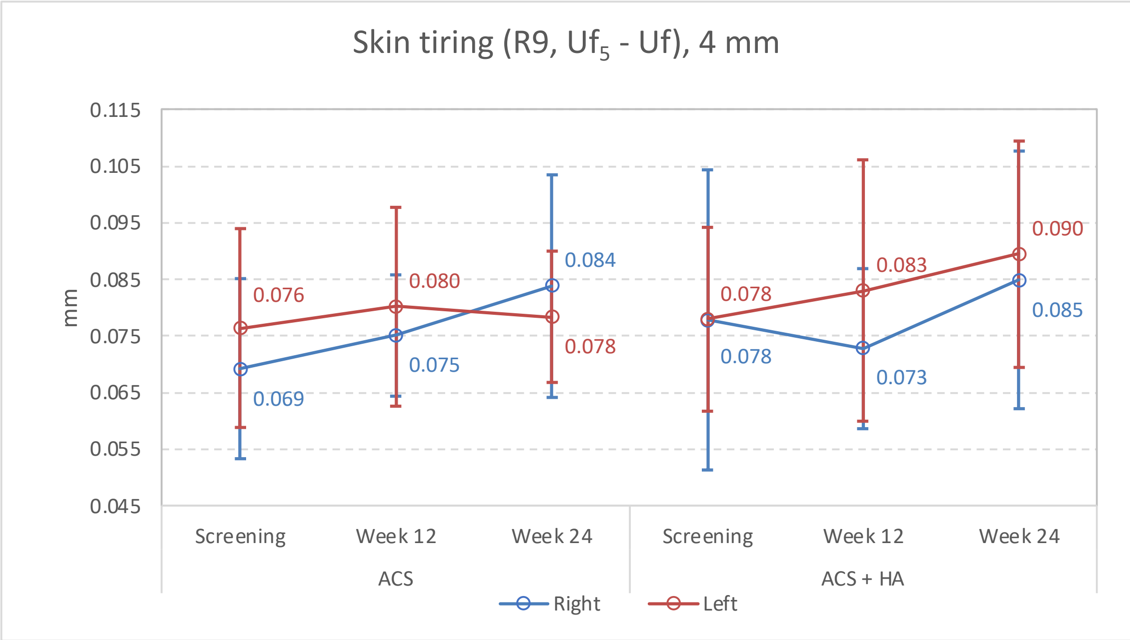


Figure 62: Skin tiring, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

Skin tiring showed no statistically significant difference for the visits, the sides nor between the treatment groups or the probe sizes.

Figure 61 shows the skin tiring of the 2 mm probe. Here, for the ACS group, the values decreased slightly by rounded 12.71 % ($p = 0.236$ right side, $p = 0.104$ left side) from screening to week 12. Then increased again by 14.95 % ($p = 0.352$ right side, $p = 0.107$ left side) from week 12 to week 24. The final values in week 24 equaled the screening values, the average difference was 0.34 % ($p = 1.000$ for both sides). The results of the ANOVA for the difference between the visit was for the right side, $F(1.921, 17.293) = 2.129$, $p = 0.150$, partial $\eta^2 = 0.191$ and $F(1.522, 13.697) = 3.042$, $p = 0.091$, partial $\eta^2 = 0.253$ for the left side.

The left side of the ACS + HA group showed a greater decrease, by – 25.44 % ($p = 0.065$), than the right side (– 12.90 % $p = 0.667$) from screening to week 12. And a stronger increase between week 12 and week 24, by 38.32 % ($p = 0.064$). The right side increased by 16.05 % ($p = 0.605$) between week 12 and week 24. Like in the ACS group, the final values in week 24 equaled the screening values, with 1.08 % ($p = 1.000$) for the right side and 3.14 % ($p = 1.000$) for the left side above screening values. The results of the ANOVA for the difference between the visits was for the right side, $F(1.125, 5.627) = 1.977$, $p = 0.215$, partial $\eta^2 = 0.283$ and $F(1.350, 6.752) = 9.763$, $p = 0.014$, partial $\eta^2 = 0.661$ for the left side. The left side showed a statistically significant overall difference, but as described above, the pairwise comparison was insignificant.

Figure 62 shows the skin tiring of the 4 mm probe. Here, for the ACS group, the right side values continuously increased by 21.24 % (p = 0.322) from screening to week 24. The left side stayed about the same, the final value was 2.62 % (p = 1.000) above screening. The results of the ANOVA for the difference between the visits was for the right side, $F(1.936, 17.422) = 1.913$, $p = 0.178$, partial $\eta^2 = 0.175$ and $F(1.752, 15.770) = 0.112$, $p = 0.870$, partial $\eta^2 = 0.012$ for the left side.

In the ACS + HA group, the left side showed a continuous increase by 14.74 % (p = 0.788) from screening to week 24. The right side decreased by 6.42 % (p = 1.000) from screening to week 12, then increased again. The final value was 8.99 % (p = 1.000) above screening. The results of the ANOVA for the difference between the visits was for the right side, $F(1.355, 6.776) = 0.936$, $p = 0.399$, partial $\eta^2 = 0.158$ and $F(1.058, 5.292) = 0.841$, $p = 0.406$, partial $\eta^2 = 0.144$ for the left side.

3.2.2.3.4 Skin recovery (R8, Ua)

The first minimum amplitude R8 (Ua) shows the maximum recovery of the skin during the relaxation phase and allows conclusions about the recovery ability of the skin. The closer the value is to 0, the higher the skin's recovery ability [168]. Skin recovery (R8, Ua) is, like the skin firmness (R0, Uf), an absolute parameter and consists of an elastic and viscoelastic part.

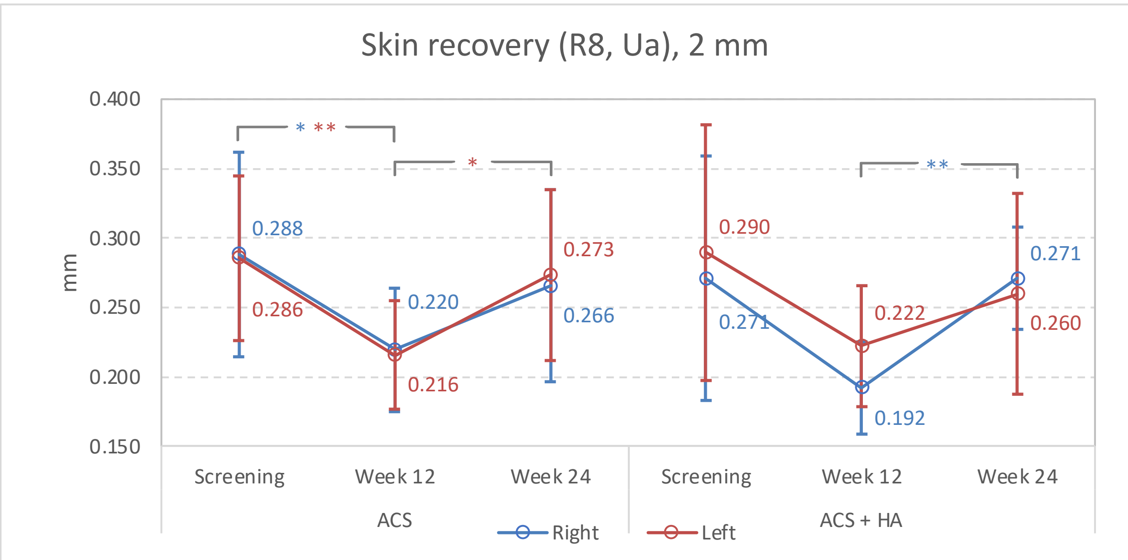


*Figure 63: Skin recovery, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

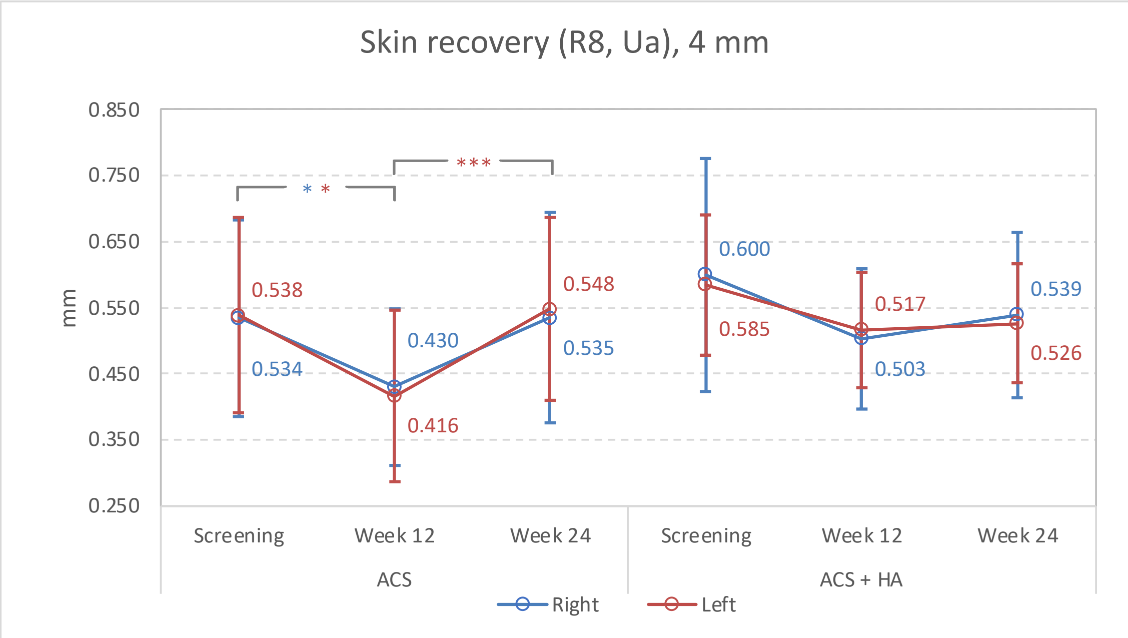


*Figure 64: Skin recovery, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Skin recovery developed similarly to skin firmness (R0, Uf) for both treatment groups and probe sizes. First with a decrease from screening to week 12 and then an increase to week 24. Also, right and left side mean values and SDs were comparable during each visit and for both treatment groups, which was confirmed by the insignificant paired samples t-tests. There was no statistically significant difference between the ACS and ACS + HA treatment group (data in appendix).

Figure 63 shows the skin recovery results of the 2 mm probe. Here, the ACS group significantly decreased until week 12 by 23.79 % (p = 0.030) and 24.41 % (p = 0.005) for the right and left side, respectively. After week 12 the skin recovery significantly increased for the left side only, by 26.69 % (p = 0.011). The right side increased by 20.79 % (p = 0.083). However, the final difference between screening and week 24 was insignificant, with – 7.94 % (p = 1.000) for the right side and – 4.24 % (p = 1.000) for the left side compared to screening. The results of the ANOVA for the difference between the visits was for the right side, F(1.648, 14.835) = 5.118, p = 0.025, partial η^2 = 0.363 and F(1.971, 17.739) = 12.532, p < 0.001, partial η^2 = 0.582 for the left side.

In the ACS + HA group, skin recovery decreased until week 12 by 29.09 % (p = 0.204) and 23.25 % (p = 0.500) for the right and left side, respectively. Skin recovery significantly increased for the right side only, by 41.02 % (p = 0.007) comparing week 12 with week 24. The left side increased by 16.87 % ($p = 1.000$). However, the final difference between screening and week 24 was insignificant, with no change ($p = 1.000$) for the right side and – 10.30 % ($p = 1.000$) for the left side. The results of the ANOVA for the difference between the visits was for the right side, $F(1.192, 5.960) = 6.120$, $p = 0.045$, partial $\eta^2 = 0.550$ and $F(1.830, 9.151) = 1.213$, $p = 0.336$, partial $\eta^2 = 0.195$ for the left side. The effect size was large for both treatment groups.

The 4 mm probe measurements are shown in *Figure 64*. There was a significant decrease in skin recovery for the ACS group until week 12 by 19.53 % ($p = 0.030$) and 22.73 % ($p = 0.036$) for the right and left side, respectively. After week 12, skin recovery significantly increased for the left side only, by 31.73 % ($p < 0.001$). The right side increased by 24.43 % ($p = 0.101$). However, the final difference between screening and week 24 was not significant, with 0.13 % ($p = 1.000$, right side) and 1.78 % ($p = 1.000$, left side) above the screening value. The results of the ANOVA for the difference between the visits was for the right side, $F(1.584, 14.258) = 6.256$, $p = 0.015$, partial $\eta^2 = 0.410$ and $F(1.268, 11.414) = 10.693$, $p = 0.005$, partial $\eta^2 = 0.543$ for the left side.
For the ACS + HA group, skin recovery showed no statistically significant difference between the visits for the right side, $F(1.978, 9.890) = 2.701$, $p = 0.116$, partial $\eta^2 = 0.351$ or the left side, $F(1.369, 6.847) = 1.562$, $p = 0.265$, partial $\eta^2 = 0.238$. The values decreased until week 12 for the right side by 16.15 % ($p = 0.181$) and 11.58 % ($p = 0.789$) for the left side. After week 12 the values increased by 7.16 % ($p = 1.000$) for the right side and 1.84 % ($p = 1.000$) for the left side. The final values were 10.14 % ($p = 0.673$, right side) and 9.95 % ($p = 0.466$, left side) below the screening values.
The values of the ACS + HA group were during screening on average 10.41 % higher than the ACS group (p = . In week 12 the difference was 20.56 %, in week 24 the ACS group was 1.63 % higher than the ACS + HA group. The difference was not significant (data in appendix).

3.2.2.3.5 Skin gross elasticity (R2, Ua/Uf)

The skin gross elasticity (Ua/Uf) is a relative parameter. It is the ratio of the first minimum amplitude of the relaxation phase (Ua) divided by the first maximal amplitude of the suction phase (Uf). The closer the value is to 1 (100 % retraction) the more elastic is the skin [168]. As the amplitude also includes the viscoelastic parts of the curve, the skin gross elasticity gives additionally information about the skin viscosity.

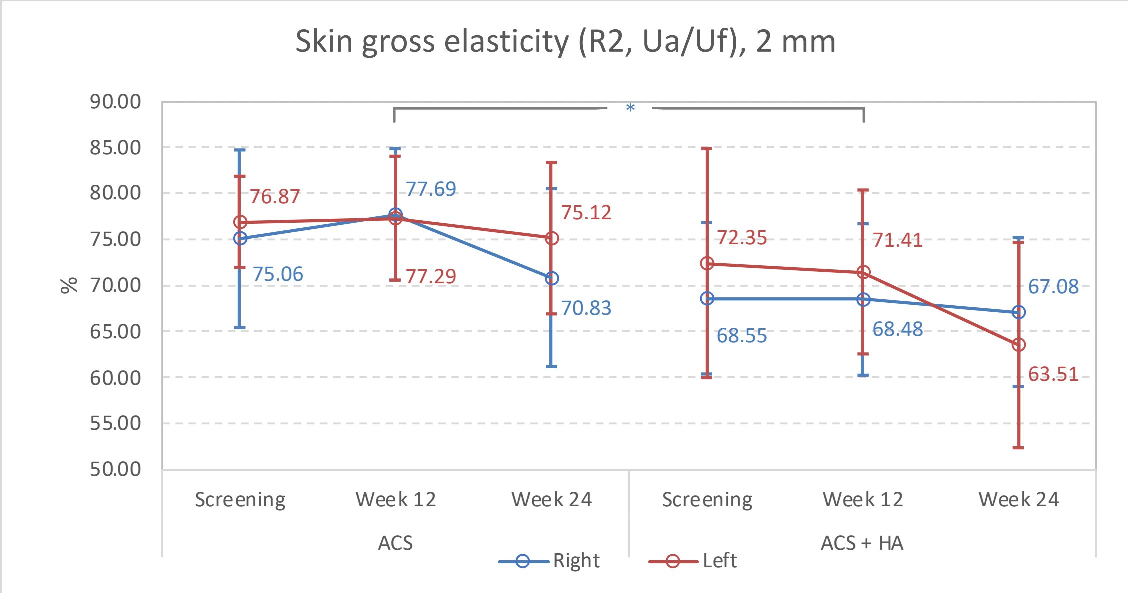


*Figure 65: Skin gross elasticity, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

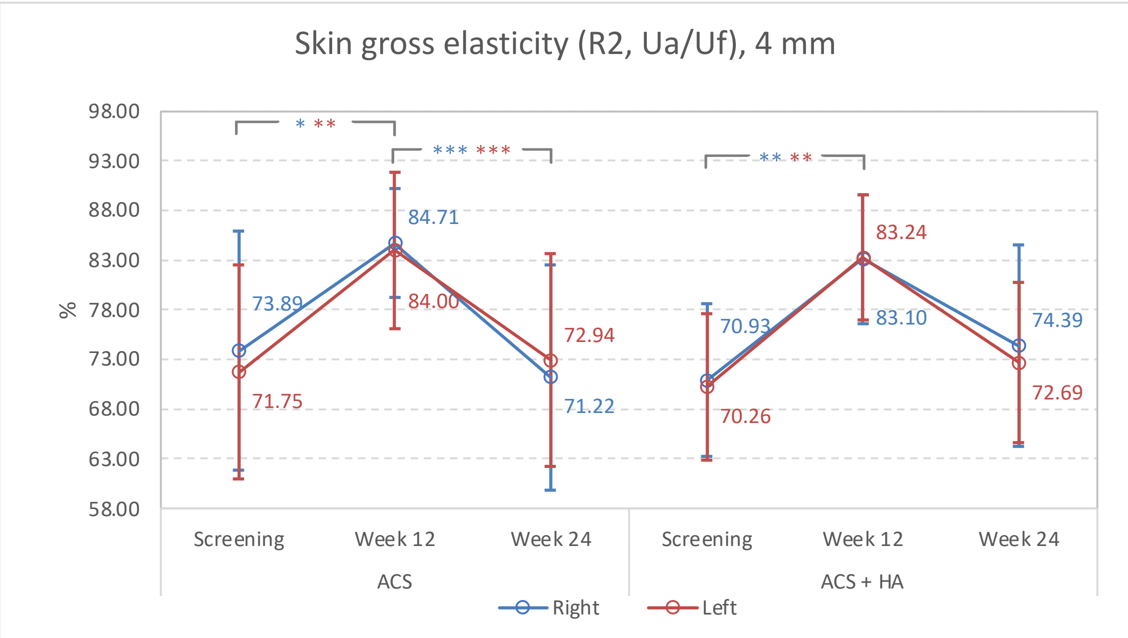


*Figure 66: Skin gross elasticity, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The gross elasticity measurements of the 2 mm probe are shown in *Figure 65*. Both treatment groups decreased slightly but insignificantly. Additionally, there was no difference between the right and left side values of the two groups.

There was no statistically significant difference between the visits of the ACS group for the right side, $F(1.694, 15.247) = 1.635$, $p = 0.227$, partial $\eta^2 = 0.154$ or the left side, $F(1.452, 13.068) = 0.447$, $p = 0.588$, partial $\eta^2 = 0.047$. The ACS + HA group also showed no statistically significant

differences between the visits for the right side, $F(1.894, 9.471) = 0.076$, $p = 0.927$, partial $\eta^2 = 0.015$ or the left side, $F(1.830, 9.152) = 1.001$, $p = 0.397$, partial $\eta^2 = 0.167$.
The ACS group values stayed above 70 % for all visits. The right side showed the strongest but also insignificant decrease form 75.06 % during screening to 70.83 % in week 24. In the ACS + HA group, the left side showed the strongest but insignificant decrease from 72.35 % during screening to 63.51 % in week 24. The right side was 68.55 % during screening and 67.08 % in week 24.
The only significant difference was between the treatment groups right sides in week 12 ($p = 0.047$), with 77.69 % for the ACS group and 68,48 % for the ACS +HA group.

Figure 66 shows the results of the skin gross elasticity measurements of the 4 mm probe. There was a statistically significant difference between the visits for the ACS group for the right side $F(1.877, 16.894) = 12.158$, $p < 0.001$, partial $\eta^2 = 0.575$ and $F(1.803, 16.225) = 17.498$, $p < 0.001$, partial $\eta^2 = 0.660$ for the left side. The ACS + HA group also showed significant differences between the visits for the right side $F(1.131, 5.653) = 13.223$, $p = 0.011$, partial $\eta^2 = 0.726$ and $F(1.589, 7.944) = 10.820$, $p = 0.007$, partial $\eta^2 = 0.684$ for the left side.
Both groups increased significantly until week 12 (ACS group: $p = 0.022$ right side, $p = 0.003$ left side; ACS + HA group: $p = 0.008$ right side, $p = 0.007$ left side), then decreased again, the ACS group again significantly ($p = 0.001$ right side, $p < 0.001$ left side), the ACS + HA insignificantly. Screening and week 24 values were insignificantly different and above 70 %, the week 12 values were above 80 %.
Both sides values were similarly for all visits and both treatment groups and therefore insignificantly different. The comparison between the ACS and ACS + HA group was also insignificant (data in appendix).

3.2.2.3.6 Skin net elasticity (R5, Ur/Ue)

The parameter R5 (Ur/Ue) is the ratio of the elastic part of the suction phase (Ur) and the elastic part of the relaxation phase (Ue). It gives information about the elastic fibers in the skin. Like the gross skin elasticity (R2), the closer the value is to 1 (100 %) the more elastic is the skin [168].

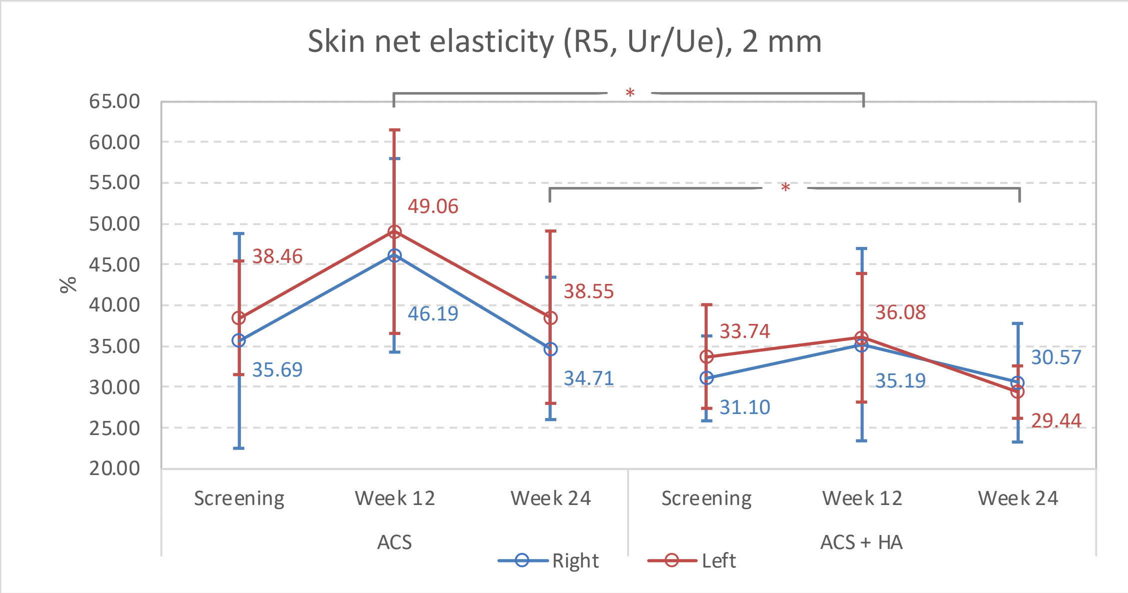


*Figure 67: Skin net elasticity, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

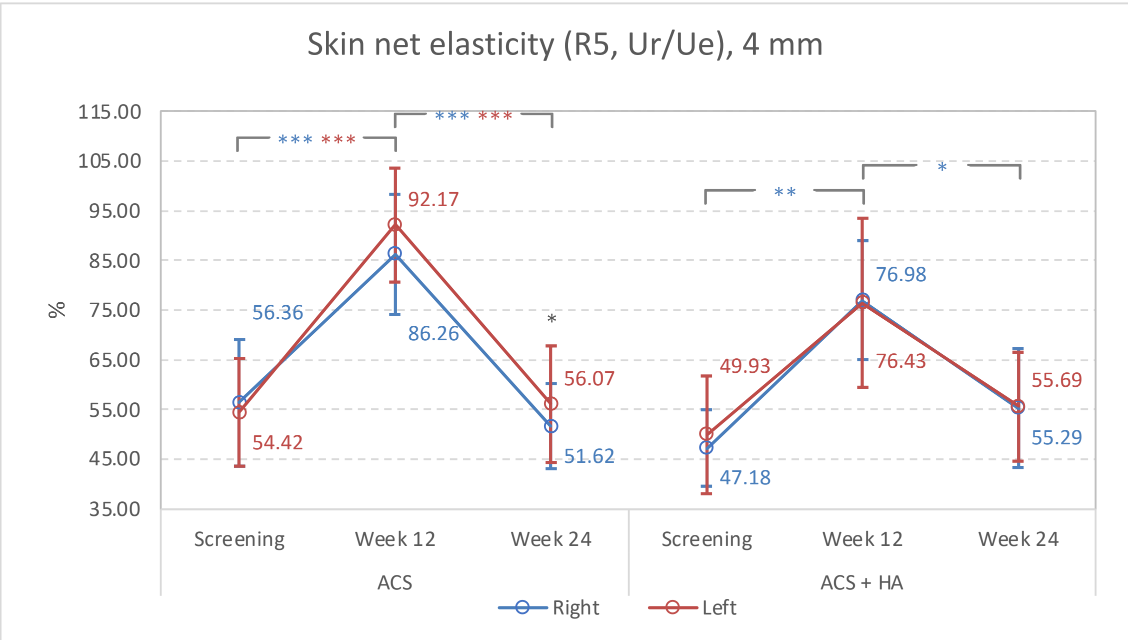


*Figure 68: Skin net elasticity, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Figure 67 visualizes the results of the 2 mm probe skin net elasticity. The right and left side values developed similarly and were insignificantly different, for both groups.

The results of the ANOVA for the difference between the visits was for the right side, $F(1.556, 14.005) = 5.017$, $p = 0.029$, partial $\eta^2 = 0.358$ and $F(1.887, 16.985) = 5.638$, $p = 0.014$, partial $\eta^2 = 0.385$ for the left side. Skin net elasticity increased from 37.07 % during screening to 47.63 % in week 12, then decreased again to 36.63 % in week 24 (average of both sides). There was an overall significant difference between the visits, but the pairwise comparison showed no significant difference between the visits anymore.

The ACS + HA group showed no statistically significant difference between the visits for the right side, $F(1.656, 8.278) = 0.523$, $p = 0.578$, partial $\eta^2 = 0.095$ or the left side, $F(1.589, 7.944) = 2.030$, $p = 0.195$, partial $\eta^2 = 0.289$. The values stayed throughout the study about the same and were on average for both sides 32.42 % during screening, 35.64 % in week 12 and 31.01 % at the end of the study in week 24. All measurements of the ACS group were higher compared to the ACS + HA group and were significantly higher for the left side measurements in week 12 ($p = 0.023$, ACS: 49.06 % vs ACS + HA: 36.08 %) and week 24 ($p = 0.027$, ACS: 38.55 % vs ACS + HA: 30.57 %).

The 4 mm probe results are shown in *Figure 68*. The ACS group showed statistically significant differences between the visits for the right side, $F(1.304, 11.735) = 45.879$, $p < 0.001$, partial $\eta^2 = 0.836$ and the left side, $F(1.419, 12.767) = 77.410$, $p < 0.001$, partial $\eta^2 = 0.896$.

The ACS + HA group also showed statistically significant differences between the visits for the right side, $F(1.461, 7.303) = 24.800$, $p < 0.001$, partial $\eta^2 = 0.832$ and the left side, $F(1.560, 7.799) = 7.371$, $p = 0.019$, partial $\eta^2 = 0.596$. All measurements, but the left side ACS + HA group, significantly increased from screening to week 12 (ACS right side: from 56.36 % to 86.26 %, $p < .001$; ACS left side: from 54.43 % to 92.17 %, $p < 0.001$; ACS + HA right side: from 47.18 % to 76.98 %, $p = 0.003$; ACS + HA left side: from 49.93 % to 76.43 %, $p = 0.056$), then decreased from week 12 to week 24 again. The final values were close to the screening values and statistically not significant different compared to screening (ACS right side: 51.62 %, $p = 0.225$; ACS left side: 56.07 %, $p = 1.000$; ACS + HA right side: 55.29 %, $p = 0.135$; ACS + HA left side: 55.69 %, $p = 0.922$). The week 24 left and right side measurements of the ACS group were significantly different ($p = 0.038$). There was no statistically difference between the treatment groups (data in appendix).

3.2.2.3.7 Ratio of viscoelastic to elastic extension (R6, Uv/Ue)

The relative parameter R6 (Uv/Ue) describes the viscoelastic versus the elastic ratio of the curve in the suction phase. The smaller the value the higher the elasticity and the more elastic fibers are in the skin [168].

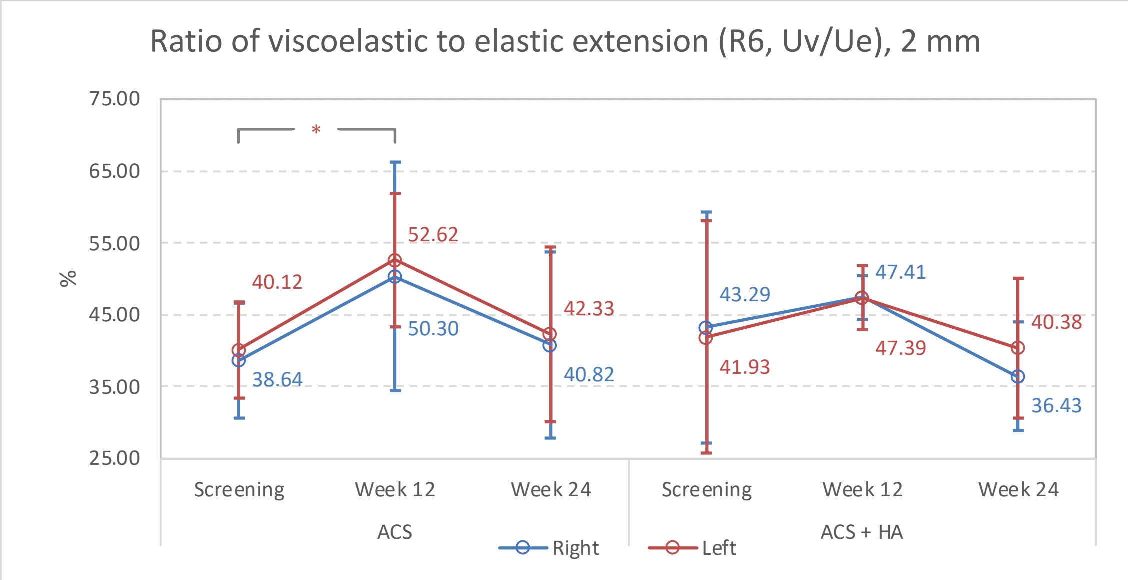


*Figure 69: Ratio of viscoelastic to elastic extension, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

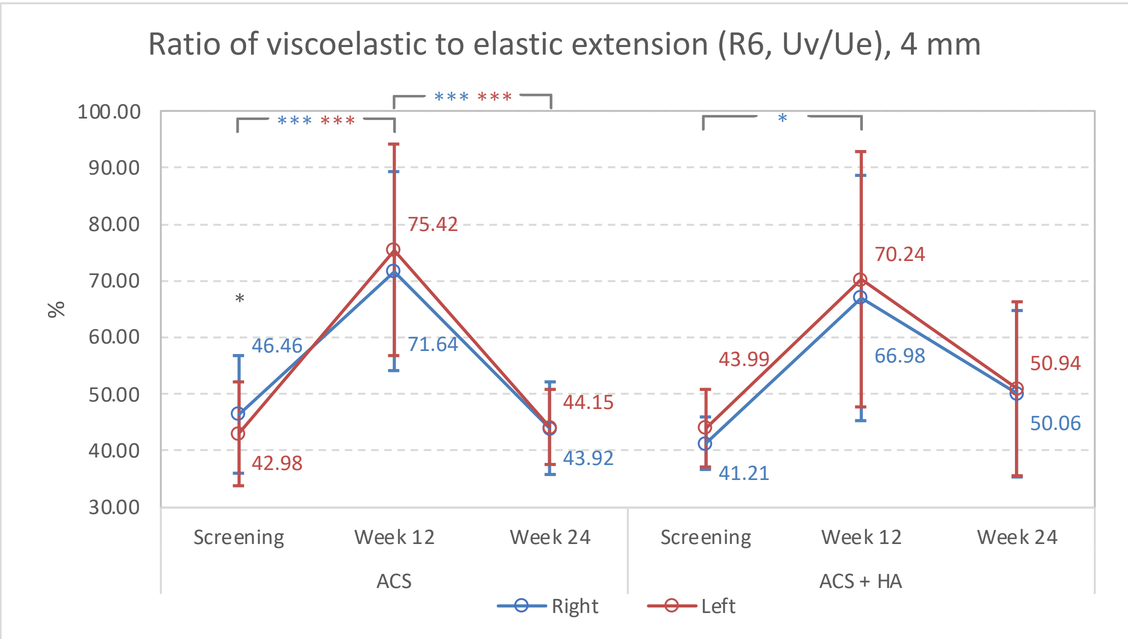


*Figure 70: Ratio of viscoelastic to elastic extension, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The ratio of viscoelastic to elastic extension of the 2 mm probe is visualized in *Figure 69*. The right and left side values developed similarly and were insignificantly different for both groups (data in appendix). The development of this parameter was similarly to the skin net elasticity parameter (R5, Ur/Ue). For the ACS group, the repeated measures ANOVA determined no statistically significant difference between the visits for the right side, $F(1.285, 11.562) = 3.979$, $p = 0.063$, partial $\eta^2 = 0.307$ but for the left side, $F(1.774, 15.963) = 6.536$, $p = 0.010$, partial $\eta^2 =$

0.421. The left side of the ACS group increased significantly from 40.12 % during screening to 52.62 % in week 12 ($p = 0.013$). The value decreased again and was just above the screening measurement in week 24 with 42.33 % ($p = 1.000$). The right side developed similarly but not significantly, from 38.64 % during screening to 50.30 % in week 12 ($p = 0.182$) and 40.82 % at the end of the study in week 24 ($p = 1.000$).
The ACS + HA showed no statistically significant difference between the visits for the right side, $F(1.342, 6.711) = 1.767$, $p = 0.235$, partial $\eta^2 = 0.261$ and the left side, $F(1.215, 6.074) = 0.652$, $p = 0.480$, partial $\eta^2 = 0.115$. In the ACS + HA group the increase to week 12 was smaller as well as the decrease to week 24 compared to the ACS group. On average both sides were 42.61 % during screening, increased to 47.40 % in week 12 and decreased to 38.40 % in week 24.

Figure 70 visualizes the results of the ratio of viscoelastic to elastic extension parameter of the 4 mm probe. Like the 2 mm probe measurements, the 4 mm probe developed similarly to the 4 mm probe skin net elasticity (R5, Ur/Ue) parameter. For the ACS treatment group the results of the repeated measures ANOVA for the difference between the visits was for the right side, $F(1.486, 13.372) = 30.810$, $p < 0.001$, partial $\eta^2 = 0.774$ and for the left side, $F(1.322, 11.898) = 46.958$, $p < 0.001$, partial $\eta^2 = 0.839$. The right and left side screening values of the ACS group were significantly different ($p = 0.026$), but the increase to week 12 and decrease to week 24 was identically significant. They increased from 46.46 % and 42.98 % during screening to 71.92 % and 75.42 % in week 12 ($p < 0.001$ for both sides) and decreased to 43.92 % and 44.15 % in week 24 ($p < 0.001$, for both sides, comparing week 12 and week 24) for the right and left side, respectively. The difference between screening and week 24 was insignificant ($p = 1.000$ for both sides).
The ACS + HA group developed similarly to the ACS group, but the only significant difference was for the right side from screening to week 12 ($p = 0.045$). Both sides values were alike and increased on average from 42.60 % during screening to 68.61 % (right side: $p = 0.045$, left side: $p = 0.067$) in week 12, then decreased to 50.50 % (right side: $p = 0.435$, left side: $p = 0.472$) in week 24. The results of the repeated measures ANOVA for the difference between the visits was for the right side, $F(1.763, 8.817) = 8.262$, $p = 0.011$, partial $\eta^2 = 0.623$ and for the left side, $F(1.420, 7.100) = 8.648$, $p = 0.016$, partial $\eta^2 = 0.634$. The left side ANOVA showed an overall

significance, but the pairwise comparison was not significance anymore, due to the Bonferroni adjustments for multiple comparisons.

3.2.2.3.8 Ratio of elastic recovery to total extension (R7, Ur/Uf)

The parameter R7 is another relative parameter (Ur/Uf). It is the ratio of the elastic recovery (Ur) of the relaxation phase and the maximum firmness (Uf). The closer the value is to 1 (100 %) the more elastic is the skin [168]. This parameter is discussed to decrease with age [173].

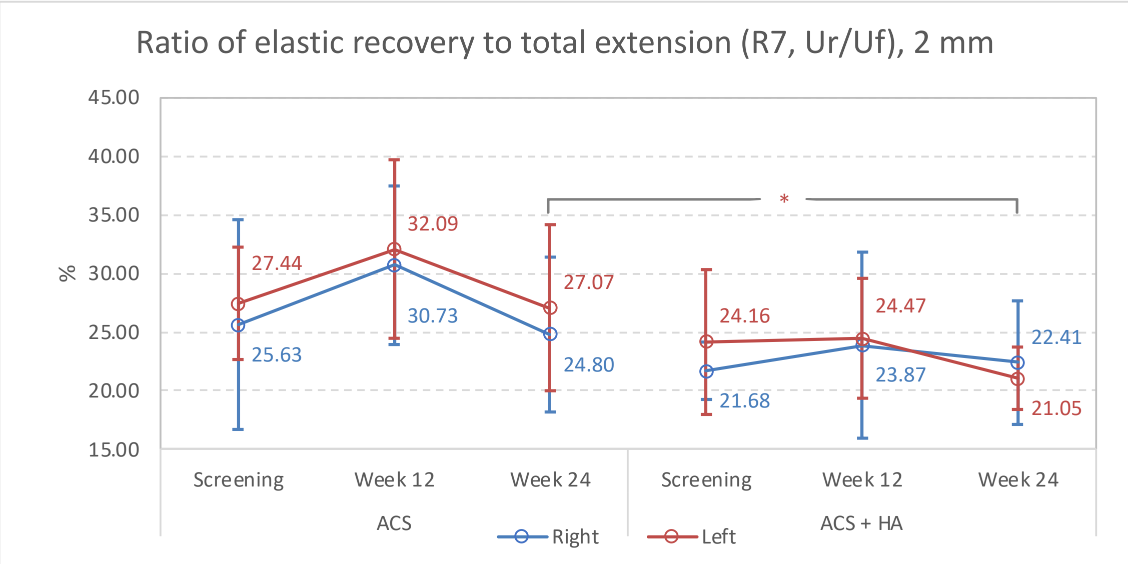


*Figure 71: Ratio of elastic recovery to total extension, ACS in vivo study II, 2 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

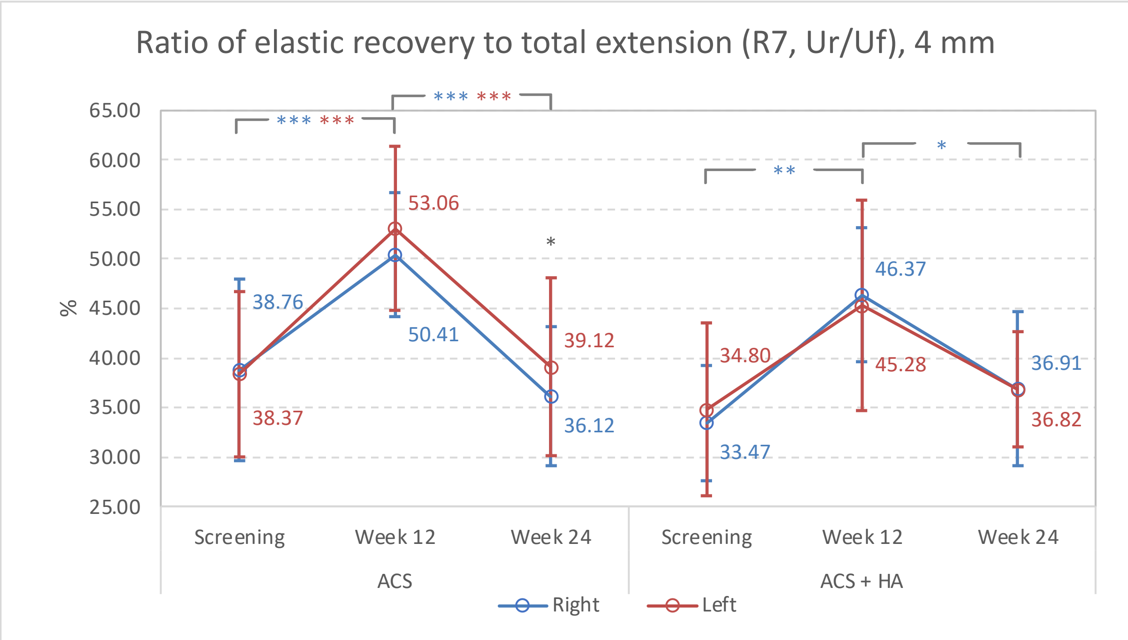


*Figure 72: Ratio of elastic recovery to total extension, ACS in vivo study II, 4 mm probe, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Figure 71 visualizes the results of the 2 mm probe ratio of elastic recovery to total extension and is comparable to the results of the skin net elasticity (R5, Ur/Ue). The right and left side values developed similarly and were insignificantly different, for both groups. For the ACS group the results of the repeated measures ANOVA for the difference between the visits was for the right side, F(1.867, 16.804) = 4.124, p = 0.037, partial η^2 = 0.314 and for the left side, F(1.968, 17.713) = 4.304, p = 0.030, partial η^2 = 0.324. For both sides together the parameter increased from rounded 26.53 % during screening to 31.41 % (right side: p = in week 12, then decreased again to 25.94 % in week 24. There was an overall significance, but the pairwise comparison showed no significant difference between the visits anymore.

For the ACS + HA group the results of the repeated measures ANOVA for the difference between the visits was for the right side, F(1.616, 8.080) = 0.324, p = 0.688, partial η^2 = 0.061 and for the left side, F(1.809, 9.046) = 1.220, p = 0.334, partial η^2 = 0.196. The values stayed throughout the study about the same and were on average for both sides 22.92 % during screening, 24.17 % in week 12 and 21.73 % at the end of the study in week 24.

All measurements of the ACS group were higher compared to the ACS + HA group and showed a significantly higher value for the left side measurement in week 24 (p = 0.031, ACS: 27.07 % vs ACS + HA: 21,05 %).

The 4 mm probe results are shown in *Figure 72*. There was no statistical difference between the treatments groups (data in appendix), but for the ACS group the left and right side measurements were significantly different in week 24 ($p = 0.038$).

The ACS group showed a statistically significant difference between the visits for the right side, $F(1.539, 13.853) = 42.995$, $p < 0.001$, partial $\eta^2 = 0.827$ and for the left side, $F(1.517, 13.657) = 54.671$, $p < 0.001$, partial $\eta^2 = 0.859$. The ACS + HA showed a statistically significant difference between the visits for the right side, $F(1.485, 7.427) = 30.479$, $p < 0.001$, partial $\eta^2 = 0.859$ and for the left side, $F(1.624, 8.119) = 5.213$, $p = 0.040$, partial $\eta^2 = 0.510$. The pairwise comparison showed for all measurements, but the left side ACS + HA group, significantly increased from screening to week 12 (ACS right side: from 38.76 % to 50.41 %, $p <.001$; ACS left side: from 38.37 % to 53.06 %, $p < 0.001$; ACS + HA right side: from 33.47 % to 46.37 %, $p = 0.002$; ACS + HA left side: from 34.80 % to 45.28 %, $p = 0.104$), then significantly decreased from week 12 to week 24 again (ACS right side: $p <.001$; ACS left side: $p < 0.001$; ACS + HA: $p = 0.020$; ACS + HA left side: $p = 0.263$). The final values were close to the screening values and statistically not significant different (ACS right side: 39.12 %, $p = 0.258$; ACS left side: from 36.12 %, $p = 1.000$; ACS + HA right side: from 36.91 %, $p = 0.117$; ACS + HA left side: from 36.82 %, $p = 1.000$).

3.2.2.4 Sonography

The skin density and thickness was measured with the ultrasound DUB Skin Scanner (Tpm taberna pro medicus) device with a 22 MHz transducer (compare *2.3.4.3.1 Sonography measurements and evaluation method*). Each side was measured one time at each visit. The results of the skin density and thickness are listed in the graphs below with the means and SDs of all 16 patients of the right (blue) and left (red) side, separated after treatment group.

3.2.2.4.1 Skin density

A high skin density is visualized by a high echogenicity of the skin connective tissue and can be seen as a bright colored image in the sonogram in the b mode. In aged skin (> 70 years) the echogenicity decreases especially in sun damaged skin areas and is visualized with a less brighter image and lower values [177].

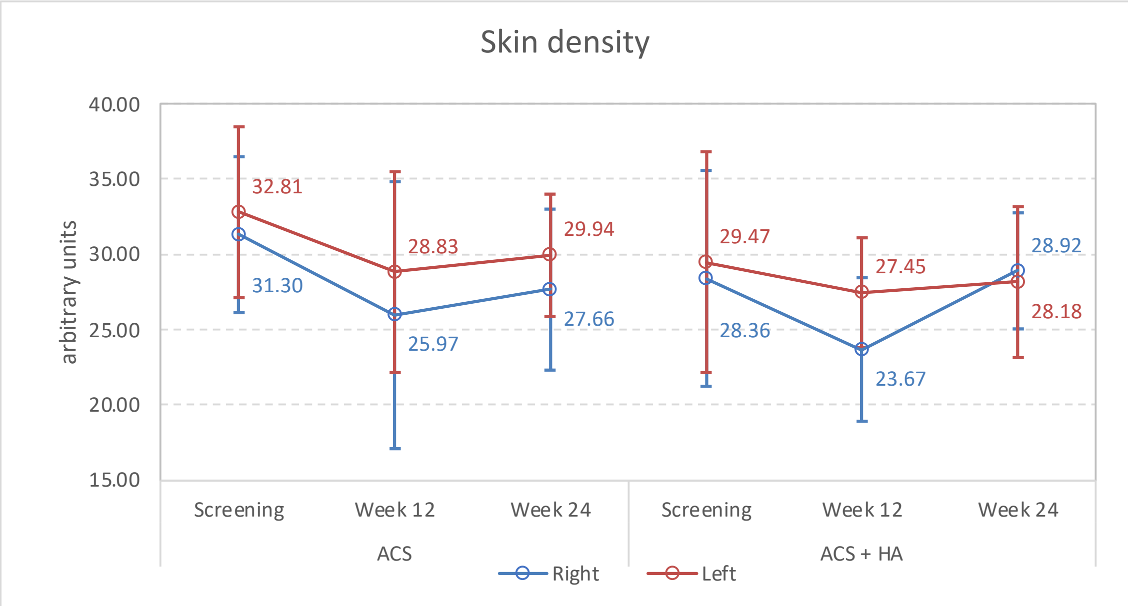


Figure 73: Skin density, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

The results of the skin density measurements are shown in *Figure 73*. For the ACS group, there was no statistically significant difference between the visits, $F(1.502, 13.516) = 2.418$, $p = 0.135$, partial $\eta^2 = 0.212$ and no overall difference between the left and right side, $F(1.000, 9.000) = 2.276$, $p = 0.166$, partial $\eta^2 = 0.202$. Neither was the interaction of the sides and visits statistically significant, $F(1.819, 16.370) = 0.098$, $p = 0.891$, partial $\eta^2 = 0.011$.

Although the difference between the right and left side measurements were statistically insignificant, the left side was throughout the study on average 7.83 % higher. Both sides decreased slightly from 31.30 a.u. and 32.81 a.u. at screening to 27,66 a.u. and 29.94 a.u. in week 24 for the right and left side, respectively.

Skin density of the ACS + HA group was also statistically not significant different between the visits, $F(1.321, 6.607) = 2.553$, $p = 0.156$, partial $\eta^2 = 0.338$, between the left and right side, $F(1.000, 5.000) = 0.638$, $p = 0.461$, partial $\eta^2 = 0.113$, nor was the interaction of the sides and visits statistically significant, $F(1.238, 6.191) = 1.066$, $p = 0.360$, partial $\eta^2 = 0.176$.

However, the right side showed a stronger decease form 28.36 a.u. during screening to 23.67 a.u. in week 12. Then the skin density increased again just above the screening value to 28.92 a.u. in week 24. The left side just slightly decrease from 29.47 a.u. during screening to 28.18 a.u. at the end of the study in week 24.

The comparison of the two treatment groups included the mean of both sides together and showed no significant difference either (screening: p = 0.264, week 12: p = 0.488, week 24: p = 0.907).

3.2.2.4.2 Skin thickness

Skin thickness is calculated in µm and measured from the skin entrance echo to the last echogenic area seen in the image of the B-mode. In ultrasound images skin thickness decreases with age

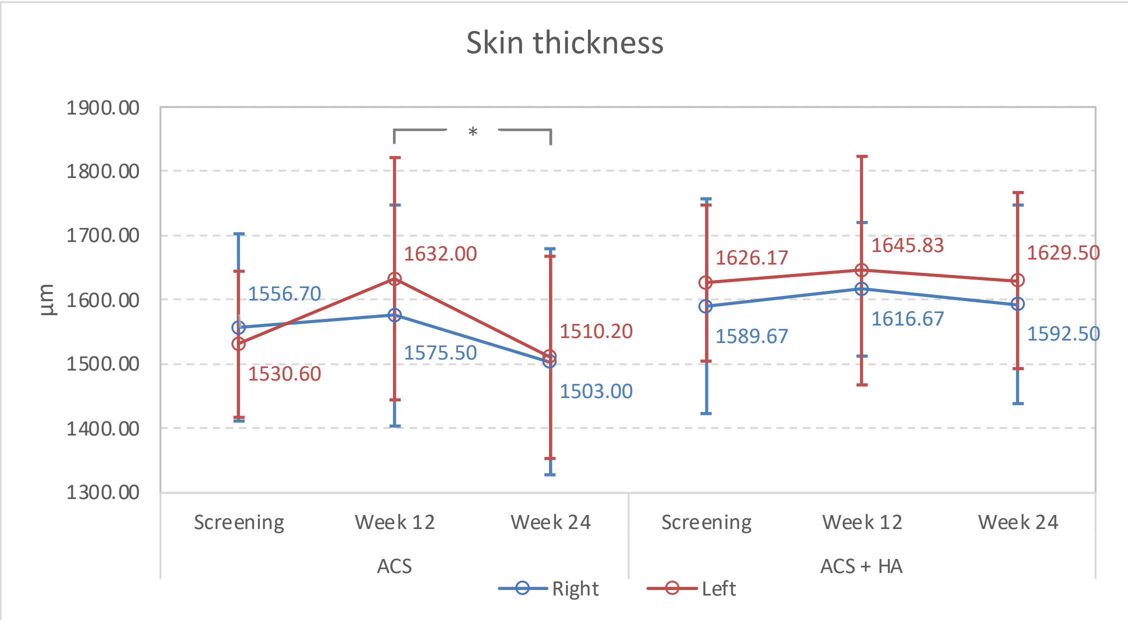


*Figure 74: Skin thickness, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The results of the skin thickness measurements are shown in *Figure 74*. For the ACS group, there was a statistically significant difference between the visits, $F(1.957, 17.610) = 4.755$, $p = 0.023$, partial $\eta^2 = 0.346$, but no overall difference between the left and right side, $F(1.000, 9.000) = 0.197$, $p = 0.668$, partial $\eta^2 = 0.021$. Neither was the interaction of the sides and visits statistically significant, $F(1.447, 13.022) = 1.061$, $p = 0.351$, partial $\eta^2 = 0.105$. The mean of both sides was calculated and slightly increase from 1543.65 µm during screening to 1603.75 µm in week 12. Then significantly decreased to 1506.60 µm in week 24 (p = 0.027, comparing week 12 and week 24). The final value in week 24 was slightly, but not significantly, lower than the screening value (p = 0.883, – 2.40 %).

Skin thickness of the ACS + HA group did not change throughout the study. There was no statistically significant difference between the visits, $F(1.523, 7.617) = 0.123$, $p = 0.834$, partial $\eta^2 = 0.024$ or between the left and right side, $F(1.000, 5.000) = 1.110$, $p = 0.340$, partial $\eta^2 = 0.182$. Neither was the interaction of the sides and visits statistically significant, $F(1.986, 9.932) = 0.018$, $p = 0.982$, partial $\eta^2 = 0.004$. Although the difference between the right and left side measurements were statistically insignificant, the left side was throughout the study slightly higher, on average by 2.14 %.
The comparison of the two treatment groups included the mean of both sides together and showed no significant difference either (screening: $p = 0.361$, week 12: $p = 0.728$, week 24: $p = 0.175$).

3.2.2.5 PRIMOS – skin topography

3.2.2.5.1 Mean skin roughness (Ra)

The mean skin roughness (Ra) is the average roughness of the set star lines separated into five sections each. The higher the values, the rougher the skins surface and an increase is associated with skin aging [191, 199].

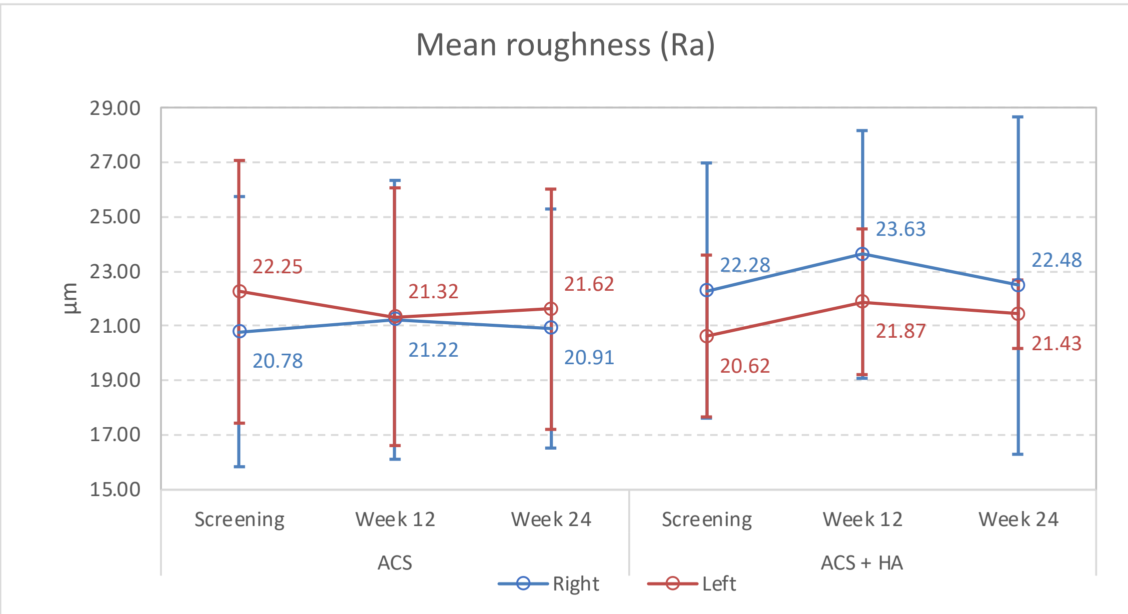


Figure 75: Mean roughness, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

The mean skin roughness of the ACS group was 20.78 µm for the right side and 22.25 µm for the left side at the beginning of the study, 20.91 µm (right side) and 21.62 µm (left side) at the end of the study and showed no statistically significant difference between the visits for the right side, $F(1.288, 11.935) = 0.080$, $p = 0.924$, partial $\eta^2 = 0{,}009$ or the left side, $F(1.925, 17.329) = 0.750$, $p = 0.482$, partial $\eta^2 = 0.077$ (*Figure 75*). As the partial η^2 were below 0.06 for both sides the effect size was small. The percentage difference between screening and week 24 was only 0.63 % and – 2.83 % for the right and left side, respectively. The left side was on average 3.50 % higher than the right side.

The mean skin roughness of the ACS + HA group was 22.28 µm for the right side and 20.62 µm for the left side at the beginning of the study, 22.48 µm (right side) and 21.43 µm (left side) at the end of the study and showed no statistically significant difference between the visits for the right side, $F(1.542, 7.710) = 0.201$, $p = 0.768$, partial $\eta^2 = 0.039$ or the left side, $F(1.410, 7.052) = 1.611$, $p = 0.257$, partial $\eta^2 = 0.244$. The effect size was small, as the partial η^2 was below 0.06 for both sides. The percentage difference between screening and week 24 was 0.90 % and 3.96 % for the right and left side, respectively. In contrast to the ACS group, the right side was on average 6.55 % higher than the left side. There was no statistically significant difference between the ACS and ACS + HA treatment groups (data in appendix).

3.2.2.5.2 Maximum roughness (Rmax)

The maximum roughness (Rmax or Rm) is the greatest minimum-maximum difference of all segments of the measured profile. Rmax has also been shown to increases with age [195].

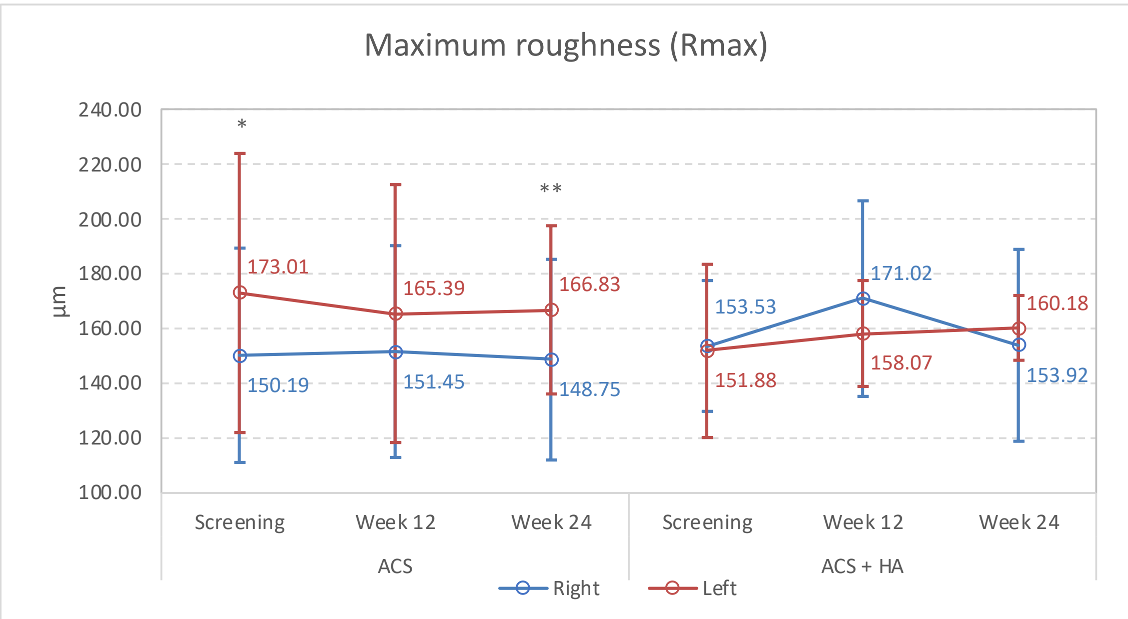


*Figure 76: Maximum roughness, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The change of the maximum roughness was like the mean skin roughness insignificant throughout the study period for both treatment groups. There was no statistically significant difference between the two treatment groups, either.

The mean values of the ACS group showed no statistically significant difference between the visits for the right side, $F(1.269, 11.422) = 0.039$, $p = 0.897$ partial $\eta^2 = 0.004$ or the left side, $F(1.761, 15.847) = 0.512$, $p = 0.586$, partial $\eta^2 = 0.054$. The effect size was small, as the partial η^2 was below 0.06 for both sides.

The maximum roughness of the ACS group was 150.19 µm for the right side and 173.01 µm for the left side at the beginning of the study, 148.75 µm (right side) and 166.83 µm (left side) at the end of the study and showed no significant change. The percentage difference between screening and week 24 was – 0.96 % and – 3.57 % for the right and left side, respectively.

The left side was on average 10.85 % higher than the right side, significantly during screening ($p = 0.038$, 15.19 %) and in week 24 ($p = 0.002$, 12.15 %).

The values of the ACS + HA group were 153.53 µm for the right side and 151.88 µm for the left side at the beginning of the study. At the end of the study the right side value was 153.92 µm, the left side value 160.18 µm. The measurements showed no significant difference between the visits for the right side, $F(1.501, 7.606) = 0.623$, $p = 0.518$, partial $\eta^2 = 0.111$ nor the left side, $F(1.262, 6.308) = 0.620$, $p = 0.496$, partial $\eta^2 = 0.110$. The percentage difference between screening and week 24 was 0.25 % and 5.46 % for the right and left side, respectively. Both sides showed similar values throughout the study.

3.2.2.5.3 Mean depth of roughness (Rz)

The mean depth of roughness is calculated as the average from all minimum-maximum difference within each measurement section [211]. An age correlation has been found here as well, with higher values with increasing age [195].

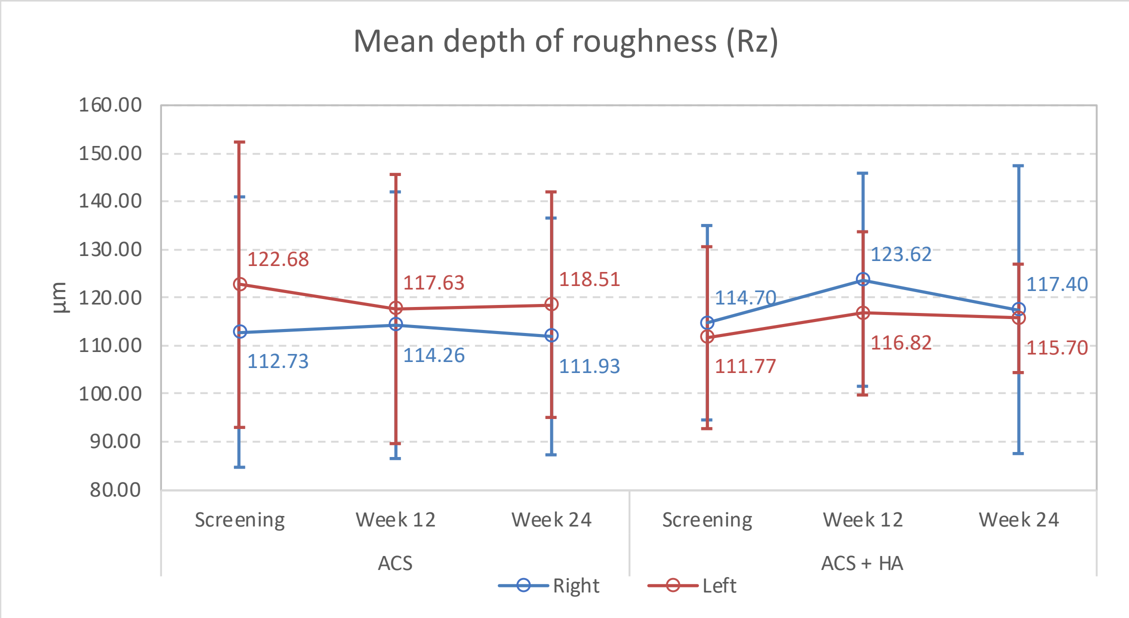


Figure 77: Mean depth of roughness, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

The change of the mean depth of roughness was, like the other two skin roughness parameters, insignificant throughout the study period for both treatment groups. There was no statistically significant difference between the left and right side, nor between the two treatment groups (*Figure 77*).

For the ACS group, there was no statistically significant difference between the visits for the right side, $F(1.298, 11.678) = 0.070$, $p = 0.857$, partial $\eta^2 = 0.008$ or the left side, $F(1.816, 16.346) = 0.671$, $p = 0.511$, partial $\eta^2 = 0.069$. The mean depth of roughness for the ACS group was 112.73 µm for the right side and 122.68 µm for the left side at the beginning of the study. The final values were 111.93 µm for the right side and 118.51 µm for the left side. The percentage difference between screening and week 24 was – 0.71 % and – 3.40 % for the right and left side, respectively. The left side was on average 5.55 % higher than the right side.

The statistical analyzation of the ACS + HA group showed no significant difference between the visits for the right side, $F(1.630, 8.150) = 0.310$, $p = 0.700$, partial $\eta^2 = 0.058$ or the left side, $F(1.261, 6.305) = 0.964$, $p = 0.387$, partial $\eta^2 = 0.162$. The mean depth of roughness of the ACS + HA group was 114.70 µm for the right side and 111.77 µm for the left side at the beginning of the study. The final values were 117.40 µm for the right side and 115.70 µm for the left side. The percentage difference between screening and week 24 was 2.35 % and 3.52 % for the right and left side, respectively. In contrast to the ACS group, the right side values were on average 3.21 % higher than the left side values.

3.2.2.5.4 Maximum profile peak (Rp)

Rp represents the highest peak within the measurement profile and is part of the DIN roughness parameters [194].

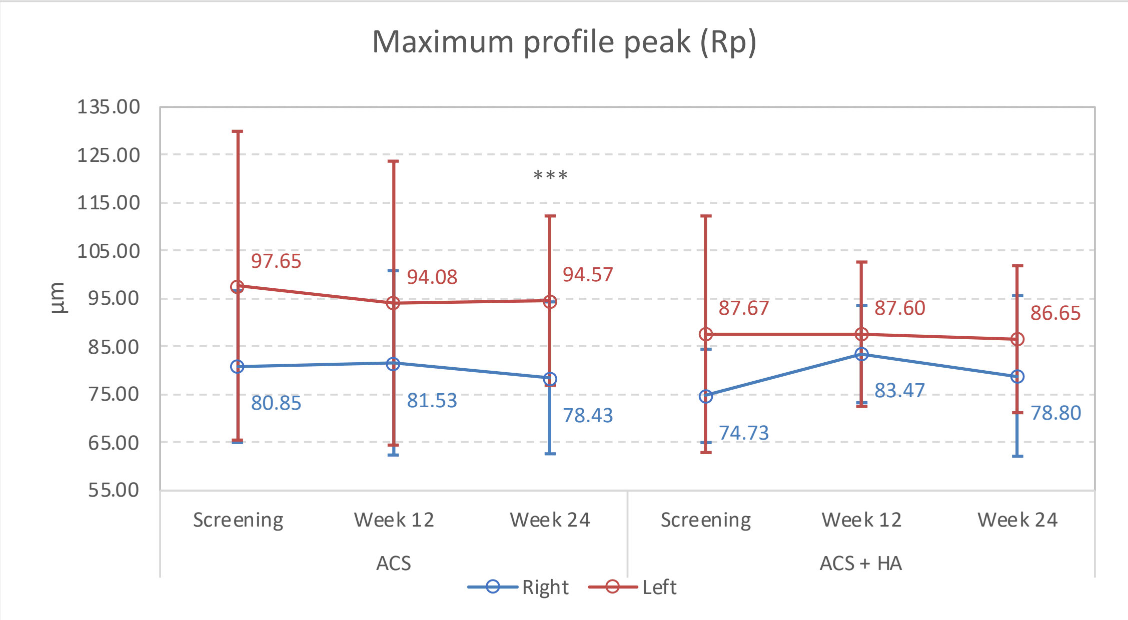


*Figure 78: Maximum profile peak, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The change of the maximum profile peak was throughout the study period insignificant for both treatment groups. And there was no statistically significant difference between the two treatment groups, either (*Figure 78*).

The statistical analyzation of the ACS group evaluating the difference between the visits was for the right side, $F(1.424, 12.814) = 0.180$, $p = 0.764$, partial $\eta^2 = 0.020$ and $F(1.728, 15.553) = 0.172$, $p = 0.813$, partial $\eta^2 = 0.019$ for the left side. The maximum profile peak was 80.85 µm for the right side and 97.65 µm for the left side at the beginning of the study. The final values were 78.43 µm for the right side and 94.57 µm for the left side. The percentage difference between screening and week 24 was only – 2.99 % and – 3.15 % for the right and left side, respectively. The left side was on average 15.89 % higher than the right side and at week 24 significantly higher ($p < 0.001$, + 20.58 %).

The statistical analyzation of the ACS + HA treatment group evaluating the differences between the visits was for the right side $F(1.684, 8.418) = 1.402$, $p = 0.292$, partial $\eta^2 = 0.219$ and $F(1.091, 5.456) = 0.015$, $p = 0.922$, partial $\eta^2 = 0.003$ for the left side. The maximum profile peak of the ACS + HA group was 74.73 µm for the right side and 87.67 µm for the left side at the beginning of the study. The final values were 78.80 µm for the right side and 86.65 µm for the left side. The percentage difference between screening and week 24 was 5.44 % and – 1.16 % for the right and left side, respectively. The left side values were, like in the ACS group, on average higher than the right side values (9.51 %) but the difference as not significant.

3.2.2.5.5 Waviness (Wt)

The waviness of the skin is the sum of the largest profile peak and valley and is an indicator to deeper furrows and changes of the cutaneous turgor [192, 196].

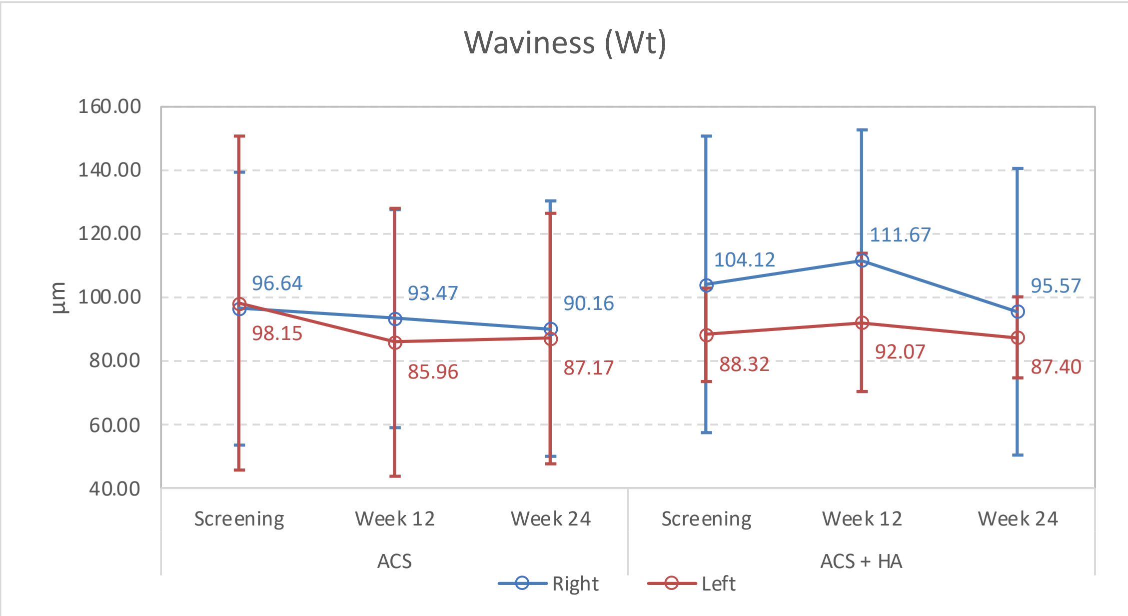


Figure 79: Waviness, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD.

The change of the skin waviness was like the skin roughness parameters insignificant throughout the study period for both treatment groups. And there was no statistically significant difference between the left and right side, nor between the two treatment groups, either.

For the ACS group there was no significant difference between the visits for the right side, $F(1.466, 13.196) = 0.654$, $p = 0.490$, partial $\eta^2 = 0.068$ or the left side, $F(1.726, 15.535) = 3.673$, $p = 0.055$, partial $\eta^2 = 0.290$. The mean skin waviness was 96.64 µm for the right side and 98.15 µm for the left side at the beginning of the study. The final values were 90.16 µm for the right side and 87.17 µm for the left side. The percentage difference between screening and week 24 was – 6.71 % and – 11.19 % for the right and left side, respectively.

The skin waviness of the ACS + HA group showed no statistically significant difference between the visits for the right side, $F(1.387, 6.935) = 0.805$, $p = 0.441$, partial $\eta^2 = 0.139$ nor the left side, F(1.955, 9.774) = 0.385, p = 0.686, partial η^2 = 0.072. The values were 104.12 µm for the right side and 88.32 µm for the left side at the beginning of the study. The final values were 95.57 µm for the right side and 88.32 µm for the left side. The percentage difference between screening and week 24 was – 8.21 % and – 1.04 % for the right and left side, respectively.

The right side values were on average 13.99 % higher than the left side values.

3.2.2.5.6 Number of peaks (PC)

PC is the number of peaks within the measurement profile and part of the surface roughness parameters [194].

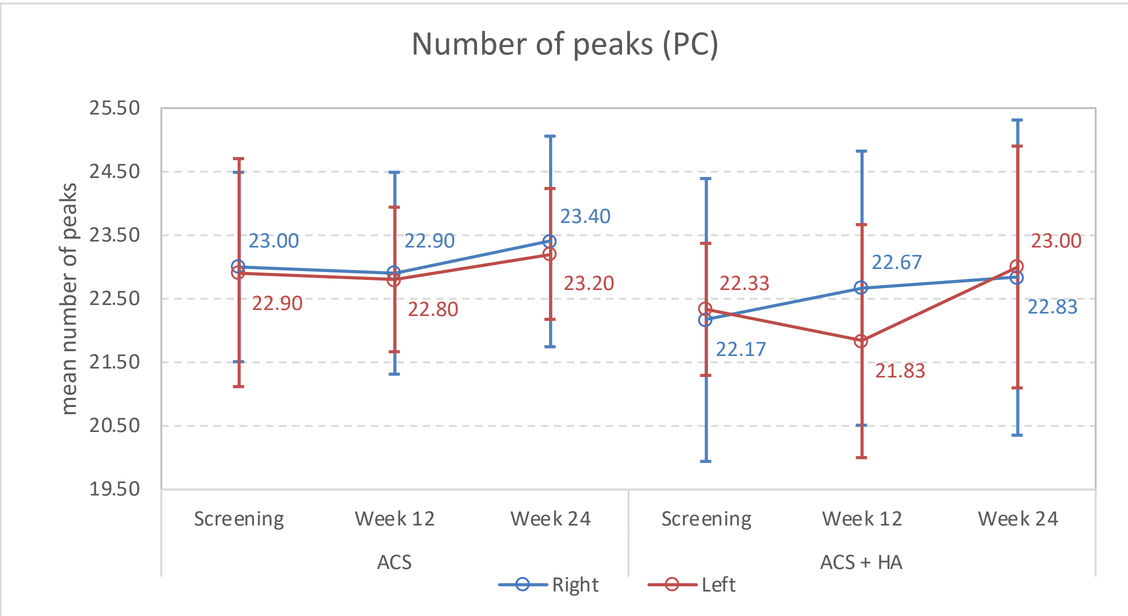


*Figure 80: Number of peaks, ACS in vivo study II, ACS treatment: n = 10, ACS + HA treatment: n = 6, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The number of peaks did not change significantly throughout the study period for both treatment groups. And there was no statistically significant difference between the left and right side, nor between the two treatment groups, either (*Figure 80*).

The statistical analyzation evaluating the difference between the visits for the right side was F(1.913, 17.219) = 0.904, p = 0.419, partial η^2 = 0.091 and F(1.353, 12.180) = 0.474, p = 0.561, partial η^2 = 0.050 for the left side. The mean number of peaks for the ACS group was 23.00 for the right side and 22.90 for the left side at the beginning of the study. The final values were 23.40 for the right side and 23.20 for the left side and increased therefore from screening to week 24 by 1.74 % for the right side and 1.31 % for the left side.

The statistical analyzation for the ACS + HA treatment group also revealed no statistically significant difference between the visits for the right side, $F(1{,}739, 8{,}696) = 0{,}844$, $p = 0{,}447$, partial $\eta^2 = 0{,}144$ or the left side, $F(1.460, 7.301) = 1.294$, $p = 0.314$, partial $\eta^2 = 0.206$ (*Figure 80*). The skin number of peaks of the ACS + HA group was 22.17 for the right side and 22.33 for the left side at the beginning of the study. The right side increased by 3.01 % to 22.83. The left side decreased at week 12 to 21.83, then increased again to 23.00. The final percentage increase for the left side was 2.99 %.

3.2.3 ACS in vivo study III

For the third in vivo ACS study, 20 female patients were enrolled. Of this multicenter study, the results of the patients treated at the University of Hamburg, Institute of cosmetic sciences, were evaluated. After screening all 20 female patients were included. There were no dropouts during the study period. The patients attended nine visits: Screening, day one (week 0, first treatment), week 2 (second treatment), week 4 (third treatment), week 6 (fourth treatment), week 12, week 24, week 36 and week 48. Measurements were taken at screening and week 0 before the treatment started. During the treatment sessions in week 0, 2, 4 and 6 no measurements were taken. They were recorded again during the follow up visits in week 12, 24, 36 and 48. Skin condition, hydration and mechanical properties of the skin were evaluated. Like in the other two studies, some parameters of the Cutometer® measurements showed significant differences between the right and left side measurements in the combined analysis of variance. Therefore, a separate repeated measures ANOVA for the right and left side was calculated. In a second step, a t-test for paired samples was computed to determine significant differences between the sides. In case of significant results, they were visualized in the graphs with whiskers, for the right side in blue and for the left side in red. A significant difference between the visits is marked in grey. All statistical results are listed in full detail in the appendix.

3.2.3.1 Age and skin condition

The age ranged from 30 to 64 years with a mean age of 47.5 ± 8.89 years. The skin condition was rated as not sensitive by 85 % of the patients and as sensitive by 15 %. 85 % of the patients characterized their skin as being normal and 15 % as dry. The results are visualized in *Figure 81*.

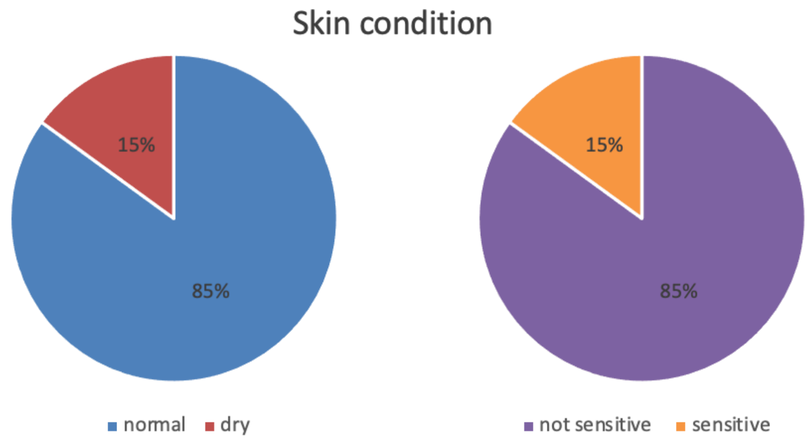


Figure 81: Skin condition, ACS in vivo study III, n = 20.

3.2.3.2 Skin hydration

The skin hydration was measured at each visit on both sides three times. The mean of these three measurements was calculated for each patient. Mean and SD were then calculated from all 20 patients and is shown in *Figure 82*.

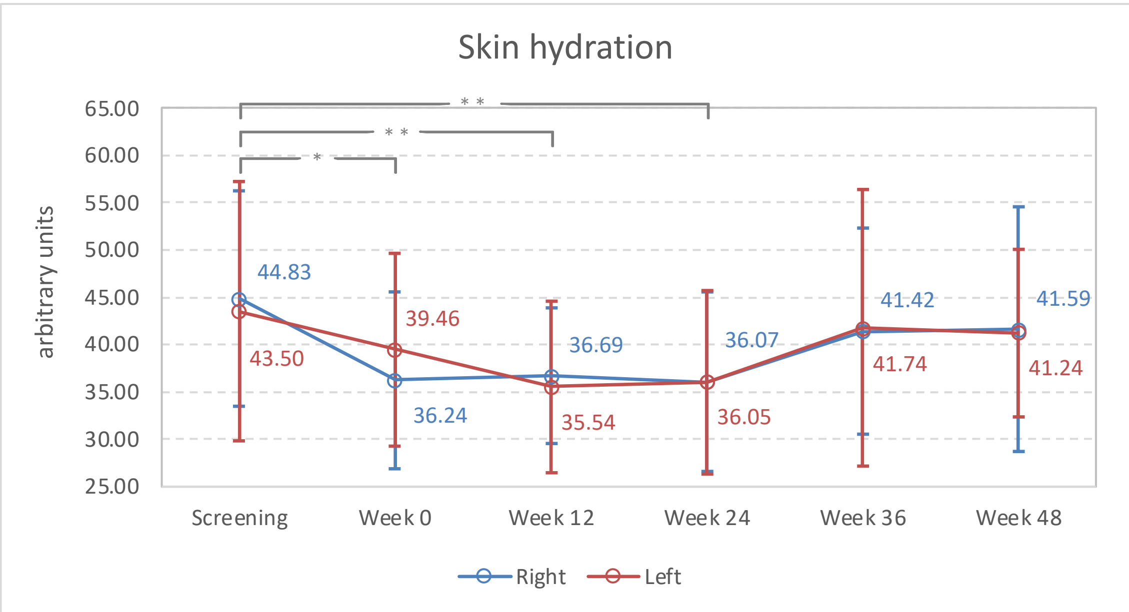


*Figure 82: Skin hydration, ACS in vivo study III, n = 20, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The screening values for the skin hydration were 44.83 ± 11.37 a.u. for the right face side and 43.50 ± 13.71 a.u. for the left face side, respectively. A second measurement was taken just before the first treatment with values significantly below the screening skin hydration (36.24 ± 9.32 a.u. right side and 39.46 ± 10.24 a.u. left side). During the treatment sessions in week 2, 4 and 6 there were no measurements taken. In week 12 and 24, six and 18 weeks after the last treatment, the skin hydration was with 36.69 ± 7.13 a.u. (right side), 35.54 ± 9.03 a.u. (left side) and 36.07 ± 9.48 a.u. (right side), 36.05 ± 9.66 a.u. (left side) at the lowest point. The skin hydration increased again and was 41.59 ± 12.9 a.u. for the right side and 41.24 ± 8,85 a.u. for the left side at the end of the study in week 48 (*Figure 82*).

There was a statistically significant overall difference for the visits with $F(3.707; 70.428) = 5.612$, $p < 0.001$, partial $\eta^2 = 0.228$. No statistically significant difference was found for the comparison of the left and right side ($F(1.000; 19.000) = 0.0131$, $p = 0.910$, partial $\eta^2 = 0.001$) or the interaction visit and side, $F(3.134; 59.537) = 0.916$, $p = 0.442$, partial $\eta^2 = 0.046$.

The skin hydration values in week 0 (p = 0.030), week 12 (p = 0.002) and week 24 (p = 0.002) were statistically significant lower compare to the values of the screening visit. Compared to the mean of the right and left screening values the skin hydration decreased by 14.29 %, 18.23 %, and 18.35 % for week 0, 12 and, 24, respectively. In week 48 it was still 6.22 % (p = 1.000) below the screening visit but 9.42 % higher than the value of the second measurement just before the first treatment in week 0. Considering the week 0 value as starting point, the post hoc tests were insignificant for all of the following visits (week 12, p = 1.000; week 24, p = 1.000; week 36, p = 0.851; week 48, p = 0.80). All values stayed within the range of dry (30 – 40 a.u.) to normal (> 40 a.u.) skin hydration [162]. The effect size was above 0.14 and therefore large.

3.2.3.3 Cutometry

Skin mechanical properties were measured with the 2 mm Cutometer® Dual MPA 580 (Courage & Khazaka electronic GmbH, Cologne) probe on each side of the face for one time in modus 1 (compare *2.3.4.2.1. Cutometer® measurements and evaluation* method).

3.2.3.3.1 Skin firmness (R0, Uf)

The maximum amplitude R0 (Uf = Ue + Uv) of the cutometer curve describes the maximum expansion of the skin during the suction phase and gives information about the skin's firmness. The lower the amplitude, the firmer or less distensible the skin [168].

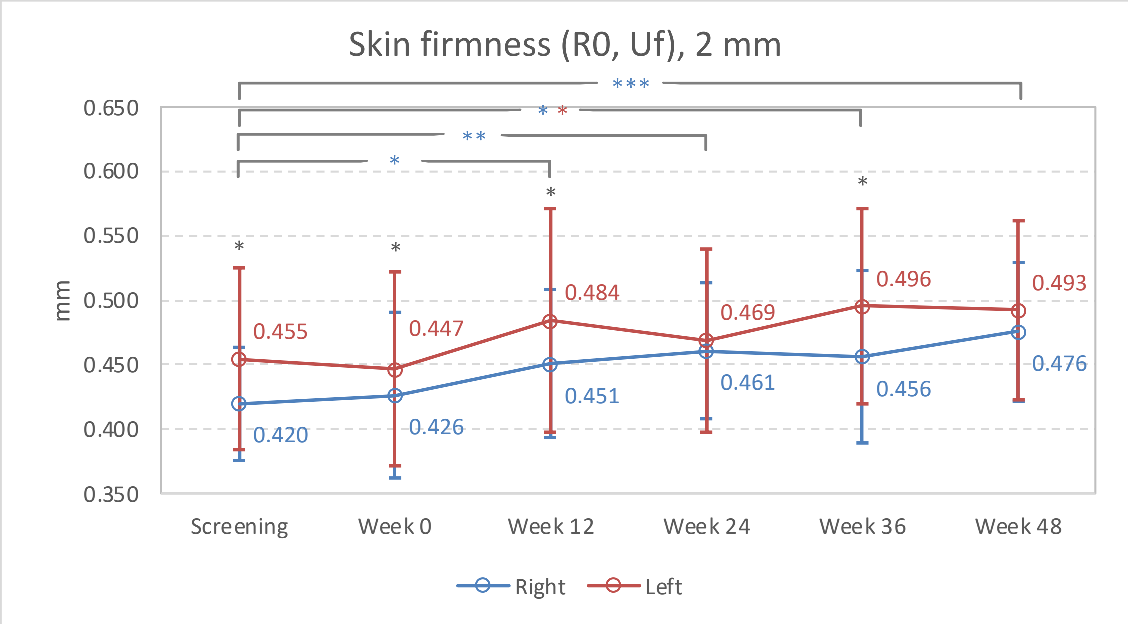


*Figure 83: Skin firmness, ACS in vivo study III, 2 mm probe, n = 20, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Figure 83 shows the results of the skin firmness. The statistical analyzation showed significant differences between the visits for the right side, F(4.173, 79.280) = 9.420, p < 0.001, partial η^2 = 0.331 and for the left side, F(3.775, 71.733) = 6.290, p < 0.001, partial η^2 = 0.249. Also the left side was significantly higher during screening (p = 0.013, 8.29 %), in week 0 (p = 0.048, 4.76 %), week 12 (p = 0.017, 7.42 %) and week 36 (p = 0.012, 8.65 %) compared to the right side. The two measurements before the treatments (screening and week 0) were for both sides similar and showed no significant difference (p = 1.000).

The left side value was 0.455 mm at the beginning of the study, showed a significantly increase in week 36 to 0.496 mm (p = 0.018, 9.05 %) and was at the end of the study 8.37 % (0.493 mm, p = 0.057) above the screening value. The right side showed significantly higher values in all four follow up visits (week 12: p = 0.042, 7.42 %, week 24: p = 0.004, 9.77 %, week 36: p = 0.040 8.69 % and week 48: p < 0.001, 13.30 %) compared to screening. The initial skin firmness value was 0.420 mm and in week 48 it increased to 0.476 mm. Therefore, the skin firmness decreased for both sides and also showed an lager effect size for both sides.

3.2.3.3.2 Skin firmness after repeated suction (R3, Uf_5)

The last maximum amplitude R3 (Uf_5) is the last maximum amplitude of the suction phase after 5 repetitions of one measurement cycle. It gives information about the skin tiring effects. The closer it is to R0 (Uf), the lower the tiring effect [168].

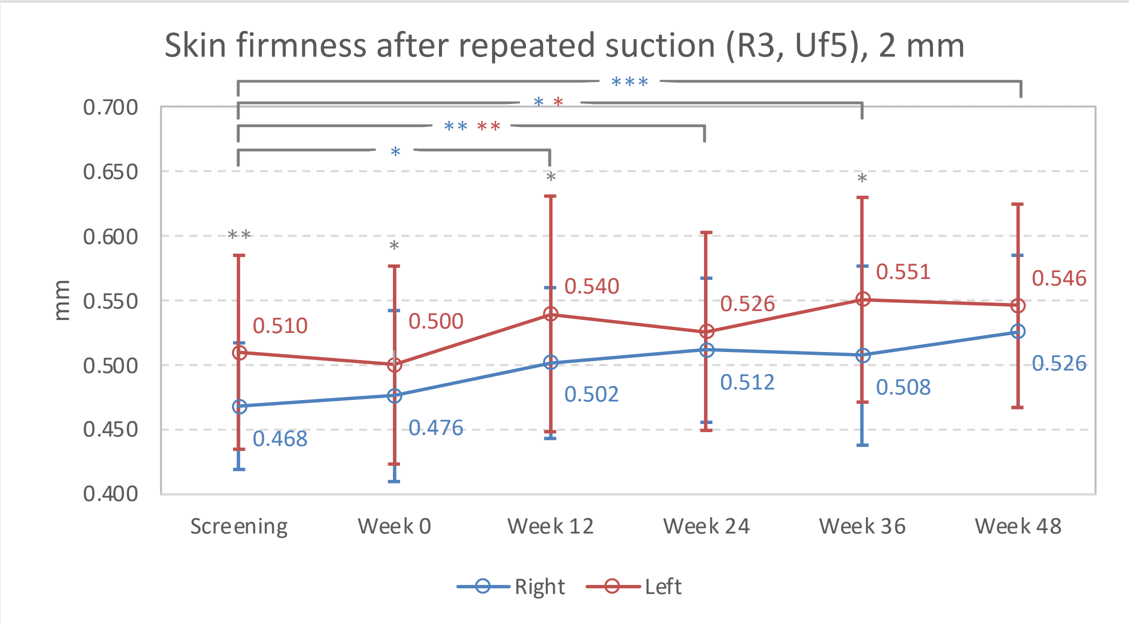


*Figure 84: Skin firmness after repeated suction, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The development of the skin firmness after repeated suction (R3, Uf_5) was for both sides similar compared to the skin firmness (R0, Uf) measurements and they showed similar significant results. The mean skin firmness after repeated suction was statistically significant different between the visits for the right side, $F(4.351, 82.676) = 9.668$, $p < 0.001$, partial $\eta^2 = 0.337$ and for the left side, $F(3.832, 72.806) = 6.185$, $p < 0.001$, partial $\eta^2 = 0.246$ (*Figure 84*).

Like the R0 (Uf) values, the left side of the R3 (Uf_5) measurements were during screening (p = 0.006, 8.91 %), in week 0 (p = 0.024, 4.99 %), week 12 (p = 0.02, 7.50 %), and week 36 (p = 0.011, 8.47 %) significantly higher compared to the right side. The left side was on average 6.03 % above the values of the right side.

The two measurements before the treatments (screening and week 0) were for both sides similar and showed no significant difference (p = 1.000).

The left side value was 0.510 mm at the beginning of the study, showed a significantly increase in week 24 to 0.526 mm (p = 0.009, 3.13 %) and week 36 to 0.551 mm (p = 0.035, 8.01 %) and was at the end of the study 7.09 % (0.546 mm, p = 0.057) above the screening value. The right side showed significantly higher values in all four follow up visits (week 12: p = 0.013, 7.24 %, week 24: p = 0.005, 9.34 %, week 36: p = 0.021 8.45 % and week 48: p < 0.001, 12.31 %) compared to screening. These percentage differences were almost identical to the R0 (Uf) values.

The initial value was 0.468 mm and increased to 0.526 mm in week 48. Therefore, the skin firmness decreased for both sides and also showed an lager effect size for both sides.

3.2.3.3.3 Skin tiring (R9, $Uf_5 – Uf$)

The difference of the last maximum amplitude and the first maximum amplitude is described as skin tiring R9 ($Uf_5 – Uf$). The smaller the value, the smaller the tiring effect. R9 visualizes therefore the difference between R0 (Uf) and R3 (Uf_5) [168].

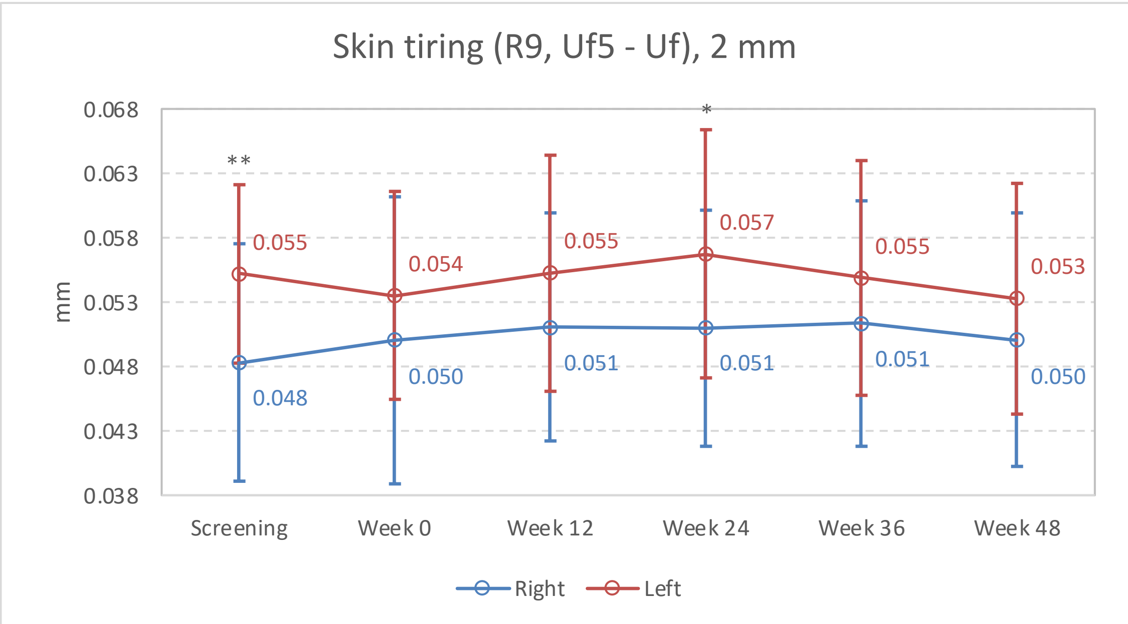


*Figure 85: Skin tiring, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The skin tiring measurements stayed throughout the study for both sides about the same. At the end of the study, the percentage increase was 3.62 % for the right side, from 0.048 mm to 0.050 mm. The left side decreased by 3.44 %, from 0.055 mm to 0.053 mm (*Figure 85*).

The values of the left side were during screening and week 24 significantly higher than the right side with an overall average of 8.98 % (screening: $p = 0.003$, 14,29 %, week 24: $p = 0.023$, 11.27 %). There was no statistically significant difference between the visits for the right side, $F(3.870, 73.527) = 0.439$, $p = 0.776$, partial $\eta^2 = 0.022$ or the left side, $F(3.980, 75.625) = 0.628$, $p = 0.678$, partial $\eta^2 = 0.032$. The effect size was small for both sides.

3.2.3.3.4 Skin recovery (R8, Ua)

The first minimum amplitude R8 (Ua) shows the maximum recovery of the skin during the relaxation phase and allows conclusions about the recovery ability of the skin. The closer the value is to 0, the higher the skin's recovery ability [168]. Skin recovery (R8, Ua) is, like the skin firmness (R0, Uf), an absolute parameter and consists of an elastic and viscoelastic part.

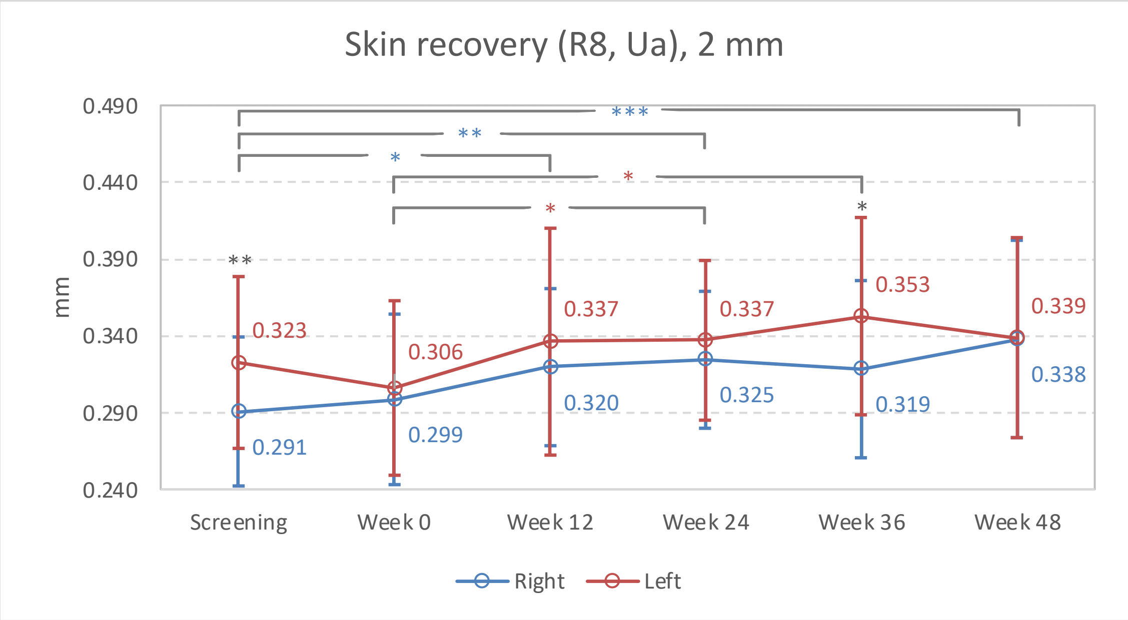


*Figure 86: Skin recovery, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The statistical analysis showed a statistically significant difference between the visits for the right side, $F(4.063, 77.201) = 6.435$, $p < 0.001$, partial $\eta^2 = 0.253$ and for the left side, $F(3.474, 66.006) = 3.546$, $p = 0.015$, partial $\eta^2 = 0.157$. As the partial η^2 was above 0.14 for both sides the effect size was large. The mean skin recovery was 0.291 mm for the right side and 0.323 mm for the left side at the beginning of the study, 0.338 mm (right side) and 0.339 mm (left side) at the end of the study. The percentage difference between screening and week 48 was significantly 16.19 % ($p < 0.001$) for the right side. Week 12 ($p = 0.03$, 9.94 %) and week 24 ($p = 0.004$, 11.69 %) were also significantly higher than screening. The left side increased by 4.99 % ($p = 1.000$) comparing screening and week 48. The left side was throughout the study higher than the right side and showed a significant difference during screening ($p = 0.009$, 10.97 %) and in week 36 ($p = 0.022$, 10.78 %). There was no significant difference for the two visits before treatment during screening and week 0 for both sides. But the left side showed a significant increase comparing week 0 with week 24 and 36.

3.2.3.3.5 Skin gross elasticity (R2, Ua/Uf)

The skin gross elasticity (Ua/Uf) is a relative parameter. It is the ratio of the first minimum amplitude of the relaxation phase (Ua) divided by the first maximal amplitude of the suction phase (Uf). The closer the value is to 1 (100 % retraction) the more elastic is the skin [168]. As the amplitude also includes the viscoelastic parts of the curve, the skin gross elasticity gives additionally information about the skin viscosity.

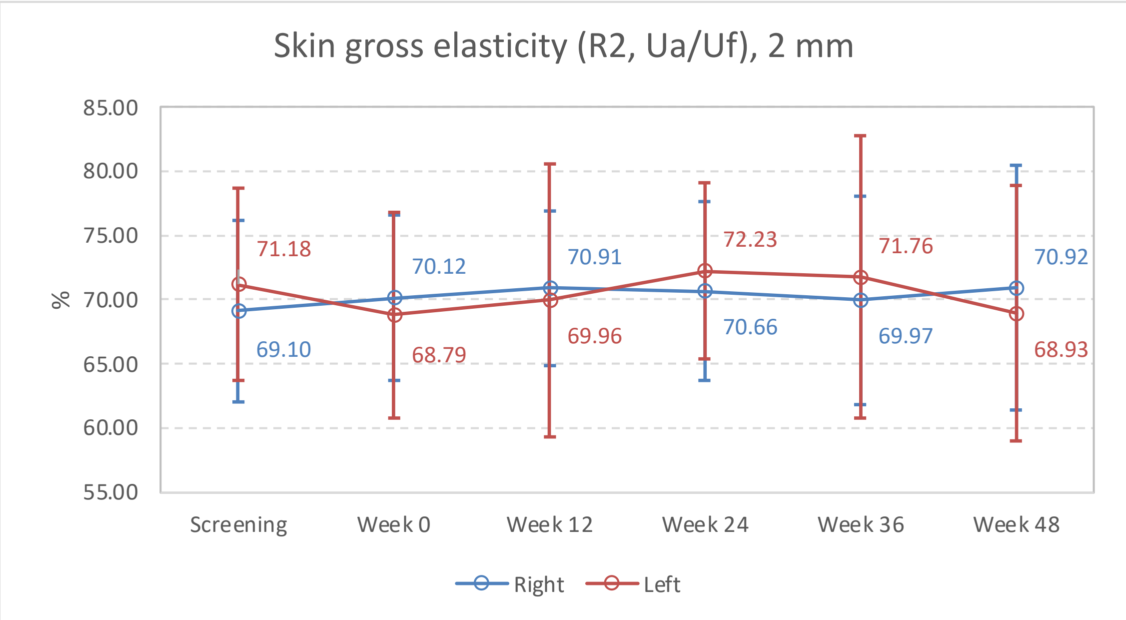


*Figure 87: Skin gross elasticity, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

The skin gross elasticity stayed throughout the study for both sides about the same. At the end of the study, the percentage increase was 2.63 % (p = 1.000) for the right side, from 69.10 % to 70.92 %. The left side decreased by 3.16 %, from 71.18 % to 68.93 %. The values of the left and right side were throughout the study very close and showed no statistically significant difference. There was no statistically significant difference between the visits for the right side, $F(3.542, 67.307) = 0.437$, $p = 0.759$, partial $\eta^2 = 0.022$ or the left side, $F(3.806, 72.316) = 1.634$, $p = 0.178$, partial $\eta^2 = 0.079$. The effect size was small for the right side and medium for the left side.

3.2.3.3.6 Skin net elasticity (R5, Ur/Ue)

The parameter R5 (Ur/Ue) is the ratio of the elastic part of the suction phase (Ur) and the elastic part of the relaxation phase (Ue). It gives information about the elastic fibers in the skin. Like the gross skin elasticity (R2), the closer the value is to 1 (100 %) the more elastic is the skin [168].

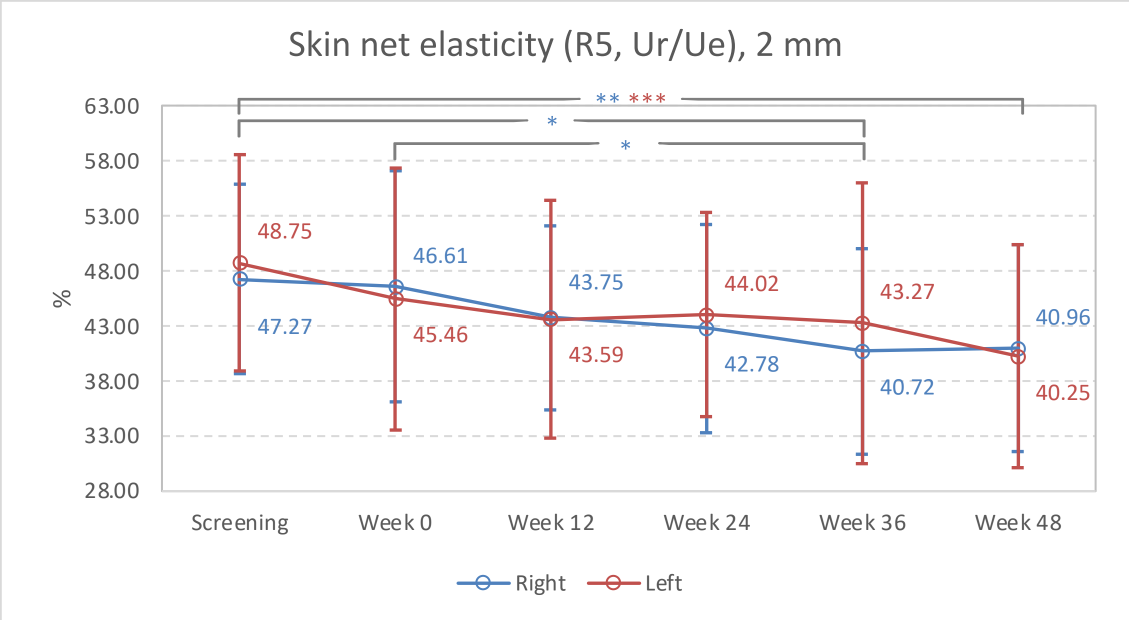


*Figure 88: Skin net elasticity, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Like the close values for the right and left side of the skin gross elasticity (R2, Ua/Uf), the sides of the skin net elasticity (R5, Ur/Ue) were nearly the same, too. The overall difference between both sides was only 1.24 %. There was a statistically significant difference between the visits for the right side, $F(4.211, 80.006) = 5.193$, $p < 0.001$, partial $\eta^2 = 0.215$ and for the left side, $F(4.518, 85.836) = 5.204$, $p < 0.001$, partial $\eta^2 = 0.215$. As the partial η^2 was higher than 0.14 for both sides the effect size was large. Both sides decreased during the study period. The right side was significantly lower with – 13.86 (p = 0.021) in week 36 and – 13.34 % (p = 0.008) at the end of the study in week 48, both compared to screening. Week 36 was with – 12.64 (p = 0.036) also significantly lower compared to week 0. The left side was only significantly lower in week 48 with – 17.43 % (p < 0.001) compared to screening.

3.2.3.3.7 Ratio of viscoelastic to elastic extension (R6, Uv/Ue)

The relative parameter R6 (Uv/Ue) describes the viscoelastic versus the elastic ratio of the curve in the suction phase. The smaller the value the higher the elasticity and the more elastic fibers are in the skin [168].

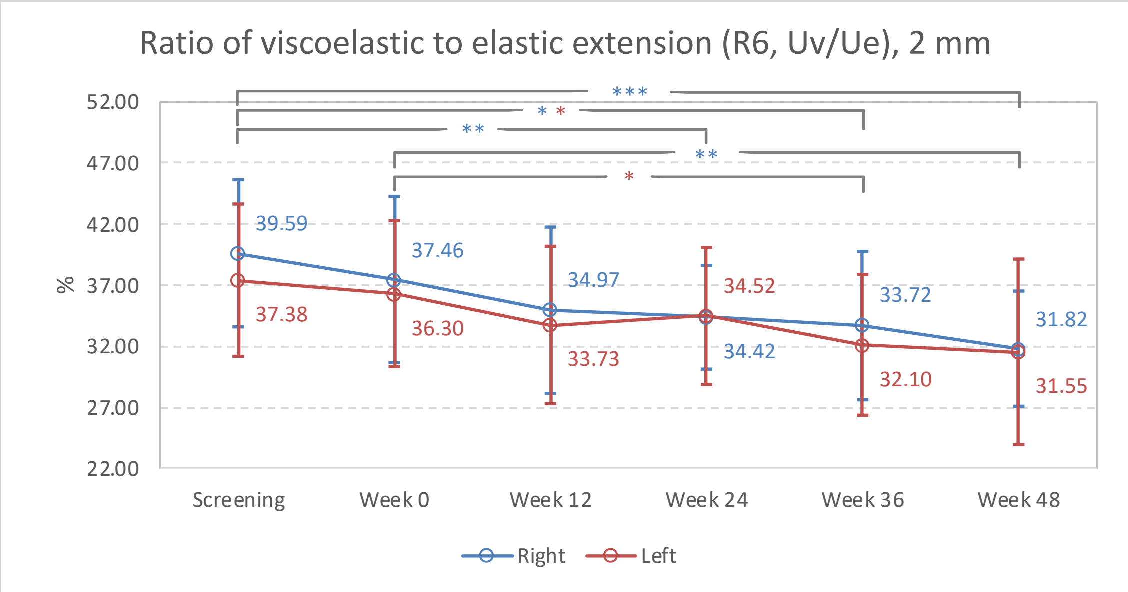


*Figure 89: Ratio of viscoelastic to elastic extension, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Both sides of the parameter ratio of viscoelastic to elastic extension (R6, Uv/Ue) developed similarly and showed alike values. There was a statistically significant difference between the visits for the right side, $F(4.142, 78.699) = 8.728$, $p < 0.001$, partial $\eta^2 = 0.315$ and for the left side, $F(3.420, 64.982) = 3.694$, $p = 0.013$, partial $\eta^2 = 0.163$. Both sides showed an large effect size, as the partial η^2 are both above 0.14. The left side was on average 3.01 % below the right side. The initial values were 39.59 % and 37.38 % for the right and left side, respectively.

For the right side there was a significant decrease from screening to week 24 (p = 0.007), week 36 (p = 0.02) and week 48 (p < 0.001). Also week 48 was significantly lower compared to week 0 (p = 0.002). The left side was significant only between screening and week 24 (p = 0.039) and week 0 and week 36 (p = 0.041). The final results were 31.82 % for the right side and 31.55 % for the left side. There was no statistically significant difference before the treatment started, between the screening and week 0 visits (p = 1.000).

3.2.3.3.8 Ratio of elastic recovery to total extension (R7, Ur/Uf)

The parameter R7 is another relative parameter (Ur/Uf). It is the ratio of the elastic recovery (Ur) of the relaxation phase and the maximum firmness (Uf). The closer the value is to 1 (100 %) the more elastic is the skin [168]. This parameter is discussed to decrease with age [173].

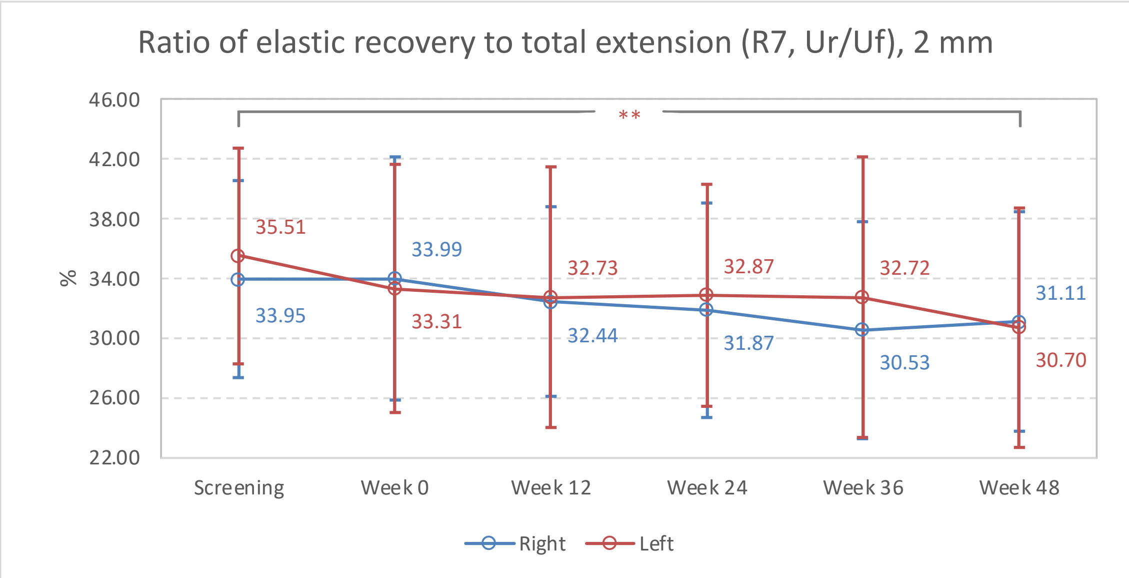


*Figure 90: Ratio of elastic recovery to total extension, ACS in vivo study III, 2 mm probe, n = 21, mean and SD, * p≤0.05, ** p≤0.01, *** p≤0.001.*

Like the other three relative parameters, both sides of the parameter ratio of elastic recovery to total extension (R7, Ur/Uf) showed similar values and slightly decreased during the study. There was a statistically significant difference between the visits for the right side, $F(3.781, 71.845) = 2.603$, $p = 0.046$, partial $\eta^2 = 0.120$ and for the left side, $F(4.407, 83.726) = 3.439$, $p = 0.010$, partial $\eta^2 = 0.153$. There was an medium effect size for the right side and an lager effect size for the left side. The initial values were 33.95 % and 35.51 % for the right and left side, respectively. The final values were 31.11 % for the right side and 30.70 % for the left side. Only the left side showed a statistically significant value between screening and the end of the study ($p = 0.002$).

4 Discussion

The discussion is divided into an in vitro part, considering the results of the influence of ACS and ACS + HA on human dermal fibroblasts vitality and protein synthesis. And an in vivo part. This part is separated into the four different biophysical measurement devices used in the three in vivo studies. They were used to evaluate skin hydration with the Corneometer, mechanical properties of the skin with the Cutometer, skin density and thickness via sonographic measurements and skin topography to evaluate skin roughness parameters with the PRIMOS device. Following these discussions, a comparison is drawn between ACS, PRP and HA. This comparison is based on the results concerning skin firmness after the categorization system developed by Goldie et al. for skin quality [212]. Two papers and two posters have been published on the basis of the three studies of this work [151, 152, 200, 201].

4.1 Discussion in vitro

Human dermal fibroblast are the main cell type within the dermal layer of the skin. They synthesize collagen, elastin and other fibril structures as well as the extracellular substance with its proteoglycan HA [31]. They are involved in multiple physiological processes such as maintaining a healthy skin function or the wound healing process [54, 68]. Consequently, viable and healthy fibroblasts are essential for a proper functioning and youthfulness of the skin.
Within this work, the influence of ACS and ACS + HA to human dermal fibroblast was tested. With the assumption that ACS and ACS + HA preparations have positive effects to the proper functioning of the cells. Therefore, human dermal fibroblast were incubated with serum-free DMEM and 10 % ACS and ACS + HA (4:1) of six different volunteers for 2, 6 and 24 hours, respectively.
The results of the **MTT assays** for cell viability clearly showed positive results. Here, all incubated samples increased between 10 % and nearly 30 % after 24 hours of incubation with ACS and ACS + HA, respectively (*Figure 22 and Figure 23*). The addition with HA did now show a superiority in cell viability.
Kakudo et al. found similar positive cell viability results in human dermal fibroblasts incubated with different PRP preparations. These cells were assayed for proliferation using a cell counting test, with the results shown in cell number per well. Cells were incubated for 1, 4, and 7 days. The highest increase was achieved with a 5 % solution of activated platelet-rich plasma. Higher

concentrations, with 10 % and 20 % activated platelet-rich plasma showed poorer promotion of cell proliferation. The control with serum-free DMEM also showed only little cell number increase [213]. Kim et al. investigated the cell proliferation after 3 and 5 days in a similar manner, with the best results also shown by a 5 % solution of activated platelet-rich plasma [15].
Anitua et al. studied the fibroblastic response to treatment with different preparations rich in growth factors. Medium with 20 % of the different preparations were used and evaluated after 3 days of incubation. The highest proliferation rate was seen in the most concentrated solution (called PRGF4x) [214].
All three studies also evaluated the concentration and synthesis of different proteins which are important for cell proliferation, cell differentiation and for tissue regeneration. Those proteins were either assayed within the platelet-rich plasma or the cultured cell medium. High concentrations of PDGF-AB and TGF-ß1 were found in activated platelet-rich plasma (aPRP) but not in nonactivated platelet-rich plasma [213]. Angiogenic factors like vascular endothelial growth factor (VEGF) was not elevated in cultured cells but hepatocyte growth factor (HGF), type I collagen (COL1A) and MMP-1 were elevated [15, 214]. The procollagen type I carboxy-terminal peptide production reflects the overall collagen levels within the cultures [145]. It was not increased in the study by Anitua et al. [214]. However, it was found highest in cells treated with 5 % aPRP, the same concentration of PRP that induced maximum cellular proliferation in the study by Kim et al. [15].
Like in the study by Kim et al., **procollagen type I carboxy-terminal peptide** could be detected within the two in vitro assays of this work. The first in vitro study showed increasing concentrations over incubation time in the extracellular supernatant, from average 104.5 ± 12.1 ng/ml to 397.3 ng/ml for all samples. The concentrations of the lysates stayed about the same (535.8 ± 57.7 ng/ml). In the second in vitro study no obvious increase could be detected. The values varied between 188.0 ± 67.4 ng/ml and 399.0 ng/ml (*Figure 25* and *Figure 26*).
Furthermore, **TGF-ß1** could be detected via western blot in both in vitro tests. It was found in the intracellular lysate and extracellular supernatant of the incubated cells, but not in the control cells incubated with FCS. Again, the combination of ACS + HA did not show superior results (*Figure 27*). The finding of TGF-ß1 is in accordance with other findings by the already mentioned Anitua et al. and Kakudo et al., but also by Magalon et al., who compared different PRP preparation systems. All found concentrations of TGF-ß1 within the PRP preparations [108, 213, 214].

Conclusions about the presence of **type I collagen** (COL1A) could not be verified via western blot. Here, the question remains whether collagen production was induced by ACS after 24 hours of incubation. As procollagen was detected within the cell cultures, a presence of collagen type I could have been assumed as well. But there seems to be a complex mechanism, which was also mentioned by Anitua at al., with contradictory results concerning the collagen I production [214]. Kim et al. on the other hand found both, procollagen type I carboxy-terminal peptide and collagen type I production, increased [15].

One reason for these different findings could be the varying incubation times. The western blots of the in vitro tests were conducted after 24 hours incubation with ACS and ACS + HA, respectively. Kim et al. evaluated their cell cultures after 3 and 5 days of incubation, Anitua et al. after 3 days. Also, the settings concerning the used concentrations of the investigated material and fibroblast concentrations could be a source influencing the different results.

All these factors reduce the comparability of the in vitro results of this study to the studies mentioned above. Especially the concentration of the investigated autologous blood product seems to influence the results. A higher concentration did not necessarily lead to better results [15, 213]. The results of the used 10 % ACS solutions of the two in vitro studies could be further examined with 5 % and 20 % solutions to increase the comparability and new investigations with varying concentrations of ACS in the medium could give knowledge of a dose dependency. Also further collagen and HA inducing proteins, like VEGF or EGF could be examined. The presents of pro-inflammatory proteins could also have an influence on the ACS treatment outcome and should be further questioned [132].

One further aspect, not explicitly mentioned within the discussed studies, is the small sample size with only 2 to 10 autologous test products [15, 108, 213, 214]. The results of the in vitro assays included six ACS probes and could also be expanded for more statistical power analyzations [215].

Nevertheless, a positive conclusion can be drawn to the cell viability, procollagen, and TGF-ß1 concentration, which are indicators for viable and cell synthesis performing fibroblast.

4.2 Discussion in vivo

The first ACS in vivo study was an investigator-initiated trial to examine the influence of ACS on human female facial skin. The two following studies were initiated to verify the first study results and elaborate further information about combined treatments with HA, treatment sessions, and incubation times for the ACS processing. As ACS has been used for different orthopedic indications reducing inflammations and heal injuries, an anti-inflammatory and anti-aging correlation was assumed for the face as well [24]. The following discussion is based on this assumption and draws correlations to studies conducted with PRP and HA.

4.2.1 Skin hydration (corneometry)

Skin hydration is a crucial parameter for healthy and radiant skin [216-218]. To be able to evaluate skin hydration the corneometry is a fast and reproducible method [155, 162]. It measures within seconds the stratum corneum hydration without infringing the skin, and is a tolerated and widely applied device in clinical and cosmetic studies [161, 218, 219].
Dry skin is often treated with moisturizing factors to rebalance the skin's hydration rate. Skin moisturizing factors such as urea, HA or glycerin are therefore part of many topical cosmetic products [216]. But as long as they are applied topical, they only have a limited moisturizing duration and need to be reapplied consequently [220, 221]. Injections with HA for skin revitalization have a longer durability on skin hydration of up to nine months [175, 222]. Studies with autologous conditioned products also showed increasing skin hydration after injection, but in contrast to HA, these studies duration were only 45 days and 4 months long, respectively [115, 136].
In the three studies of this work, skin hydration was evaluated over 6 and 12 months, respectively. The **first two ACS in vivo studies** showed constant to increasing skin hydration, with values above 40 a.u. at the end of the study period after 6 months. The first study showed nearly 30 % (p = 0.009) increase in skin hydration, the second nearly 33 % (p = 0.239) for the ACS + HA group, but only 2.29 % (p = 0.793) for the ACS group. The **third ACS in vivo study** showed an initial decrease and then increase to a steady level. All values were above 40 a.u. at the end of the studies after 6 and 12 months, respectively. The second and third ACS in vivo study could not confirm the significantly positive development of the first study over 6 months. Nevertheless, the initial and final values were within the range of normal, and therefore healthy, hydrated skin [162]. Additionally, there was no statistically significant difference between the ACS and ACS + HA group of the second in vivo study, even though, the final value of the ACS + HA group was with 48.47 a.u. higher compared to the ACS group with 42.53 a.u. (*Figure 56*). As HA is known to be a strong water binding agent, a more profound difference could have been assumed [93]. However, the corneometer device only measures up to a depth of around 10 – 20

µm, which resembles the stratum corneum thickness [31, 161]. The highest concentration of HA is found in the stratum spinosum of the epidermis and in the papillary dermis. A diffusion of water from these layers is prevented by the keratinosomes of the stratum granulosum [94, 223, 224]. It is therefore possible, that ACS contributes indirectly to a healthy skin barrier, but the stratum corneum hydration is not directly influenced by ACS injections. The missing additional effect of HA could be due to the small amount of 0.5 ml per side and session or the small sample size of this group, with just 6 patients. The usual volume of injected HA is around 1 – 2.5 ml per side and session and also depends on the indication [138, 139, 222, 225]. The sample size influences the significance level and is also dependent on the sample distribution [203]. A small sample size decreases the statistical power and reduces the informative value of the data [202]. But it remains challenging to increase the sample size in investigator-initiated trials, often due to capacities. Most of the PRP studies for aesthetic indications also showed sample sizes of around 10 to 20 patients [114, 116-118, 122]. But, as mentioned above, only few investigated the skin hydration objectively [115, 136]. An overall increase of small studies with comparable evaluation methods would increase the informativeness of the use of autologous blood products and would make comparisons easier. Interestingly, the skin condition was rated as dry by more than ¾ of the patients in the second ACS in vivo study. This subjective impression did not resemble the objective measurement with values around 40 a.u. Here again, further comparisons concerning subjective and objective measurements could give further insight in the patient's impressions and objective reassurance. The healthy skin condition could have also influenced the fast regeneration of the skin after injection. The stress for the skin after injections was only of short duration as an exemplarily documentation of the regeneration of the skin after injection shows in *Figure 91*.

Figure 91: Injection example of ACS in vivo study I.
***a**: Immediately after injection, **b**: after cleaning (5 minutes after injection) **c**: 6 hours after injection*

4.2.2 Mechanical properties of the skin (cutometry)

Mechanical properties of the skin include skin firmness, elasticity and skin recovery. All these parameters can be evaluated with the Cutometer® Dual MPA 580 device (Courage & Khazaka electronic GmbH, Cologne). They are important parameters describing the proper functioning, stability and indirectly the appearance of the skin. Skin aging processes are accomplished with changes of the biochemical and cellular composition of the skin. Collagen and elastic fibers decrease in intrinsically and extrinsically aged skin [51, 226, 227]. Consequently, this leads to decreased skin firmness, elasticity and increased skin tiring [165, 172, 173]. The cutometry can therefore be used to evaluate the skin's aging status and the influence of anti-aging substances regarding improvements of the respective parameters.

Skin firmness (R0, Uf), skin firmness after repeated suction (R3, Uf_x), skin tiring (R9, Uf – Uf_x), and skin recovery (R8, Ua) are all absolute parameters, measured in mm. These parameters describe the resilience of the skin to force and the ability of the skin to recover to its initial status [167]. One can conclude, that the lower the values and the smaller the difference between the first maximum amplitude (R0, Uf) and the last maximum amplitude R3, (Uf_x), the firmer the skin, the lower the tiring effect and the greater the recovery. This is in correlation with a higher degree of collagen and elastin in the skin and a strong extracellular matrix [228].

Skin gross elasticity (R2, Ua/Uf), net elasticity (R5, Ur/Ue), the ratio of viscoelastic to elastic extension (R6, Uv/Ue) and the ratio of elastic recovery to total extension (R7, Ur/Uf) are the relative parameters, measured in %. These parameters can be divided into informativeness concerning elastic behavior (R2, Ua/Uf; R5,Ur/Ue; R7, Ur/Uf) and viscoelastic behavior (R6, Uv/Ue) of the skin [167]. The parameter R6 (Uv/Ue) describes the extension behavior, R2 (Ua/Uf), R5 (Ur/Ue) and R7 (Ur/Uf) the relaxation behavior of the skin [166]. Particularly the gross elasticity (R2, Ua/Uf) and net elasticity (R5, Ur/Ue) parameters are discussed in clinical investigations concerning anti-aging therapies, location differences, and age related changes of the skin [9, 165, 172, 175, 229, 230]. All parameters but the ratio of viscoelastic to elastic extension (R6, Uv/Ue) are found to decrease with age, meaning that skin elasticity and the ability to recover decreases. An increasing value for R6 (Uv/Ue) is, on the other hand, a sign for skin elasticity loss [167].

In the **first ACS in vivo study** the skin firmness (R0, Uf), skin firmness after repeated suction (R3, Uf_5) and skin tiring (R9, Uf_5 – Uf) showed an overall improvement for the 2 mm as well as the 4 mm probe until week 24 compared to screening. Both sides were evaluated separately as especially the 4 mm probe showed higher values for the left side, with significant differences in week 2, 4 and 12 for R0 (Uf) and R3 (Uf_5) (*Figure 31* and *Figure 33)*. Interestingly, this changed from week 12 to week 24. Here, the values of the left side decreased stronger than the right side and were below the right side at the end of the study in week 24. Only skin tiring (R9, Uf_5 – Uf) for the 4 mm probe showed a stronger decrease of the right side and significantly higher values for the left side in week 12 and 24 (*Figure 35*). In general, an overall improvement of skin firmness (R0, Uf and R3, Uf_5) and skin tiring (R9, Uf_5 – Uf) are ascertained for both sides and probe sizes, assuming that decreasing values are associated with an increase in skin fibers (Ue increases), which is also depending on the resilience of the skin to force [167]. This can especially be seen in the overall improvement of 51.06 % for the right side and 53.74 % for the left side from screening to week 24 for the skin firmness (R0, Uf).

Skin recovery (R8, Ua) also improves with decreasing values, meaning the smaller the values the higher the skin's recovery ability, recoil capacity of fibers, and the smaller the hysteresis effect [163, 166]. For the 2 mm probe measurements the skin recovery significantly improved for both sides accordingly (*Figure 36*). The 4 mm probe values significantly increased from screening to week 2, indicating an initial decrease in the skin's recovery ability after one treatment with ACS. But showed improving values afterwards until the end of the study. The left side values were again higher than the right side values, with a change from week 12 to 24, where the left side decreased just below the right side (*Figure 37*).

Skin gross elasticity (R2, Ua/Uf), improved for both sides significantly until week 8 for the 2 mm probe but decreased afterwards and in week 24 the screening values were reached again (*Figure 38*). The 4 mm probe on the other hand significantly increased until the end of the study, improving from 72.59 % for the right side and 75.48 % for the left side to 84.85 % for the right side and 85.41 % for the left side (*Figure 39*). Skin net elasticity (R5, Ur/Ue) was also significantly increased for both sides and probes sizes in week 8 compared to screening (*Figure 40* and *Figure 41*). After week 8 there was a change for the skin net elasticity, which was also found in the R6 (Uv/Ue) and R7 (Ur/Uf) parameters. For all three parameters the right side values decreased after week 8 until week 12 and then increased again until week 24. The left side 2 mm probe values decreased slightly or stayed the same and the 4 mm probe values decreased until week 12 and then increases again (*Figure 42 – Figure 45)*.

Taken together, skin net elasticity (R5, Ur/Ue) and the ratio of elastic recovery to extension (R7, Ur/Uf)) improved thorough the study. The R6 (Uv/Ue) parameter values however also increased, which indicates a loss of extension ability and is associated with skin aging [164].
Assuming, that the treatment with ACS induces the fibroblast's collagen synthesis, one can conclude that the skin firmness improves, the strength of the skin against force, and the recovery of the skin. This improvement can be seen in the first ACS in vivo study with enhanced skin firmness (R0, Uf), skin recovery (R8, Ua) and skin tiring (R9 Uf_5 – Uf). But the increase of R6 (Uv/Ue) also allows the assumption, that the elastic part of the curve (Ue) does not increase as pronounced as the before mentioned parameters suggest.

In the **second ACS in vivo study** there was an overall significant improvement until week 12 for the absolute parameters skin firmness (R0, Uf), skin firmness after repeated suction (R3, Uf_5), skin tiring (R9, Uf_5 – Uf) and skin recovery (R8, Ua) for the 2 mm and 4 mm probes *(Figure 57* – *Figure 64*). From week 12 to week 24 the values significantly increased again to the level of the baseline values. Skin tiring (R9, Uf_5 - Uf) of the 4 mm probe did not improve throughout the study but slightly increased (*Figure 62*). Both treatment groups, ACS and ACS + HA, showed similar developments. A superiority was not found for any of the treatment groups, even though the ACS + HA group showed slightly higher values (significantly higher in week 12 for the 4 mm probe R0 (Uf) ($p = 0.025$) and R3 (Uf_5) ($p = 0.03$) values). The positive development until week 12 could not be seen in week 24 anymore, where no value was significantly different compared to the screening value. In contrast to the first in vivo study, there was no significant difference between the right and left side for the absolute parameters.
Skin gross elasticity (R2, Ua/Uf) for the 2 mm probe did not improve throughout the study period and even slightly decreased (*Figure 65*). In week 12 the right side of the ACS group was significantly higher than the right side of the ACS + HA group ($p = 0.047$) but the overall trend was similar for both treatment groups. The values of the ACS group stayed between 70 % and 80 % for all visits, the overall decrease was from 75.97 % during screening to 72.97 % in week 24 (average of both sides). The ACS + HA was altogether lower and decreased from 70.45 % during screening to 65.30 % in week 24 (average of both sides). The skin gross elasticity (R2, Ua/Uf) of the 4 mm probe followed the same significant improvement until week 12 and

decreased after week 12, similar to the absolute parameters (*Figure 66*). Here, the values even significantly increased to above 80 % in week 12 before decreasing again to around 70 %.
The skin net elasticity (R5, Ur/Ue) and the ratio of elastic recovery to total extension (R7, Ur/Uf) followed a similar significant improvement compared to the absolute parameters until week 12. Additionally, there was a similar decrease to the baseline values after week 12 to week 24 for both probe sizes and treatment groups (*Figure 67, Figure 68, Figure 71* and *Figure 72*). Skin net elasticity (R5, Ur/Uf) of the 4 mm probe even increased from 54.42 % to above 90 % (92.17 %, $p < 0.001$, compared to screening) for the left side ACS group, before decreasing to 56.07 % ($p = 1.000$ compared to screening) in week 24 again. The 2 mm probe measurements of the ACS treatment group for both parameters (R5, Ur/Uf and R7, Ur/Uf) were already at screening above those of the ACS + HA group and were significantly higher in week 12 and 24 for the left side (R5: week 12 $p = 0.023$, week 24 $p =0.027$; R7 week 24 $p = 0.031$).
The ratio of viscoelastic to elastic extension (R6, Uv/Ue) values increased until week 12 and then decreased again, resembling a decrease in elastic fibers in the skin [164]. This finding is similar to the results of the first in vivo study where the R6 (Uv/Ue) values also increased.

The first two ACS in vivo studies had the same injection regime at day 0, week 2, week 4 and week 12. Rather continuous improvements, except for the ratio of viscoelastic to elastic extension parameter (R6, Uv/Ue), could be seen in the first study until week 12. A changing point seemed to be between week 8 / 12 and week 24, where some values deteriorated (R2, Ua/Uf and R6, Uv/Ue) but others even stronger improved (R0, Uf; R3, Uf_5; R9, Uf_5 – Uf; R8, Ua; R5, Ur/Uf and R7, Ur/Uf). As the long time effect was of importance for the second ACS in vivo study, the measurements were reduced to screening, week 12 and week 24, evaluating data after 8 and 20 weeks after the injection regime. The difference between baseline and week 12 but no improvement in week 24 anymore was very pronounced in the second study for nearly all parameters. The first study suggested a different development and made the data of the third study all the more interesting.

The **third ACS in vivo study** focused on the data from the 2 mm probe but for a prolonged time period over 48 weeks. The injection regime was slightly adjusted with four injections at day 0, week 2, week 4 and week 6. Therefore, the evaluated data refers to measurements 6, 18, 30, and 42 weeks after the treatment regime.

Interestingly, here the measurements did not develop in any way similar to the first two studies. The skin firmness (R0, Uf) and skin firmness after repeated suction (R3, Uf_5) even decreased by about 10 % (*Figure 83* and *Figure 84*). Skin tiring (R9, Uf_5 – Uf) stayed constant throughout the study but showed higher values for the left side compared to the right side, significantly at screening ($p = 0.003$) and in week 24 ($p = 0.023$) (*Figure 85*).

A significant decrease for both sides was seen in the skin net elasticity (R5, Ur/Ue) parameter from 48.01 % to 40.61 % (average of both sides) (*Figure 88*).

In this study the decreasing values of the ratio of viscoelastic to elastic extension (R6, Uv/Ue) (*Figure 89*) can be seen as positive development, but the skin gross elasticity (R2, Ua/Uf) and ratio of elastic recovery to total extension (R7, Ur/Uf) did not improve after the ACS treatments (*Figure 87* and *Figure 90*).

Particularly notable was the pronounced difference between the right and left side values. The right side values were throughout the study below the left side values, in parts significantly even at screening for the absolute parameters. This was seen in the first ACS in vivo study as well, but mainly for the 4 mm probe and only slightly in the 2 mm probe measurements.

The data of the first study indicated an improvement of the mechanical properties of the skin for up to 20 weeks after the last injection, with a booster in week 12.

The possible underling effect of collagen synthesis due to the influence of ACS was therefore assumed and the positive results of the in vitro assays confirmed this assumption. The second ACS in vivo study only showed improvements until week 12, which was four weeks after the treatment regime. The third ACS in vivo study had no impact on the skin's mechanical properties but revealed a difference between the right and left side's skin firmness (R0, Uf) and skin tiring (R3, Uf_5 and R9, Uf_5 – Uf) status.

A difference between the facial sides can be possible, especially for photoaged skin. A famous photograph of a truck driver with pronounced unilateral dermatoheliosis was published in 2012 by Gordon et al. It shows a strong difference between the left and right side face concerning wrinkling and sagging, multiple open comedones, and areas of nodular elastosis due to severe UVA exposure of only the window assigned left side of the face [231]. Other findings concerning variations between different locations of the whole body also suggest differences [232, 233]. But the focus is more often on the skin barrier function with transepidermal water loss or skin hydration but not on the mechanical properties of the skin [234, 235]. One study by Kawalkie-wicz et al. investigates different locations of the face in 72 patients divided into two age groups with the cutometry and could not find statistically significant differences between the sides but the age. Skin firmness showed constant values and was therefore interpreted as independent of age, whereas the skin elasticity and ability to recover after suction were greater in younger patients [236]. This is not completely in accordance to other literature but Luebberding et al. also found a grater correlation of skin recovery than skin firmness to age [165]. Further data concerning the mapping of the face with the Cutometer® are difficult as often only the cheek area is measured as basis for comparisons of locations [170].

Nevertheless, an evaluation of the three ACS in vivo studies with a study by Pinto et al. and Pierre et al. on the basis of the age related changes in skin net elasticity (R5, Ur/Ue) by Lueb-berding et al. is drawn [136, 165, 233]. The result of this comparison is shown in *Figure 92*.

Pinto et al. investigated the effect of so called “autologous antiaging serum”, which equals ACS, on 14 female patients receiving two treatments. Measurements were taken before and 45 days after treatments. The cutometry was applied but the exact location of the measurements is not mentioned within the study.

Pierre et al. investigated the skin net elasticity (R5, Ur/Ue) along 24 different sites of the face, with the right or left side randomly chosen. Here, cheekbone and jawbone measurements are visualized, showing rather low values compared to the other studies and resembling an age around 70 years when taking the data by Luebberding et al. as basis. This does not resemble the age range of 50 – 65 years of the study [233]. Altogether, there is a wide variety with the highest value seen in the first ACS in vivo study after week 24 with 48.49 %, which is comparable with an age of around 35 years when taking the data by Luebberding et al. as basis.

A study by Cameli et al. reported an improvement of skin gross elasticity after PRP injections [115]. However, the reported value is called R5 – which is after the definition of Courage + Khazaka the skin net elasticity (R2, Ua/Uf) [168]. This inaccuracy limits the conclusiveness of this study. A study by Everst et al. also investigated the effects of pure PRP for facial skin reju-venation and found improved skin firmness parameters [118]. Unfortunately, only the change in % to baseline was reported and a comparison was therefore not possible. Hersant et al.

studied the synergistic effect of PRP with HA for facial skin rejuvenation. In total 93 patients were included and received three treatments of either activated PRP, HA or a mixture of activated PRP and HA [237]. This study also did not state actual numbers for the cutometer values and could therefore also not be visualized within *Figure 92*. Nevertheless, they found a synergistic effect and better improvement for the PRP and HA combination. This is in contradictions to the findings of the second in vivo study, where no obvious differences were found between the ACS and ACS + HA treatment groups. This could of cause be due to the small sample size and low amount of injected HA. Also the blending of ACS and HA has not been investigated in detail yet and a possible interaction cannot be excluded.

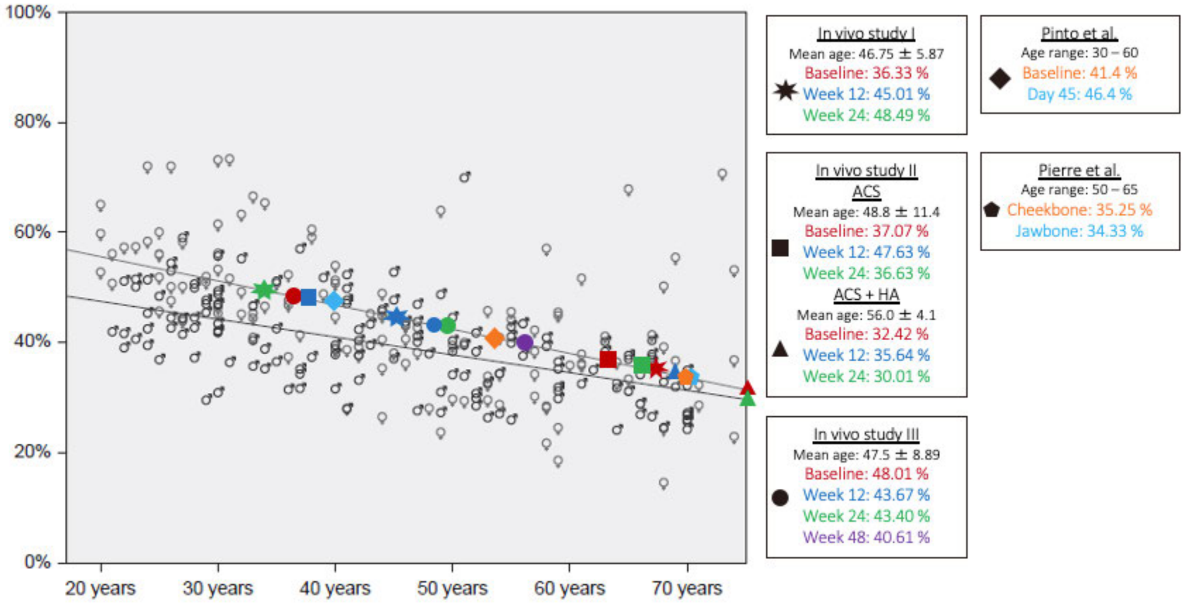


Figure 92: Age correlation of the skin net elasticity (R5, Ur/Ue) based on the data by Luebberding et al. [165]. Comparison of all three ACS in vivo studies, data from Pinto et al. and Pierre et al.[136, 233]

With increasing age, skin is characterized by reduced elasticity and firmness or, in other words, increased distensibility, tiring and hysteresis [163].

The elastic resistance during an applied force to the skin is decreases in UV-exposed areas like the facial skin, while the viscoelastic part increases [173, 238]. Accordingly, aged facial skin is characterized by reduced elasticity and increased viscosity. Furthermore, the ability of the skin to recover after force is stronger influenced by age than the skin firmness, possibly due to the ratio of around 70 – 80 % collagen and only 2 – 4 % elastin in the skin [165, 239]. Indeed, elastin fibers were found to be especially important for the recovery of the skin after a force has been applied, but not as much for the overall firmness of the skin [240].

Injections of products high in collagen synthesis inducing proteins and anti-inflammatory cytokines can increase skin firmness. Furthermore, the strength of the skin can positively be influenced. But the used amount and injection regimes still need further investigations and could not be entirely standardized yet [115, 118, 136, 237].

To what degree ACS injections in vivo influence this process and constantly improve the mechanical properties of the skin could not finally be verified within these three in vivo studies.

The first ACS in vivo study used the highest amount of ACS in a small injection area with the best results, thus a potential increase of the injected amount of ACS might be crucial for a stronger influence. The second and third ACS in vivo study used the same amount but on the whole face. The needed amount for a positive effect must therefore still be explored and verified. Additionally, more comparable studies with similar injection regimes and measurement methods would help to identify the needed amount with the strongest impact for the overall performance of ACS.

4.2.3 Skin density and thickness

Skin density and thickness were measured sonographically with the DUB® Skin Scanner device (taberna pro medicum, Lüneburg, Germany). These two parameters were evaluated in the first two ACS in vivo studies. Skin density is a parameter visualizing the amount of fibers in the skin. Skin thickness is associated with a decrease during aging [45]. The 20 MHz transducer was used, which can visualize the skin up to 0.8 – 1 mm and is commonly used in literature for skin mapping [30, 176, 241-243].

Within the **first ACS in vivo study** skin density did not change noticeably. Yet, the left side had a higher skin density until week 8, in week 2 even significantly with a 19.72 % higher density ($p = 0.003$), decreasing and aligning to the right side values in the following visits. In week 8, 12 and 24 skin density was for both sides similar. All values were within the range of 20 to just above 30 a.u. throughout the study (*Figure 46*). Skin thickness decreased on average by 6.22 % in the first ACS in vivo study, calculating both sides together from screening to week 24. This difference was not significant and the values varied between 1650 and 1550 µm altogether (*Figure 47*).

In the **second ACS in vivo study** skin density values were comparably to the first in vivo study. They ranged between 20 to just above 30 a.u. Here, skin density decreased insignificantly until week 12 and 24. The left side also showed a higher skin density compared to the right side. In the ACS group, the left side was on average 7.83 % higher throughout the study compared to the right side. In the ACS + HA group screening and week 12 left side values were higher, but were nearly the same in week 24, with 28.92 a.u. for the right side and 28.18 a.u. for the left side (*Figure 73*).

Skin thickness values of the second in vivo study were within a similar range compared with the first study. The ACS group values varied between 1500 to just above 1600 µm, the ACS + HA group values were slightly higher around 1600 µm. In the ACS + HA group the left side was on average 2.14 % higher than the right side (*Figure 74*).

In conclusion, skin density and thickness were comparable for both studies but an improving effect, especially for skin density could not be seen. An increase in skin density is associated with an increasing number of collagen and elastin fibers in the skin and therefore an anticipated effect. High concentrations of TGF-ß1 showed in vivo and in vitro increasing collagen synthesis [75, 111]. Therefore, an increase in skin density could have been associated with high TGF-ß1 concentrations within the ACS. In a study by Diaz-Ley et al. facial skin biopsies were analyzed before and after three treatments of plasma rich in growth factors (PRGF). They found an significant increase in epidermis and papillary dermis thickness of the histological biopsies. Additionally, the volume of collagen fibers increased and were better organized. The specific composition of the PRGF was not evaluated, leaving the question what component of the PRGF in particular initiated the improvements [116]. Everts et al. evaluated the thickness and density of the subepidermal low echogenic band (SLEB) with a 20 MHz Dermascan-C ultrasound device of 11 female volunteers after three treatments of neutrophile-poor purePRP in the face. The in vivo assessments showed a decrease in SLEB thickness with an simultaneous increase in SLEB density, which reflects an improvement in collagen production and the skin's regeneration ability. But here again, no evaluation was conducted concerning the platelets and further composition of the purePRP. This was also stated as weakness by the authors themselves [118]. Furthermore, the results of the ultrasound measurements cannot be compared with the two ACS in vivo studies, as the epidermis and dermis was measured and not the SLEB area.

Evaluating the composition of autologous conditioned preparations in combination with stated objective and subjective improvements after PRP treatments would be helpful for a decisive conclusion concerning the biochemical effects. Concerning facial treatments, there are still few studies combining both research questions. One study by Cameli et al. evaluated the platelets concentration via flow cytometry besides the in vivo treatment effects, providing objective improvements in skin biostimulation [115]. But more often the specific biochemical compositions of RPR are studied separately [108].

The in vitro assessments of this work did not specifically evaluate the composition of the ACS preparations either, but showed the influence on dermal fibroblasts vitality, TGF-β1 and pro-collagen production. The positive effects of the in vitro assays could not be seen in the skin's density parameter in vivo. Therefore, other influences need to be considered to draw conclusions about the in vitro and in vivo relations. In a study by Rutgers et al., beside the increased concentration of TGF-β1 in the ACS probes, there was also an increase in IL-6 and TNF-α, which are strong pro-inflammatory cytokines [4, 132]. Whether theses pro-inflammatory cytokines counteract with the anti-inflammatory and collagen activating proteins of the ACS would be a question for further research protocols.

Even though the results of the two ACS in vivo studies did not show an improvement in skin density and skin thickness, the comparison to studies, evaluating age and side depend changes of skin density and thickness, showed comparable values and verified the accuracy of the measurements of this study. Seidenari et al. compared two age groups, 27 – 31 (n = 24) and 60 – 90 (n = 24) years with values for the cheek of 1.64 ± 0.20 mm for the young group and 1.48 ± 0.12 mm for the aged group, but these values only include the dermis thickness [50]. Firooz et al. found values for the cheek of female volunteers of 1608.94 ± 492.94 µm for the dermis and 88.24 ± 16.14 µm for the epidermis (17 female, age range 24 – 61) [30]. Meng et al. studied three female age groups from 18 – 44 years (n = 37), 45 – 59 years (n = 27), and ≥ 60 years (n = 5). Here, the skin thickness of the cheeks were also separated into epidermis and dermis with 0.18 ± 0.02 mm (epidermis) + 0.97 ± 0.33 mm (dermis) for the youngest group, 0.23 ± 0.28 mm (epidermis) + 0.78 ± 0.36 mm (dermis) for the middle aged group, and 0.18 ± 0.04 mm (epidermis) + 0.63 ± 0.20 mm (dermis) for the oldest group [244].

The mean age of the patients in the first ACS in vivo study was 46.75 ± 5.87, in the second ACS in vivo study 48.8 ± 11.4 (ACS group) and 56.0 ± 4.1 years (ACS + HA group), and the skin thickness (epidermis + dermis) of both studies was around 1600 µm. Therefore, the skin thickness was just below the results of Firooz et al. and just above Meng et al. [30, 244]. These variations could be due to different evaluation methods and skin locations as visualize in *Figure 93* and *Figure 94*. Firooz et al. measured thickness and echo-density of the dermis (in an area with highest density), and separately the epidermal entrance echo thickness of the cheek [30]. Meng et al. measured the epidermal thickness as distance between the thin strip and strong echo at the entrance of the epidermis (yellow arrows). Dermal thickness included the wide medium echo area between the back of the epidermis and the hypoechogenic subcutaneous fat layer (red arrows) of the zygoma [244]. However, similarities can be seen and generally comparable results were obtained in all above mentioned studies.

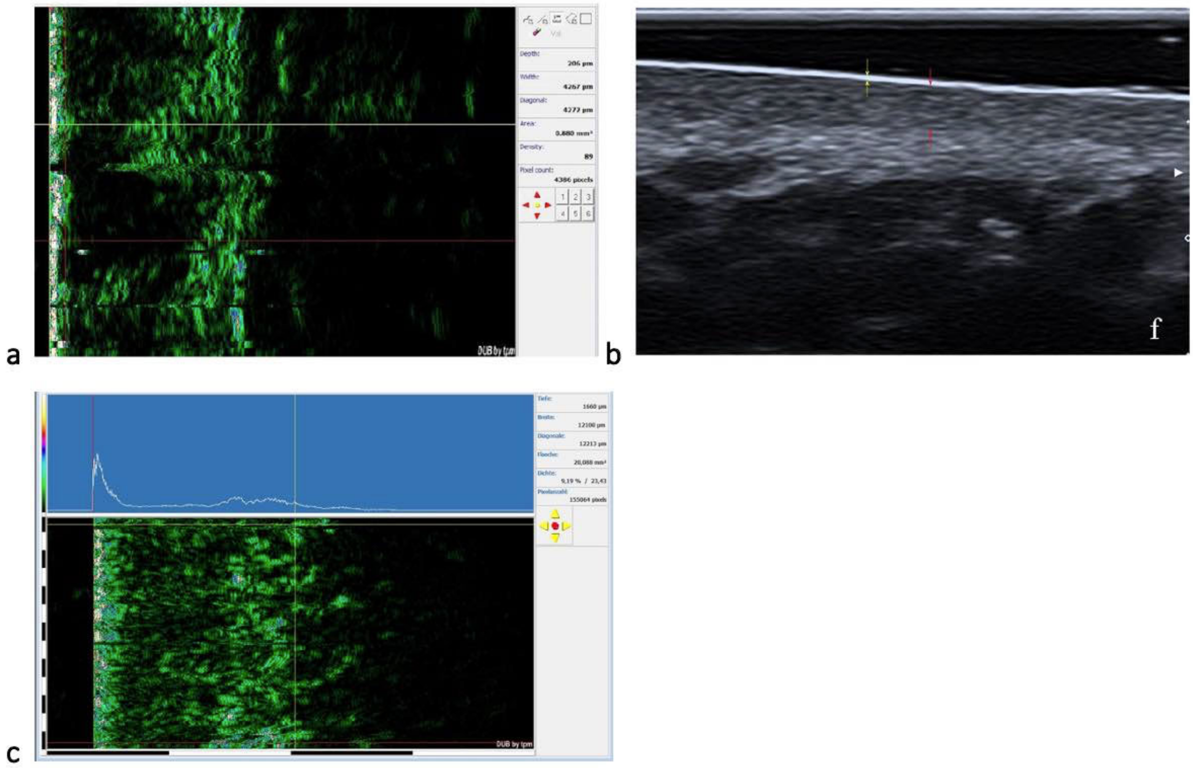


Figure 93: Comparison of sonographic evaluation methods.
***a:** evaluation by Firooz et al. [30], **b:** evaluation of the zygoma by Meng et al. [244], **c:** evaluation method of this work.*

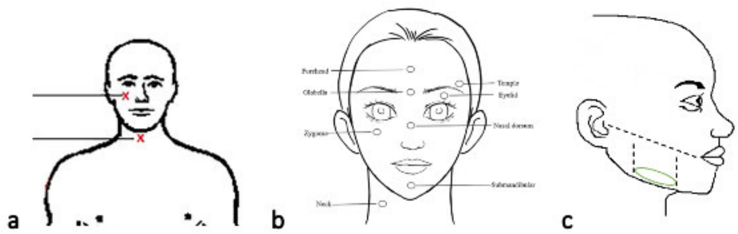


Figure 94: Comparison of sonographic measurement areas.
***a:** cheek area by Firooz et al. [30], **b:** zygoma by Meng et al. [244], **c:** mandibular area of this work.*

4.2.4 Skin topography

The skin surface structure was measured with the PRIMOS device (GFMesstechnik GmbH, Teltow, Germany). This measurement method can verify finest lines and surface variations, like roughness and waviness [191]. A smooth and even skin without reflections caused by fine lines and wrinkles is a sign for youthfulness and healthy skin [245-248]. The skin roughness and waviness allows conclusions about the evenness of the skin and thus also relates to aged skin. Within the first and second in vivo ACS study, the PRIMOS device was used to measure the surface effects of ACS and ACS + HA treatments over 24 weeks.

The data collected, evaluating the parameters of the skin roughness, waviness and surface peaks, however showed no changes for any of the studies. Within the **first in vivo study** the values varied only marginal. Though, the left side values were in all parameters and during most visits even significantly higher compared to the right side (*Figure 48* – *Figure 53*).

The **second in vivo study** also showed no significant change throughout the study period for any of the parameters. Most values varied by less than 5 % comparing the screening values with week 24. Only the skin waviness parameter of the ACS treatment group decreased by 11.19 % for the left side and by 6.71 % for the right side, but with high SDs (*Figure 79*). The pronounced higher values for the left side of the first ACS in vivo study were not found in the second ACS in vivo study. Here, both sides varied equally concerning higher values. Only the maximum profile peak (Rp) showed constantly and for the ACS treatment group also significantly higher values for the left side compared to the right side (*Figure 78*). Also, the maximum roughness (Rmax) and mean depth of roughness (Rz) values of the left side of the ACS treatment group (*Figure 76* and *Figure 77*) were (significantly) higher compared to the right side.

Measurement examples of the skin topography with the PRIMOS device are visualized in *Figure 95* and *Figure 96* for the right and left side, respectively. The evaluated areal (compare with *Figure 13* and *Figure 21*) was not affected by pronounced wrinkles, since only the skin texture was of interest. All measurements assessed the skin roughness but not changes within wrinkle depth or width. Wrinkle depth or width is often of interest for the evaluation concerning age related changes in wrinkle severity or treatment efficacy with hyaluronic acid fillers [139, 249]. However, as the composition of ACS is a liquid serum and not like hyaluronic acid viscous and water binding, a direct wrinkle reduction by a filing effect was not assumed. The influence on skin roughness parameters were however assumed to improve with ACS treatment due to the supposed involvement in anti-inflammatory and cell regenerative processes [17, 24]. But just like the results of skin thickness and density, an direct influence of the ACS treatments could not be verified.

The skins microrelief is influenced by a well hydrated and intact epidermis, which could be shown on mouse and human skin models [250, 251]. There are investigations that confirm a correlation between skin hydration and skin roughness but also others with contradictory results [252, 253]. Comparing for example healthy and clinically unaffected skin with atopic skin, an increased roughness for the atopic skin sites were found [196, 254]. Considering that aging is also associated with dryer skin [218, 255], a decrease in skin roughness parameters after treatment with ACS would be anticipated. However, all patients included in the ACS in vivo studies had a healthy skin status without skin diseases such as atopic dermatitis or neurodermitis, as these were also exclusion criteria. This was also confirmed by the skin hydration measurements. The patients included in all three studies showed a good range, with values above 40 a.u. [162]. This circumstances could have influenced the skin topography leading to unnoticeable changes recorded by the PRIMOS device.

A study by Gold et al. investigated the influence of a skin cream with human growth factors and cytokines in a double-blind, placebo controlled study design on 18 patients aged 52 ± 8 years. The evaluation method with the PRIMOS device was comparable to the three ACS in vivo studies with 16 profile lines arranged in a radial display. The average skin roughness (Ra), mean roughness depth (Rz) and maximum roughness depth (Rmax) in the periorbital skin area was evaluated. All parameters decreased significantly between 10 % and 18 %. The placebo controlled side also showed significantly improvements in average skin roughness (Ra) and mean roughness depth (Rz). A difference between the sides was not found before the treatment but after the treatment of active and placebo, receptively [256]. A topical application of growths factors and cytokines therefore seems to have positive effects on the skin topography. However, the distinct composition of the investigated cream was not described.

A positive superficial influence on skin smoothness with a similar device could also be seen after PRP injections for facial skin rejuvenation [115]. Another publication reported positive outcomes of wrinkle count, depth and volume of the periocular area after PRP injections, also evaluated with the PRIMOS device [118]. If ACS has this potential as well could not be proven within the two ACS in vivo studies. However, only skin roughness parameters were evaluated and not the depth of wrinkles within the face. As the measurement area of the PRIMOS device is limited to a maximum of 30 x 40 mm, it was not possible to assess and evaluate the whole injection area [192]. Multiple recordings of different areas could increase completeness of the topography analyzation but involves a considerably increased time consumption and is not always in compliance with the volunteers patience and time.

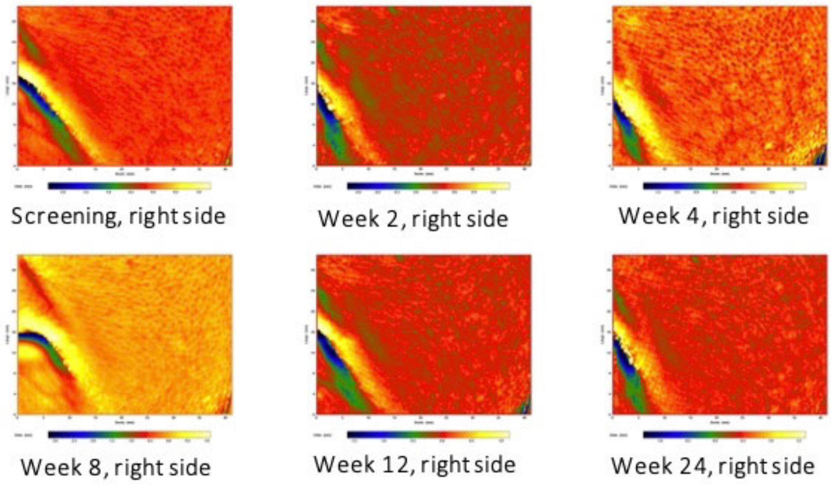


Figure 95: PRIMOS measurement, ACS in vivo study I, Patient 20, right side.

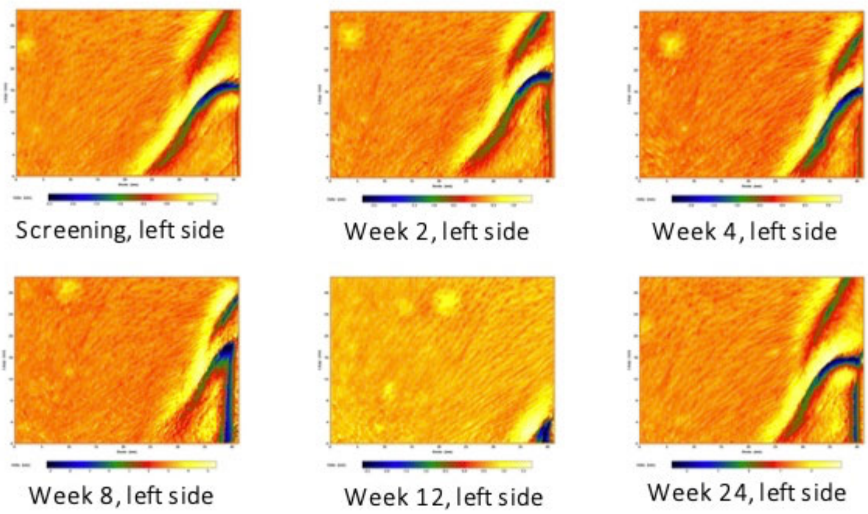


Figure 96: PRIMOS measurement, ACS in vivo study I, Patient 20, left side.

Even if no change in skin topography was found within the two ACS in vivo studies, the differences between the left and right side measurements, which were noticeable for the first ACS in vivo study in particular, should be pointed out. This side difference was also found in the cutometer data for the skin firmness (R0, Uf) and recovery (R8, Ua). A possible difference can occur due to unequally UV exposure, like described in the section mechanical properties of the skin (*4.2.2 Mechanical properties of the skin (cutometry)*). Besides the idea of an unequal UVA exposure to the sides of the face resulting in different degrees of photoaging, sleep lines could also be a reason for a left and right side difference [257]. It is therefore always recommended to mention and describe the exact location of the investigated area, and a right and left side comparison before the beginning of a treatment could in some cases be of importance [155, 258].

4.2.5 Comparison of ACS, PRP and skin quality booster with HA

Parameters describing the appearance and aging status of the skin include skin quality, wrinkle severity or volume loss. They can visually be categorized by photonumerical scales and are common tools in aesthetic clinical trials [140, 248, 259-262]. An additional categorization system based on biophysical measurement methods has recently been developed by Goldie et al. An advisory board of ten dermatologists and physicians defined skin quality based on four emergent perceptual categories (EPCs): skin tone evenness, skin surface evenness, skin firmness and skin glow [212].

To be able to classify the treatment potential of ACS, an overview of comparable publications with HA and PRP treatments was worked out with the focus on the EPC “skin firmness”. Skin firmness is often evaluated by cutometry for skin elasticity and tightness and corneometry for skin hydration. Both devices were assessed in the three ACS in vivo studies and therefore are a suitable basis for the comparison.

Clinical trials with similar injection regimes and evaluation methods with HA in the lower facial cheeks were also conducted at the University of Hamburg, Institute of cosmetic sciences in recent years. A study by Reuther et al. evaluated the effects of a three-session treatment into the lower facial cheeks using stabilized HA-based gel of non-animal origin on 19 female patients. The three treatments were four weeks apart with 50 x 0.02 ml (1 ml per side) per treatment session. Measurements for skin elasticity with the Cutometer® MPA 580 were conducted before the first, second and third treatment sessions (weeks 0, 4 and 8), and during follow-up visits in week 12 and 24. Skin firmness (R0, Uf) increased significantly until the end of the study. Skin gross elasticity (R2, Ua/Uf) and skin net elasticity (R5, Ur/Ue) also both increased significantly until the end of the study. Skin hydration was not evaluated within this publication [166]. In a pervious publication of the same pilot study by Kerscher et al. skin surface roughness, dermal thickness and density were additionally evaluated. Here, skin surface roughness gradually decreased, whereas skin thickness stayed unchanged during the study period. Skin density decreased form baseline to week 8 and then increased again [263].

A study, published in cooperation among myself investigated the performance and safety of a new cohesive polydensified matrix HA filler, additionally containing glycerol, on 21 female patients over 36 weeks. The patients received three treatments with 1 ml HA injections per side into the lower cheek area each four weeks apart. Mechanical properties of the skin and skin hydration were measured during each treatment session and in week 9, 12, 24, 28 and 36. Skin gross elasticity (R2, Ua/Uf) significantly increased from baseline to week 9 and week 12 and remained increased up to week 28 with at least 25 %. Skin firmness (R0, Uf) improved significantly up to 24 weeks. Skin hydration significantly increased throughout the whole study period [175].

A randomized multicenter clinical study for skin revitalization with the same cohesive polydensified matrix HA filler, was conducted in 2021 with a total of 159 subjects. The patients were randomized in a 2:1 ratio to three- or single-dose treatment. The multiple-dose group was treated over three sessions each four weeks apart with 1 ml HA per side. The single-dose group received 1.5 ml HA per side. Measurements were performed at day 1 and weeks 4, 8, 12, 16, 24, 32, and 44 in the multiple-dose group and at day 1 and weeks 4, 8, 16, 24, and 36 in the single-dose group. Skin hydration, elasticity, firmness and roughness measurements as well as investigator- and subject-assessed Global Aesthetic Improvement Scales and different questionnaires were assessed. Skin hydration improved from baseline to all follow-up visits in a subset of subjects with dry or very dry skin. For the skin elasticity, data from 28 and 14 subjects in the multiple- and single- dose groups was analyzed, respectively. Both groups showed improvements in skin gross elasticity (R2, Ua/Uf). However, in the multiple-dose group (n = 28) skin gross elasticity (R2, Ua/Uf) returned to baseline in week 44. In the single-dose group (n = 14), the value remained improved to week 36. Skin firmness showed slight improvements in the multi-dose group only [222].

Studies evaluating the effectiveness of PRP or similar called autologous conditioned products in aesthetic medicine predominantly assess skin biopsies or photographs, use questionnaires or visual scaling systems [114, 116, 122, 123, 125, 128, 264-267]. However, the application of biophysical measurement devices was also conducted by several studies. Within those studies the cutometry was the most common measurement device. Furthermore, studies with three treatment sessions were conducted by Aust et al., Cameli et al., Everst et al. and Hersant et al [115, 118, 126, 237].

Aust et al. conducted a study with 20 subjects (male and female) with three treatment session each one month apart. Per treatment 2 ml PRP was injected into the lower eyelid region per side. Photographs, cutometer measurement and different questionnaires were assessed during the visits. Relevant for the EPC, skin firmness (R0, Uf) significantly increased as well as skin elasticity (measured as area parameters F0 and F1). Skin hydration was not evaluated [126].
Cameli et al. treated twelve female patients in three sessions each one month apart. Different areas of the face were treated with 4 ml PRP (compare *1.1.8.2.1 Platelet rich plasma for skin rejuvenation*). Transepidermal water loss, skin hydration, skin elasticity and smoothness were measured during baseline and after four month (one month after the last treatment). Improvements were reported for all assessed parameters but inaccuracies in the reporting were found as described above (compare *4.2.2 Mechanical properties of the skin (cutometry)*) [115].
A publication by Everst et al. evaluated the effect of PRP injections to the whole face in eleven female subjects. The patients received 7 ml PRP at three sessions each one month apart with a follow up visit after 6 month. Full-aligned facial images were taken to asses skin profile changes, wrinkle count, depth, and volume was measured by the PRIMOS 3D Skin Device. Skin firmness measurements were performed with a Cutometer® dual MPA 580. Skin color, thickness and density of the subepidermal low echogenic band (SLEB) measurements were also performed. Improvements were reported for all measurements in this study as well [118].
Hersant et al. compared PRP, HA and PRP + HA in a randomized controlled prospective study in 93 patients over nine month. Each patient was randomly assigned to receive three treatments of one kind, each one month apart. Follow-up visits were one, three and six month after the last treatment. Photographs, questionnaires and skin elasticity parameters with the Cutometer® were assessed. All three treatment groups showed improvements, but the best result was found in the combined PRP + HA treatment group. Skin firmness was reported to improve as well, but no data was published within the paper [237].

The results of the above described studies concerning the available data for the EPC "skin firmness" are listed in *Table 10.* The data of the three ACS in vivo studies of this work are listed below the other publications.

Table 10: Overview of publications with similar study designs of HA and PRP compared to the ACS in vivo studies

Reference	Patients	Injection area	Injection substance / Amount per session	Total injected amount	Injection regime	Study duration	Elasticity	Tautness / Tightness	Hydration	
Reuther et al. 2010 [166]	19	Lower cheeks	HA 1 ml per side	6 mL	Day 0, week 4, week 8	24 weeks	✓	✓	–	HA
Hertz-Kleptow et al. 2018 [175]	25	Lower cheeks	HA 1 ml per side	6 mL	Day 0, week 4, week 8	36 weeks	✓	✓	✓	HA
Kerscher et al. 2021 [222]	159	Lower cheeks	HA Single dose: 1.5 ml per side	3 ml	Day 0	36 weeks	✓	✗	✓	HA
			Multi-dose: 1 ml per side	6 ml	Day 0, week 4, week 8	44 weeks	✓	✓	✓	HA
Aust et al. 2018 [126]	20	Lower eyelid region	PRP 2 ml per side	6 ml	Day 0, week 4, week 8	12 weeks	✓	✓	–	PRP
Cameli et al. 2017 [115]	12	Whole face	PRP 4 ml per session	12 ml	Day 0, week 4, week 8	12 weeks	(✓)	–	✓	PRP
Everst et al. 2018 [118]	11	Whole face	PRP 7 ml per session	21 ml	Day 0, week 4, week 8	24 weeks	–	✓	–	PRP
Hersant et al. 2017 [237]	93	Whole cheeks	PRP HA PRP + HA 4 ml pre session	12 ml	Day 0, week 4, week 8	36 weeks	✓	–	–	PRP
							✓	–	–	PRP
							✓	–	–	PRP
ACS in vivo I	21	Lower cheeks	ACS 1 ml per side	8 ml	Day 0, week 2, week 4, week 12	24 weeks	✓	✓	✓	ACS
ACS in vivo II	16	Whole cheeks	ACS 2.5 ml per side ACS + HA 2.5 ml per side (4:1)	20 ml	Day 0, week 2, week 4, Week 12	24 weeks	✓	✓	✗	ACS
ACS in vivo III	20	Wholes cheeks	ACS 2 ml per side	16 ml	Day 0, week 2, week 4, week 6	48 weeks	✗	✗	✗	ACS

✓ improvement, ✗ no change , – not done, (✓) data inaccuracy)

A tendency for the application of three treatment sessions can be seen from the data of the publications listed in *Table 10*. This seems to be a tolerated regime by the patients and is well suited to achieve a good patients compliance. However, the study duration varies between three month to one year, which can influence the measurement results and following conclusions. The frequency of a treatment regime for a continual improvement depends therefore on the available data and should be considered carefully. The injected amount of product also needs to be considered carefully. The highest concentration of ACS was administered in the first ACS in vivo study with 1 ml per side in a 4 x 4 ml areal (compare *2.3.2.1 ACS in vivo study I*). The outcome of this study showed the most promising results. An increase in product could thus increases the efficacy of the treatments. Although, this could not necessarily be confirmed by in vitro data published by Kim et al. and Kakudo et al. [15, 213]. A does depended in vivo and in vitro comparison with ACS could be a next step for further research. Also the application of the three other EPCs: skin surface evenness, skin tone and skin glow could be part of further investigations [212].

Different biochemical pathways within the cells of the dermis and epidermis concerning collagen and hyaluronic acid synthesis need to be stimulated by ACS injections to enhance skin revitalization and skin rejuvenation. Some of these pathways can be stimulated, considering the high amount of TGF-ß1 in ACS, others might not be stimulated as needed. ACS might therefore be one key to skin revitalization and rejuvenation but it cannot address the whole complexity of the natural aging process.

5 Conclusion

A fundamental part of the cosmetic industry are the minimally invasive cosmetic procedures. Besides the treatment with botulinum toxin, soft tissue fillers are the second most common minimally invasive non-surgical procedures with over 3 million interventions per year. In the American society of Plastic Surgeons statistics report, PRP is part of the soft tissue fillers [12, 13]. Therefore, ACS as a counterpart to PRP can be used for a comparison with PRP as well as the leading soft tissue filler HA.

The main difference between ACS and PRP is, that the serum is cell free, contains no clotting factors, no additives and no anticoagulant. The processing is standardized and needs to be done only once for multiple treatment sessions [17, 24, 129]. Primarily, ACS has been used for orthopedic diseases in animals and humans till now [25, 26, 131, 268]. The three ACS in vivo studies were to explore the potential of ACS as a regenerative substance for facial skin revitalization and rejuvenation.

The first two in vivo studies showed improvements, especially in the mechanical properties of the skin. Possible induction of fibroblast proliferation and an increased synthesis of collagen, as underlying mechanisms, were supported by the two in vitro studies. They showed increased cell viability, TGF-ß1 and procollagen production. Yet, the results of the first two ACS in vivo studies could not be confirmed by the third study, where no evident improvements in the mechanical properties of the skin were seen. However, skin hydration was consistent in the well hydrated range at the end of the investigations for all three studies.

In order to classify the potential of ACS treatments to the already known PRP and skin quality booster with HA, a comparison based on the newly developed emergent perceptual categories (EPCs) for skin quality was conducted [212]. The EPC “skin firmness” was used to compare the results of the three studies from this work with outcomes from PRP and skin quality booster with HA studies [115, 118, 126, 166, 175, 222, 237].

The comparison was difficult as most study designs were different and only parts of the EPC “skin firmness” were evaluated. The available data for skin quality booster with HA was more consistent and showed superiority over ACS and PRP. This superiority can be attributed to the experience of HA in the cosmetic industry. The first known clinical trials with PRP application for cosmetic anti-aging purposes were around 2010 [14, 122]. HA as filler and for skin revitalization has been studied since the 1970s and has therefore a wide-ranging database [28, 98, 225, 269, 270].

Nevertheless, the comparison of the different treatment products is promising and could induce more studies with an adaption to the EPCs to allow more comparability.

Interestingly, there is a wide range of in vitro data for PRP and ACS. PRP showed good effects on cell proliferation processes, concentrations of anti-inflammatory cytokines and also collagen synthesis activation [108, 271]. In case of ACS, positive effects can be confirmed by the in vitro results of this study as well as from research concerning cytokine and protein concentrations [24, 132, 136]. In vitro studies with HA also showed improvement in collagen synthesis and fibroblast activation [272, 273]. Studies with the focus on skin rejuvenation comparing ACS and HA products in vitro in a parallel design do not exist until now. The combined ACS + HA incubation in this study did not show superior results over the ACS incubated cells. The influence of merely HA hasn't been tested in this study. Therefore, comparing the effects of ACS, PRP and skin quality booster with HA solely in vitro on skin cells or skin models could be a next step of investigation. Also, clinical trials with comparable study designs for all three products and an adaption in the used ACS amount could help to enhance comprehensive insights into their in vivo effects on skin revitalization and rejuvenation.

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Appendix

List of hazardous substances according to GHS (hazard symbols H- und P) used in this study

Chemicals	Pictogram	H statement	P statement
Hydrochloric acid		H290, H314, H335	P234, P261, P271, P280
Isopropanol		H225, H319, H336	P210, P305+P351+P338, P312,P370+P378, P403+P233, P403+P235, P501
Tris		H225, H302-H314-H332	P261, P280, P302+P352, P305+P351+P338, P304+P340

Hazard statement(s)

H225 Highly flammable liquid and vapor

H290 May be corrosive to metals.

H302 Harmful if swallowed.

H314 Causes severe skin burns and eye damage.

H319 Causes serious eye irritation

H332 Harmful if inhaled.

H335 May cause respiratory irritation.

H336 May cause drowsiness or dizziness

Precautionary statement(s)

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking

P233 Keep container tightly closed

P234 Keep only in original packaging.

P261 Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.

P271 Use only outdoors or in a well-ventilated area.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

P302 + P352 IF ON SKIN: Wash with plenty of soap and water.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

P312 Call a POISON CENTER or doctor/physician if you feel unwell

P370+P378 In case of fire: Use sand, carbon dioxide or powder extinguisher for extinction

P403+P233 Store in a well-ventilated place. Keep container tightly closed

P403+P235 Store in a well-ventilated place. Keep cool

P501 Dispose of contents/container to industrial combustion plant

In vitro I MTT assay

Dual wavelength measurement with measuring wavelength 550 nm

	aACS	aACS+HA	yACS	yACS+HA	FCS
2 h	0.0807	0.1066	0.0880	0.1290	0.2347
	0.0962	0.1276	0.0931	0.1287	0.2321
6 h	0.0942	0.1299	0.1243	0.1540	0.2496
	0.1209	0.2777	0.1211	0.1389	0.2807
24 h	0.1773	0.1587	0.1368	0.1613	0.2438
	0.1670	0.1874	0.1501	0.1772	0.2736

FCS - normalized MTT values in %

	yACS	yACS+HA	aACS	aACS+HA
2h	37.49	54.96	34.38	45.42
	40.11	55.45	41.45	54.98
MW	**38.80**	**55.21**	**37.92**	**50.20**
SD	**1.85**	**0.34**	**4.99**	**6.76**
6h	49.80	61.70	37.74	52.04
	43.14	49.48	43.07	98.93
MW	**46.47**	**55.59**	**40.41**	**75.49**
SD	**4.71**	**8.64**	**3.77**	**33.15**
24h	56.11	66.16	72.72	65.09
	54.86	64.77	61.04	68.49
MW	**55.49**	**65.46**	**66.88**	**66.79**
SD	**0.88**	**0.99**	**8.26**	**2.40**

In vitro I PIP assay

Absorption wavelength 450 nm

		Supernatant			Lysate			
PIP (ng/ml)		2h	6h	24h	2h	6h	24h	
	1	2	3	4	5	6	7	
0	0.048300002	0.487899989	0.668799996	0.945500016	1.055299997	1.002699971	1.284000039	aACS
10	0.0973	0.498600006	0.76849997	1.127099991	1.368199944	1.300699949	1.395599961	
20	0.142800003	0.432900012	0.617799997	1.013700008	1.097300053	1.005300045	1.278100014	aACS+HA
40	0.241300002	0.460700005	0.679700017	1.01970005	1.281700015	1.195600033	1.322299957	
80	0.40259999	0.364800006	0.689100027	0.944899976	1.002099991	1.256899953	1.273200035	yACS
160	0.58160001	0.4278	0.532599986	1.095299959	1.271600008	1.279099941	1.376999974	
320	0.906400025	0.329600006	0.62650001	0.941399992	1.01970005	1.175999999	1.174000025	yACS+HA
640	1.364899993	0.407499999	0.665400028	0.975600004	1.265699983	1.183200002	1.418099999	

Fit of lg (PIP) and A450 values in Sigma-Plot with the exponential equation y = y0 + a * e ^ (b * x), with $r^2 = 0.99$

y0=-0,03662
a=0,03365
b=1,329

Mean PIP concentration (ng/ml) – extracellular (2 ml Supernatant) und intracellular (100 µl Lysate)

	Supernatant			Lysate		
	2h	6h	24h	2h	6h	24h
aACS	118.6	219.8	404.6	528.8	485.6	620.8
aACS+HA	108.5	208.0	425.9	542.9	486.4	618.6
yACS	101.3	185.5	390.1	509.4	447.4	589.7
yACS+HA	89.8	202.3	368.6	478.1	534.4	587.8
mean	**104.5**	**203.9**	**397.3**	**514.8**	**488.4**	**604.2**
SD	**12.1**	**14.3**	**24.1**	**28.0**	**35.6**	**17.9**

Standard deviation of PIP concentration (ng/ml) – extracellular (2 ml Supernatant) und intracellular (100 µl Lysate)

	Supernatant			Lysate		
	2h	6h	24h	2h	6h	24h
aACS	2.9	35.4	83.5	160.7	147.6	61.6
aACS+HA	17.3	52.2	53.4	140.9	146.5	64.8
yACS	7.1	20.5	2.7	93.5	91.3	23.9
yACS+HA	23.3	3.2	33.1	137.7	31.8	26.5

Difference of PIP concentration (ng/ml) increase over incubation time

	Supernatant		Lysate	
	2 to 6 h	2 to 24 h	2 to 6 h	2 to 24 h
aACS	1.9	3.4	0.9	1.2
aACS+HA	1.9	3.9	0.9	1.1
yACS	1.8	3.9	0.9	1.2
yACS+HA	2.3	4.1	1.1	1.2

In vitro II MTT assay

Measurement with measuring wavelength 550 nm

		550 nm			Background (no cells)		
		1	2	3	1	2	3
yACS#1	2h	0.1047	0.0952	0.0914	0.0446	0.0436	0.0436
	6h	0.1027	0.1041	0.1032	0.0446	0.0436	0.0436
	24h	0.1626	0.192	0.1577	0.0446	0.0436	0.0436
yACS#2	2h	0.069	0.0742	0.0711	0.0454	0.065	0.0438
	6h	0.0806	0.0728	0.0806	0.0454	0.065	0.0438
	24h	0.1339	0.1203	0.1353	0.0454	0.065	0.0438
yACS#3	2h	0.0887	0.0857	0.0813	0.0441	0.0434	0.043
	6h	0.0968	0.0953	0.0953	0.0441	0.0434	0.043
	24h	0.1294	0.1223	0.1103	0.0441	0.0434	0.043
aACS#1	2h	0.2272*	0.1003	0.1008	0.0605	0.0449	0.0464

	6h	0.1094	0.1618	0.104	0.0605	0.0449	0.0464
	24h	0.1486	0.1378	0.1452	0.0605	0.0449	0.0464
aACS#2	2h	0.0906	0.0933	0.09	0.0458	0.0447	0.0452
	6h	0.095	0.1051	0.09	0.0458	0.0447	0.0452
	24h	0.1453	0.2134	0.1717	0.0458	0.0447	0.0452
aACS#3	2h	0.0933	0.0854	0.0885	0.0458	0.0448	0.0446
	6h	0.0944	0.1305	0.0939	0.0458	0.0448	0.0446
	24h	0.1616	0.1402	0.1324	0.0458	0.0448	0.0446
FCS	2h	0.2089	0.1885	0.2141	0.1144	0.0446	0.0669
	6h	0.2297	0.2113	0.1871	0.1144	0.0446	0.0669
	24h	0.2422	0.2646	0.2435	0.1144	0.0446	0.0669

* measurement error – data not included

FCS - normalized MTT values in %

mean	yACS#1	yACS#2	yACS#3	aACS#1	aACS#2	aACS#3
2h	43.98	16.64	34.20	37.73*	37.21	36.10
6h	45.42	21.94	40.12	53.49	38.73	44.86
24h	74.80	48.73	46.91	55.70	75.39	61.23
delta 2h to 24h	30.82	32.09	12.70	17.98	38.18	25.13
SD	yACS#1	yACS#2	yACS#3	aACS#1	aACS#2	aACS#3
2h	17.08	9.44	11.38	1.09	8.99	12.29
6h	7.92	14.95	7.86	14.67	3.46	5.70
24h	15.25	22.22	17.22	13.36	3.31	25.64

* Mean data from two measurements only

In vitro II PIP assay

Absorption wavelength 450 nm

	Supernatant			Lysate		
	2h	6h	24h	2h	6h	24h
aACS#1	2.5882	3.0748	2.6499	OVER	OVER	3.9947
	1.687	2.8504	2.436	OVER	OVER	OVER
aACS#2	3.2494	3.5559	3.4011	3.5581	3.6937	3.862
	3.0097	3.1452	3.1973	3.8388	3.907	3.9481
aACS#3	3.8747	3.9918	3.8262	3.9404	3.8617	3.5284
	3.5869	3.7181	3.0316	3.9569	3.9662	3.7763
yACS#1	2.0184	2.8618	2.8095	3.5873	OVER	3.9986
	3.233	3.1076	2.9815	3.9574	3.9685	OVER
yACS#2	2.617	3.4776	3.3316	3.5474	3.211	3.6675
	3.1573	3.7147	3.4767	3.1064	3.1735	3.6017
yACS#3	3.8919	3.7993	3.7585	OVER	3.8934	3.627
	3.8762	3.8383	3.7525	OVER	3.8692	3.9084

PIP (ng/ml)	**#1**	**#2**	**#3**
0	0.057	0.0582	0.0596
10	0.211	0.2136	0.2137
20	0.283	0.2813	0.2854
40	0.5689	0.5577	0.6127
80	1.1896	1.2776	1.2209
160	2.1244	1.4592	
320	3.3772	3.2864	
640	3.922	3.9405	

Fit of lg (PIP) and A450 values in Excel with the exponential equation y = m * e ^ (b * x), with r^2 = 0.99 (x=ln(y/0.0284)/1.9021)

m	0.0284
b	1.9021

Mean of PIP concentration (ng/ml) – mean of duplicates

	Supernatant			Lysate		
	2h	6h	24h	2h	6h	24h
aACS#1	188.0	277.6	230.7	OVER	OVER	398.5*
aACS#2	296.6	322.3	316.2	363.1	375.2	387.7
aACS#3	367.0	381.8	331.8	393.0	388.8	357.6
yACS#1	241.4	280.1	270.0	371.9	395.4*	399.0*
yACS#2	269.3	351.0	328.4	319.5	303.8	355.5
yACS#3	385.2	377.4	369.8	OVER	384.9	371.3

* only one value available

Standard deviation of PIP concentration (ng/ml) – of mean of duplicates

	Supernatant			Lysate		
	2h	6h	24h	2h	6h	24h
aACS#1	67.4	18.0	16.6	OVER	OVER	
aACS#2	19.4	33.8	16.7	23.6	18.0	7.3
aACS#3	24.2	23.2	65.7	1.4	8.9	20.8
yACS#1	94.8	19.7	13.7	31.2		
yACS#2	43.1	19.8	12.0	36.2	3.1	5.5
yACS#3	1.3	3.3	0.5	OVER	2.1	23.7

ACS in vivo study I data and statistical analyses

All of the following SPSS datasets are in German formatting. The comma in decimal numbers corresponds to the point in English.

Patient data and skin condition

Pateint-number	Age	Skin condition				
		normal	dry	oily	not sensitive	sensitive
1	46			x	x	
2	49		x		x	
3	46		x		x	
4	47		x		x	
5	41			x	x	
7	45		x			x
9	52	x				x
11	48	x			x	
12	53		x		x	
13	55	x				x
14	41			x	x	
15	38	x				x
16	35		x			x
17	51	x				x
18	38		x		x	
19	54	x			x	
20	54	x			x	
21	41		x		x	
22	51		x		x	
23	50		x			x
24	46	x			x	

Corneometry – skin hydration

Corneometer data, mean of three measurements

Patient	ScreeningR	ScreeningL	Week2R	Week2L	Week4R	Week4L	Week8R	Week8L	Week12R	Week12L	Week24R	Week24L
1	48.767	44.367	35.167	36.367	50.200	52.067	60.367	67.167	62.833	67.367	64.400	59.133
2	36.533	38.000	35.000	40.267	50.133	40.433	44.333	28.833	40.867	39.633	48.833	43.700
3	52.367	50.133	43.333	41.267	47.567	36.333	89.033	62.033	55.100	65.433	62.400	67.267
4	50.167	53.700	49.033	55.767	50.133	35.833	54.633	60.667	50.067	44.967	52.633	55.367
5	73.967	59.700	44.867	49.867	48.133	43.967	56.333	59.267	41.233	55.033	56.000	65.267
7	26.367	25.233	31.233	35.633	37.933	43.933	49.267	40.267	74.600	73.767	56.367	60.700
9	42.700	48.467	46.333	50.000	58.567	50.667	66.200	69.333	57.233	67.900	49.367	51.867
11	46.533	34.567	30.467	34.833	54.833	38.100	56.300	58.733	59.800	57.067	57.400	62.800
12	51.900	54.200	47.267	58.367	59.333	47.100	80.900	78.833	50.000	54.700	59.300	60.567
13	49.567	48.067	63.533	59.967	37.300	50.400	51.900	56.167	49.767	64.000	61.633	75.933
14	52.033	43.933	57.600	40.200	42.367	40.933	35.167	26.833	64.700	62.100	64.567	62.100
15	26.033	13.167	31.833	29.633	43.833	34.200	39.067	50.133	32.267	39.367	38.800	50.067
16	31.200	21.167	25.900	22.167	15.533	18.500	20.800	9.533	58.133	40.900	95.000	51.400
17	52.333	55.433	66.467	65.300	38.467	44.333	77.600	78.067	72.967	68.867	36.567	33.400
18	50.633	37.967	55.133	38.667	57.167	43.400	59.800	42.467	43.100	47.667	55.233	55.933
19	17.500	20.667	30.500	36.900	41.633	37.967	28.100	34.400	49.500	36.833	42.500	47.833
20	55.267	47.300	45.367	47.333	55.333	47.600	44.567	41.033	49.900	52.333	50.933	49.000
21	43.267	40.900	52.200	41.667	51.100	56.533	22.933	38.567	66.300	71.933	52.133	58.167
22	30.467	34.133	50.433	39.933	41.767	32.833	26.500	25.333	48.933	46.800	42.233	40.333
23	46.000	43.733	42.900	48.900	42.167	45.067	44.200	51.067	53.800	63.167	46.000	57.600
24	42.700	47.967	52.567	57.467	40.800	45.033	53.533	64.633	41.567	54.667	54.800	53.167
mean	**44.110**	**41.086**	**44.625**	**44.310**	**45.919**	**42.154**	**50.549**	**49.684**	**53.460**	**55.929**	**54.624**	**55.314**
SD	**12.609**	**12.520**	**11.353**	**10.887**	**9.830**	**8.219**	**18.533**	**18.434**	**10.903**	**11.580**	**12.259**	**9.615**

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,935	21	,171
ScreeningL	,937	21	,187
Week2R	,962	21	,563
Week2L	,969	21	,717

Week4R	,891	21	,024
Week4L	,943	21	,248
Week8R	,969	21	,704
Week8L	,972	21	,772
Week12R	,973	21	,801
Week12L	,945	21	,268
Week24R	,868	21	,009
Week24L	,988	21	,994

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,325	20,358	14	,122	,675	,828	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,409	16,194	14	,306	,763	,966	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Greenhouse-Geisser	6461,836	3,374	1915,000	6,551	<,001	,247
Error(Visit)	Greenhouse-Geisser	19729,277	67,487	292,344			
Side	Greenhouse-Geisser	40,507	1,000	40,507	,520	,479	,025
Error(Side)	Greenhouse-Geisser	1557,461	20,000	77,873			
Visit * Side	Greenhouse-Geisser	282,226	3,817	73,931	1,688	,164	,078
Error(Visit*Side)	Greenhouse-Geisser	3343,384	76,348	43,791			

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
1	2	-1,870	1,952	1.000	-8.370	4.630
	3	-1,439	2,341	1.000	-9.237	6.360
	4	-7,519	3,063	.351	-17.720	2.682
	5	-12,097*	3,019	.010	-22.151	-2.042
	6	-12,371*	3,029	.009	-22.461	-2.282
2	3	,431	2,234	1.000	-7.010	7.872
	4	-5,649	3,346	1.000	-16.792	5.494
	5	-10,227*	2,805	.024	-19.571	-.883
	6	-10,502	3,351	.078	-21.664	.661
3	4	-6,080	3,319	1.000	-17.135	4.975
	5	-10,658*	2,544	.007	-19.131	-2.185
	6	-10,933*	2,929	.020	-20.689	-1.176
4	5	-4,578	3,920	1.000	-17.634	8.478
	6	-4,852	4,428	1.000	-19.599	9.894
5	6	-,275	2,764	1.000	-9.479	8.930

Based on estimated marginal means
*. The mean difference is significant at the .05 level.
b. Adjustment for multiple comparisons: Bonferroni.

Cutometry – mechanical properties of the skin

Means and SDs

Mean 2 mm								
Parameter	R0	R2	R3	R5	R6	R7	R8	R9
n	21	21	21	21	21	21	21	21
ScreeningR2	0.337	0.701	0.389	0.363	0.398	0.260	0.236	0.052
ScreeningL2	0.339	0.689	0.393	0.364	0.376	0.263	0.234	0.054
Week2R2	0.304	0.730	0.353	0.394	0.432	0.274	0.222	0.048
Week2L2	0.316	0.734	0.368	0.404	0.405	0.287	0.232	0.051
Week4R2	0.285	0.752	0.335	0.435	0.454	0.299	0.213	0.050
Week4L2	0.298	0.756	0.348	0.464	0.462	0.316	0.223	0.051
Week8R2	0.252	0.749	0.296	0.460	0.494	0.307	0.189	0.044
Week8L2	0.263	0.748	0.313	0.489	0.506	0.324	0.197	0.050
Week12R2	0.238	0.728	0.284	0.421	0.457	0.288	0.173	0.046
Week12L2	0.243	0.743	0.296	0.479	0.497	0.318	0.181	0.053
Week24R2	0.165	0.705	0.208	0.513	0.558	0.322	0.117	0.043
Week24L2	0.157	0.686	0.191	0.457	0.512	0.298	0.108	0.034
SD	R0	R2	R3	R5	R6	R7	R8	R9
ScreeningR2	0.043	0.082	0.046	0.059	0.061	0.044	0.041	0.008
ScreeningL2	0.036	0.082	0.038	0.083	0.065	0.052	0.037	0.010
Week2R2	0.036	0.080	0.040	0.081	0.051	0.052	0.036	0.006
Week2L2	0.047	0.082	0.051	0.093	0.065	0.060	0.042	0.007
Week4R2	0.051	0.079	0.055	0.081	0.067	0.052	0.039	0.009
Week4L2	0.065	0.080	0.072	0.108	0.097	0.063	0.044	0.010
Week8R2	0.046	0.055	0.049	0.070	0.081	0.039	0.036	0.008
Week8L2	0.038	0.069	0.047	0.094	0.087	0.054	0.038	0.010
Week12R2	0.055	0.069	0.064	0.103	0.092	0.060	0.041	0.013
Week12L2	0.054	0.076	0.060	0.113	0.078	0.064	0.044	0.012
Week24R2	0.033	0.086	0.042	0.188	0.213	0.078	0.029	0.016
Week24L2	0.032	0.064	0.038	0.122	0.177	0.048	0.025	0.012

Mean 4 mm								
Parameter	R0	R2	R3	R5	R6	R7	R8	R9
n	21	21	21	21	21	21	21	21
ScreeningR4	0.662	0.726	0.753	0.593	0.492	0.396	0.482	0.092
Week2R4	0.712	0.800	0.799	0.645	0.483	0.434	0.567	0.087
Week4R4	0.653	0.840	0.738	0.718	0.542	0.463	0.544	0.085
Week8R4	0.560	0.870	0.643	0.822	0.621	0.507	0.489	0.082
Week12R4	0.540	0.875	0.614	0.783	0.573	0.496	0.472	0.073
Week24R4	0.505	0.848	0.574	0.787	0.574	0.496	0.424	0.069
ScreeningL4	0.688	0.755	0.777	0.563	0.446	0.390	0.524	0.089
Week2L4	0.765	0.809	0.857	0.617	0.435	0.430	0.618	0.092
Week4L4	0.703	0.844	0.791	0.678	0.480	0.456	0.589	0.088
Week8L4	0.599	0.874	0.680	0.756	0.537	0.490	0.526	0.081
Week12L4	0.616	0.869	0.699	0.692	0.479	0.464	0.533	0.083
Week24L4	0.476	0.854	0.551	0.824	0.636	0.502	0.407	0.075
SD	R0	R2	R3	R5	R6	R7	R8	R9
ScreeningR4	0.099	0.072	0.103	0.113	0.077	0.062	0.092	0.011
Week2R4	0.101	0.074	0.105	0.138	0.104	0.081	0.079	0.013
Week4R4	0.136	0.053	0.147	0.156	0.125	0.078	0.093	0.015
Week8R4	0.114	0.049	0.127	0.144	0.181	0.064	0.110	0.020
Week12R4	0.091	0.042	0.097	0.162	0.171	0.074	0.082	0.013
Week24R4	0.108	0.084	0.119	0.187	0.156	0.087	0.077	0.016
ScreeningL4	0.123	0.070	0.130	0.073	0.068	0.052	0.135	0.013
Week2L4	0.089	0.072	0.095	0.096	0.077	0.063	0.081	0.013
Week4L4	0.130	0.072	0.142	0.147	0.143	0.072	0.099	0.020
Week8L4	0.135	0.040	0.147	0.143	0.190	0.060	0.125	0.017
Week12L4	0.117	0.064	0.130	0.182	0.133	0.090	0.097	0.018
Week24L4	0.099	0.051	0.107	0.155	0.177	0.063	0.090	0.015

Skin firmness (R0, Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR2	,893	21	,025
ScreeningR4	,963	21	,586
ScreeningL2	,946	21	,290
ScreeningL4	,775	21	<,001
Week2R2	,978	21	,888
Week2R4	,946	21	,291
Week2L2	,956	21	,433

Week2L4	,953	21	,380
Week4R2	,942	21	,244
Week4R4	,927	21	,117
Week4L2	,954	21	,403
Week4L4	,971	21	,745
Week8R2	,943	21	,245
Week8R4	,961	21	,529
Week8L2	,980	21	,927
Week8L4	,944	21	,260
Week12R2	,942	21	,242
Week12R4	,951	21	,358
Week12L2	,906	21	,045
Week12L4	,989	21	,995
Week24R2	,974	21	,826
Week24R4	,955	21	,424
Week24L2	,969	21	,716
Week24L4	,906	21	,047

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon^c Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,389	17,090	14	,255	,695	,860	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,378	17,624	14	,228	,755	,952	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,821	5	,164	71,824	<,001	,782
	Greenhouse-Geisser	,821	3,477	,236	71,824	<,001	,782
Error(Visit)	Sphericity Assumed	,229	100	,002			
	Greenhouse-Geisser	,229	69,535	,003			
Side	Sphericity Assumed	,002	1	,002	1,624	,217	,075
	Greenhouse-Geisser	,002	1,000	,002	1,624	,217	,075
Error(Side)	Sphericity Assumed	,025	20	,001			
	Greenhouse-Geisser	,025	20,000	,001			
Visit * Side	Sphericity Assumed	,003	5	,001	1,031	,404	,049
	Greenhouse-Geisser	,003	3,773	,001	1,031	,394	,049
Error(Visit*Side)	Sphericity Assumed	,066	100	,001			
	Greenhouse-Geisser	,066	75,459	,001			

a. Parameter = R0

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon^c Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,553	10,727	14	,710	,810	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,378	5	,076	55,389	<,001	,735
	Greenhouse-Geisser	,378	4,048	,093	55,389	<,001	,735
Error(Visits)	Sphericity Assumed	,136	100	,001			
	Greenhouse-Geisser	,136	80,959	,002			

a. Parameter = R0

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c] Lower Bound	Upper Bound
1	2	,033*	,009	,019	,004	,062
	3	,053*	,010	<,001	,018	,087
	4	,085*	,011	<,001	,049	,121
	5	,099*	,011	<,001	,061	,137
	6	,172*	,011	<,001	,136	,209
2	1	-,033*	,009	,019	-,062	-,004
	3	,020	,013	1,000	-,022	,062
	4	,052*	,011	,001	,016	,088
	5	,066*	,011	<,001	,029	,103
	6	,139*	,010	<,001	,107	,171
3	1	-,053*	,010	<,001	-,087	-,018
	2	-,020	,013	1,000	-,062	,022
	4	,032	,014	,486	-,014	,079
	5	,046*	,010	,002	,014	,079
	6	,120*	,012	<,001	,080	,159
4	1	-,085*	,011	<,001	-,121	-,049
	2	-,052*	,011	,001	-,088	-,016
	3	-,032	,014	,486	-,079	,014
	5	,014	,014	1,000	-,031	,059
	6	,087*	,012	<,001	,047	,128
5	1	-,099*	,011	<,001	-,137	-,061
	2	-,066*	,011	<,001	-,103	-,029
	3	-,046*	,010	,002	-,079	-,014
	4	-,014	,014	1,000	-,059	,031
	6	,073*	,012	<,001	,034	,113
6	1	-,172*	,011	<,001	-,209	-,136
	2	-,139*	,010	<,001	-,171	-,107
	3	-,120*	,012	<,001	-,159	-,080
	4	-,087*	,012	<,001	-,128	-,047
	5	-,073*	,012	<,001	-,113	-,034

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[c] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,333	19,918	14	,136	,719	,897	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,446	5	,089	56,555	<,001	,739
	Greenhouse-Geisser	,446	3,597	,124	56,555	<,001	,739
Error(Visits)	Sphericity Assumed	,158	100	,002			
	Greenhouse-Geisser	,158	71,937	,002			

a. Parameter = R0

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c] Lower Bound	Upper Bound
1	2	,023	,011	,812	-,015	,061

	3	,042	,014	,126	-,006	,089
	4	,076*	,009	<,001	,048	,105
	5	,096*	,013	<,001	,054	,139
	6	,182*	,010	<,001	,149	,216
2	1	-,023	,011	,812	-,061	,015
	3	,019	,017	1,000	-,038	,075
	4	,053*	,011	,002	,016	,090
	5	,073*	,012	<,001	,032	,114
	6	,159*	,011	<,001	,122	,196
3	1	-,042	,014	,126	-,089	,006
	2	-,019	,017	1,000	-,075	,038
	4	,034	,014	,363	-,013	,082
	5	,055*	,013	,008	,010	,099
	6	,141*	,013	<,001	,096	,185
4	1	-,076*	,009	<,001	-,105	-,048
	2	-,053*	,011	,002	-,090	-,016
	3	-,034	,014	,363	-,082	,013
	5	,020	,012	1,000	-,020	,060
	6	,106*	,007	<,001	,083	,129
5	1	-,096*	,013	<,001	-,139	-,054
	2	-,073*	,012	<,001	-,114	-,032
	3	-,055*	,013	,008	-,099	-,010
	4	-,020	,012	1,000	-,060	,020
	6	,086*	,012	<,001	,044	,128
6	1	-,182*	,010	<,001	-,216	-,149
	2	-,159*	,011	<,001	-,196	-,122
	3	-,141*	,013	<,001	-,185	-,096
	4	-,106*	,007	<,001	-,129	-,083
	5	-,086*	,012	<,001	-,128	-,044

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

a. Parameter = R0

c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R0

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,002048	,038139	,008323	-,019408	,015313	-,246	20	,404	,808
Pair 2	Week2R2 - Week2L2	-,011952	,047045	,010266	-,033367	,009462	-1,164	20	,129	,258
Pair 3	Week4R2 - Week4L2	-,013000	,032789	,007155	-,027925	,001925	-1,817	20	,042	,084
Pair 4	Week8R2 - Week8L2	-,010762	,034580	,007546	-,026503	,004979	-1,426	20	,085	,169
Pair 5	Week12R2 - Week12L2	-,004667	,043110	,009407	-,024290	,014957	-,496	20	,313	,625
Pair 6	Week24R2 - Week24L2	,008095	,036267	,007914	-,008413	,024604	1,023	20	,159	,319

a. Parameter = R0

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilon[c]		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,308	21,339	14	,096	,631	,763	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,481	13,256	14	,511	,783	,997	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1,698	5	,340	41,129	<,001	,673
	Greenhouse-Geisser	1,698	3,155	,538	41,129	<,001	,673
Error(Visit)	Sphericity Assumed	,826	100	,008			
	Greenhouse-Geisser	,826	63,097	,013			
Side	Sphericity Assumed	,081	1	,081	9,673	,006	,326
	Greenhouse-Geisser	,081	1,000	,081	9,673	,006	,326
Error(Side)	Sphericity Assumed	,168	20	,008			
	Greenhouse-Geisser	,168	20,000	,008			
Visit * Side	Sphericity Assumed	,067	5	,013	2,667	,026	,118
	Greenhouse-Geisser	,067	3,915	,017	2,667	,039	,118
Error(Visit*Side)	Sphericity Assumed	,502	100	,005			
	Greenhouse-Geisser	,502	78,304	,006			

a. Parameter = R0

General linear model 4 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[c] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,323	20,471	14	,119	,688	,848	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,695	5	,139	23,803	<,001	,543
	Greenhouse-Geisser	,695	3,438	,202	23,803	<,001	,543
Error(Visits)	Sphericity Assumed	,584	100	,006			
	Greenhouse-Geisser	,584	68,758	,008			

a. Parameter = R0

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c] Lower Bound	Upper Bound
1	2	-,050	,025	,889	-,134	,033
	3	,009	,033	1,000	-,102	,120
	4	,102*	,023	,004	,024	,179
	5	,121*	,022	<,001	,050	,193
	6	,157*	,030	<,001	,057	,256
2	1	,050	,025	,889	-,033	,134
	3	,059	,020	,133	-,009	,127
	4	,152*	,018	<,001	,091	,212
	5	,172*	,016	<,001	,119	,224
	6	,207*	,023	<,001	,130	,284
3	1	-,009	,033	1,000	-,120	,102
	2	-,059	,020	,133	-,127	,009
	4	,092*	,027	,041	,002	,183
	5	,112*	,025	,004	,029	,196
	6	,148*	,025	<,001	,064	,231
4	1	-,102*	,023	,004	-,179	-,024
	2	-,152*	,018	<,001	-,212	-,091
	3	-,092*	,027	,041	-,183	-,002
	5	,020	,016	1,000	-,033	,073
	6	,055	,023	,358	-,020	,130
5	1	-,121*	,022	<,001	-,193	-,050
	2	-,172*	,016	<,001	-,224	-,119
	3	-,112*	,025	,004	-,196	-,029
	4	-,020	,016	1,000	-,073	,033
	6	,035	,020	1,000	-,033	,103

6	1	-,157*	,030	<,001	-,256	-,057
	2	-,207*	,023	<,001	-,284	-,130
	3	-,148*	,025	<,001	-,231	-,064
	4	-,055	,023	,358	-,130	,020
	5	-,035	,020	1,000	-,103	,033

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[c] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,430	15,260	14	,365	,745	,936	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	1,070	5	,214	28,755	<,001	,590
	Greenhouse-Geisser	1,070	3,723	,287	28,755	<,001	,590
Error(Visits)	Sphericity Assumed	,744	100	,007			
	Greenhouse-Geisser	,744	74,464	,010			

a. Parameter = R0

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c] Lower Bound	Upper Bound
1	2	-,078	,025	,089	-,162	,006
	3	-,015	,034	1,000	-,128	,097
	4	,088	,030	,118	-,011	,188
	5	,072	,025	,128	-,010	,154
	6	,211*	,024	<,001	,131	,292
2	1	,078	,025	,089	-,006	,162
	3	,062	,026	,399	-,024	,149
	4	,166*	,033	<,001	,056	,275
	5	,149*	,022	<,001	,076	,223
	6	,289*	,021	<,001	,217	,361
3	1	,015	,034	1,000	-,097	,128
	2	-,062	,026	,399	-,149	,024
	4	,104	,035	,113	-,012	,220
	5	,087*	,022	,011	,014	,160
	6	,227*	,024	<,001	,147	,306
4	1	-,088	,030	,118	-,188	,011
	2	-,166*	,033	<,001	-,275	-,056
	3	-,104	,035	,113	-,220	,012
	5	-,016	,028	1,000	-,109	,076
	6	,123*	,026	,002	,036	,210
5	1	-,072	,025	,128	-,154	,010
	2	-,149*	,022	<,001	-,223	-,076
	3	-,087*	,022	,011	-,160	-,014
	4	,016	,028	1,000	-,076	,109
	6	,140*	,019	<,001	,076	,203
6	1	-,211*	,024	<,001	-,292	-,131
	2	-,289*	,021	<,001	-,361	-,217
	3	-,227*	,024	<,001	-,306	-,147
	4	-,123*	,026	,002	-,210	-,036
	5	-,140*	,019	<,001	-,203	-,076

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R0

		Paired Differences							Significance	
					95% Confidence Interval of the Difference				One-Sided p	Two-Sided p
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df		
Pair 1	ScreeningR4 - ScreeningL4	-,025857	,120206	,026231	-,080574	,028860	-,986	20	,168	,336
Pair 2	Week2R4 - Week2L4	-,053333	,106912	,023330	-,101999	-,004667	-2,286	20	,017	,033
Pair 3	Week4R4 - Week4L4	-,050381	,105664	,023058	-,098479	-,002283	-2,185	20	,020	,041
Pair 4	Week8R4 - Week8L4	-,039238	,125308	,027344	-,096278	,017801	-1,435	20	,083	,167
Pair 5	Week12R4 - Week12L4	-,075429	,078957	,017230	-,111370	-,039488	-4,378	20	<,001	<,001
Pair 6	Week24R4 - Week24L4	,028857	,089719	,019578	-,011982	,069697	1,474	20	,078	,156

a. Parameter = R0

Skin gross elasticity (R2, Ua/Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR2	,950	21	,335
ScreeningR4	,937	21	,188
ScreeningL2	,985	21	,980
ScreeningL4	,919	21	,084
Week2R2	,907	21	,047
Week2R4	,974	21	,818
Week2L2	,971	21	,752
Week2L4	,934	21	,166
Week4R2	,976	21	,860
Week4R4	,885	21	,018
Week4L2	,907	21	,048
Week4L4	,734	21	<,001
Week8R2	,951	21	,352
Week8R4	,956	21	,448
Week8L2	,993	21	1,000
Week8L4	,954	21	,403
Week12R2	,931	21	,141
Week12R4	,923	21	,099
Week12L2	,967	21	,670
Week12L4	,725	21	<,001
Week24R2	,932	21	,150
Week24R4	,722	21	<,001
Week24L2	,931	21	,145
Week24L4	,962	21	,554

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,406	16,312	14	,299	,710	,882	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,521	11,817	14	,624	,814	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,139	5	,028	6,278	<,001	,239
	Greenhouse-Geisser	,139	3,550	,039	6,278	<,001	,239
Error(Visit)	Sphericity Assumed	,443	100	,004			
	Greenhouse-Geisser	,443	70,998	,006			
Side	Sphericity Assumed	,000	1	,000	,037	,849	,002
	Greenhouse-Geisser	,000	1,000	,000	,037	,849	,002
Error(Side)	Sphericity Assumed	,056	20	,003			
	Greenhouse-Geisser	,056	20,000	,003			
Visit * Side	Sphericity Assumed	,008	5	,002	,649	,663	,031
	Greenhouse-Geisser	,008	4,069	,002	,649	,631	,031
Error(Visit*Side)	Sphericity Assumed	,250	100	,003			
	Greenhouse-Geisser	,250	81,380	,003			
	Huynh-Feldt	,250	100,000	,003			
	Lower-bound	,250	20,000	,013			

a. Parameter = R2

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[c]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,321	20,559	14	,116	,665	,814	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,047	5	,009	2,669	,026	,118
	Greenhouse-Geisser	,047	3,327	,014	2,669	,049	,118
	Huynh-Feldt	,047	4,071	,012	2,669	,037	,118
	Lower-bound	,047	1,000	,047	2,669	,118	,118
Error(Visits)	Sphericity Assumed	,356	100	,004			
	Greenhouse-Geisser	,356	66,540	,005			
	Huynh-Feldt	,356	81,410	,004			
	Lower-bound	,356	20,000	,018			

a. Parameter = R2

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c]	
					Lower Bound	Upper Bound
1	2	-,029	,016	1,000	-,081	,023
	3	-,051*	,014	,019	-,096	-,006
	4	-,048*	,014	,030	-,094	-,003
	5	-,028	,018	1,000	-,088	,032
	6	-,005	,024	1,000	-,086	,077
2	1	,029	,016	1,000	-,023	,081
	3	-,022	,018	1,000	-,082	,037
	4	-,019	,015	1,000	-,068	,029
	5	,001	,016	1,000	-,053	,056
	6	,024	,021	1,000	-,046	,095
3	1	,051*	,014	,019	,006	,096
	2	,022	,018	1,000	-,037	,082
	4	,003	,012	1,000	-,038	,043
	5	,023	,016	1,000	-,030	,077
	6	,046	,024	1,000	-,034	,127
4	1	,048*	,014	,030	,003	,094
	2	,019	,015	1,000	-,029	,068
	3	-,003	,012	1,000	-,043	,038

	5	,021	,014	1,000	-,026	,068
	6	,044	,023	1,000	-,033	,120
5	1	,028	,018	1,000	-,032	,088
	2	-,001	,016	1,000	-,056	,053
	3	-,023	,016	1,000	-,077	,030
	4	-,021	,014	1,000	-,068	,026
	6	,023	,024	1,000	-,058	,103
6	1	,005	,024	1,000	-,077	,086
	2	-,024	,021	1,000	-,095	,046
	3	-,046	,024	1,000	-,127	,034
	4	-,044	,023	1,000	-,120	,033
	5	-,023	,024	1,000	-,103	,058

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[c] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,338	19,637	14	,145	,732	,916	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,100	5	,020	5,913	<,001	,228
	Greenhouse-Geisser	,100	3,660	,027	5,913	<,001	,228
Error(Visits)	Sphericity Assumed	,338	100	,003			
	Greenhouse-Geisser	,338	73,197	,005			

a. Parameter = R2

Pairwise Comparisons

					Lower Bound	Upper Bound
1	2	-,045*	,013	,046	-,090	-,001
	3	-,067*	,019	,031	-,131	-,004
	4	-,059*	,017	,044	-,117	-,001
	5	-,054*	,016	,040	-,107	-,001
	6	,003	,023	1,000	-,072	,079
2	1	,045*	,013	,046	,001	,090
	3	-,022	,015	1,000	-,070	,027
	4	-,014	,018	1,000	-,074	,046
	5	-,009	,016	1,000	-,063	,046
	6	,049	,019	,283	-,015	,112
3	1	,067*	,019	,031	,004	,131
	2	,022	,015	1,000	-,027	,070
	4	,008	,017	1,000	-,049	,065
	5	,013	,022	1,000	-,059	,085
	6	,071*	,018	,016	,009	,132
4	1	,059*	,017	,044	,001	,117
	2	,014	,018	1,000	-,046	,074
	3	-,008	,017	1,000	-,065	,049
	5	,005	,019	1,000	-,058	,068
	6	,062*	,015	,006	,013	,112
5	1	,054*	,016	,040	,001	,107
	2	,009	,016	1,000	-,046	,063
	3	-,013	,022	1,000	-,085	,059
	4	-,005	,019	1,000	-,068	,058
	6	,057	,019	,118	-,007	,122

6	1	-,003	,023	1,000	-,079	,072
	2	-,049	,019	,283	-,112	,015
	3	-,070*	,018	,016	-,132	-,009
	4	-,062*	,015	,006	-,112	-,013
	5	-,057	,019	,118	-,122	,007

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R2

		Paired Differences					t	df	Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	,011586	,073852	,016116	-,022031	,045203	,719	20	,240	,481
Pair 2	Week2R2 - Week2L2	-,004881	,068875	,015030	-,036232	,026470	-,325	20	,374	,749
Pair 3	Week4R2 - Week4L2	-,004590	,061563	,013434	-,032614	,023433	-,342	20	,368	,736
Pair 4	Week8R2 - Week8L2	,000762	,063452	,013846	-,028121	,029645	,055	20	,478	,957
Pair 5	Week12R2 - Week12L2	-,014805	,079638	,017378	-,051056	,021446	-,852	20	,202	,404
Pair 6	Week24R2 - Week24L2	,019605	,078869	,017211	-,016296	,055505	1,139	20	,134	,268

a. Parameter = R2

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilon[c]		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,126	37,538	14	<,001	,550	,647	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,348	19,099	14	,165	,666	,815	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,539	5	,108	37,902	<,001	,655
	Greenhouse-Geisser	,539	2,751	,196	37,902	<,001	,655
Error(Visit)	Sphericity Assumed	,284	100	,003			
	Greenhouse-Geisser	,284	55,019	,005			
Side	Sphericity Assumed	,004	1	,004	1,835	,191	,084
	Greenhouse-Geisser	,004	1,000	,004	1,835	,191	,084
Error(Side)	Sphericity Assumed	,040	20	,002			
	Greenhouse-Geisser	,040	20,000	,002			
Visit * Side	Sphericity Assumed	,007	5	,001	,868	,505	,042
	Greenhouse-Geisser	,007	3,331	,002	,868	,472	,042
Error(Visit*Side)	Sphericity Assumed	,161	100	,002			
	Greenhouse-Geisser	,161	66,625	,002			

a. Parameter = R2

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilon[c]		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,219	27,486	14	,017	,607	,727	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,330	5	,066	32,981	<,001	,623
	Greenhouse-Geisser	,330	3,034	,109	32,981	<,001	,623
Error(Visits)	Sphericity Assumed	,200	100	,002			
	Greenhouse-Geisser	,200	60,678	,003			

a. Parameter = R2

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c] Lower Bound	Upper Bound
1	2	-,074*	,013	<,001	-,118	-,029
	3	-,114*	,016	<,001	-,169	-,060
	4	-,144*	,014	<,001	-,191	-,098
	5	-,149*	,013	<,001	-,191	-,106
	6	-,123*	,022	<,001	-,195	-,050
2	1	,074*	,013	<,001	,029	,118
	3	-,041*	,010	,009	-,074	-,007
	4	-,071*	,012	<,001	-,111	-,030
	5	-,075*	,010	<,001	-,109	-,041
	6	-,049	,015	,073	-,100	,003
3	1	,114*	,016	<,001	,060	,169
	2	,041*	,010	,009	,007	,074
	4	-,030	,011	,255	-,068	,008
	5	-,034	,011	,059	-,069	,001
	6	-,008	,016	1,000	-,060	,044
4	1	,144*	,014	<,001	,098	,191
	2	,071*	,012	<,001	,030	,111
	3	,030	,011	,255	-,008	,068
	5	-,004	,008	1,000	-,031	,022
	6	,022	,015	1,000	-,029	,072
5	1	,149*	,013	<,001	,106	,191
	2	,075*	,010	<,001	,041	,109
	3	,034	,011	,059	-,001	,069
	4	,004	,008	1,000	-,022	,031
	6	,026	,014	1,000	-,022	,074
6	1	,123*	,022	<,001	,050	,195
	2	,049	,015	,073	-,003	,100
	3	,008	,016	1,000	-,044	,060
	4	-,022	,015	1,000	-,072	,029
	5	-,026	,014	1,000	-,074	,022

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[c] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,222	27,226	14	,019	,684	,842	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,215	5	,043	17,554	<,001	,467
	Greenhouse-Geisser	,215	3,421	,063	17,554	<,001	,467
Error(Visits)	Sphericity Assumed	,245	100	,002			

	Greenhouse-Geisser	,245	68,413	,004			

a. Parameter = R2

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c] Lower Bound	Upper Bound
1	2	-,054*	,013	,007	-,097	-,011
	3	-,089*	,018	,001	-,149	-,029
	4	-,120*	,013	<,001	-,164	-,075
	5	-,114*	,017	<,001	-,171	-,057
	6	-,099*	,019	<,001	-,164	-,035
2	1	,054*	,013	,007	,011	,097
	3	-,035	,014	,318	-,082	,012
	4	-,066*	,015	,003	-,114	-,017
	5	-,060*	,016	,015	-,112	-,008
	6	-,045	,019	,382	-,108	,017
3	1	,089*	,018	,001	,029	,149
	2	,035	,014	,318	-,012	,082
	4	-,031	,014	,682	-,078	,017
	5	-,025	,009	,149	-,054	,004
	6	-,010	,017	1,000	-,068	,048
4	1	,120*	,013	<,001	,075	,164
	2	,066*	,015	,003	,017	,114
	3	,031	,014	,682	-,017	,078
	5	,006	,012	1,000	-,033	,044
	6	,020	,013	1,000	-,024	,065
5	1	,114*	,017	<,001	,057	,171
	2	,060*	,016	,015	,008	,112
	3	,025	,009	,149	-,004	,054
	4	-,006	,012	1,000	-,044	,033
	6	,015	,016	1,000	-,039	,068
6	1	,099*	,019	<,001	,035	,164
	2	,045	,019	,382	-,017	,108
	3	,010	,017	1,000	-,048	,068
	4	-,020	,013	1,000	-,065	,024
	5	-,015	,016	1,000	-,068	,039

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R2

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,028938	,054907	,011982	-,053931	-,003945	-2,415	20	,013	,025
Pair 2	Week2R4 - Week2L4	-,009205	,053487	,011672	-,033552	,015142	-,789	20	,220	,440
Pair 3	Week4R4 - Week4L4	-,003543	,054688	,011934	-,028437	,021351	-,297	20	,385	,770
Pair 4	Week8R4 - Week8L4	-,004229	,049211	,010739	-,026629	,018172	-,394	20	,349	,698
Pair 5	Week12R4 - Week12L4	,005733	,057963	,012649	-,020651	,032118	,453	20	,328	,655
Pair 6	Week24R4 - Week24L4	-,005690	,074182	,016188	-,039458	,028077	-,352	20	,364	,729

a. Parameter = R2

Skin firmness after repeated suction (R3, Uf_5)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	,899	21	,034

ScreeningR4	,959	21	,493
ScreeningL2	,903	21	,040
ScreeningL4	,760	21	<,001
Week2R2	,981	21	,934
Week2R4	,965	21	,611
Week2L2	,954	21	,397
Week2L4	,926	21	,116
Week4R2	,943	21	,254
Week4R4	,924	21	,104
Week4L2	,941	21	,223
Week4L4	,970	21	,731
Week8R2	,946	21	,288
Week8R4	,956	21	,447
Week8L2	,982	21	,950
Week8L4	,945	21	,273
Week12R2	,967	21	,673
Week12R4	,961	21	,532
Week12L2	,934	21	,169
Week12L4	,978	21	,898
Week24R2	,963	21	,588
Week24R4	,953	21	,385
Week24L2	,917	21	,076
Week24L4	,899	21	,033

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,297	21,955	14	,082	,669	,820	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,313	21,048	14	,103	,708	,880	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,953	5	,191	75,514	<,001	,791
	Greenhouse-Geisser	,953	3,345	,285	75,514	<,001	,791
Error(Visit)	Sphericity Assumed	,252	100	,003			
	Greenhouse-Geisser	,252	66,902	,004			
Side	Sphericity Assumed	,003	1	,003	1,944	,179	,089
	Greenhouse-Geisser	,003	1,000	,003	1,944	,179	,089
Error(Side)	Sphericity Assumed	,035	20	,002			
	Greenhouse-Geisser	,035	20,000	,002			
Visit * Side	Sphericity Assumed	,009	5	,002	2,085	,073	,094
	Greenhouse-Geisser	,009	3,542	,002	2,085	,100	,094
Error(Visit*Side)	Sphericity Assumed	,083	100	,001			
	Greenhouse-Geisser	,083	70,848	,001			

a. Parameter = R3

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,535	11,325	14	,664	,816	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,420	5	,084	55,691	<,001	,736
	Greenhouse-Geisser	,420	4,078	,103	55,691	<,001	,736
Error(Visits)	Sphericity Assumed	,151	100	,002			
	Greenhouse-Geisser	,151	81,556	,002			

a. Parameter = R3

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	,036*	,009	,010	,006	,067
	3	,054*	,011	<,001	,019	,089
	4	,093*	,011	<,001	,055	,131
	5	,105*	,012	<,001	,064	,146
	6	,181*	,011	<,001	,143	,219
2	1	-,036*	,009	,010	-,067	-,006
	3	,018	,013	1,000	-,026	,061
	4	,057*	,011	<,001	,021	,092
	5	,068*	,012	<,001	,028	,108
	6	,145*	,010	<,001	,113	,177
3	1	-,054*	,011	<,001	-,089	-,019
	2	-,018	,013	1,000	-,061	,026
	4	,039	,014	,210	-,009	,087
	5	,051*	,011	,002	,014	,087
	6	,127*	,013	<,001	,084	,170
4	1	-,093*	,011	<,001	-,131	-,055
	2	-,057*	,011	<,001	-,092	-,021
	3	-,039	,014	,210	-,087	,009
	5	,012	,014	1,000	-,035	,059
	6	,088*	,013	<,001	,045	,132
5	1	-,105*	,012	<,001	-,146	-,064
	2	-,068*	,012	<,001	-,108	-,028
	3	-,051*	,011	,002	-,087	-,014
	4	-,012	,014	1,000	-,059	,035
	6	,076*	,013	<,001	,034	,118
6	1	-,181*	,011	<,001	-,219	-,143
	2	-,145*	,010	<,001	-,177	-,113
	3	-,127*	,013	<,001	-,170	-,084
	4	-,088*	,013	<,001	-,132	-,045
	5	-,076*	,013	<,001	-,118	-,034

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,199	29,181	14	,010	,655	,799	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,542	5	,108	58,727	<,001	,746
	Greenhouse-Geisser	,542	3,276	,165	58,727	<,001	,746
	Huynh-Feldt	,542	3,994	,136	58,727	<,001	,746
	Lower-bound	,542	1,000	,542	58,727	<,001	,746
Error(Visits)	Sphericity Assumed	,185	100	,002			

	Greenhouse-Geisser	,185	65,516	,003			
	Huynh-Feldt	,185	79,875	,002			
	Lower-bound	,185	20,000	,009			

a. Parameter = R3

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,026	,011	,470	-,011	,063
	3	,045	,015	,120	-,006	,096
	4	,080*	,009	<,001	,052	,109
	5	,097*	,014	<,001	,052	,143
	6	,203*	,009	<,001	,171	,234
2	1	-,026	,011	,470	-,063	,011
	3	,019	,019	1,000	-,044	,083
	4	,054*	,012	,005	,013	,096
	5	,072*	,013	<,001	,029	,114
	6	,177*	,011	<,001	,140	,214
3	1	-,045	,015	,120	-,096	,006
	2	-,019	,019	1,000	-,083	,044
	4	,035	,016	,597	-,018	,088
	5	,052*	,016	,050	1,119E-5	,105
	6	,158*	,014	<,001	,111	,205
4	1	-,080*	,009	<,001	-,109	-,052
	2	-,054*	,012	,005	-,096	-,013
	3	-,035	,016	,597	-,088	,018
	5	,017	,013	1,000	-,028	,062
	6	,123*	,007	<,001	,099	,147
5	1	-,097*	,014	<,001	-,143	-,052
	2	-,072*	,013	<,001	-,114	-,029
	3	-,052*	,016	,050	-,105	-1,119E-5
	4	-,017	,013	1,000	-,062	,028
	6	,105*	,014	<,001	,060	,151
6	1	-,203*	,009	<,001	-,234	-,171
	2	-,177*	,011	<,001	-,214	-,140
	3	-,158*	,014	<,001	-,205	-,111
	4	-,123*	,007	<,001	-,147	-,099
	5	-,105*	,014	<,001	-,151	-,060

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,004333	,039923	,008712	-,022506	,013839	-,497	20	,312	,624
Pair 2	Week2R2 - Week2L2	-,014905	,050921	,011112	-,038084	,008274	-1,341	20	,097	,195
Pair 3	Week4R2 - Week4L2	-,013238	,038828	,008473	-,030912	,004436	-1,562	20	,067	,134
Pair 4	Week8R2 - Week8L2	-,017143	,037526	,008189	-,034225	-,000061	-2,093	20	,025	,049
Pair 5	Week12R2 - Week12L2	-,011762	,053571	,011690	-,036147	,012623	-1,006	20	,163	,326
Pair 6	Week24R2 - Week24L2	,017286	,042637	,009304	-,002122	,036694	1,858	20	,039	,078

a. Parameter = R3

General linear model 4 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc

					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,296	22,061	14	,080	,624	,753	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,388	17,148	14	,252	,744	,935	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1,970	5	,394	39,731	<,001	,665
	Greenhouse-Geisser	1,970	3,120	,632	39,731	<,001	,665
Error(Visit)	Sphericity Assumed	,992	100	,010			
	Greenhouse-Geisser	,992	62,394	,016			
Side	Sphericity Assumed	,096	1	,096	10,423	,004	,343
	Greenhouse-Geisser	,096	1,000	,096	10,423	,004	,343
Error(Side)	Sphericity Assumed	,185	20	,009			
	Greenhouse-Geisser	,185	20,000	,009			
Visit * Side	Sphericity Assumed	,070	5	,014	2,398	,042	,107
	Greenhouse-Geisser	,070	3,719	,019	2,398	,062	,107
Error(Visit*Side)	Sphericity Assumed	,587	100	,006			
	Greenhouse-Geisser	,587	74,389	,008			

a. Parameter = R3

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,325	20,348	14	,122	,690	,852	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,837	5	,167	24,785	<,001	,553
	Greenhouse-Geisser	,837	3,452	,242	24,785	<,001	,553
Error(Visits)	Sphericity Assumed	,675	100	,007			
	Greenhouse-Geisser	,675	69,039	,010			

a. Parameter = R3

Pairwise Comparisons

					95% Confidence Interval for Differencec	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	Lower Bound	Upper Bound
1	2	-,046	,026	1,000	-,133	,042
	3	,016	,036	1,000	-,106	,137
	4	,111*	,026	,006	,025	,197
	5	,140*	,023	<,001	,063	,217
	6	,180*	,032	<,001	,072	,287
2	1	,046	,026	1,000	-,042	,133
	3	,061	,022	,165	-,012	,134
	4	,157*	,020	<,001	,091	,223
	5	,186*	,016	<,001	,134	,238
	6	,226*	,024	<,001	,147	,304
3	1	-,016	,036	1,000	-,137	,106
	2	-,061	,022	,165	-,134	,012
	4	,095*	,028	,048	,000	,190
	5	,124*	,027	,002	,035	,213
	6	,164*	,028	<,001	,071	,257
4	1	-,111*	,026	,006	-,197	-,025
	2	-,157*	,020	<,001	-,223	-,091
	3	-,095*	,028	,048	-,190	,000
	5	,029	,018	1,000	-,031	,089

	6	,069	,024	,147	-,011	,149
5	1	-,140*	,023	<,001	-,217	-,063
	2	-,186*	,016	<,001	-,238	-,134
	3	-,124*	,027	,002	-,213	-,035
	4	-,029	,018	1,000	-,089	,031
	6	,040	,022	1,000	-,034	,114
6	1	-,180*	,032	<,001	-,287	-,072
	2	-,226*	,024	<,001	-,304	-,147
	3	-,164*	,028	<,001	-,257	-,071
	4	-,069	,024	,147	-,149	,011
	5	-,040	,022	1,000	-,114	,034

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,399	16,639	14	,280	,735	,921	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	1,204	5	,241	26,638	<,001	,571
	Greenhouse-Geisser	1,204	3,674	,328	26,638	<,001	,571
Error(Visits)	Sphericity Assumed	,904	100	,009			
	Greenhouse-Geisser	,904	73,486	,012			

a. Parameter = R3

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,080	,028	,133	-,171	,012
	3	-,014	,037	1,000	-,139	,110
	4	,097	,032	,104	-,010	,203
	5	,078	,027	,125	-,011	,167
	6	,226*	,026	<,001	,138	,314
2	1	,080	,028	,133	-,012	,171
	3	,065	,028	,464	-,028	,159
	4	,176*	,036	,002	,055	,298
	5	,158*	,026	<,001	,073	,243
	6	,306*	,024	<,001	,226	,385
3	1	,014	,037	1,000	-,110	,139
	2	-,065	,028	,464	-,159	,028
	4	,111	,038	,130	-,016	,238
	5	,092*	,023	,011	,015	,170
	6	,240*	,027	<,001	,151	,329
4	1	-,097	,032	,104	-,203	,010
	2	-,176*	,036	,002	-,298	-,055
	3	-,111	,038	,130	-,238	,016
	5	-,018	,030	1,000	-,119	,082
	6	,129*	,029	,004	,032	,227
5	1	-,078	,027	,125	-,167	,011
	2	-,158*	,026	<,001	-,243	-,073
	3	-,092*	,023	,011	-,170	-,015
	4	,018	,030	1,000	-,082	,119
	6	,148*	,022	<,001	,074	,222
6	1	-,226*	,026	<,001	-,314	-,138

	2	-,306*	,024	<,001	-,385	-,226
	3	-,240*	,027	<,001	-,329	-,151
	4	-,129*	,029	,004	-,227	-,032
	5	-,148*	,022	<,001	-,222	-,074

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,023429	,129500	,028259	-,082376	,035519	-,829	20	,208	,417
Pair 2	Week2R4 - Week2L4	-,057381	,113219	,024706	-,108918	-,005844	-2,323	20	,015	,031
Pair 3	Week4R4 - Week4L4	-,053381	,108437	,023663	-,102741	-,004021	-2,256	20	,018	,035
Pair 4	Week8R4 - Week8L4	-,037857	,142190	,031028	-,102581	,026867	-1,220	20	,118	,237
Pair 5	Week12R4 - Week12L4	-,085190	,082544	,018013	-,122764	-,047617	-4,730	20	<,001	<,001
Pair 6	Week24R4 - Week24L4	,022524	,094085	,020531	-,020303	,065351	1,097	20	,143	,286

a. Parameter = R3

Skin net elasticity (R5, Ur/Ue)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR2	,968	21	,684
ScreeningR4	,866	21	,008
ScreeningL2	,858	21	,006
ScreeningL4	,920	21	,088
Week2R2	,981	21	,935
Week2R4	,955	21	,427
Week2L2	,945	21	,272
Week2L4	,930	21	,137
Week4R2	,936	21	,179
Week4R4	,938	21	,198
Week4L2	,957	21	,457
Week4L4	,951	21	,363
Week8R2	,949	21	,320
Week8R4	,970	21	,738
Week8L2	,948	21	,313
Week8L4	,930	21	,139
Week12R2	,962	21	,550
Week12R4	,938	21	,198
Week12L2	,977	21	,883
Week12L4	,952	21	,377
Week24R2	,868	21	,009
Week24R4	,959	21	,497
Week24L2	,926	21	,112
Week24L4	,980	21	,929

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilon[c]		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,120	38,373	14	<,001	,546	,641	,200
Side	1,000	,000	0	.	1,000	1,000	1,000

Visit * Side	,244	25,527	14	,031	,621	,748	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,457	5	,091	8,227	<,001	,291
	Greenhouse-Geisser	,457	2,730	,168	8,227	<,001	,291
Error(Visit)	Sphericity Assumed	1,112	100	,011			
	Greenhouse-Geisser	1,112	54,607	,020			
Side	Sphericity Assumed	,009	1	,009	1,728	,204	,080
	Greenhouse-Geisser	,009	1,000	,009	1,728	,204	,080
Error(Side)	Sphericity Assumed	,103	20	,005			
	Greenhouse-Geisser	,103	20,000	,005			
Visit * Side	Sphericity Assumed	,078	5	,016	3,661	,004	,155
	Greenhouse-Geisser	,078	3,105	,025	3,661	,016	,155
Error(Visit*Side)	Sphericity Assumed	,424	100	,004			
	Greenhouse-Geisser	,424	62,108	,007			

a. Parameter = R5

General linear model 2 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,042	57,513	14	<,001	,405	,451	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,286	5	,057	7,387	<,001	,270
	Greenhouse-Geisser	,286	2,025	,141	7,387	,002	,270
Error(Visits)	Sphericity Assumed	,774	100	,008			
	Greenhouse-Geisser	,774	40,496	,019			

a. Parameter = R5

Pairwise Comparisons

					95% Confidence Interval for Differencec	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	Lower Bound	Upper Bound
1	2	-,031	,015	,861	-,083	,020
	3	-,072*	,016	,004	-,126	-,018
	4	-,097*	,012	<,001	-,137	-,057
	5	-,058	,020	,112	-,123	,007
	6	-,150*	,040	,021	-,284	-,015
2	1	,031	,015	,861	-,020	,083
	3	-,041	,014	,109	-,087	,005
	4	-,066	,020	,056	-,132	,001
	5	-,027	,018	1,000	-,088	,033
	6	-,119	,042	,155	-,258	,021
3	1	,072*	,016	,004	,018	,126
	2	,041	,014	,109	-,005	,087
	4	-,025	,018	1,000	-,086	,037
	5	,014	,018	1,000	-,045	,073
	6	-,078	,041	1,000	-,214	,059
4	1	,097*	,012	<,001	,057	,137
	2	,066	,020	,056	-,001	,132
	3	,025	,018	1,000	-,037	,086
	5	,039	,022	1,000	-,035	,112
	6	-,053	,038	1,000	-,181	,075
5	1	,058	,020	,112	-,007	,123
	2	,027	,018	1,000	-,033	,088

	3	-,014	,018	1,000	-,073	,045
	4	-,039	,022	1,000	-,112	,035
	6	-,092	,038	,369	-,217	,034
6	1	,150*	,040	,021	,015	,284
	2	,119	,042	,155	-,021	,258
	3	,078	,041	1,000	-,059	,214
	4	,053	,038	1,000	-,075	,181
	5	,092	,038	,369	-,034	,217

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,324	20,409	14	,121	,737	,924	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,249	5	,050	6,542	<,001	,246
	Greenhouse-Geisser	,249	3,684	,068	6,542	<,001	,246
Error(Visits)	Sphericity Assumed	,762	100	,008			
	Greenhouse-Geisser	,762	73,686	,010			

a. Parameter = R5

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,040	,021	1,000	-,112	,031
	3	-,100*	,025	,011	-,184	-,016
	4	-,125*	,022	<,001	-,197	-,054
	5	-,115*	,026	,004	-,202	-,028
	6	-,093	,032	,139	-,201	,015
2	1	,040	,021	1,000	-,031	,112
	3	-,060*	,016	,019	-,113	-,007
	4	-,085	,026	,052	-,171	,001
	5	-,075	,024	,074	-,154	,004
	6	-,053	,031	1,000	-,157	,050
3	1	,100*	,025	,011	,016	,184
	2	,060*	,016	,019	,007	,113
	4	-,025	,029	1,000	-,122	,072
	5	-,015	,029	1,000	-,110	,080
	6	,007	,031	1,000	-,096	,109
4	1	,125*	,022	<,001	,054	,197
	2	,085	,026	,052	-,001	,171
	3	,025	,029	1,000	-,072	,122
	5	,010	,028	1,000	-,083	,103
	6	,032	,025	1,000	-,052	,116
5	1	,115*	,026	,004	,028	,202
	2	,075	,024	,074	-,004	,154
	3	,015	,029	1,000	-,080	,110
	4	-,010	,028	1,000	-,103	,083
	6	,022	,034	1,000	-,090	,134
6	1	,093	,032	,139	-,015	,201
	2	,053	,031	1,000	-,050	,157
	3	-,007	,031	1,000	-,109	,096
	4	-,032	,025	1,000	-,116	,052

5	-,022	,034	1,000	-,134	,090

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,000738	,076192	,016626	-,035420	,033944	-,044	20	,483	,965
Pair 2	Week2R2 - Week2L2	-,009952	,070516	,015388	-,042051	,022146	-,647	20	,263	,525
Pair 3	Week4R2 - Week4L2	-,028824	,070091	,015295	-,060729	,003081	-1,885	20	,037	,074
Pair 4	Week8R2 - Week8L2	-,029433	,073380	,016013	-,062836	,003969	-1,838	20	,040	,081
Pair 5	Week12R2 - Week12L2	-,057862	,100263	,021879	-,103501	-,012223	-2,645	20	,008	,016
Pair 6	Week24R2 - Week24L2	,055567	,146712	,032015	-,011216	,122349	1,736	20	,049	,098

a. Parameter = R5

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,131	36,797	14	<,001	,586	,697	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,218	27,552	14	,017	,699	,865	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1,680	5	,336	18,471	<,001	,480
	Greenhouse-Geisser	1,680	2,929	,574	18,471	<,001	,480
Error(Visit)	Sphericity Assumed	1,820	100	,018			
	Greenhouse-Geisser	1,820	58,581	,031			
Side	Sphericity Assumed	,082	1	,082	5,616	,028	,219
	Greenhouse-Geisser	,082	1,000	,082	5,616	,028	,219
Error(Side)	Sphericity Assumed	,291	20	,015			
	Greenhouse-Geisser	,291	20,000	,015			
Visit * Side	Sphericity Assumed	,098	5	,020	2,860	,019	,125
	Greenhouse-Geisser	,098	3,495	,028	2,860	,036	,125
Error(Visit*Side)	Sphericity Assumed	,685	100	,007			
	Greenhouse-Geisser	,685	69,907	,010			

a. Parameter = R5

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,137	35,934	14	,001	,599	,716	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,853	5	,171	13,445	<,001	,402
	Greenhouse-Geisser	,853	2,995	,285	13,445	<,001	,402
Error(Visits)	Sphericity Assumed	1,269	100	,013			
	Greenhouse-Geisser	1,269	59,890	,021			

a. Parameter = R5

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,052	,020	,253	-,118	,014
	3	-,125*	,035	,025	-,240	-,010
	4	-,229*	,036	<,001	-,350	-,109
	5	-,190*	,041	,002	-,325	-,055
	6	-,195*	,047	,008	-,352	-,037
2	1	,052	,020	,253	-,014	,118
	3	-,073	,023	,077	-,151	,004
	4	-,177*	,036	,001	-,297	-,058
	5	-,138*	,031	,003	-,241	-,036
	6	-,143*	,042	,042	-,282	-,003
3	1	,125*	,035	,025	,010	,240
	2	,073	,023	,077	-,004	,151
	4	-,104	,037	,171	-,229	,020
	5	-,065	,029	,549	-,162	,032
	6	-,069	,038	1,000	-,198	,059
4	1	,229*	,036	<,001	,109	,350
	2	,177*	,036	,001	,058	,297
	3	,104	,037	,171	-,020	,229
	5	,039	,034	1,000	-,074	,152
	6	,035	,034	1,000	-,079	,148
5	1	,190*	,041	,002	,055	,325
	2	,138*	,031	,003	,036	,241
	3	,065	,029	,549	-,032	,162
	4	-,039	,034	1,000	-,152	,074
	6	-,004	,028	1,000	-,097	,088
6	1	,195*	,047	,008	,037	,352
	2	,143*	,042	,042	,003	,282
	3	,069	,038	1,000	-,059	,198
	4	-,035	,034	1,000	-,148	,079
	5	,004	,028	1,000	-,088	,097

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,273	23,476	14	,055	,725	,905	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,925	5	,185	14,974	<,001	,428
	Greenhouse-Geisser	,925	3,623	,255	14,974	<,001	,428
Error(Visits)	Sphericity Assumed	1,236	100	,012			
	Greenhouse-Geisser	1,236	72,467	,017			

a. Parameter = R5

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,055	,017	,059	-,110	,001

	3	-,116*	,030	,013	-,214	-,017
	4	-,193*	,036	<,001	-,312	-,075
	5	-,130	,040	,064	-,264	,004
	6	-,262*	,035	<,001	-,379	-,144
2	1	,055	,017	,059	-,001	,110
	3	-,061	,029	,675	-,156	,034
	4	-,139*	,034	,009	-,252	-,026
	5	-,075	,038	,940	-,202	,052
	6	-,207*	,033	<,001	-,318	-,097
3	1	,116*	,030	,013	,017	,214
	2	,061	,029	,675	-,034	,156
	4	-,078	,041	1,000	-,216	,060
	5	-,014	,034	1,000	-,127	,099
	6	-,146*	,027	<,001	-,238	-,055
4	1	,193*	,036	<,001	,075	,312
	2	,139*	,034	,009	,026	,252
	3	,078	,041	1,000	-,060	,216
	5	,064	,036	1,000	-,055	,183
	6	-,068	,040	1,000	-,201	,064
5	1	,130	,040	,064	-,004	,264
	2	,075	,038	,940	-,052	,202
	3	,014	,034	1,000	-,099	,127
	4	-,064	,036	1,000	-,183	,055
	6	-,132*	,037	,029	-,256	-,009
6	1	,262*	,035	<,001	,144	,379
	2	,207*	,033	<,001	,097	,318
	3	,146*	,027	<,001	,055	,238
	4	,068	,040	1,000	-,064	,201
	5	,132*	,037	,029	,009	,256

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,030038	,083832	,018294	-,008122	,068198	1,642	20	,058	,116
Pair 2	Week2R4 - Week2L4	,027329	,073681	,016079	-,006211	,060868	1,700	20	,052	,105
Pair 3	Week4R4 - Week4L4	,039400	,133987	,029238	-,021590	,100390	1,348	20	,096	,193
Pair 4	Week8R4 - Week8L4	,065805	,151612	,033085	-,003208	,134818	1,989	20	,030	,061
Pair 5	Week12R4 - Week12L4	,090510	,128746	,028095	,031905	,149114	3,222	20	,002	,004
Pair 6	Week24R4 - Week24L4	-,037167	,166183	,036264	-,112812	,038479	-1,025	20	,159	,318

a. Parameter = R5

Ratio of viscoelastic to elastic extension (R6, Uv/Ue)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR2	,901	21	,037
ScreeningR4	,858	21	,006
ScreeningL2	,950	21	,337
ScreeningL4	,981	21	,944
Week2R2	,957	21	,451
Week2R4	,928	21	,125

Week2L2	,960	21	,511
Week2L4	,970	21	,731
Week4R2	,902	21	,038
Week4R4	,948	21	,314
Week4L2	,921	21	,091
Week4L4	,874	21	,011
Week8R2	,957	21	,465
Week8R4	,970	21	,739
Week8L2	,910	21	,056
Week8L4	,899	21	,034
Week12R2	,978	21	,892
Week12R4	,905	21	,044
Week12L2	,958	21	,483
Week12L4	,894	21	,027
Week24R2	,890	21	,023
Week24R4	,969	21	,710
Week24L2	,903	21	,040
Week24L4	,930	21	,136

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,043	57,088	14	<,001	,431	,485	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,222	27,269	14	,019	,597	,714	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,607	5	,121	8,059	<,001	,287
	Greenhouse-Geisser	,607	2,157	,282	8,059	<,001	,287
Error(Visit)	Sphericity Assumed	1,507	100	,015			
	Greenhouse-Geisser	1,507	43,145	,035			
Side	Sphericity Assumed	,002	1	,002	,386	,541	,019
	Greenhouse-Geisser	,002	1,000	,002	,386	,541	,019
Error(Side)	Sphericity Assumed	,117	20	,006			
	Greenhouse-Geisser	,117	20,000	,006			
Visit * Side	Sphericity Assumed	,053	5	,011	2,420	,041	,108
	Greenhouse-Geisser	,053	2,987	,018	2,420	,075	,108
Error(Visit*Side)	Sphericity Assumed	,439	100	,004			
	Greenhouse-Geisser	,439	59,745	,007			

a. Parameter = R6

General linear model 2 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,041	57,898	14	<,001	,425	,476	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,320	5	,064	6,107	<,001	,234
	Greenhouse-Geisser	,320	2,123	,151	6,107	,004	,234
Error(Visits)	Sphericity Assumed	1,049	100	,010			

	Greenhouse-Geisser	1,049	42,460	,025				

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,034	,013	,240	-,078	,009
	3	-,056*	,017	,047	-,112	-,001
	4	-,095*	,019	<,001	-,158	-,033
	5	-,058	,026	,533	-,145	,028
	6	-,160*	,043	,020	-,303	-,017
2	1	,034	,013	,240	-,009	,078
	3	-,022	,014	1,000	-,067	,024
	4	-,061	,020	,105	-,129	,007
	5	-,024	,026	1,000	-,109	,061
	6	-,126	,047	,228	-,284	,032
3	1	,056*	,017	,047	,001	,112
	2	,022	,014	1,000	-,024	,067
	4	-,039	,024	1,000	-,120	,042
	5	-,002	,025	1,000	-,086	,082
	6	-,104	,049	,717	-,268	,060
4	1	,095*	,019	<,001	,033	,158
	2	,061	,020	,105	-,007	,129
	3	,039	,024	1,000	-,042	,120
	5	,037	,027	1,000	-,054	,128
	6	-,065	,041	1,000	-,200	,070
5	1	,059	,026	,533	-,028	,145
	2	,024	,026	1,000	-,061	,109
	3	,002	,025	1,000	-,082	,086
	4	-,037	,027	1,000	-,128	,054
	6	-,102	,046	,574	-,255	,051
6	1	,160*	,043	,020	,017	,303
	2	,126	,047	,228	-,032	,284
	3	,104	,049	,717	-,060	,268
	4	,065	,041	1,000	-,070	,200
	5	,102	,046	,574	-,051	,255

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,136	36,142	14	,001	,518	,602	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,340	5	,068	7,582	<,001	,275
	Greenhouse-Geisser	,340	2,590	,131	7,582	<,001	,275
Error(Visits)	Sphericity Assumed	,897	100	,009			
	Greenhouse-Geisser	,897	51,796	,017			

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,029	,016	1,000	-,083	,025

	3	-,086*	,021	,007	-,154	-,017
	4	-,130*	,023	<,001	-,207	-,052
	5	-,121*	,022	<,001	-,194	-,048
	6	-,135*	,040	,044	-,269	-,002
2	1	,029	,016	1,000	-,025	,083
	3	-,057*	,017	,043	-,113	-,001
	4	-,101*	,025	,010	-,184	-,017
	5	-,092*	,023	,010	-,168	-,016
	6	-,107	,043	,321	-,249	,036
3	1	,086*	,021	,007	,017	,154
	2	,057*	,017	,043	,001	,113
	4	-,044	,030	1,000	-,142	,055
	5	-,035	,025	1,000	-,119	,048
	6	-,050	,041	1,000	-,187	,088
4	1	,130*	,023	<,001	,052	,207
	2	,101*	,025	,010	,017	,184
	3	,044	,030	1,000	-,055	,142
	5	,009	,023	1,000	-,068	,085
	6	-,006	,034	1,000	-,118	,106
5	1	,121*	,022	<,001	,048	,194
	2	,092*	,023	,010	,016	,168
	3	,035	,025	1,000	-,048	,119
	4	-,009	,023	1,000	-,085	,068
	6	-,014	,037	1,000	-,139	,110
6	1	,135*	,040	,044	,002	,269
	2	,107	,043	,321	-,036	,249
	3	,050	,041	1,000	-,088	,187
	4	,006	,034	1,000	-,106	,118
	5	,014	,037	1,000	-,110	,139

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference				One-Sided p	Two-Sided p
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df		
Pair 1	ScreeningR2 - ScreeningL2	,021886	,068891	,015033	-,009473	,053245	1,456	20	,080	,161
Pair 2	Week2R2 - Week2L2	,027529	,062638	,013669	-,000984	,056041	2,014	20	,029	,058
Pair 3	Week4R2 - Week4L2	-,007495	,078449	,017119	-,043205	,028214	-,438	20	,333	,666
Pair 4	Week8R2 - Week8L2	-,012200	,091227	,019907	-,053726	,029326	-,613	20	,273	,547
Pair 5	Week12R2 - Week12L2	-,040595	,087758	,019150	-,080542	-,000648	-2,120	20	,023	,047
Pair 6	Week24R2 - Week24L2	,046743	,157237	,034312	-,024830	,118316	1,362	20	,094	,188

a. Parameter = R6

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,182	30,816	14	,006	,659	,805	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,263	24,140	14	,046	,690	,851	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,718	5	,144	8,154	<,001	,290

	Greenhouse-Geisser	,718	3,296	,218	8,154	<,001	,290
Error(Visit)	Sphericity Assumed	1,761	100	,018			
	Greenhouse-Geisser	1,761	65,927	,027			
Side	Sphericity Assumed	,129	1	,129	7,262	,014	,266
	Greenhouse-Geisser	,129	1,000	,129	7,262	,014	,266
Error(Side)	Sphericity Assumed	,355	20	,018			
	Greenhouse-Geisser	,355	20,000	,018			
Visit * Side	Sphericity Assumed	,164	5	,033	3,697	,004	,156
	Greenhouse-Geisser	,164	3,448	,048	3,697	,012	,156
Error(Visit*Side)	Sphericity Assumed	,888	100	,009			
	Greenhouse-Geisser	,888	68,967	,013			

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,248	25,250	14	,033	,704	,874	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,297	5	,059	4,952	<,001	,198
	Greenhouse-Geisser	,297	3,522	,084	4,952	,002	,198
Error(Visits)	Sphericity Assumed	1,199	100	,012			
	Greenhouse-Geisser	1,199	70,447	,017			

Pairwise Comparisons

					95% Confidence Interval for Differencec	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	Lower Bound	Upper Bound
1	2	,009	,024	1,000	-,070	,088
	3	-,050	,029	1,000	-,147	,047
	4	-,130*	,038	,038	-,255	-,005
	5	-,081	,040	,860	-,215	,053
	6	-,082	,037	,547	-,205	,040
2	1	-,009	,024	1,000	-,088	,070
	3	-,059	,025	,400	-,141	,023
	4	-,139*	,037	,021	-,263	-,014
	5	-,090	,032	,163	-,197	,017
	6	-,091	,036	,309	-,212	,030
3	1	,050	,029	1,000	-,047	,147
	2	,059	,025	,400	-,023	,141
	4	-,080	,041	,996	-,216	,057
	5	-,031	,027	1,000	-,121	,059
	6	-,032	,037	1,000	-,155	,090
4	1	,130*	,038	,038	,005	,255
	2	,139*	,037	,021	,014	,263
	3	,080	,041	,996	-,057	,216
	5	,049	,035	1,000	-,067	,164
	6	,047	,035	1,000	-,068	,162
5	1	,081	,040	,860	-,053	,215
	2	,090	,032	,163	-,017	,197
	3	,031	,027	1,000	-,059	,121
	4	-,049	,035	1,000	-,164	,067
	6	-,001	,029	1,000	-,098	,095
6	1	,082	,037	,547	-,040	,205
	2	,091	,036	,309	-,030	,212
	3	,032	,037	1,000	-,090	,155
	4	-,047	,035	1,000	-,162	,068
	5	,001	,029	1,000	-,095	,098

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,358	18,598	14	,184	,756	,954	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,585	5	,117	8,073	<,001	,288
	Greenhouse-Geisser	,585	3,779	,155	8,073	<,001	,288
Error(Visits)	Sphericity Assumed	1,450	100	,015			
	Greenhouse-Geisser	1,450	75,574	,019			

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,011	,020	1,000	-,056	,078
	3	-,034	,032	1,000	-,141	,072
	4	-,091	,041	,567	-,228	,045
	5	-,033	,034	1,000	-,147	,081
	6	-,190*	,038	<,001	-,316	-,064
2	1	-,011	,020	1,000	-,078	,056
	3	-,046	,029	1,000	-,142	,051
	4	-,102	,042	,358	-,242	,037
	5	-,044	,029	1,000	-,139	,051
	6	-,201*	,035	<,001	-,318	-,084
3	1	,034	,032	1,000	-,072	,141
	2	,046	,029	1,000	-,051	,142
	4	-,057	,047	1,000	-,214	,101
	5	,001	,030	1,000	-,099	,102
	6	-,156*	,039	,011	-,286	-,025
4	1	,091	,041	,567	-,045	,228
	2	,102	,042	,358	-,037	,242
	3	,057	,047	1,000	-,101	,214
	5	,058	,045	1,000	-,090	,206
	6	-,099	,047	,740	-,256	,059
5	1	,033	,034	1,000	-,081	,147
	2	,044	,029	1,000	-,051	,139
	3	-,001	,030	1,000	-,102	,099
	4	-,058	,045	1,000	-,206	,090
	6	-,157*	,038	,008	-,284	-,030
6	1	,190*	,038	<,001	,064	,316
	2	,201*	,035	<,001	,084	,318
	3	,156*	,039	,011	,025	,286
	4	,099	,047	,740	-,059	,256
	5	,157*	,038	,008	,030	,284

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm

	Paired Differences					t	df	Significance	
				95% Confidence Interval of the Difference					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1 ScreeningR4 - ScreeningL4	,045743	,100576	,021948	-,000039	,091525	2,084	20	,025	,050
Pair 2 Week2R4 - Week2L4	,048110	,096222	,020997	,004310	,091909	2,291	20	,016	,033
Pair 3 Week4R4 - Week4L4	,061338	,110217	,024051	,011168	,111508	2,550	20	,010	,019
Pair 4 Week8R4 - Week8L4	,084281	,189715	,041399	-,002076	,170638	2,036	20	,028	,055
Pair 5 Week12R4 - Week12L4	,093838	,161439	,035229	,020352	,167324	2,664	20	,007	,015
Pair 6 Week24R4 - Week24L4	-,061919	,175309	,038256	-,141719	,017881	-1,619	20	,061	,121

Ratio of elastic recovery to total extension (R7, Ur/Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR2	,967	21	,659
ScreeningR4	,956	21	,438
ScreeningL2	,910	21	,054
ScreeningL4	,943	21	,249
Week2R2	,967	21	,663
Week2R4	,963	21	,568
Week2L2	,909	21	,053
Week2L4	,966	21	,643
Week4R2	,962	21	,558
Week4R4	,926	21	,114
Week4L2	,967	21	,662
Week4L4	,918	21	,079
Week8R2	,961	21	,531
Week8R4	,893	21	,026
Week8L2	,960	21	,507
Week8L4	,950	21	,335
Week12R2	,957	21	,467
Week12R4	,972	21	,784
Week12L2	,952	21	,367
Week12L4	,977	21	,880
Week24R2	,906	21	,047
Week24R4	,873	21	,011
Week24L2	,968	21	,683
Week24L4	,961	21	,544

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,324	20,387	14	,121	,712	,886	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,481	13,258	14	,510	,786	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,091	5	,018	7,276	<,001	,267
	Greenhouse-Geisser	,091	3,562	,026	7,276	<,001	,267

Error(Visit)	Sphericity Assumed	,250	100	,003			
	Greenhouse-Geisser	,250	71,234	,004			
Side	Sphericity Assumed	,006	1	,006	3,966	,060	,165
	Greenhouse-Geisser	,006	1,000	,006	3,966	,060	,165
Error(Side)	Sphericity Assumed	,028	20	,001			
	Greenhouse-Geisser	,028	20,000	,001			
Visit * Side	Sphericity Assumed	,017	5	,003	2,581	,031	,114
	Greenhouse-Geisser	,017	3,928	,004	2,581	,044	,114
Error(Visit*Side)	Sphericity Assumed	,136	100	,001			
	Greenhouse-Geisser	,136	78,569	,002			

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,183	30,732	14	,006	,578	,686	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,052	5	,010	5,946	<,001	,229
	Greenhouse-Geisser	,052	2,891	,018	5,946	,001	,229
Error(Visits)	Sphericity Assumed	,176	100	,002			
	Greenhouse-Geisser	,176	57,827	,003			

a. Parameter = R7

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,014	,010	1,000	-,047	,018
	3	-,039*	,011	,028	-,075	-,003
	4	-,047*	,007	<,001	-,070	-,024
	5	-,028	,012	,462	-,067	,012
	6	-,061*	,018	,043	-,122	-,001
2	1	,014	,010	1,000	-,018	,047
	3	-,024	,010	,305	-,057	,008
	4	-,033	,011	,086	-,068	,002
	5	-,013	,009	1,000	-,043	,017
	6	-,047	,017	,197	-,105	,011
3	1	,039*	,011	,028	,003	,075
	2	,024	,010	,305	-,008	,057
	4	-,008	,010	1,000	-,041	,025
	5	,011	,011	1,000	-,025	,048
	6	-,023	,018	1,000	-,082	,036
4	1	,047*	,007	<,001	,024	,070
	2	,033	,011	,086	-,002	,068
	3	,008	,010	1,000	-,025	,041
	5	,020	,012	1,000	-,021	,060
	6	-,014	,017	1,000	-,071	,043
5	1	,028	,012	,462	-,012	,067
	2	,013	,009	1,000	-,017	,043
	3	-,011	,011	1,000	-,048	,025
	4	-,020	,012	1,000	-,060	,021
	6	-,034	,015	,586	-,085	,017
6	1	,061*	,018	,043	,001	,122
	2	,047	,017	,197	-,011	,105
	3	,023	,018	1,000	-,036	,082
	4	,014	,017	1,000	-,043	,071
	5	,034	,015	,586	-,017	,085

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.
a. Parameter = R7
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,482	13,221	14	,513	,802	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,056	5	,011	5,355	<,001	,211
	Greenhouse-Geisser	,056	4,012	,014	5,355	<,001	,211
Error(Visits)	Sphericity Assumed	,209	100	,002			
	Greenhouse-Geisser	,209	80,233	,003			

a. Parameter = R7

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,023	,014	1,000	-,070	,024
	3	-,052*	,015	,040	-,103	-,002
	4	-,061*	,011	<,001	-,098	-,023
	5	-,055*	,014	,012	-,101	-,008
	6	-,035	,015	,520	-,086	,016
2	1	,023	,014	1,000	-,024	,070
	3	-,029	,010	,157	-,063	,005
	4	-,037	,015	,279	-,086	,011
	5	-,031	,014	,515	-,077	,015
	6	-,012	,014	1,000	-,059	,036
3	1	,052*	,015	,040	,002	,103
	2	,029	,010	,157	-,005	,063
	4	-,008	,016	1,000	-,060	,043
	5	-,002	,016	1,000	-,055	,051
	6	,018	,014	1,000	-,028	,063
4	1	,061*	,011	<,001	,023	,098
	2	,037	,015	,279	-,011	,086
	3	,008	,016	1,000	-,043	,060
	5	,006	,015	1,000	-,043	,056
	6	,026	,012	,611	-,014	,065
5	1	,055*	,014	,012	,008	,101
	2	,031	,014	,515	-,015	,077
	3	,002	,016	1,000	-,051	,055
	4	-,006	,015	1,000	-,056	,043
	6	,020	,016	1,000	-,032	,072
6	1	,035	,015	,520	-,016	,086
	2	,012	,014	1,000	-,036	,059
	3	-,018	,014	1,000	-,063	,028
	4	-,026	,012	,611	-,065	,014
	5	-,020	,016	1,000	-,072	,032

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,003352	,049638	,010832	-,025947	,019242	-,309	20	,380	,760
Pair 2	Week2R2 - Week2L2	-,012205	,047180	,010296	-,033681	,009271	-1,185	20	,125	,250
Pair 3	Week4R2 - Week4L2	-,016824	,044441	,009698	-,037053	,003405	-1,735	20	,049	,098
Pair 4	Week8R2 - Week8L2	-,016933	,040624	,008865	-,035425	,001559	-1,910	20	,035	,071
Pair 5	Week12R2 - Week12L2	-,030386	,063762	,013914	-,059410	-,001361	-2,184	20	,021	,041
Pair 6	Week24R2 - Week24L2	,023305	,063097	,013769	-,005417	,052026	1,693	20	,053	,106

a. Parameter = R7

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,138	35,883	14	,001	,545	,640	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,257	24,557	14	,041	,725	,905	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,363	5	,073	23,194	<,001	,537
	Greenhouse-Geisser	,363	2,727	,133	23,194	<,001	,537
Error(Visit)	Sphericity Assumed	,313	100	,003			
	Greenhouse-Geisser	,313	54,540	,006			
Side	Sphericity Assumed	,006	1	,006	2,046	,168	,093
	Greenhouse-Geisser	,006	1,000	,006	2,046	,168	,093
Error(Side)	Sphericity Assumed	,059	20	,003			
	Greenhouse-Geisser	,059	20,000	,003			
Visit * Side	Sphericity Assumed	,009	5	,002	1,293	,273	,061
	Greenhouse-Geisser	,009	3,624	,002	1,293	,282	,061
Error(Visit*Side)	Sphericity Assumed	,138	100	,001			
	Greenhouse-Geisser	,138	72,479	,002			

a. Parameter = R7

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,285	22,744	14	,067	,646	,786	,200

Tests of Within-Subjects Effects

Tests of Within-Subjects Effectsa
Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,196	5	,039	17,640	<,001	,469
	Greenhouse-Geisser	,196	3,232	,061	17,640	<,001	,469
Error(Visits)	Sphericity Assumed	,222	100	,002			
	Greenhouse-Geisser	,222	64,636	,003			

a. Parameter = R7

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,037*	,010	,024	-,071	-,003
	3	-,066*	,015	,003	-,116	-,017
	4	-,110*	,015	<,001	-,160	-,060
	5	-,100*	,015	<,001	-,149	-,050
	6	-,100*	,021	,002	-,169	-,030
2	1	,037*	,010	,024	,003	,071
	3	-,029	,011	,262	-,066	,008
	4	-,073*	,015	,001	-,122	-,024
	5	-,062*	,012	<,001	-,102	-,022
	6	-,062*	,018	,041	-,123	-,002
3	1	,066*	,015	,003	,017	,116
	2	,029	,011	,262	-,008	,066
	4	-,044*	,013	,038	-,087	-,001
	5	-,033	,013	,226	-,075	,008
	6	-,033	,016	,722	-,086	,019
4	1	,110*	,015	<,001	,060	,160
	2	,073*	,015	,001	,024	,122
	3	,044*	,013	,038	,001	,087
	5	,011	,014	1,000	-,035	,056
	6	,011	,016	1,000	-,041	,063
5	1	,100*	,015	<,001	,050	,149
	2	,062*	,012	<,001	,022	,102
	3	,033	,013	,226	-,008	,075
	4	-,011	,014	1,000	-,056	,035
	6	,000	,013	1,000	-,042	,042
6	1	,100*	,021	,002	,030	,169
	2	,062*	,018	,041	,002	,123
	3	,033	,016	,722	-,019	,086
	4	-,011	,016	1,000	-,063	,041
	5	,000	,013	1,000	-,042	,042

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,187	30,387	14	,007	,660	,806	,200

Tests of Within-Subjects Effects

Tests of Within-Subjects Effectsa
Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,176	5	,035	15,362	<,001	,434
	Greenhouse-Geisser	,176	3,300	,053	15,362	<,001	,434
Error(Visits)	Sphericity Assumed	,229	100	,002			
	Greenhouse-Geisser	,229	65,992	,003			

a. Parameter = R7

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,040*	,009	,003	-,070	-,011
	3	-,066*	,014	,001	-,112	-,021

	4	-,101*	,014	<,001	-,147	-,054
	5	-,074*	,019	,016	-,138	-,009
	6	-,112*	,016	<,001	-,165	-,059
2	1	,040*	,009	,003	,011	,070
	3	-,026	,013	1,000	-,070	,019
	4	-,060*	,013	,002	-,103	-,017
	5	-,033	,018	1,000	-,095	,028
	6	-,072*	,016	,003	-,124	-,019
3	1	,066*	,014	,001	,021	,112
	2	,026	,013	1,000	-,019	,070
	4	-,035	,015	,512	-,085	,016
	5	-,008	,014	1,000	-,054	,039
	6	-,046*	,012	,013	-,085	-,007
4	1	,101*	,014	<,001	,054	,147
	2	,060*	,013	,002	,017	,103
	3	,035	,015	,512	-,016	,085
	5	,027	,013	,768	-,016	,070
	6	-,011	,015	1,000	-,060	,038
5	1	,074*	,019	,016	,009	,138
	2	,033	,018	1,000	-,028	,095
	3	,008	,014	1,000	-,039	,054
	4	-,027	,013	,768	-,070	,016
	6	-,038	,018	,621	-,097	,020
6	1	,112*	,016	<,001	,059	,165
	2	,072*	,016	,003	,019	,124
	3	,046*	,012	,013	,007	,085
	4	,011	,015	1,000	-,038	,060
	5	,038	,018	,621	-,020	,097

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm

		Paired Differences					t	df	Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,006424	,035674	,007785	-,009815	,022663	,825	20	,210	,419
Pair 2	Week2R4 - Week2L4	,003362	,041051	,008958	-,015324	,022048	,375	20	,356	,711
Pair 3	Week4R4 - Week4L4	,006576	,067876	,014812	-,024321	,037473	,444	20	,331	,662
Pair 4	Week8R4 - Week8L4	,016033	,061708	,013466	-,012056	,044122	1,191	20	,124	,248
Pair 5	Week12R4 - Week12L4	,032200	,057199	,012482	,006163	,058237	2,580	20	,009	,018
Pair 6	Week24R4 - Week24L4	-,005948	,071240	,015546	-,038376	,026481	-,383	20	,353	,706

a. Parameter = R7

Skin recovery (R8, Ua)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	,991	21	,998
ScreeningR4	,950	21	,336
ScreeningL2	,968	21	,692
ScreeningL4	,778	21	<,001
Week2R2	,951	21	,363
Week2R4	,940	21	,222

Week2L2	,969	21	,716
Week2L4	,971	21	,759
Week4R2	,968	21	,696
Week4R4	,950	21	,341
Week4L2	,981	21	,937
Week4L4	,980	21	,927
Week8R2	,978	21	,890
Week8R4	,974	21	,825
Week8L2	,959	21	,501
Week8L4	,950	21	,346
Week12R2	,964	21	,598
Week12R4	,930	21	,137
Week12L2	,951	21	,352
Week12L4	,986	21	,986
Week24R2	,985	21	,977
Week24R4	,940	21	,221
Week24L2	,948	21	,312
Week24L4	,913	21	,062

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,442	14,776	14	,398	,784	,998	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,433	15,129	14	,374	,723	,903	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,432	5	,086	48,115	<,001	,706
	Greenhouse-Geisser	,432	3,918	,110	48,115	<,001	,706
Error(Visit)	Sphericity Assumed	,180	100	,002			
	Greenhouse-Geisser	,180	78,355	,002			
Side	Sphericity Assumed	,001	1	,001	,999	,330	,048
	Greenhouse-Geisser	,001	1,000	,001	,999	,330	,048
Error(Side)	Sphericity Assumed	,021	20	,001			
	Greenhouse-Geisser	,021	20,000	,001			
Visit * Side	Sphericity Assumed	,003	5	,001	1,131	,349	,054
	Greenhouse-Geisser	,003	3,617	,001	1,131	,347	,054
Error(Visit*Side)	Sphericity Assumed	,061	100	,001			
	Greenhouse-Geisser	,061	72,334	,001			

a. Parameter = R8

General linear model 2 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,540	11,167	14	,676	,832	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,195	5	,039	35,626	<,001	,640
	Greenhouse-Geisser	,195	4,158	,047	35,626	<,001	,640
Error(Visits)	Sphericity Assumed	,109	100	,001			
	Greenhouse-Geisser	,109	83,168	,001			

a. Parameter = R8

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,015	,009	1,000	-,014	,043
	3	,023	,010	,436	-,010	,056
	4	,047*	,010	,002	,013	,082
	5	,063*	,010	<,001	,029	,098
	6	,119*	,011	<,001	,082	,156
2	1	-,015	,009	1,000	-,043	,014
	3	,008	,012	1,000	-,033	,050
	4	,033*	,010	,039	,001	,065
	5	,049*	,011	,004	,012	,085
	6	,105*	,009	<,001	,075	,135
3	1	-,023	,010	,436	-,056	,010
	2	-,008	,012	1,000	-,050	,033
	4	,024	,010	,447	-,010	,059
	5	,040*	,009	,005	,009	,071
	6	,096*	,011	<,001	,059	,133
4	1	-,047*	,010	,002	-,082	-,013
	2	-,033*	,010	,039	-,065	-,001
	3	-,024	,010	,447	-,059	,010
	5	,016	,010	1,000	-,017	,049
	6	,072*	,010	<,001	,037	,107
5	1	-,063*	,010	<,001	-,098	-,029
	2	-,049*	,011	,004	-,085	-,012
	3	-,040*	,009	,005	-,071	-,009
	4	-,016	,010	1,000	-,049	,017
	6	,056*	,009	<,001	,025	,087
6	1	-,119*	,011	<,001	-,156	-,082
	2	-,105*	,009	<,001	-,135	-,075
	3	-,096*	,011	<,001	-,133	-,059
	4	-,072*	,010	<,001	-,107	-,037
	5	-,056*	,009	<,001	-,087	-,025

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,354	18,771	14	,177	,761	,962	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,241	5	,048	36,638	<,001	,647
	Greenhouse-Geisser	,241	3,806	,063	36,638	<,001	,647
Error(Visits)	Sphericity Assumed	,131	100	,001			
	Greenhouse-Geisser	,131	76,121	,002			

a. Parameter = R8

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound

1	2	,001	,010	1,000	-,032	,035
	3	,011	,012	1,000	-,030	,052
	4	,036*	,009	,014	,005	,067
	5	,053*	,012	,003	,014	,091
	6	,126*	,010	<,001	,093	,159
2	1	-,001	,010	1,000	-,035	,032
	3	,009	,015	1,000	-,040	,059
	4	,035	,011	,081	-,002	,072
	5	,051*	,013	,010	,009	,093
	6	,124*	,010	<,001	,090	,159
3	1	-,011	,012	1,000	-,052	,030
	2	-,009	,015	1,000	-,059	,040
	4	,025	,011	,487	-,011	,062
	5	,042	,014	,096	-,004	,088
	6	,115*	,010	<,001	,083	,148
4	1	-,036*	,009	,014	-,067	-,005
	2	-,035	,011	,081	-,072	,002
	3	-,025	,011	,487	-,062	,011
	5	,017	,012	1,000	-,023	,056
	6	,090*	,006	<,001	,069	,111
5	1	-,053*	,012	,003	-,091	-,014
	2	-,051*	,013	,010	-,093	-,009
	3	-,042	,014	,096	-,088	,004
	4	-,017	,012	1,000	-,056	,023
	6	,073*	,010	<,001	,039	,108
6	1	-,126*	,010	<,001	-,159	-,093
	2	-,124*	,010	<,001	-,159	-,090
	3	-,115*	,010	<,001	-,148	-,083
	4	-,090*	,006	<,001	-,111	-,069
	5	-,073*	,010	<,001	-,108	-,039

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference				One-Sided p	Two-Sided p
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df		
Pair 1	ScreeningR2 - ScreeningL2	,002714	,038802	,008467	-,014948	,020377	,321	20	,376	,752
Pair 2	Week2R2 - Week2L2	-,010381	,041325	,009018	-,029192	,008430	-1,151	20	,132	,263
Pair 3	Week4R2 - Week4L2	-,009619	,032978	,007196	-,024630	,005392	-1,337	20	,098	,196
Pair 4	Week8R2 - Week8L2	-,008524	,029067	,006343	-,021755	,004707	-1,344	20	,097	,194
Pair 5	Week12R2 - Week12L2	-,007762	,043847	,009568	-,027721	,012197	-,811	20	,213	,427
Pair 6	Week24R2 - Week24L2	,009333	,033355	,007279	-,005850	,024516	1,282	20	,107	,214

a. Parameter = R8

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,368	18,107	14	,206	,689	,850	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,397	16,726	14	,275	,800	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,793	5	,159	23,811	<,001	,543
	Greenhouse-Geisser	,793	3,444	,230	23,811	<,001	,543
Error(Visit)	Sphericity Assumed	,666	100	,007			
	Greenhouse-Geisser	,666	68,882	,010			
Side	Sphericity Assumed	,084	1	,084	11,381	,003	,363
	Greenhouse-Geisser	,084	1,000	,084	11,381	,003	,363
Error(Side)	Sphericity Assumed	,147	20	,007			
	Greenhouse-Geisser	,147	20,000	,007			
Visit * Side	Sphericity Assumed	,039	5	,008	1,811	,118	,083
	Greenhouse-Geisser	,039	4,002	,010	1,811	,135	,083
Error(Visit*Side)	Sphericity Assumed	,429	100	,004			
	Greenhouse-Geisser	,429	80,031	,005			

a. Parameter = R8

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,427	15,422	14	,354	,738	,926	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,280	5	,056	13,833	<,001	,409
	Greenhouse-Geisser	,280	3,692	,076	13,833	<,001	,409
Error(Visits)	Sphericity Assumed	,405	100	,004			
	Greenhouse-Geisser	,405	73,836	,005			

a. Parameter = R8

Pairwise Comparisons

					95% Confidence Interval for Differencec	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	Lower Bound	Upper Bound
1	2	-,085*	,019	,004	-,148	-,021
	3	-,062	,028	,541	-,154	,030
	4	-,007	,021	1,000	-,076	,062
	5	,010	,017	1,000	-,047	,067
	6	,058	,020	,118	-,007	,124
2	1	,085*	,019	,004	,021	,148
	3	,023	,021	1,000	-,047	,092
	4	,078*	,016	,002	,023	,133
	5	,094*	,014	<,001	,049	,140
	6	,143*	,018	<,001	,084	,201
3	1	,062	,028	,541	-,030	,154
	2	-,023	,021	1,000	-,092	,047
	4	,055	,024	,527	-,026	,136
	5	,072*	,020	,032	,004	,140
	6	,120*	,021	<,001	,049	,192
4	1	,007	,021	1,000	-,062	,076
	2	-,078*	,016	,002	-,133	-,023
	3	-,055	,024	,527	-,136	,026
	5	,017	,015	1,000	-,032	,065
	6	,065	,021	,073	-,003	,134
5	1	-,010	,017	1,000	-,067	,047
	2	-,094*	,014	<,001	-,140	-,049
	3	-,072*	,020	,032	-,140	-,004
	4	-,017	,015	1,000	-,065	,032
	6	,049	,016	,082	-,003	,100
6	1	-,058	,020	,118	-,124	,007

	2	-,143*	,018	<,001	-,201	-,084
	3	-,120*	,021	<,001	-,192	-,049
	4	-,065	,021	,073	-,134	,003
	5	-,049	,016	,082	-,100	,003

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,369	18,028	14	,209	,737	,924	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,552	5	,110	15,995	<,001	,444
	Greenhouse-Geisser	,552	3,685	,150	15,995	<,001	,444
Error(Visits)	Sphericity Assumed	,691	100	,007			
	Greenhouse-Geisser	,691	73,696	,009			

a. Parameter = R8

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,094*	,024	,014	-,175	-,013
	3	-,065	,034	1,000	-,178	,048
	4	-,002	,030	1,000	-,100	,097
	5	-,009	,026	1,000	-,096	,079
	6	,117*	,029	,010	,020	,213
2	1	,094*	,024	,014	,013	,175
	3	,029	,026	1,000	-,058	,115
	4	,092	,030	,100	-,009	,194
	5	,085*	,022	,013	,013	,158
	6	,211*	,024	<,001	,131	,290
3	1	,065	,034	1,000	-,048	,178
	2	-,029	,026	1,000	-,115	,058
	4	,064	,028	,502	-,029	,156
	5	,057	,018	,075	-,003	,116
	6	,182*	,023	<,001	,105	,259
4	1	,002	,030	1,000	-,097	,100
	2	-,092	,030	,100	-,194	,009
	3	-,064	,028	,502	-,156	,029
	5	-,007	,022	1,000	-,080	,066
	6	,118*	,026	,002	,033	,203
5	1	,009	,026	1,000	-,079	,096
	2	-,085*	,022	,013	-,158	-,013
	3	-,057	,018	,075	-,116	,003
	4	,007	,022	1,000	-,066	,080
	6	,125*	,018	<,001	,066	,184
6	1	-,117*	,029	,010	-,213	-,020
	2	-,211*	,024	<,001	-,290	-,131
	3	-,182*	,023	<,001	-,259	-,105
	4	-,118*	,026	,002	-,203	-,033
	5	-,125*	,018	<,001	-,184	-,066

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,042048	,110638	,024143	-,092410	,008314	-1,742	20	,048	,097
Pair 2	Week2R4 - Week2L4	-,051190	,098371	,021466	-,095968	-,006413	-2,385	20	,014	,027
Pair 3	Week4R4 - Week4L4	-,045095	,098131	,021414	-,089764	-,000426	-2,106	20	,024	,048
Pair 4	Week8R4 - Week8L4	-,036667	,122200	,026666	-,092291	,018958	-1,375	20	,092	,184
Pair 5	Week12R4 - Week12L4	-,060286	,069353	,015134	-,091855	-,028717	-3,983	20	<,001	<,001
Pair 6	Week24R4 - Week24L4	,016524	,079448	,017337	-,019640	,052688	,953	20	,176	,352

a. Parameter = R8

Skin tiring (R9, Uf5 – Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR2	,946	21	,283
ScreeningR4	,951	21	,358
ScreeningL2	,939	21	,205
ScreeningL4	,957	21	,453
Week2R2	,958	21	,484
Week2R4	,944	21	,265
Week2L2	,950	21	,347
Week2L4	,954	21	,412
Week4R2	,958	21	,471
Week4R4	,970	21	,727
Week4L2	,879	21	,014
Week4L4	,887	21	,020
Week8R2	,937	21	,192
Week8R4	,887	21	,020
Week8L2	,983	21	,961
Week8L4	,945	21	,276
Week12R2	,982	21	,949
Week12R4	,989	21	,997
Week12L2	,958	21	,486
Week12L4	,921	21	,092
Week24R2	,965	21	,618
Week24R4	,986	21	,985
Week24L2	,880	21	,015
Week24L4	,939	21	,208

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,299	21,831	14	,084	,700	,867	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,357	18,627	14	,183	,725	,905	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,006	5	,001	16,237	<,001	,448
	Greenhouse-Geisser	,006	3,501	,002	16,237	<,001	,448
Error(Visit)	Sphericity Assumed	,007	100	6,968E-5			

	Greenhouse-Geisser	,007	70,026	9,951E-5			
Side	Sphericity Assumed	,000	1	,000	3,301	,084	,142
	Greenhouse-Geisser	,000	1,000	,000	3,301	,084	,142
Error(Side)	Sphericity Assumed	,001	20	5,052E-5			
	Greenhouse-Geisser	,001	20,000	5,052E-5			
Visit * Side	Sphericity Assumed	,002	5	,000	5,808	<,001	,225
	Greenhouse-Geisser	,002	3,625	,001	5,808	<,001	,225
Error(Visit*Side)	Sphericity Assumed	,006	100	6,278E-5			
	Greenhouse-Geisser	,006	72,503	8,659E-5			

a. Parameter = R9

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,093	43,013	14	<,001	,567	,671	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,001	5	,000	4,282	,001	,176
	Greenhouse-Geisser	,001	2,837	,000	4,282	,010	,176
Error(Visits)	Sphericity Assumed	,007	100	6,587E-5			
	Greenhouse-Geisser	,007	56,730	,000			

a. Parameter = R9

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,003	,001	,145	-,001	,007
	3	,001	,002	1,000	-,004	,007
	4	,008*	,002	<,001	,003	,013
	5	,006	,003	,679	-,003	,015
	6	,009	,003	,202	-,002	,020
2	1	-,003	,001	,145	-,007	,001
	3	-,002	,001	1,000	-,007	,003
	4	,005*	,001	,026	,000	,009
	5	,002	,003	1,000	-,006	,011
	6	,006	,003	1,000	-,005	,016
3	1	-,001	,002	1,000	-,007	,004
	2	,002	,001	1,000	-,003	,007
	4	,007	,002	,060	,000	,014
	5	,004	,003	1,000	-,005	,014
	6	,008	,003	,399	-,003	,018
4	1	-,008*	,002	<,001	-,013	-,003
	2	-,005*	,001	,026	-,009	,000
	3	-,007	,002	,060	-,014	,000
	5	-,002	,002	1,000	-,011	,006
	6	,001	,003	1,000	-,010	,012
5	1	-,006	,003	,679	-,015	,003
	2	-,002	,003	1,000	-,011	,006
	3	-,004	,003	1,000	-,014	,005
	4	,002	,002	1,000	-,006	,011
	6	,003	,003	1,000	-,008	,014
6	1	-,009	,003	,202	-,020	,002
	2	-,006	,003	1,000	-,016	,005
	3	-,008	,003	,399	-,018	,003
	4	-,001	,003	1,000	-,012	,010
	5	-,003	,003	1,000	-,014	,008

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.
a. Parameter = R9
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,506	12,343	14	,582	,772	,980	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,006	5	,001	18,230	<,001	,477
	Greenhouse-Geisser	,006	3,862	,002	18,230	<,001	,477
Error(Visits)	Sphericity Assumed	,007	100	6,659E-5			
	Greenhouse-Geisser	,007	77,244	8,621E-5			

a. Parameter = R9

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,003	,002	1,000	-,005	,010
	3	,003	,002	1,000	-,005	,012
	4	,004	,002	,512	-,002	,010
	5	,001	,002	1,000	-,007	,009
	6	,021*	,002	<,001	,014	,027
2	1	-,003	,002	1,000	-,010	,005
	3	,001	,003	1,000	-,009	,011
	4	,001	,003	1,000	-,007	,010
	5	-,002	,002	1,000	-,008	,005
	6	,018*	,003	<,001	,009	,027
3	1	-,003	,002	1,000	-,012	,005
	2	-,001	,003	1,000	-,011	,009
	4	,001	,003	1,000	-,009	,010
	5	-,002	,003	1,000	-,013	,008
	6	,017*	,002	<,001	,009	,025
4	1	-,004	,002	,512	-,010	,002
	2	-,001	,003	1,000	-,010	,007
	3	-,001	,003	1,000	-,010	,009
	5	-,003	,003	1,000	-,012	,005
	6	,016*	,002	<,001	,009	,024
5	1	-,001	,002	1,000	-,009	,007
	2	,002	,002	1,000	-,005	,008
	3	,002	,003	1,000	-,008	,013
	4	,003	,003	1,000	-,005	,012
	6	,019*	,003	<,001	,010	,029
6	1	-,021*	,002	<,001	-,027	-,014
	2	-,018*	,003	<,001	-,027	-,009
	3	-,017*	,002	<,001	-,025	-,009
	4	-,016*	,002	<,001	-,024	-,009
	5	-,019*	,003	<,001	-,029	-,010

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R9
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,002286	,008289	,001809	-,006059	,001488	-1,264	20	,110	,221
Pair 2	Week2R2 - Week2L2	-,002952	,008164	,001781	-,006668	,000764	-1,657	20	,057	,113
Pair 3	Week4R2 - Week4L2	-,000238	,009762	,002130	-,004682	,004205	-,112	20	,456	,912
Pair 4	Week8R2 - Week8L2	-,006381	,009892	,002159	-,010884	-,001878	-2,956	20	,004	,008
Pair 5	Week12R2 - Week12L2	-,007095	,015700	,003426	-,014242	,000051	-2,071	20	,026	,052
Pair 6	Week24R2 - Week24L2	,009190	,012404	,002707	,003544	,014837	3,395	20	,001	,003

a. Parameter = R9

General linear model 4 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,550	10,824	14	,703	,826	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,499	12,568	14	,565	,773	,981	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,011	5	,002	9,604	<,001	,324
	Greenhouse-Geisser	,011	4,129	,003	9,604	<,001	,324
Error(Visit)	Sphericity Assumed	,023	100	,000			
	Greenhouse-Geisser	,023	82,579	,000			
Side	Sphericity Assumed	,001	1	,001	3,152	,091	,136
	Greenhouse-Geisser	,001	1,000	,001	3,152	,091	,136
Error(Side)	Sphericity Assumed	,004	20	,000			
	Greenhouse-Geisser	,004	20,000	,000			
Visit * Side	Sphericity Assumed	,001	5	,000	1,778	,124	,082
	Greenhouse-Geisser	,001	3,863	,000	1,778	,144	,082
Error(Visit*Side)	Sphericity Assumed	,013	100	,000			
	Greenhouse-Geisser	,013	77,266	,000			

a. Parameter = R9

General linear model 4 mm R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,576	9,976	14	,766	,812	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,008	5	,002	9,980	<,001	,333
	Greenhouse-Geisser	,008	4,058	,002	9,980	<,001	,333
Error(Visits)	Sphericity Assumed	,016	100	,000			
	Greenhouse-Geisser	,016	81,155	,000			

a. Parameter = R9

Pairwise Comparisons

					95% Confidence Interval for Differencec	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	Lower Bound	Upper Bound
1	2	,004	,003	1,000	-,007	,016

	3	,007	,004	1,000	-,007	,021
	4	,009	,005	1,000	-,007	,026
	5	,019*	,004	<,001	,007	,030
	6	,023*	,003	<,001	,012	,035
2	1	-,004	,003	1,000	-,016	,007
	3	,002	,004	1,000	-,010	,015
	4	,005	,004	1,000	-,009	,019
	5	,014*	,003	,007	,003	,026
	6	,019*	,003	<,001	,008	,030
3	1	-,007	,004	1,000	-,021	,007
	2	-,002	,004	1,000	-,015	,010
	4	,003	,004	1,000	-,010	,016
	5	,012*	,003	,030	,001	,023
	6	,017*	,004	,019	,002	,031
4	1	-,009	,005	1,000	-,026	,007
	2	-,005	,004	1,000	-,019	,009
	3	-,003	,004	1,000	-,016	,010
	5	,009	,004	,715	-,005	,023
	6	,014	,004	,064	,000	,028
5	1	-,019*	,004	<,001	-,030	-,007
	2	-,014*	,003	,007	-,026	-,003
	3	-,012*	,003	,030	-,023	-,001
	4	-,009	,004	,715	-,023	,005
	6	,005	,004	1,000	-,009	,018
6	1	-,023*	,003	<,001	-,035	-,012
	2	-,019*	,003	<,001	-,030	-,008
	3	-,017*	,004	,019	-,031	-,002
	4	-,014	,004	,064	-,028	,000
	5	-,005	,004	1,000	-,018	,009

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R9
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,422	15,596	14	,343	,765	,969	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,004	5	,001	4,195	,002	,173
	Greenhouse-Geisser	,004	3,826	,001	4,195	,005	,173
Error(Visits)	Sphericity Assumed	,019	100	,000			
	Greenhouse-Geisser	,019	76,525	,000			

a. Parameter = R9

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,002	,004	1,000	-,015	,011
	3	,001	,005	1,000	-,016	,018
	4	,008	,004	,482	-,004	,020
	5	,006	,004	1,000	-,008	,020
	6	,014*	,004	,024	,001	,028
2	1	,002	,004	1,000	-,011	,015
	3	,003	,004	1,000	-,009	,016
	4	,011	,005	,500	-,005	,026
	5	,008	,005	1,000	-,007	,024
	6	,017*	,004	,003	,004	,029

3	1	-,001	,005	1,000	-,018	,016
	2	-,003	,004	1,000	-,016	,009
	4	,007	,005	1,000	-,010	,025
	5	,005	,004	1,000	-,008	,019
	6	,013	,005	,125	-,002	,029
4	1	-,008	,004	,482	-,020	,004
	2	-,011	,005	,500	-,026	,005
	3	-,007	,005	1,000	-,025	,010
	5	-,002	,004	1,000	-,015	,011
	6	,006	,004	1,000	-,007	,019
5	1	-,006	,004	1,000	-,020	,008
	2	-,008	,005	1,000	-,024	,007
	3	-,005	,004	1,000	-,019	,008
	4	,002	,004	1,000	-,011	,015
	6	,008	,005	1,000	-,008	,024
6	1	-,014*	,004	,024	-,028	-,001
	2	-,017*	,004	,003	-,029	-,004
	3	-,013	,005	,125	-,029	,002
	4	-,006	,004	1,000	-,019	,007
	5	-,008	,005	1,000	-,024	,008

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R9
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,002429	,016741	,003653	-,005192	,010049	,665	20	,257	,514
Pair 2	Week2R4 - Week2L4	-,004048	,014372	,003136	-,010590	,002494	-1,291	20	,106	,212
Pair 3	Week4R4 - Week4L4	-,003000	,016358	,003570	-,010446	,004446	-,840	20	,205	,411
Pair 4	Week8R4 - Week8L4	,001381	,023018	,005023	-,009097	,011859	,275	20	,393	,786
Pair 5	Week12R4 - Week12L4	-,009762	,014693	,003206	-,016450	-,003074	-3,045	20	,003	,006
Pair 6	Week24R4 - Week24L4	-,006333	,013047	,002847	-,012272	-,000394	-2,224	20	,019	,038

a. Parameter = R9

Sonography –skin density and skin thickness

Means and SDs

Mean	Parameter		SD	
	density	thickness	density	thickness
n	21	21	21	21
ScreeningR	27.02	1647.05	6.77	211.57
ScreeningL	31.11	1653.29	9.97	229.23
Week2R	25.29	1621.33	6.74	218.62
Week2L	30.27	1624.00	8.67	182.36
Week4R	23.78	1625.76	8.08	154.62
Week4L	26.17	1665.95	8.11	205.05
Week8R	23.15	1636.29	8.35	129.26
Week8L	24.40	1596.71	7.92	211.96
Week12R	24.64	1536.10	7.71	128.37
Week12L	24.19	1587.76	9.15	193.01
Week24R	24.95	1546.38	8.58	179.10
Week24L	27.54	1548.52	9.73	139.18

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,979	21	,917
ScreeningL	,937	21	,191
Week2R	,972	21	,770
Week2L	,923	21	,101
Week4R	,937	21	,187
Week4L	,972	21	,781
Week8R	,969	21	,710
Week8L	,953	21	,391
Week12R	,978	21	,895
Week12L	,956	21	,441
Week24R	,977	21	,876
Week24L	,888	21	,021

General linear model density RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,283	22,875	14	,064	,745	,937	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,727	5,781	14	,972	,894	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	886,120	5	177,224	3,771	,004	,159
	Greenhouse-Geisser	886,120	3,726	237,816	3,771	,009	,159
Error(Visit)	Sphericity Assumed	4700,113	100	47,001			
	Greenhouse-Geisser	4700,113	74,521	63,071			
Side	Sphericity Assumed	386,558	1	386,558	8,915	,007	,308
	Greenhouse-Geisser	386,558	1,000	386,558	8,915	,007	,308
Error(Side)	Sphericity Assumed	867,218	20	43,361			
	Greenhouse-Geisser	867,218	20,000	43,361			
Visit * Side	Sphericity Assumed	198,951	5	39,790	1,208	,311	,057
	Greenhouse-Geisser	198,951	4,469	44,515	1,208	,313	,057

Error(Visit*Side)	Sphericity Assumed	3292,657	100	32,927			
	Greenhouse-Geisser	3292,657	89,386	36,836			

a. Parameter = density

General linear model density R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,592	9,476	14	,802	,858	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	188,175	5	37,635	1,314	,264	,062
	Greenhouse-Geisser	188,175	4,290	43,859	1,314	,270	,062
Error(Visit)	Sphericity Assumed	2863,813	100	28,638			
	Greenhouse-Geisser	2863,813	85,810	33,374			

a. Parameter = density

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	1,731	1,112	1,000	-1,972	5,434
	3	3,240	1,646	,946	-2,242	8,721
	4	3,864	1,464	,236	-1,013	8,742
	5	2,382	1,296	1,000	-1,933	6,697
	6	2,065	1,727	1,000	-3,686	7,815
2	1	-1,731	1,112	1,000	-5,434	1,972
	3	1,509	1,596	1,000	-3,809	6,826
	4	2,133	1,590	1,000	-3,161	7,428
	5	,651	1,588	1,000	-4,639	5,941
	6	,334	1,741	1,000	-5,465	6,133
3	1	-3,240	1,646	,946	-8,721	2,242
	2	-1,509	1,596	1,000	-6,826	3,809
	4	,625	1,895	1,000	-5,688	6,937
	5	-,858	1,934	1,000	-7,300	5,585
	6	-1,175	1,739	1,000	-6,968	4,619
4	1	-3,864	1,464	,236	-8,742	1,013
	2	-2,133	1,590	1,000	-7,428	3,161
	3	-,625	1,895	1,000	-6,937	5,688
	5	-1,482	1,660	1,000	-7,013	4,048
	6	-1,800	1,792	1,000	-7,767	4,168
5	1	-2,382	1,296	1,000	-6,697	1,933
	2	-,651	1,588	1,000	-5,941	4,639
	3	,858	1,934	1,000	-5,585	7,300
	4	1,482	1,660	1,000	-4,048	7,013
	6	-,317	1,791	1,000	-6,282	5,647
6	1	-2,065	1,727	1,000	-7,815	3,686
	2	-,334	1,741	1,000	-6,133	5,465
	3	1,175	1,739	1,000	-4,619	6,968
	4	1,800	1,792	1,000	-4,168	7,767
	5	,317	1,791	1,000	-5,647	6,282

Based on estimated marginal means
a. Parameter = density
b. Adjustment for multiple comparisons: Bonferroni.

General linear model density L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,689	6,745	14	,945	,871	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	896,895	5	179,379	3,497	,006	,149
	Greenhouse-Geisser	896,895	4,357	205,858	3,497	,009	,149
Error(Visit)	Sphericity Assumed	5128,956	100	51,290			
	Greenhouse-Geisser	5128,956	87,137	58,861			

a. Parameter = density

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,835	1,725	1,000	-4,912	6,581
	3	4,936	1,854	,224	-1,239	11,111
	4	6,704	2,333	,141	-1,067	14,476
	5	6,915	2,166	,069	-,300	14,130
	6	3,569	2,389	1,000	-4,388	11,526
2	1	-,835	1,725	1,000	-6,581	4,912
	3	4,101	1,873	,608	-2,137	10,339
	4	5,870	2,293	,280	-1,768	13,507
	5	6,080	2,195	,177	-1,230	13,390
	6	2,734	2,298	1,000	-4,918	10,387
3	1	-4,936	1,854	,224	-11,111	1,239
	2	-4,101	1,873	,608	-10,339	2,137
	4	1,768	2,012	1,000	-4,934	8,470
	5	1,979	2,189	1,000	-5,311	9,268
	6	-1,367	2,212	1,000	-8,735	6,001
4	1	-6,704	2,333	,141	-14,476	1,067
	2	-5,870	2,293	,280	-13,507	1,768
	3	-1,768	2,012	1,000	-8,470	4,934
	5	,210	2,115	1,000	-6,834	7,255
	6	-3,135	2,709	1,000	-12,157	5,887
5	1	-6,915	2,166	,069	-14,130	,300
	2	-6,080	2,195	,177	-13,390	1,230
	3	-1,979	2,189	1,000	-9,268	5,311
	4	-,210	2,115	1,000	-7,255	6,834
	6	-3,346	2,570	1,000	-11,907	5,215
6	1	-3,569	2,389	1,000	-11,526	4,388
	2	-2,734	2,298	1,000	-10,387	4,918
	3	1,367	2,212	1,000	-6,001	8,735
	4	3,135	2,709	1,000	-5,887	12,157
	5	3,346	2,570	1,000	-5,215	11,907

Based on estimated marginal means
a. Parameter = density
b. Adjustment for multiple comparisons: Bonferroni.

T-Test density

		Paired Differences							Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-4,09000	9,02906	1,97030	-8,19998	,01998	-2,076	20	,026	,051
Pair 2	Week2R - Week2L	-4,98619	6,86703	1,49851	-8,11202	-1,86036	-3,327	20	,002	,003
Pair 3	Week4R - Week4L	-2,39333	9,19016	2,00546	-6,57664	1,78998	-1,193	20	,123	,247
Pair 4	Week8R - Week8L	-1,25000	8,03797	1,75403	-4,90884	2,40884	-,713	20	,242	,484
Pair 5	Week12R - Week12L	,44286	8,82643	1,92608	-3,57489	4,46060	,230	20	,410	,820

Pair 6	Week24R - Week24L	-2,58571	7,76748	1,69500	-6,12143	,95000	-1,525	20	,071	,143

a. Parameter = density

General linear model thickness RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,377	17,649	14	,227	,795	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,287	22,604	14	,069	,716	,892	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	389977,714	5	77995,543	5,442	<,001	,214
	Greenhouse-Geisser	389977,714	3,976	98072,652	5,442	<,001	,214
Error(Visit)	Sphericity Assumed	1433222,119	100	14332,221			
	Greenhouse-Geisser	1433222,119	79,528	18021,529			
Side	Sphericity Assumed	7019,444	1	7019,444	,535	,473	,026
	Greenhouse-Geisser	7019,444	1,000	7019,444	,535	,473	,026
Error(Side)	Sphericity Assumed	262228,056	20	13111,403			
	Greenhouse-Geisser	262228,056	20,000	13111,403			
Visit * Side	Sphericity Assumed	54943,508	5	10988,702	,857	,513	,041
	Greenhouse-Geisser	54943,508	3,581	15342,244	,857	,484	,041
Error(Visit*Side)	Sphericity Assumed	1282312,992	100	12823,130			
	Greenhouse-Geisser	1282312,992	71,624	17903,443			

a. Parameter = thickness

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	27,500	20,772	1,000	-41,684	96,684
	3	4,310	24,779	1,000	-78,220	86,839
	4	33,667	22,097	1,000	-39,930	107,263
	5	88,238	30,029	,122	-11,777	188,253
	6	102,714	31,820	,063	-3,265	208,694
2	1	-27,500	20,772	1,000	-96,684	41,684
	3	-23,190	27,863	1,000	-115,992	69,611
	4	6,167	24,146	1,000	-74,256	86,590
	5	60,738	27,942	,629	-32,327	153,803
	6	75,214	26,810	,164	-14,081	164,510
3	1	-4,310	24,779	1,000	-86,839	78,220
	2	23,190	27,863	1,000	-69,611	115,992
	4	29,357	23,146	1,000	-47,733	106,448
	5	83,929	26,338	,070	-3,793	171,650
	6	98,405*	28,891	,042	2,180	194,630
4	1	-33,667	22,097	1,000	-107,263	39,930
	2	-6,167	24,146	1,000	-86,590	74,256
	3	-29,357	23,146	1,000	-106,448	47,733
	5	54,571	23,694	,482	-24,343	133,486
	6	69,048*	19,730	,034	3,336	134,760
5	1	-88,238	30,029	,122	-188,253	11,777
	2	-60,738	27,942	,629	-153,803	32,327
	3	-83,929	26,338	,070	-171,650	3,793
	4	-54,571	23,694	,482	-133,486	24,343
	6	14,476	30,260	1,000	-86,310	115,262
6	1	-102,714	31,820	,063	-208,694	3,265
	2	-75,214	26,810	,164	-164,510	14,081
	3	-98,405*	28,891	,042	-194,630	-2,180
	4	-69,048*	19,730	,034	-134,760	-3,336

5	-14,476	30,260	1,000	-115,262	86,310

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = thickness
c. Adjustment for multiple comparisons: Bonferroni.

PRIMOS –skin topography

Means and SDs

Mean						
Parameter	**PC**	**Ra**	**Rmax**	**Rp**	**Rz**	**Wt**
n	21	21	21	21	21	21
ScreeningL	21.95	28.00	217.88	116.53	150.72	139.85
ScreeningR	21.57	26.69	193.14	93.56	139.40	112.55
Week2L	22.05	27.71	212.10	110.68	149.21	137.58
Week2R	21.29	26.77	193.71	96.80	139.41	116.64
Week4L	22.19	27.31	206.99	109.44	146.88	134.14
Week4R	21.43	25.25	184.40	90.92	132.57	108.18
Week8L	21.71	28.17	210.50	111.71	149.23	141.81
Week8R	21.62	26.47	192.27	95.55	138.03	117.55
Week12L	21.90	28.36	213.91	111.30	150.96	136.46
Week12R	21.48	27.09	192.90	93.85	140.28	132.74
Week24L	21.95	29.04	227.40	120.29	155.43	145.91
Week24R	21.48	25.95	192.00	94.79	135.03	112.74
SD	**PC**	**Ra**	**Rmax**	**Rp**	**Rz**	**Wt**
ScreeningL	2.22	8.53	66.69	28.40	38.96	85.70
ScreeningR	2.25	7.70	54.93	20.36	34.95	51.78
Week2L	2.38	6.59	56.69	23.31	32.25	82.15
Week2R	2.49	7.37	50.09	22.11	32.16	53.56
Week4L	2.20	6.98	58.45	23.87	33.63	76.95
Week4R	2.20	6.16	45.53	18.25	29.68	42.61
Week8L	2.39	8.63	72.71	33.52	41.55	96.05
Week8R	1.91	7.31	50.46	19.38	34.05	50.99
Week12L	2.17	7.77	63.76	27.11	35.03	88.90
Week12R	2.16	7.31	48.98	21.16	33.31	66.23
Week24L	2.38	7.86	65.90	34.14	35.26	94.74
Week24R	2.02	7.49	63.38	31.87	36.78	48.45

Mean skin roughness (Ra)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,898	21	,033
ScreeningL	,760	21	,000
Week2R	,851	21	,004
Week2L	,884	21	,017
Week4R	,940	21	,215
Week4L	,883	21	,017
Week8R	,937	21	,194
Week8L	,856	21	,005
Week12R	,935	21	,171
Week12L	,833	21	,002
Week24R	,940	21	,218
Week24L	,824	21	,002

General linear model RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,434	15,093	14	,376	,758	,957	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,321	20,580	14	,116	,690	,851	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	51,750	5	10,350	1,092	,369	,052
	Greenhouse-Geisser	51,750	3,789	13,657	1,092	,365	,052
Error(Visit)	Sphericity Assumed	947,373	100	9,474			
	Greenhouse-Geisser	947,373	75,788	12,500			
Side	Sphericity Assumed	189,107	1	189,107	5,431	,030	,214
	Greenhouse-Geisser	189,107	1,000	189,107	5,431	,030	,214
Error(Side)	Sphericity Assumed	696,366	20	34,818			
	Greenhouse-Geisser	696,366	20,000	34,818			
Visit * Side	Sphericity Assumed	31,042	5	6,208	,881	,497	,042
	Greenhouse-Geisser	31,042	3,449	9,001	,881	,467	,042
Error(Visit*Side)	Sphericity Assumed	704,770	100	7,048			
	Greenhouse-Geisser	704,770	68,974	10,218			

a. Parameter = Ra

General linear model R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,530	11,502	14	,650	,831	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	46,550	5	9,310	1,131	,349	,054
	Greenhouse-Geisser	46,550	4,154	11,205	1,131	,348	,054
Error(Visit)	Sphericity Assumed	823,526	100	8,235			
	Greenhouse-Geisser	823,526	83,087	9,912			

a. Parameter = Ra

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,081	,779	1,000	-2,676	2,514
	3	1,438	,814	1,000	-1,272	4,149
	4	,219	1,075	1,000	-3,363	3,801
	5	-,400	,877	1,000	-3,320	2,520
	6	,738	,982	1,000	-2,533	4,009
2	1	,081	,779	1,000	-2,514	2,676
	3	1,519	,732	,767	-,920	3,958
	4	,300	,887	1,000	-2,654	3,254
	5	-,319	1,031	1,000	-3,754	3,116
	6	,819	,861	1,000	-2,050	3,688
3	1	-1,438	,814	1,000	-4,149	1,272
	2	-1,519	,732	,767	-3,958	,920
	4	-1,219	,902	1,000	-4,222	1,784
	5	-1,838	,832	,584	-4,608	,932
	6	-,700	,672	1,000	-2,939	1,539

4	1	-,219	1,075	1,000	-3,801	3,363
	2	-,300	,887	1,000	-3,254	2,654
	3	1,219	,902	1,000	-1,784	4,222
	5	-,619	,933	1,000	-3,725	2,487
	6	,519	,935	1,000	-2,594	3,632
5	1	,400	,877	1,000	-2,520	3,320
	2	,319	1,031	1,000	-3,116	3,754
	3	1,838	,832	,584	-,932	4,608
	4	,619	,933	1,000	-2,487	3,725
	6	1,138	,882	1,000	-1,798	4,074
6	1	-,738	,982	1,000	-4,009	2,533
	2	-,819	,861	1,000	-3,688	2,050
	3	,700	,672	1,000	-1,539	2,939
	4	-,519	,935	1,000	-3,632	2,594
	5	-1,138	,882	1,000	-4,074	1,798

Based on estimated marginal means
a. Parameter = Ra
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,426	15,465	14	,351	,772	,980	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	36,241	5	7,248	,875	,501	,042
	Greenhouse-Geisser	36,241	3,862	9,384	,875	,480	,042
Error(Visit)	Sphericity Assumed	828,617	100	8,286			
	Greenhouse-Geisser	828,617	77,237	10,728			

a. Parameter = Ra

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,290	1,056	1,000	-3,228	3,809
	3	,690	1,117	1,000	-3,029	4,410
	4	-,162	1,042	1,000	-3,633	3,310
	5	-,352	,970	1,000	-3,583	2,878
	6	-1,033	1,093	1,000	-4,674	2,608
2	1	-,290	1,056	1,000	-3,809	3,228
	3	,400	,547	1,000	-1,421	2,221
	4	-,452	,810	1,000	-3,151	2,246
	5	-,643	,869	1,000	-3,538	2,252
	6	-1,324	,862	1,000	-4,196	1,549
3	1	-,690	1,117	1,000	-4,410	3,029
	2	-,400	,547	1,000	-2,221	1,421
	4	-,852	,817	1,000	-3,574	1,869
	5	-1,043	,748	1,000	-3,536	1,450
	6	-1,724	,847	,830	-4,546	1,098
4	1	,162	1,042	1,000	-3,310	3,633
	2	,452	,810	1,000	-2,246	3,151
	3	,852	,817	1,000	-1,869	3,574
	5	-,190	,738	1,000	-2,649	2,268
	6	-,871	,798	1,000	-3,528	1,785
5	1	,352	,970	1,000	-2,878	3,583
	2	,643	,869	1,000	-2,252	3,538
	3	1,043	,748	1,000	-1,450	3,536

	4	,190	,738	1,000	-2,268	2,649
	6	-,681	,819	1,000	-3,409	2,047
6	1	1,033	1,093	1,000	-2,608	4,674
	2	1,324	,862	1,000	-1,549	4,196
	3	1,724	,847	,830	-1,098	4,546
	4	,871	,798	1,000	-1,785	3,528
	5	,681	,819	1,000	-2,047	3,409

Based on estimated marginal means
a. Parameter = Ra
b. Adjustment for multiple comparisons: Bonferroni.

T-Test mean skin roughness (Ra)

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-1,31905	4,44866	,97078	-3,34406	,70596	-1,359	20	,095	,189
Pair 2	Week2R - Week2L	-,94762	4,17368	,91077	-2,84746	,95222	-1,040	20	,155	,311
Pair 3	Week4R - Week4L	-2,06667	3,97043	,86642	-3,87399	-,25935	-2,385	20	,014	,027
Pair 4	Week8R - Week8L	-1,70000	4,91019	1,07149	-3,93509	,53509	-1,587	20	,064	,128
Pair 5	Week12R - Week12L	-1,27143	5,37347	1,17259	-3,71740	1,17454	-1,084	20	,146	,291
Pair 6	Week24R - Week24L	-3,09048	5,84422	1,27531	-5,75073	-,43022	-2,423	20	,012	,025

a. Parameter = Ra

Maximum roughness (Rmax)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,928	21	,125
ScreeningL	,846	21	,004
Week2R	,911	21	,056
Week2L	,808	21	,001
Week4R	,930	21	,136
Week4L	,849	21	,004
Week8R	,916	21	,071
Week8L	,852	21	,005
Week12R	,937	21	,188
Week12L	,861	21	,007
Week24R	,915	21	,069
Week24L	,905	21	,043

General linear model RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,242	25,659	14	,030	,688	,848	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,166	32,505	14	,004	,645	,783	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	4504,943	5	900,989	1,202	,314	,057
	Greenhouse-Geisser	4504,943	3,440	1309,538	1,202	,317	,057
Error(Visit)	Sphericity Assumed	74951,330	100	749,513			
	Greenhouse-Geisser	74951,330	68,802	1089,377			
Side	Sphericity Assumed	34470,545	1	34470,545	15,584	<,001	,438
	Greenhouse-Geisser	34470,545	1,000	34470,545	15,584	<,001	,438

Error(Side)	Sphericity Assumed	44239,411	20	2211,971			
	Greenhouse-Geisser	44239,411	20,000	2211,971			
Visit * Side	Sphericity Assumed	2143,164	5	428,633	,704	,621	,034
	Greenhouse-Geisser	2143,164	3,224	664,764	,704	,563	,034
Error(Visit*Side)	Sphericity Assumed	60847,015	100	608,470			
	Greenhouse-Geisser	60847,015	64,479	943,673			

a. Parameter = Rmax

General linear model R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,196	29,518	14	,009	,698	,864	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1277,138	5	255,428	,370	,868	,018
	Greenhouse-Geisser	1277,138	3,492	365,720	,370	,805	,018
Error(Visit)	Sphericity Assumed	69125,282	100	691,253			
	Greenhouse-Geisser	69125,282	69,842	989,733			

a. Parameter = Rmax

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,567	6,926	1,000	-23,633	22,500
	3	8,748	6,662	1,000	-13,443	30,938
	4	,871	8,472	1,000	-27,346	29,089
	5	,238	7,799	1,000	-25,736	26,212
	6	1,148	10,872	1,000	-35,064	37,359
2	1	,567	6,926	1,000	-22,500	23,633
	3	9,314	4,826	1,000	-6,760	25,388
	4	1,438	6,974	1,000	-21,789	24,665
	5	,805	8,925	1,000	-28,923	30,532
	6	1,714	8,308	1,000	-25,958	29,386
3	1	-8,748	6,662	1,000	-30,938	13,443
	2	-9,314	4,826	1,000	-25,388	6,760
	4	-7,876	6,682	1,000	-30,133	14,380
	5	-8,510	6,212	1,000	-29,201	12,182
	6	-7,600	8,544	1,000	-36,056	20,856
4	1	-,871	8,472	1,000	-29,089	27,346
	2	-1,438	6,974	1,000	-24,665	21,789
	3	7,876	6,682	1,000	-14,380	30,133
	5	-,633	7,744	1,000	-26,426	25,160
	6	,276	10,821	1,000	-35,763	36,316
5	1	-,238	7,799	1,000	-26,212	25,736
	2	-,805	8,925	1,000	-30,532	28,923
	3	8,510	6,212	1,000	-12,182	29,201
	4	,633	7,744	1,000	-25,160	26,426
	6	,910	9,510	1,000	-30,765	32,584
6	1	-1,148	10,872	1,000	-37,359	35,064
	2	-1,714	8,308	1,000	-29,386	25,958
	3	7,600	8,544	1,000	-20,856	36,056
	4	-,276	10,821	1,000	-36,316	35,763
	5	-,910	9,510	1,000	-32,584	30,765

Based on estimated marginal means
a. Parameter = Rmax
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,225	27,028	14	,020	,712	,885	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	5370,969	5	1074,194	1,611	,164	,075
	Greenhouse-Geisser	5370,969	3,561	1508,303	1,611	,187	,075
Error(Visit)	Sphericity Assumed	66673,063	100	666,731			
	Greenhouse-Geisser	66673,063	71,219	936,174			

a. Parameter = Rmax

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	5,786	9,450	1,000	-25,688	37,260
	3	10,890	8,734	1,000	-18,199	39,980
	4	7,386	8,225	1,000	-20,008	34,780
	5	3,971	8,038	1,000	-22,799	30,742
	6	-9,514	11,214	1,000	-46,864	27,836
2	1	-5,786	9,450	1,000	-37,260	25,688
	3	5,105	5,178	1,000	-12,140	22,349
	4	1,600	6,691	1,000	-20,686	23,886
	5	-1,814	7,027	1,000	-25,219	21,590
	6	-15,300	9,209	1,000	-45,973	15,373
3	1	-10,890	8,734	1,000	-39,980	18,199
	2	-5,105	5,178	1,000	-22,349	12,140
	4	-3,505	6,768	1,000	-26,045	19,036
	5	-6,919	4,419	1,000	-21,637	7,799
	6	-20,405	8,782	,462	-49,655	8,845
4	1	-7,386	8,225	1,000	-34,780	20,008
	2	-1,600	6,691	1,000	-23,886	20,686
	3	3,505	6,768	1,000	-19,036	26,045
	5	-3,414	5,685	1,000	-22,348	15,519
	6	-16,900	8,873	1,000	-46,453	12,653
5	1	-3,971	8,038	1,000	-30,742	22,799
	2	1,814	7,027	1,000	-21,590	25,219
	3	6,919	4,419	1,000	-7,799	21,637
	4	3,414	5,685	1,000	-15,519	22,348
	6	-13,486	8,344	1,000	-41,275	14,304
6	1	9,514	11,214	1,000	-27,836	46,864
	2	15,300	9,209	1,000	-15,373	45,973
	3	20,405	8,782	,462	-8,845	49,655
	4	16,900	8,873	1,000	-12,653	46,453
	5	13,486	8,344	1,000	-14,304	41,275

Based on estimated marginal means
a. Parameter = Rmax
b. Adjustment for multiple comparisons: Bonferroni.

T-Test maximum roughness (Rmax)

		Paired Differences							Significance	
		Mean	Std. Devi-ation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-24,73810	30,31385	6,61502	-38,53679	-10,93940	-3,740	20	<,001	,001

Pair 2	Week2R - Week2L	-18,38571	41,15741	8,98128	-37,12034	,34891	-2,047	20	,027	,054
Pair 3	Week4R - Week4L	-22,59524	36,14620	7,88775	-39,04879	-6,14169	-2,865	20	,005	,010
Pair 4	Week8R - Week8L	-18,22381	41,35940	9,02536	-37,05038	,60276	-2,019	20	,029	,057
Pair 5	Week12R - Week12L	-21,00476	38,86917	8,48195	-38,69780	-3,31173	-2,476	20	,011	,022
Pair 6	Week24R - Week24L	-35,40000	58,03293	12,66382	-61,81627	-8,98373	-2,795	20	,006	,011

a. Parameter = Rmax

Mean depth of roughness (Rz)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,939	21	,210
ScreeningL	,877	21	,013
Week2R	,881	21	,015
Week2L	,918	21	,078
Week4R	,962	21	,560
Week4L	,916	21	,073
Week8R	,952	21	,372
Week8L	,920	21	,088
Week12R	,947	21	,304
Week12L	,916	21	,071
Week24R	,944	21	,266
Week24L	,924	21	,107

General linear model RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,367	18,133	14	,204	,753	,949	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,328	20,169	14	,128	,720	,897	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	998,352	5	199,670	,765	,577	,037
	Greenhouse-Geisser	998,352	3,765	265,159	,765	,544	,037
Error(Visit)	Sphericity Assumed	26093,471	100	260,935			
	Greenhouse-Geisser	26093,471	75,302	346,516			
Side	Sphericity Assumed	10566,553	1	10566,553	13,030	,002	,394
	Greenhouse-Geisser	10566,553	1,000	10566,553	13,030	,002	,394
Error(Side)	Sphericity Assumed	16218,654	20	810,933			
	Greenhouse-Geisser	16218,654	20,000	810,933			
Visit * Side	Sphericity Assumed	820,380	5	164,076	,899	,485	,043
	Greenhouse-Geisser	820,380	3,599	227,925	,899	,461	,043
Error(Visit*Side)	Sphericity Assumed	18256,533	100	182,565			
	Greenhouse-Geisser	18256,533	71,987	253,609			

a. Parameter = Rz

General linear model R

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,431	15,228	14	,367	,806	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	958,775	5	191,755	,827	,533	,040
	Greenhouse-Geisser	958,775	4,032	237,810	,827	,513	,040
Error(Visit)	Sphericity Assumed	23188,215	100	231,882			
	Greenhouse-Geisser	23188,215	80,634	287,575			

a. Parameter = Rz

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,005	4,273	1,000	-14,237	14,228
	3	6,833	4,295	1,000	-7,472	21,139
	4	1,371	5,486	1,000	-16,900	19,643
	5	-,876	4,572	1,000	-16,102	14,350
	6	4,371	5,679	1,000	-14,543	23,286
2	1	,005	4,273	1,000	-14,228	14,237
	3	6,838	3,364	,834	-4,367	18,044
	4	1,376	4,519	1,000	-13,676	16,428
	5	-,871	5,455	1,000	-19,039	17,296
	6	4,376	4,872	1,000	-11,851	20,604
3	1	-6,833	4,295	1,000	-21,139	7,472
	2	-6,838	3,364	,834	-18,044	4,367
	4	-5,462	4,346	1,000	-19,935	9,012
	5	-7,710	4,034	1,000	-21,146	5,727
	6	-2,462	3,877	1,000	-15,375	10,451
4	1	-1,371	5,486	1,000	-19,643	16,900
	2	-1,376	4,519	1,000	-16,428	13,676
	3	5,462	4,346	1,000	-9,012	19,935
	5	-2,248	4,937	1,000	-18,690	14,195
	6	3,000	5,371	1,000	-14,887	20,887
5	1	,876	4,572	1,000	-14,350	16,102
	2	,871	5,455	1,000	-17,296	19,039
	3	7,710	4,034	1,000	-5,727	21,146
	4	2,248	4,937	1,000	-14,195	18,690
	6	5,248	4,761	1,000	-10,611	21,106
6	1	-4,371	5,679	1,000	-23,286	14,543
	2	-4,376	4,872	1,000	-20,604	11,851
	3	2,462	3,877	1,000	-10,451	15,375
	4	-3,000	5,371	1,000	-20,887	14,887
	5	-5,248	4,761	1,000	-21,106	10,611

Based on estimated marginal means
a. Parameter = Rz
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,483	13,153	14	,518	,827	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	859,958	5	171,992	,813	,543	,039
	Greenhouse-Geisser	859,958	4,135	207,949	,813	,524	,039
Error(Visit)	Sphericity Assumed	21161,789	100	211,618			
	Greenhouse-Geisser	21161,789	82,709	255,859			

a. Parameter = Rz

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	1,514	4,694	1,000	-14,121	17,149
	3	3,848	4,724	1,000	-11,886	19,581
	4	1,490	5,237	1,000	-15,951	18,932
	5	-,238	4,860	1,000	-16,425	15,949
	6	-4,710	5,700	1,000	-23,694	14,275
2	1	-1,514	4,694	1,000	-17,149	14,121
	3	2,333	2,845	1,000	-7,142	11,809
	4	-,024	3,925	1,000	-13,098	13,050
	5	-1,752	4,386	1,000	-16,360	12,855
	6	-6,224	4,751	1,000	-22,046	9,599
3	1	-3,848	4,724	1,000	-19,581	11,886
	2	-2,333	2,845	1,000	-11,809	7,142
	4	-2,357	3,916	1,000	-15,399	10,685
	5	-4,086	3,445	1,000	-15,560	7,389
	6	-8,557	4,728	1,000	-24,304	7,190
4	1	-1,490	5,237	1,000	-18,932	15,951
	2	,024	3,925	1,000	-13,050	13,098
	3	2,357	3,916	1,000	-10,685	15,399
	5	-1,729	3,950	1,000	-14,884	11,426
	6	-6,200	4,752	1,000	-22,028	9,628
5	1	,238	4,860	1,000	-15,949	16,425
	2	1,752	4,386	1,000	-12,855	16,360
	3	4,086	3,445	1,000	-7,389	15,560
	4	1,729	3,950	1,000	-11,426	14,884
	6	-4,471	4,625	1,000	-19,874	10,931
6	1	4,710	5,700	1,000	-14,275	23,694
	2	6,224	4,751	1,000	-9,599	22,046
	3	8,557	4,728	1,000	-7,190	24,304
	4	6,200	4,752	1,000	-9,628	22,028
	5	4,471	4,625	1,000	-10,931	19,874

Based on estimated marginal means
a. Parameter = Rz
b. Adjustment for multiple comparisons: Bonferroni.

T-Test mean depth of roughness (Rz)

		Paired Differences							Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-11,31905	18,81403	4,10556	-19,88309	-2,75501	-2,757	20	,006	,012
Pair 2	Week2R - Week2L	-9,80000	21,55474	4,70363	-19,61160	,01160	-2,083	20	,025	,050
Pair 3	Week4R - Week4L	-14,30476	21,20525	4,62736	-23,95727	-4,65225	-3,091	20	,003	,006
Pair 4	Week8R - Week8L	-11,20000	24,10618	5,26040	-22,17300	-,22700	-2,129	20	,023	,046
Pair 5	Week12R - Week12L	-10,68095	26,01868	5,67774	-22,52451	1,16261	-1,881	20	,037	,075
Pair 6	Week24R - Week24L	-20,40000	30,35131	6,62320	-34,21575	-6,58425	-3,080	20	,003	,006

a. Parameter = Rz

Maximum profile peak (Rp)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,976	21	,855
ScreeningL	,924	21	,104
Week2R	,946	21	,292

Week2L	,914	21	,065
Week4R	,971	21	,765
Week4L	,964	21	,603
Week8R	,916	21	,072
Week8L	,933	21	,157
Week12R	,937	21	,194
Week12L	,934	21	,164
Week24R	,850	21	,004
Week24L	,941	21	,226

General linear model RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,245	25,468	14	,031	,706	,876	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,273	23,467	14	,055	,731	,915	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1266,739	5	253,348	,951	,452	,045
	Greenhouse-Geisser	1266,739	3,530	358,807	,951	,432	,045
Error(Visit)	Sphericity Assumed	26638,107	100	266,381			
	Greenhouse-Geisser	26638,107	70,608	377,265			
Side	Sphericity Assumed	22933,397	1	22933,397	44,342	<,001	,689
	Greenhouse-Geisser	22933,397	1,000	22933,397	44,342	<,001	,689
Error(Side)	Sphericity Assumed	10343,873	20	517,194			
	Greenhouse-Geisser	10343,873	20,000	517,194			
Visit * Side	Sphericity Assumed	997,968	5	199,594	,977	,435	,047
	Greenhouse-Geisser	997,968	3,657	272,880	,977	,420	,047
Error(Visit*Side)	Sphericity Assumed	20418,972	100	204,190			
	Greenhouse-Geisser	20418,972	73,143	279,163			

a. Parameter = Rp

General linear model R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,209	28,291	14	,014	,682	,840	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	425,108	5	85,022	,389	,855	,019
	Greenhouse-Geisser	425,108	3,411	124,618	,389	,786	,019
Error(Visit)	Sphericity Assumed	21844,157	100	218,442			
	Greenhouse-Geisser	21844,157	68,226	320,175			

a. Parameter = Rp

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-3,248	3,307	1,000	-14,261	7,766
	3	2,638	3,444	1,000	-8,831	14,107
	4	-1,995	4,565	1,000	-17,198	13,207
	5	-,295	4,565	1,000	-15,501	14,910
	6	-1,229	5,825	1,000	-20,628	18,171

2	1	3,248	3,307	1,000	-7,766	14,261
	3	5,886	3,119	1,000	-4,503	16,275
	4	1,252	4,507	1,000	-13,760	16,265
	5	2,952	5,241	1,000	-14,503	20,408
	6	2,019	4,536	1,000	-13,090	17,128
3	1	-2,638	3,444	1,000	-14,107	8,831
	2	-5,886	3,119	1,000	-16,275	4,503
	4	-4,633	3,990	1,000	-17,924	8,657
	5	-2,933	3,436	1,000	-14,377	8,510
	6	-3,867	4,683	1,000	-19,464	11,731
4	1	1,995	4,565	1,000	-13,207	17,198
	2	-1,252	4,507	1,000	-16,265	13,760
	3	4,633	3,990	1,000	-8,657	17,924
	5	1,700	4,213	1,000	-12,331	15,731
	6	,767	6,165	1,000	-19,765	21,298
5	1	,295	4,565	1,000	-14,910	15,501
	2	-2,952	5,241	1,000	-20,408	14,503
	3	2,933	3,436	1,000	-8,510	14,377
	4	-1,700	4,213	1,000	-15,731	12,331
	6	-,933	5,492	1,000	-19,226	17,360
6	1	1,229	5,825	1,000	-18,171	20,628
	2	-2,019	4,536	1,000	-17,128	13,090
	3	3,867	4,683	1,000	-11,731	19,464
	4	-,767	6,165	1,000	-21,298	19,765
	5	,933	5,492	1,000	-17,360	19,226

Based on estimated marginal means
a. Parameter = Rp
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,336	19,738	14	,142	,749	,942	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1839,599	5	367,920	1,459	,210	,068
	Greenhouse-Geisser	1839,599	3,743	491,439	1,459	,226	,068
Error(Visit)	Sphericity Assumed	25212,922	100	252,129			
	Greenhouse-Geisser	25212,922	74,866	336,775			

a. Parameter = Rp

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	5,852	4,222	1,000	-8,208	19,913
	3	7,090	4,364	1,000	-7,444	21,625
	4	4,814	5,103	1,000	-12,183	21,811
	5	5,229	5,386	1,000	-12,709	23,166
	6	-3,762	6,059	1,000	-23,942	16,418
2	1	-5,852	4,222	1,000	-19,913	8,208
	3	1,238	3,253	1,000	-9,596	12,072
	4	-1,038	4,711	1,000	-16,728	14,652
	5	-,624	5,166	1,000	-17,830	16,582
	6	-9,614	6,035	1,000	-29,716	10,487
3	1	-7,090	4,364	1,000	-21,625	7,444
	2	-1,238	3,253	1,000	-12,072	9,596
	4	-2,276	4,541	1,000	-17,401	12,848

	5	-1,862	3,701	1,000	-14,188	10,464
	6	-10,852	5,531	,957	-29,273	7,568
4	1	-4,814	5,103	1,000	-21,811	12,183
	2	1,038	4,711	1,000	-14,652	16,728
	3	2,276	4,541	1,000	-12,848	17,401
	5	,414	3,891	1,000	-12,546	13,375
	6	-8,576	4,903	1,000	-24,907	7,754
5	1	-5,229	5,386	1,000	-23,166	12,709
	2	,624	5,166	1,000	-16,582	17,830
	3	1,862	3,701	1,000	-10,464	14,188
	4	-,414	3,891	1,000	-13,375	12,546
	6	-8,990	5,620	1,000	-27,710	9,729
6	1	3,762	6,059	1,000	-16,418	23,942
	2	9,614	6,035	1,000	-10,487	29,716
	3	10,852	5,531	,957	-7,568	29,273
	4	8,576	4,903	1,000	-7,754	24,907
	5	8,990	5,620	1,000	-9,729	27,710

Based on estimated marginal means
a. Parameter = Rp
b. Adjustment for multiple comparisons: Bonferroni.

T-Test maximum profile peak (Rp)

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-22,97143	15,73061	3,43270	-30,13192	-15,81094	-6,692	20	<,001	<,001
Pair 2	Week2R - Week2L	-13,87143	16,85696	3,67849	-21,54463	-6,19823	-3,771	20	<,001	,001
Pair 3	Week4R - Week4L	-18,51905	17,08647	3,72857	-26,29672	-10,74138	-4,967	20	<,001	<,001
Pair 4	Week8R - Week8L	-16,16190	27,37699	5,97415	-28,62376	-3,70005	-2,705	20	,007	,014
Pair 5	Week12R - Week12L	-17,44762	25,05280	5,46697	-28,85152	-6,04372	-3,191	20	,002	,005
Pair 6	Week24R - Week24L	-25,50476	29,59029	6,45713	-38,97410	-12,03542	-3,950	20	<,001	<,001

a. Parameter = Rp

Waviness (Wt)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,700	21	,000
ScreeningL	,672	21	,000
Week2R	,712	21	,000
Week2L	,697	21	,000
Week4R	,728	21	,000
Week4L	,692	21	,000
Week8R	,736	21	,000
Week8L	,652	21	,000
Week12R	,789	21	,000
Week12L	,622	21	,000
Week24R	,723	21	,000
Week24L	,690	21	,000

General linear model RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,534	11,365	14	,660	,822	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,289	22,489	14	,071	,696	,861	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	4158,199	5	831,640	1,092	,370	,052
	Greenhouse-Geisser	4158,199	4,111	1011,445	1,092	,367	,052
Error(Visit)	Sphericity Assumed	76149,581	100	761,496			
	Greenhouse-Geisser	76149,581	82,223	926,135			
Side	Sphericity Assumed	32064,979	1	32064,979	5,520	,029	,216
	Greenhouse-Geisser	32064,979	1,000	32064,979	5,520	,029	,216
Error(Side)	Sphericity Assumed	116180,251	20	5809,013			
	Greenhouse-Geisser	116180,251	20,000	5809,013			
Visit * Side	Sphericity Assumed	5324,268	5	1064,854	1,698	,142	,078
	Greenhouse-Geisser	5324,268	3,482	1529,128	1,698	,168	,078
Error(Visit*Side)	Sphericity Assumed	62698,962	100	626,990			
	Greenhouse-Geisser	62698,962	69,638	900,356			

a. Parameter = Wt

General linear model R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,175	31,537	14	,005	,536	,626	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	7633,485	5	1526,697	3,513	,006	,149
	Greenhouse-Geisser	7633,485	2,679	2849,899	3,513	,025	,149
Error(Visit)	Sphericity Assumed	43452,910	100	434,529			
	Greenhouse-Geisser	43452,910	53,570	811,139			

a. Parameter = Wt

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-4,086	4,834	1,000	-20,188	12,016
	3	4,376	3,849	1,000	-8,444	17,196
	4	-5,000	5,539	1,000	-23,448	13,448
	5	-20,186	8,776	,485	-49,417	9,045
	6	-,186	4,863	1,000	-16,381	16,010
2	1	4,086	4,834	1,000	-12,016	20,188
	3	8,462	4,396	1,000	-6,178	23,102
	4	-,914	5,234	1,000	-18,349	16,520
	5	-16,100	8,690	1,000	-45,043	12,843
	6	3,900	5,392	1,000	-14,059	21,859
3	1	-4,376	3,849	1,000	-17,196	8,444
	2	-8,462	4,396	1,000	-23,102	6,178
	4	-9,376	4,680	,883	-24,962	6,210
	5	-24,562	8,489	,135	-52,837	3,713
	6	-4,562	3,711	1,000	-16,923	7,799
4	1	5,000	5,539	1,000	-13,448	23,448
	2	,914	5,234	1,000	-16,520	18,349
	3	9,376	4,680	,883	-6,210	24,962
	5	-15,186	9,342	1,000	-46,299	15,927
	6	4,814	4,794	1,000	-11,153	20,781
5	1	20,186	8,776	,485	-9,045	49,417
	2	16,100	8,690	1,000	-12,843	45,043
	3	24,562	8,489	,135	-3,713	52,837

	4	15,186	9,342	1,000	-15,927	46,299
	6	20,000	9,051	,584	-10,146	50,146
6	1	,186	4,863	1,000	-16,010	16,381
	2	-3,900	5,392	1,000	-21,859	14,059
	3	4,562	3,711	1,000	-7,799	16,923
	4	-4,814	4,794	1,000	-20,781	11,153
	5	-20,000	9,051	,584	-50,146	10,146

Based on estimated marginal means
a. Parameter = Wt
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,217	27,633	14	,017	,646	,785	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1848,983	5	369,797	,388	,856	,019
	Greenhouse-Geisser	1848,983	3,228	572,738	,388	,777	,019
Error(Visit)	Sphericity Assumed	95395,632	100	953,956			
	Greenhouse-Geisser	95395,632	64,566	1477,480			

a. Parameter = Wt

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	2,276	12,475	1,000	-39,275	43,827
	3	5,714	11,610	1,000	-32,953	44,382
	4	-1,962	12,315	1,000	-42,978	39,055
	5	3,390	10,671	1,000	-32,152	38,933
	6	-6,062	12,973	1,000	-49,270	37,146
2	1	-2,276	12,475	1,000	-43,827	39,275
	3	3,438	9,142	1,000	-27,012	33,888
	4	-4,238	7,940	1,000	-30,684	22,208
	5	1,114	6,182	1,000	-19,474	21,703
	6	-8,338	8,220	1,000	-35,717	19,041
3	1	-5,714	11,610	1,000	-44,382	32,953
	2	-3,438	9,142	1,000	-33,888	27,012
	4	-7,676	10,711	1,000	-43,352	28,000
	5	-2,324	7,661	1,000	-27,841	23,193
	6	-11,776	7,683	1,000	-37,365	13,812
4	1	1,962	12,315	1,000	-39,055	42,978
	2	4,238	7,940	1,000	-22,208	30,684
	3	7,676	10,711	1,000	-28,000	43,352
	5	5,352	6,054	1,000	-14,811	25,516
	6	-4,100	8,091	1,000	-31,050	22,850
5	1	-3,390	10,671	1,000	-38,933	32,152
	2	-1,114	6,182	1,000	-21,703	19,474
	3	2,324	7,661	1,000	-23,193	27,841
	4	-5,352	6,054	1,000	-25,516	14,811
	6	-9,452	7,181	1,000	-33,368	14,463
6	1	6,062	12,973	1,000	-37,146	49,270
	2	8,338	8,220	1,000	-19,041	35,717
	3	11,776	7,683	1,000	-13,812	37,365
	4	4,100	8,091	1,000	-22,850	31,050
	5	9,452	7,181	1,000	-14,463	33,368

Based on estimated marginal means
a. Parameter = Wt

b. Adjustment for multiple comparisons: Bonferroni.

T-Test waviness (Wt)

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-27,30000	46,08030	10,05555	-48,27550	-6,32450	-2,715	20	,007	,013
Pair 2	Week2R - Week2L	-20,93810	55,54160	12,12017	-46,22033	4,34414	-1,728	20	,050	,099
Pair 3	Week4R - Week4L	-25,96190	49,80815	10,86903	-48,63430	-3,28951	-2,389	20	,013	,027
Pair 4	Week8R - Week8L	-24,26190	59,68849	13,02510	-51,43178	2,90797	-1,863	20	,039	,077
Pair 5	Week12R - Week12L	-3,72381	52,25315	11,40257	-27,50916	20,06154	-,327	20	,374	,747
Pair 6	Week24R - Week24L	-33,17619	62,49559	13,63766	-61,62384	-4,72854	-2,433	20	,012	,024

a. Parameter = Wt

Number of peaks (PC)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,700	21	,000
ScreeningL	,672	21	,000
Week2R	,712	21	,000
Week2L	,697	21	,000
Week4R	,728	21	,000
Week4L	,692	21	,000
Week8R	,736	21	,000
Week8L	,652	21	,000
Week12R	,789	21	,000
Week12L	,622	21	,000
Week24R	,723	21	,000
Week24L	,690	21	,000

General linear model RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,431	15,231	14	,367	,778	,989	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,366	18,188	14	,202	,720	,897	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,687	5	,137	,155	,978	,008
	Greenhouse-Geisser	,687	3,888	,177	,155	,957	,008
Error(Visit)	Sphericity Assumed	88,397	100	,884			
	Greenhouse-Geisser	88,397	77,763	1,137			
Side	Sphericity Assumed	14,766	1	14,766	5,681	,027	,221
	Greenhouse-Geisser	14,766	1,000	14,766	5,681	,027	,221
Error(Side)	Sphericity Assumed	51,984	20	2,599			
	Greenhouse-Geisser	51,984	20,000	2,599			
Visit * Side	Sphericity Assumed	3,353	5	,671	,734	,600	,035
	Greenhouse-Geisser	3,353	3,599	,932	,734	,558	,035
Error(Visit*Side)	Sphericity Assumed	91,397	100	,914			
	Greenhouse-Geisser	91,397	71,971	1,270			

a. Parameter = PC

General linear model R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,502	12,467	14	,573	,798	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1,429	5	,286	,313	,904	,015
	Greenhouse-Geisser	1,429	3,988	,358	,313	,868	,015
Error(Visit)	Sphericity Assumed	91,238	100	,912			
	Greenhouse-Geisser	91,238	79,753	1,144			

a. Parameter = PC

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,286	,302	1,000	-,720	1,291
	3	,143	,270	1,000	-,756	1,041
	4	-,048	,305	1,000	-1,062	,967
	5	,095	,344	1,000	-1,052	1,242
	6	,095	,300	1,000	-,904	1,095
2	1	-,286	,302	1,000	-1,291	,720
	3	-,143	,252	1,000	-,981	,695
	4	-,333	,319	1,000	-1,395	,728
	5	-,190	,255	1,000	-1,039	,658
	6	-,190	,281	1,000	-1,127	,746
3	1	-,143	,270	1,000	-1,041	,756
	2	,143	,252	1,000	-,695	,981
	4	-,190	,335	1,000	-1,307	,927
	5	-,048	,312	1,000	-1,088	,992
	6	-,048	,327	1,000	-1,137	1,042
4	1	,048	,305	1,000	-,967	1,062
	2	,333	,319	1,000	-,728	1,395
	3	,190	,335	1,000	-,927	1,307
	5	,143	,311	1,000	-,892	1,178
	6	,143	,261	1,000	-,726	1,012
5	1	-,095	,344	1,000	-1,242	1,052
	2	,190	,255	1,000	-,658	1,039
	3	,048	,312	1,000	-,992	1,088
	4	-,143	,311	1,000	-1,178	,892
	6	,000	,218	1,000	-,727	,727
6	1	-,095	,300	1,000	-1,095	,904
	2	,190	,281	1,000	-,746	1,127
	3	,048	,327	1,000	-1,042	1,137
	4	-,143	,261	1,000	-1,012	,726
	5	,000	,218	1,000	-,727	,727

Based on estimated marginal means
a. Parameter = PC
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,410	16,138	14	,309	,759	,959	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	2,611	5	,522	,590	,708	,029
	Greenhouse-Geisser	2,611	3,794	,688	,590	,662	,029
Error(Visit)	Sphericity Assumed	88,556	100	,886			
	Greenhouse-Geisser	88,556	75,880	1,167			

a. Parameter = PC

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,095	,358	1,000	-1,287	1,097
	3	-,238	,351	1,000	-1,408	,932
	4	,238	,238	1,000	-,555	1,031
	5	,048	,288	1,000	-,913	1,008
	6	,000	,338	1,000	-1,126	1,126
2	1	,095	,358	1,000	-1,097	1,287
	3	-,143	,295	1,000	-1,126	,840
	4	,333	,326	1,000	-,753	1,419
	5	,143	,252	1,000	-,695	,981
	6	,095	,248	1,000	-,730	,921
3	1	,238	,351	1,000	-,932	1,408
	2	,143	,295	1,000	-,840	1,126
	4	,476	,245	,994	-,340	1,293
	5	,286	,302	1,000	-,720	1,291
	6	,238	,284	1,000	-,707	1,183
4	1	-,238	,238	1,000	-1,031	,555
	2	-,333	,326	1,000	-1,419	,753
	3	-,476	,245	,994	-1,293	,340
	5	-,190	,235	1,000	-,974	,593
	6	-,238	,284	1,000	-1,183	,707
5	1	-,048	,288	1,000	-1,008	,913
	2	-,143	,252	1,000	-,981	,695
	3	-,286	,302	1,000	-1,291	,720
	4	,190	,235	1,000	-,593	,974
	6	-,048	,271	1,000	-,952	,857
6	1	,000	,338	1,000	-1,126	1,126
	2	-,095	,248	1,000	-,921	,730
	3	-,238	,284	1,000	-1,183	,707
	4	,238	,284	1,000	-,707	1,183
	5	,048	,271	1,000	-,857	,952

Based on estimated marginal means
a. Parameter = PC
b. Adjustment for multiple comparisons: Bonferroni.

T-Test number of peaks (PC)

		Paired Differences							Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,38095	1,39557	,30454	-1,01621	,25430	-1,251	20	,113	,225
Pair 2	Week2R - Week2L	-,76190	1,51343	,33026	-1,45081	-,07300	-2,307	20	,016	,032
Pair 3	Week4R - Week4L	-,76190	1,86828	,40769	-1,61234	,08853	-1,869	20	,038	,076
Pair 4	Week8R - Week8L	-,09524	1,54612	,33739	-,79902	,60855	-,282	20	,390	,781
Pair 5	Week12R - Week12L	-,42857	1,43427	,31298	-1,08145	,22430	-1,369	20	,093	,186
Pair 6	Week24R - Week24L	-,47619	1,47034	,32085	-1,14548	,19310	-1,484	20	,077	,153

a. Parameter = PC

ACS in vivo study II data and statistical analyses

Patient data and skin condition

Pateint-number	Treatment	Age	Skin condition				
			normal	dry	oily	not sensitive	sensitive
1	ACS+HA	53	x			x	
2	ACS+HA	55		x			x
3	ACS+HA	53		x			x
5	ACS	38		x			x
6	ACS	36		x			x
7	ACS	35	x			x	
8	ACS	43		x			x
9	ACS	42		x			x
10	ACS+HA	56		x		x	
13	ACS+HA	64		x			x
14	ACS	63		x			x
15	ACS+HA	55	x			x	
17	ACS	61		x			x
19	ACS	60		x			x
20	ACS	49		x			x
21	ACS	61	x			x	

Corneometry – skin hydration

Corneometer data, mean of three measurements

Patient	Treatment	ScreeningR	ScreeningL	Week12R	Week12L	Week24R	Week24L
5	ACS	53.133	58.100	41.667	44.800	31.433	45.000
6	ACS	58.967	64.200	39.933	50.100	56.567	57.167
7	ACS	23.400	18.867	43.033	43.467	40.467	37.700
8	ACS	28.033	30.700	31.700	40.533	44.833	40.867
9	ACS	35.933	34.667	49.533	55.000	39.500	43.900
14	ACS	29.833	32.333	22.200	26.700	22.667	25.000
17	ACS	47.567	41.633	56.433	44.733	46.300	36.933
19	ACS	59.767	52.133	38.233	43.767	43.267	47.000
20	ACS	46.033	40.967	57.933	70.200	56.133	54.800
21	ACS	37.067	38.267	34.633	36.067	43.733	37.400
mean		41.973	41.187	41.530	45.537	42.490	42.577
SD		13.017	13.635	10.999	11.532	10.191	9.332

Patient	Treatment	ScreeningR	ScreeningL	Week12R	Week12L	Week24R	Week24L
1	ACS + HA	48.000	45.300	51.167	41.067	52.100	66.867
2	ACS + HA	39.700	45.100	37.467	39.900	50.467	40.733
3	ACS + HA	33.667	35.600	66.833	65.700	57.433	55.233
10	ACS + HA	38.933	36.433	41.900	43.767	47.567	47.500
13	ACS + HA	53.500	36.000	30.133	21.333	38.400	37.233
15	ACS + HA	31.467	22.567	43.067	39.133	41.800	46.300
mean		40.878	36.833	45.094	41.817	47.961	48.978
SD		8.435	8.318	12.695	14.179	6.967	10.718

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	ACS	,938	10	,532
	ACS + HA	,937	6	,635
ScreeningL	ACS	,967	10	,864
	ACS + HA	,867	6	,216
Week12R	ACS	,970	10	,886
	ACS + HA	,942	6	,673

Week12L	ACS	,934	10	,491
	ACS + HA	,891	6	,324
Week24R	ACS	,937	10	,515
	ACS + HA	,975	6	,927
Week24L	ACS	,958	10	,768
	ACS + HA	,939	6	,655

General linear model ACS RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,552	4,754	2	,093	,691	,775	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,981	,157	2	,924	,981	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	38,162	2	19,081	,141	,870	,015
	Greenhouse-Geisser	38,162	1,381	27,630	,141	,793	,015
Error(Visit)	Sphericity Assumed	2442,547	18	135,697			
	Greenhouse-Geisser	2442,547	12,431	196,493			
Side	Sphericity Assumed	18,223	1	18,223	,593	,461	,062
	Greenhouse-Geisser	18,223	1,000	18,223	,593	,461	,062
Error(Side)	Sphericity Assumed	276,809	9	30,757			
	Greenhouse-Geisser	276,809	9,000	30,757			
Visit * Side	Sphericity Assumed	65,175	2	32,588	2,757	,090	,235
	Greenhouse-Geisser	65,175	1,962	33,223	2,757	,092	,235
Error(Visit*Side)	Sphericity Assumed	212,736	18	11,819			
	Greenhouse-Geisser	212,736	17,656	12,049			

a. Treatment = ACS

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-1,953	4,647	1,000	-15,586	11,679
	3	-,953	3,638	1,000	-11,624	9,717
2	1	1,953	4,647	1,000	-11,679	15,586
	3	1,000	2,424	1,000	-6,111	8,111
3	1	,953	3,638	1,000	-9,717	11,624
	2	-1,000	2,424	1,000	-8,111	6,111

Based on estimated marginal means
a. Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model ACS + HA RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,208	6,281	2	,043	,558	,605	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,520	2,612	2	,271	,676	,838	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	554,904	2	277,452	1,770	,220	,261

	Greenhouse-Geisser	554,904	1,116	497,200	1,770	,239	,261
Error(Visit)	Sphericity Assumed	1567,958	10	156,796			
	Greenhouse-Geisser	1567,958	5,580	280,981			
Side	Sphericity Assumed	39,760	1	39,760	2,004	,216	,286
	Greenhouse-Geisser	39,760	1,000	39,760	2,004	,216	,286
Error(Side)	Sphericity Assumed	99,205	5	19,841			
	Greenhouse-Geisser	99,205	5,000	19,841			
Visit * Side	Sphericity Assumed	44,645	2	22,322	,732	,505	,128
	Greenhouse-Geisser	44,645	1,352	33,026	,732	,463	,128
Error(Visit*Side)	Sphericity Assumed	304,768	10	30,477			
	Greenhouse-Geisser	304,768	6,759	45,091			

a. Treatment = ACS + HA

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-4,600	7,010	1,000	-29,375	20,175
	3	-9,614	4,189	,211	-24,419	5,191
2	1	4,600	7,010	1,000	-20,175	29,375
	3	-5,014	3,421	,608	-17,105	7,077
3	1	9,614	4,189	,211	-5,191	24,419
	2	5,014	3,421	,608	-7,077	17,105

Based on estimated marginal means
a. Treatment = ACS + HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test RL ACS vs ACS + HA

Group Statistics

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Screening	ACS	10	41,5800	13,11754	4,14813
	ACS + HA	6	38,8556	7,31330	2,98564
Week12	ACS	10	43,5333	10,76273	3,40347
	ACS + HA	6	43,4556	13,19254	5,38583
Week24	ACS	10	42,5333	9,22078	2,91587
	ACS + HA	6	48,4694	8,06560	3,29277

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Screening	Equal variances assumed	2,656	,125	,463	14	,325	,650	2,72444	5,88145	-9,89001	15,33890
	Equal variances not assumed			,533	13,985	,301	,602	2,72444	5,11087	-8,23842	13,68731
Week12	Equal variances assumed	,050	,826	,013	14	,495	,990	,07778	6,03599	-12,86814	13,02369
	Equal variances not assumed			,012	8,994	,495	,991	,07778	6,37109	-14,33612	14,49168
Week24	Equal variances assumed	,007	,934	-1,302	14	,107	,214	-5,93611	4,55751	-15,71101	3,83878
	Equal variances not assumed			-1,350	11,863	,101	,202	-5,93611	4,39825	-15,53132	3,65909

Cutometry – mechanical properties of the skin

Means and SDs

Mean 2 mm	R0		R2		R3		R5		R6		R7		R8		R9	
Treatment	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA
n	10	6	10	6	10	6	10	6	10	6	10	6	10	6	10	6
ScreeningR2	0.385	0.390	0.751	0.685	0.430	0.437	0.357	0.311	0.386	0.433	0.256	0.217	0.288	0.271	0.045	0.047
Week12R2	0.283	0.283	0.777	0.685	0.323	0.324	0.462	0.352	0.503	0.474	0.307	0.239	0.220	0.192	0.040	0.041
Week24R2	0.371	0.405	0.708	0.671	0.416	0.452	0.347	0.306	0.408	0.364	0.248	0.224	0.266	0.271	0.044	0.047
ScreeningL2	0.371	0.391	0.769	0.724	0.415	0.439	0.385	0.337	0.401	0.419	0.274	0.242	0.286	0.290	0.044	0.048
Week12L2	0.278	0.311	0.773	0.714	0.316	0.346	0.491	0.361	0.526	0.474	0.321	0.245	0.216	0.222	0.038	0.036
Week24L2	0.361	0.403	0.751	0.635	0.406	0.453	0.385	0.294	0.423	0.404	0.271	0.210	0.273	0.260	0.045	0.049
SD																
ScreeningR2	0.085	0.098	0.097	0.082	0.090	0.104	0.132	0.052	0.079	0.160	0.090	0.025	0.074	0.088	0.008	0.007
Week12R2	0.048	0.053	0.071	0.082	0.049	0.056	0.118	0.118	0.159	0.031	0.068	0.079	0.044	0.033	0.007	0.008
Week24R2	0.067	0.034	0.096	0.081	0.064	0.037	0.087	0.073	0.129	0.075	0.067	0.053	0.069	0.037	0.008	0.009
ScreeningL2	0.072	0.074	0.049	0.124	0.075	0.076	0.070	0.064	0.068	0.162	0.048	0.062	0.059	0.092	0.006	0.007
Week12L2	0.037	0.039	0.067	0.089	0.040	0.039	0.125	0.079	0.092	0.045	0.077	0.051	0.039	0.043	0.005	0.011
Week24L2	0.058	0.057	0.083	0.112	0.063	0.056	0.106	0.032	0.122	0.097	0.071	0.027	0.062	0.072	0.008	0.004

Mean 4 mm	R0		R2		R3		R5		R6		R7		R8		R9	
Treatment	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA
n	10	6	10	6	10	6	10	6	10	6	10	6	10	6	10	6
ScreeningR4	0.716	0.839	0.739	0.709	0.786	0.917	0.564	0.472	0.465	0.412	0.388	0.335	0.534	0.600	0.069	0.078
Week12R4	0.505	0.603	0.847	0.831	0.580	0.676	0.863	0.770	0.716	0.670	0.504	0.464	0.430	0.503	0.075	0.073
Week24R4	0.737	0.725	0.712	0.744	0.820	0.810	0.516	0.553	0.439	0.501	0.361	0.369	0.535	0.539	0.084	0.085
ScreeningL4	0.740	0.832	0.718	0.703	0.816	0.910	0.544	0.499	0.430	0.440	0.384	0.348	0.538	0.585	0.076	0.078
Week12L4	0.489	0.620	0.840	0.832	0.569	0.703	0.922	0.764	0.754	0.702	0.531	0.453	0.416	0.517	0.080	0.083
Week24L4	0.742	0.729	0.729	0.727	0.820	0.818	0.561	0.557	0.441	0.509	0.391	0.368	0.548	0.526	0.078	0.090
SD																
ScreeningR4	0.129	0.207	0.121	0.077	0.133	0.210	0.127	0.077	0.104	0.046	0.092	0.058	0.148	0.176	0.016	0.026
Week12R4	0.126	0.103	0.055	0.065	0.134	0.112	0.121	0.120	0.176	0.217	0.063	0.068	0.118	0.107	0.011	0.014
Week24R4	0.135	0.131	0.113	0.102	0.138	0.138	0.085	0.120	0.081	0.147	0.070	0.078	0.160	0.124	0.020	0.023
ScreeningL4	0.134	0.118	0.108	0.073	0.131	0.118	0.108	0.118	0.091	0.069	0.083	0.087	0.148	0.106	0.018	0.016
Week12L4	0.125	0.083	0.078	0.063	0.120	0.097	0.115	0.170	0.187	0.226	0.083	0.106	0.130	0.087	0.018	0.023
Week24L4	0.091	0.120	0.107	0.081	0.095	0.107	0.118	0.110	0.067	0.154	0.090	0.058	0.138	0.090	0.012	0.020

Skin firmness (R0, Uf)

Test of normality – Shapiro-Wilk

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR2	ACS	,853	10	,062
	ACS+HA	,876	6	,249
ScreeningL2	ACS	,944	10	,602
	ACS+HA	,762	6	,026
Week12R2	ACS	,962	10	,811
	ACS+HA	,864	6	,203
Week12L2	ACS	,953	10	,704
	ACS+HA	,962	6	,836
Week24R2	ACS	,892	10	,180
	ACS+HA	,930	6	,580
Week24L2	ACS	,823	10	,028
	ACS+HA	,996	6	,998

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR4	ACS	,938	10	,529
	ACS+HA	,864	6	,202
ScreeningL4	ACS	,930	10	,444
	ACS+HA	,944	6	,695
Week12R4	ACS	,958	10	,760
	ACS+HA	,944	6	,690
Week12L4	ACS	,949	10	,661
	ACS+HA	,898	6	,360
Week24R4	ACS	,959	10	,779
	ACS+HA	,946	6	,704
Week24L4	ACS	,940	10	,550
	ACS+HA	,782	6	,040

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,809	1,698	2	,428	,839	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,958	,339	2	,844	,960	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,113	2	,057	18,524	<,001
	Greenhouse-Geisser	,113	1,679	,067	18,524	<,001
Error(Visit)	Sphericity Assumed	,055	18	,003		
	Greenhouse-Geisser	,055	15,110	,004		
Side	Sphericity Assumed	,001	1	,001	1,172	,307
	Greenhouse-Geisser	,001	1,000	,001	1,172	,307
Error(Side)	Sphericity Assumed	,011	9	,001		
	Greenhouse-Geisser	,011	9,000	,001		
Visit * Side	Sphericity Assumed	,000	2	,000	,296	,748
	Greenhouse-Geisser	,000	1,920	,000	,296	,739
Error(Visit*Side)	Sphericity Assumed	,006	18	,000		
	Greenhouse-Geisser	,006	17,282	,000		

a. Parameter = R0, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,810	,841	2	,657	,841	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,791	,938	2	,626	,827	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,082	2	,041	6,848	,013
	Greenhouse-Geisser	,082	1,681	,049	6,848	,020
Error(Visit)	Sphericity Assumed	,060	10	,006		
	Greenhouse-Geisser	,060	8,406	,007		
Side	Sphericity Assumed	,001	1	,001	,391	,559
	Greenhouse-Geisser	,001	1,000	,001	,391	,559
Error(Side)	Sphericity Assumed	,009	5	,002		
	Greenhouse-Geisser	,009	5,000	,002		
Visit * Side	Sphericity Assumed	,002	2	,001	1,102	,369
	Greenhouse-Geisser	,002	1,654	,001	1,102	,363
Error(Visit*Side)	Sphericity Assumed	,007	10	,001		
	Greenhouse-Geisser	,007	8,272	,001		

a. Parameter = R0, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visits	,824	1,550	2	,461	,850	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,061	2	,031	15,332	<,001	,630
	Greenhouse-Geisser	,061	1,701	,036	15,332	<,001	,630
Error(Visits)	Sphericity Assumed	,036	18	,002			
	Greenhouse-Geisser	,036	15,305	,002			

a. Parameter = R0, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	,102*	,024	,006	,033	,171
	3	,014	,019	1,000	-,042	,070
2	1	-,102*	,024	,006	-,171	-,033
	3	-,088*	,017	,001	-,137	-,040
3	1	-,014	,019	1,000	-,070	,042
	2	,088*	,017	,001	,040	,137

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,792	,935	2	,627	,828	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,053	2	,026	8,052	,008	,617
	Greenhouse-Geisser	,053	1,655	,032	8,052	,014	,617
Error(Visits)	Sphericity Assumed	,033	10	,003			
	Greenhouse-Geisser	,033	8,276	,004			

a. Parameter = R0, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	,107	,036	,089	-,019	,233
	3	-,014	,038	1,000	-,147	,119
2	1	-,107	,036	,089	-,233	,019
	3	-,121*	,025	,013	-,208	-,035
3	1	,014	,038	1,000	-,119	,147
	2	,121*	,025	,013	,035	,208

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc

					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,844	1,356	2	,508	,865	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,052	2	,026	18,444	<,001	,672
	Greenhouse-Geisser	,052	1,730	,030	18,444	<,001	,672
Error(Visits)	Sphericity Assumed	,025	18	,001			
	Greenhouse-Geisser	,025	15,573	,002			

a. Parameter = R0, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	,093*	,020	,003	,035	,151
	3	,010	,016	1,000	-,036	,056
2	1	-,093*	,020	,003	-,151	-,035
	3	-,083*	,015	<,001	-,125	-,040
3	1	-,010	,016	1,000	-,056	,036
	2	,083*	,015	<,001	,040	,125

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

a. Parameter = R0, Treatment = ACS

c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,863	,589	2	,745	,880	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,030	2	,015	4,483	,041	,473
	Greenhouse-Geisser	,030	1,759	,017	4,483	,049	,473
Error(Visits)	Sphericity Assumed	,034	10	,003			
	Greenhouse-Geisser	,034	8,795	,004			

a. Parameter = R0, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	,080	,036	,237	-,049	,209
	3	-,013	,037	1,000	-,142	,117
2	1	-,080	,036	,237	-,209	,049
	3	-,093	,027	,053	-,187	,002
3	1	,013	,037	1,000	-,117	,142
	2	,093	,027	,053	-,002	,187

Based on estimated marginal means

a. Parameter = R0, Treatment = ACS+HA

b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,384900	10	,0851854	,0269380
	ScreeningL2	,371100	10	,0723517	,0228796
Pair 2	Week12R2	,282900	10	,0481143	,0152151
	Week12L2	,278200	10	,0368535	,0116541
Pair 3	Week24R2	,371300	10	,0672641	,0212708
	Week24L2	,361000	10	,0580594	,0183600

a. Parameter = R0, Treatment = ACS

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	,0138000	,0417367	,0131983	-,0160567	,0436567	1,046	9	,162	,323
Pair 2	Week12R2 - Week12L2	,0047000	,0289215	,0091458	-,0159892	,0253892	,514	9	,310	,620
Pair 3	Week24R2 - Week24L2	,0103000	,0347373	,0109849	-,0145495	,0351495	,938	9	,186	,373

a. Parameter = R0, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,390333	6	,0979238	,0399772
	ScreeningL2	,390833	6	,0735701	,0300349
Pair 2	Week12R2	,283167	6	,0526172	,0214809
	Week12L2	,310667	6	,0388724	,0158696
Pair 3	Week24R2	,404500	6	,0336199	,0137253
	Week24L2	,403333	6	,0573748	,0234232

a. Parameter = R0, Treatment = ACS+HA

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0005000	,0410354	,0167526	-,0435640	,0425640	-,030	5	,489	,977
Pair 2	Week12R2 - Week12L2	-,0275000	,0344717	,0140730	-,0636759	,0086759	-1,954	5	,054	,108
Pair 3	Week24R2 - Week24L2	,0011667	,0602542	,0245987	-,0620662	,0643996	,047	5	,482	,964

a. Parameter = R0, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,384900	,0851854	,0269380
	ACS+HA	6	,390333	,0979238	,0399772
ScreeningL2	ACS	10	,371100	,0723517	,0228796
	ACS+HA	6	,390833	,0735701	,0300349
Week12R2	ACS	10	,282900	,0481143	,0152151
	ACS+HA	6	,283167	,0526172	,0214809
Week12L2	ACS	10	,278200	,0368535	,0116541
	ACS+HA	6	,310667	,0388724	,0158696
Week24R2	ACS	10	,371300	,0672641	,0212708
	ACS+HA	6	,404500	,0336199	,0137253
Week24L2	ACS	10	,361000	,0580594	,0183600
	ACS+HA	6	,403333	,0573748	,0234232

a. Parameter = R0

Independent Samples Testa

	Levene's Test for Equality of Variances	t-test for Equality of Means

		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
ScreeningR2	Equal variances assumed	,070	,796	-,117	14	,454	,909	-,0054333	,0464459	-,1050499	,0941833
	Equal variances not assumed			-,113	9,485	,456	,913	-,0054333	,0482061	-,1136392	,1027725
ScreeningL2	Equal variances assumed	,328	,576	-,525	14	,304	,608	-,0197333	,0375882	-,1003520	,0608853
	Equal variances not assumed			-,523	10,519	,306	,612	-,0197333	,0377567	-,1033016	,0638349
Week12R2	Equal variances assumed	,495	,493	-,010	14	,496	,992	-,0002667	,0257007	-,0553893	,0548559
	Equal variances not assumed			-,010	9,892	,496	,992	-,0002667	,0263235	-,0590058	,0584725
Week12L2	Equal variances assumed	,015	,904	-1,673	14	,058	,117	-,0324667	,0194098	-,0740966	,0091633
	Equal variances not assumed			-1,649	10,199	,065	,130	-,0324667	,0196891	-,0762210	,0112876
Week24R2	Equal variances assumed	2,716	,122	-1,117	14	,141	,283	-,0332000	,0297198	-,0969427	,0305427
	Equal variances not assumed			-1,311	13,761	,106	,211	-,0332000	,0253146	-,0875831	,0211831
Week24L2	Equal variances assumed	,002	,969	-1,418	14	,089	,178	-,0423333	,0298560	-,1063680	,0217013
	Equal variances not assumed			-1,422	10,772	,092	,183	-,0423333	,0297613	-,1080067	,0233401

a. Parameter = R0

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,893	,909	2	,635	,903	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,987	,103	2	,950	,987	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,748	2	,374	46,614	<,001	,838
	Greenhouse-Geisser	,748	1,806	,414	46,614	<,001	,838
Error(Visit)	Sphericity Assumed	,144	18	,008			
	Greenhouse-Geisser	,144	16,255	,009			
Side	Sphericity Assumed	,000	1	,000	,034	,857	,004
	Greenhouse-Geisser	,000	1,000	,000	,034	,857	,004
Error(Side)	Sphericity Assumed	,073	9	,008			
	Greenhouse-Geisser	,073	9,000	,008			
Visit * Side	Sphericity Assumed	,004	2	,002	,573	,574	,060
	Greenhouse-Geisser	,004	1,975	,002	,573	,572	,060
Error(Visit*Side)	Sphericity Assumed	,060	18	,003			

	Greenhouse-Geisser	,060		17,774	,003			

a. Parameter = R0, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,865	,580	2	,748	,881	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,472	3,003	2	,223	,654	,793	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,302	2	,151	11,898	,002	,704
	Greenhouse-Geisser	,302	1,762	,171	11,898	,004	,704
Error(Visit)	Sphericity Assumed	,127	10	,013			
	Greenhouse-Geisser	,127	8,810	,014			
Side	Sphericity Assumed	,000	1	,000	,012	,916	,002
	Greenhouse-Geisser	,000	1,000	,000	,012	,916	,002
Error(Side)	Sphericity Assumed	,082	5	,016			
	Greenhouse-Geisser	,082	5,000	,016			
Visit * Side	Sphericity Assumed	,001	2	,000	,060	,942	,012
	Greenhouse-Geisser	,001	1,309	,001	,060	,873	,012
Error(Visit*Side)	Sphericity Assumed	,072	10	,007			
	Greenhouse-Geisser	,072	6,544	,011			

a. Parameter = R0, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,495	5,631	2	,060	,664	,736	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,329	2	,165	28,916	<,001	,763
	Greenhouse-Geisser	,329	1,329	,248	28,916	<,001	,763
Error(Visits)	Sphericity Assumed	,103	18	,006			
	Greenhouse-Geisser	,103	11,957	,009			

a. Parameter = R0, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,211*	,029	<,001	,126	,297
	3	-,020	,025	1,000	-,094	,054
2	1	-,211*	,029	<,001	-,297	-,126
	3	-,232*	,044	,002	-,361	-,103
3	1	,020	,025	1,000	-,054	,094
	2	,232*	,044	,002	,103	,361

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,838	,709	2	,701	,860	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,167	2	,084	9,907	,004	,665
	Greenhouse-Geisser	,167	1,720	,097	9,907	,007	,665
Error(Visits)	Sphericity Assumed	,084	10	,008			
	Greenhouse-Geisser	,084	8,602	,010			

a. Parameter = R0, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,236*	,049	,014	,063	,409
	3	,114	,063	,384	-,107	,336
2	1	-,236*	,049	,014	-,409	-,063
	3	-,122	,046	,137	-,284	,041
3	1	-,114	,063	,384	-,336	,107
	2	,122	,046	,137	-,041	,284

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,813	1,652	2	,438	,843	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,422	2	,211	37,278	<,001	,806
	Greenhouse-Geisser	,422	1,686	,250	37,278	<,001	,806
Error(Visits)	Sphericity Assumed	,102	18	,006			
	Greenhouse-Geisser	,102	15,170	,007			

a. Parameter = R0, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,251*	,040	<,001	,135	,367
	3	-,002	,033	1,000	-,100	,096
2	1	-,251*	,040	<,001	-,367	-,135
	3	-,253*	,027	<,001	-,331	-,174
3	1	,002	,033	1,000	-,096	,100
	2	,253*	,027	<,001	,174	,331

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,827	,758	2	,685	,853	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,135	2	,068	5,908	,020	,542
	Greenhouse-Geisser	,135	1,706	,079	5,908	,028	,542
Error(Visits)	Sphericity Assumed	,114	10	,011			
	Greenhouse-Geisser	,114	8,528	,013			

a. Parameter = R0, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,212	,069	,084	-,032	,457
	3	,103	,047	,244	-,064	,271
2	1	-,212	,069	,084	-,457	,032
	3	-,109	,066	,484	-,343	,125
3	1	-,103	,047	,244	-,271	,064
	2	,109	,066	,484	-,125	,343

Based on estimated marginal means
a. Parameter = R0, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,716300	10	,1288755	,0407540
	ScreeningL4	,739700	10	,1341285	,0424152
Pair 2	Week12R4	,504800	10	,1259045	,0398145
	Week12L4	,489100	10	,1245396	,0393829
Pair 3	Week24R4	,736500	10	,1346190	,0425703
	Week24L4	,741700	10	,0912092	,0288429

a. Parameter = R0, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,0234000	,0637516	,0201600	-,0690051	,0222051	-1,161	9	,138	,276
Pair 2	Week12R4 - Week12L4	,0157000	,1243562	,0393249	-,0732590	,1046590	,399	9	,350	,699
Pair 3	Week24R4 - Week24L4	-,0052000	,1000431	,0316364	-,0767665	,0663665	-,164	9	,437	,873

a. Parameter = R0, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,839000	6	,2072400	,0846054
	ScreeningL4	,832000	6	,1183098	,0482998

Pair 2	Week12R4	,602833	6	,1028230	,0419773
	Week12L4	,619833	6	,0826303	,0337337
Pair 3	Week24R4	,724667	6	,1305077	,0532796
	Week24L4	,728833	6	,1197838	,0489015

a. Parameter = R0, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0070000	,2222890	,0907491	-,2262780	,2402780	,077	5	,471	,942
Pair 2	Week12R4 - Week12L4	-,0170000	,0716603	,0292552	-,0922029	,0582029	-,581	5	,293	,586
Pair 3	Week24R4 - Week24L4	-,0041667	,0838127	,0342164	-,0921227	,0837894	-,122	5	,454	,908

a. Parameter = R0, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,716300	,1288755	,0407540
	ACS+HA	6	,839000	,2072400	,0846054
ScreeningL4	ACS	10	,739700	,1341285	,0424152
	ACS+HA	6	,832000	,1183098	,0482998
Week12R4	ACS	10	,504800	,1259045	,0398145
	ACS+HA	6	,602833	,1028230	,0419773
Week12L4	ACS	10	,489100	,1245396	,0393829
	ACS+HA	6	,619833	,0826303	,0337337
Week24R4	ACS	10	,736500	,1346190	,0425703
	ACS+HA	6	,724667	,1305077	,0532796
Week24L4	ACS	10	,741700	,0912092	,0288429
	ACS+HA	6	,728833	,1197838	,0489015

a. Parameter = R0

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	2,361	,147	-1,473	14	,081	,163	-,1227000	,0832920	-,3013437	,0559437
	Equal variances not assumed			-1,307	7,369	,115	,231	-,1227000	,0939093	-,3425253	,0971253
ScreeningL4	Equal variances assumed	,496	,493	-1,389	14	,093	,187	-,0923000	,0664616	-,2348460	,0502460
	Equal variances not assumed			-1,436	11,790	,089	,177	-,0923000	,0642800	-,2326312	,0480312
Week12R4	Equal variances assumed	,506	,489	-1,606	14	,065	,131	-,0980333	,0610278	-,2289249	,0328582
	Equal variances not assumed			-1,694	12,447	,058	,115	-,0980333	,0578558	-,2235905	,0275238

Week12L4	Equal variances assumed	1,081	,316	-2,273	14	,020	,039	-,1307333	,0575251	-,2541124	-,0073543
	Equal variances not assumed			-2,521	13,739	,012	,025	-,1307333	,0518553	-,2421505	-,0193162
Week24R4	Equal variances assumed	,037	,850	,172	14	,433	,866	,0118333	,0687662	-,1356556	,1593223
	Equal variances not assumed			,174	10,944	,433	,865	,0118333	,0681978	-,1383630	,1620297
Week24L4	Equal variances assumed	,175	,682	,243	14	,406	,811	,0128667	,0528453	-,1004753	,1262086
	Equal variances not assumed			,227	8,512	,413	,826	,0128667	,0567739	-,1166968	,1424302

a. Parameter = R0

Skin gross elasticity (R2, Ua/Uf)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	ACS	,950	10	,674
	ACS+HA	,916	6	,474
ScreeningL2	ACS	,954	10	,717
	ACS+HA	,982	6	,962
Week12R2	ACS	,897	10	,204
	ACS+HA	,930	6	,580
Week12L2	ACS	,941	10	,563
	ACS+HA	,963	6	,845
Week24R2	ACS	,969	10	,883
	ACS+HA	,881	6	,273
Week24L2	ACS	,960	10	,790
	ACS+HA	,928	6	,565

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,954	10	,717
	ACS+HA	,902	6	,386
ScreeningL4	ACS	,856	10	,069
	ACS+HA	,983	6	,967
Week12R4	ACS	,859	10	,075
	ACS+HA	,904	6	,396
Week12L4	ACS	,918	10	,338
	ACS+HA	,897	6	,354
Week24R4	ACS	,922	10	,370
	ACS+HA	,914	6	,465
Week24L4	ACS	,924	10	,390
	ACS+HA	,885	6	,294

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visit	,544	4,871	2	,088	,687	,769	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,925	,627	2	,731	,930	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,021	2	,011	1,550	,239
	Greenhouse-Geisser	,021	1,374	,015	1,550	,245
Error(Visit)	Sphericity Assumed	,123	18	,007		
	Greenhouse-Geisser	,123	12,362	,010		
Side	Sphericity Assumed	,005	1	,005	2,747	,132
	Greenhouse-Geisser	,005	1,000	,005	2,747	,132
Error(Side)	Sphericity Assumed	,018	9	,002		
	Greenhouse-Geisser	,018	9,000	,002		
Visit * Side	Sphericity Assumed	,006	2	,003	,790	,469
	Greenhouse-Geisser	,006	1,860	,003	,790	,462
Error(Visit*Side)	Sphericity Assumed	,063	18	,003		
	Greenhouse-Geisser	,063	16,738	,004		

a. Parameter = R2, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,994	,023	2	,989	,994	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,837	,711	2	,701	,860	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,019	2	,010	1,067	,380
	Greenhouse-Geisser	,019	1,989	,010	1,067	,380
Error(Visit)	Sphericity Assumed	,091	10	,009		
	Greenhouse-Geisser	,091	9,944	,009		
Side	Sphericity Assumed	,001	1	,001	,299	,608
	Greenhouse-Geisser	,001	1,000	,001	,299	,608
Error(Side)	Sphericity Assumed	,017	5	,003		
	Greenhouse-Geisser	,017	5,000	,003		
Visit * Side	Sphericity Assumed	,010	2	,005	,467	,640
	Greenhouse-Geisser	,010	1,720	,006	,467	,614
Error(Visit*Side)	Sphericity Assumed	,104	10	,010		
	Greenhouse-Geisser	,104	8,600	,012		

a. Parameter = R2, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,819	1,593	2	,451	,847	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,024	2	,012	1,635	,223	,154
	Greenhouse-Geisser	,024	1,694	,014	1,635	,227	,154
Error(Visits)	Sphericity Assumed	,132	18	,007			
	Greenhouse-Geisser	,132	15,247	,009			

a. Parameter = R2, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	-,026	,031	1,000	-,118	,066
	3	,042	,045	1,000	-,090	,175
2	1	,026	,031	1,000	-,066	,118
	3	,069	,037	,289	-,040	,177
3	1	-,042	,045	1,000	-,175	,090
	2	-,069	,037	,289	-,177	,040

Based on estimated marginal means
a. Parameter = R2, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,944	,230	2	,892	,947	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,001	2	,000	,076	,927	,015
	Greenhouse-Geisser	,001	1,894	,000	,076	,919	,015
Error(Visits)	Sphericity Assumed	,054	10	,005			
	Greenhouse-Geisser	,054	9,471	,006			

a. Parameter = R2, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	,001	,044	1,000	-,155	,156
	3	,015	,045	1,000	-,146	,175
2	1	-,001	,044	1,000	-,156	,155
	3	,014	,037	1,000	-,117	,145
3	1	-,015	,045	1,000	-,175	,146
	2	-,014	,037	1,000	-,145	,117

Based on estimated marginal means
a. Parameter = R2, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,623	3,791	2	,150	,726	,829	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,003	2	,001	,447	,646	,047
	Greenhouse-Geisser	,003	1,452	,002	,447	,588	,047
Error(Visits)	Sphericity Assumed	,053	18	,003			
	Greenhouse-Geisser	,053	13,068	,004			

a. Parameter = R2, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,004	,016	1,000	-,051	,043
	3	,017	,030	1,000	-,070	,105
2	1	,004	,016	1,000	-,043	,051
	3	,022	,025	1,000	-,052	,096
3	1	-,017	,030	1,000	-,105	,070
	2	-,022	,025	1,000	-,096	,052

Based on estimated marginal means
a. Parameter = R2, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,907	,389	2	,823	,915	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,028	2	,014	1,001	,402	,167
	Greenhouse-Geisser	,028	1,830	,015	1,001	,397	,167
Error(Visits)	Sphericity Assumed	,141	10	,014			
	Greenhouse-Geisser	,141	9,152	,015			

a. Parameter = R2, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,009	,059	1,000	-,198	,217
	3	,088	,077	,911	-,184	,361
2	1	-,009	,059	1,000	-,217	,198
	3	,079	,069	,907	-,164	,322
3	1	-,088	,077	,911	-,361	,184
	2	-,079	,069	,907	-,322	,164

Based on estimated marginal means
a. Parameter = R2, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,750630	10	,0969155	,0306474
	ScreeningL2	,768720	10	,0492159	,0155634
Pair 2	Week12R2	,776860	10	,0713381	,0225591
	Week12L2	,772940	10	,0668670	,0211452
Pair 3	Week24R2	,708250	10	,0961397	,0304020
	Week24L2	,751220	10	,0828439	,0261975

a. Parameter = R2, Treatment = ACS

Paired Samples Testa

Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p

		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0180900	,0759933	,0240312	-,0724523	,0362723	-,753	9	,235	,471
Pair 2	Week12R2 - Week12L2	,0039200	,0738701	,0233598	-,0489235	,0567635	,168	9	,435	,870
Pair 3	Week24R2 - Week24L2	-,0429700	,0816439	,0258181	-,1013745	,0154345	-1,664	9	,065	,130

a. Parameter = R2, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,685450	6	,0823630	,0336245
	ScreeningL2	,723500	6	,1243233	,0507548
Pair 2	Week12R2	,684750	6	,0817623	,0333793
	Week12L2	,714050	6	,0892156	,0364221
Pair 3	Week24R2	,670783	6	,0807589	,0329697
	Week24L2	,635133	6	,1118759	,0456731

a. Parameter = R2, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0380500	,0844969	,0344957	-,1267240	,0506240	-1,103	5	,160	,320
Pair 2	Week12R2 - Week12L2	-,0293000	,1218217	,0497335	-,1571441	,0985441	-,589	5	,291	,581
Pair 3	Week24R2 - Week24L2	,0356500	,1625233	,0663498	-,1349077	,2062077	,537	5	,307	,614

a. Parameter = R2, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,750630	,0969155	,0306474
	ACS+HA	6	,685450	,0823630	,0336245
ScreeningL2	ACS	10	,768720	,0492159	,0155634
	ACS+HA	6	,723500	,1243233	,0507548
Week12R2	ACS	10	,776860	,0713381	,0225591
	ACS+HA	6	,684750	,0817623	,0333793
Week12L2	ACS	10	,772940	,0668670	,0211452
	ACS+HA	6	,714050	,0892156	,0364221
Week24R2	ACS	10	,708250	,0961397	,0304020
	ACS+HA	6	,670783	,0807589	,0329697
Week24L2	ACS	10	,751220	,0828439	,0261975
	ACS+HA	6	,635133	,1118759	,0456731

a. Parameter = R2

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR2	Equal variances assumed	,012	,915	1,372	14	,096	,192	,0651800	,0474997	-,0366968	,1670568
	Equal variances not assumed			1,433	12,114	,089	,177	,0651800	,0454958	-,0338438	,1642038

Screen-ingL2	Equal variances assumed	4,925	,043	1,041	14	,158	,316	,0452200	,0434426	-,0479552	,1383952
	Equal variances not assumed			,852	5,955	,214	,427	,0452200	,0530874	-,0849172	,1753572
Week12R2	Equal variances assumed	,282	,604	2,371	14	,016	,033	,0921100	,0388471	,0087913	,1754287
	Equal variances not assumed			2,286	9,509	,023	,047	,0921100	,0402876	,0017109	,1825091
Week12L2	Equal variances assumed	,566	,464	1,508	14	,077	,154	,0588900	,0390452	-,0248537	,1426337
	Equal variances not assumed			1,398	8,408	,099	,198	,0588900	,0421152	-,0374139	,1551939
Week24R2	Equal variances assumed	,238	,633	,798	14	,219	,438	,0374667	,0469641	-,0632613	,1381947
	Equal variances not assumed			,835	12,213	,210	,420	,0374667	,0448473	-,0600589	,1349923
Week24L2	Equal variances assumed	1,398	,257	2,385	14	,016	,032	,1160867	,0486678	,0117047	,2204687
	Equal variances not assumed			2,205	8,330	,029	,057	,1160867	,0526531	-,0044986	,2366720

a. Parameter = R2

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,887	,962	2	,618	,898	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,797	1,813	2	,404	,831	,997	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,189	2	,095	21,680	<,001	,707
	Greenhouse-Geisser	,189	1,796	,105	21,680	<,001	,707
Error(Visit)	Sphericity Assumed	,079	18	,004			
	Greenhouse-Geisser	,079	16,168	,005			
Side	Sphericity Assumed	,000	1	,000	,043	,841	,005
	Greenhouse-Geisser	,000	1,000	,000	,043	,841	,005
Error(Side)	Sphericity Assumed	,046	9	,005			
	Greenhouse-Geisser	,046	9,000	,005			
Visit * Side	Sphericity Assumed	,004	2	,002	,780	,473	,080
	Greenhouse-Geisser	,004	1,663	,002	,780	,454	,080
Error(Visit*Side)	Sphericity Assumed	,044	18	,002			
	Greenhouse-Geisser	,044	14,965	,003			

a. Parameter = R2, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,158	7,390	2	,025	,543	,576	,500
Side	1,000	,000	0	.	1,000	1,000	1,000

Visit * Side	,923	,318	2	,853	,929	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,104	2	,052	15,955	<,001	,761
	Greenhouse-Geisser	,104	1,086	,096	15,955	,008	,761
Error(Visit)	Sphericity Assumed	,033	10	,003			
	Greenhouse-Geisser	,033	5,428	,006			
Side	Sphericity Assumed	,000	1	,000	,676	,448	,119
	Greenhouse-Geisser	,000	1,000	,000	,676	,448	,119
Error(Side)	Sphericity Assumed	,004	5	,001			
	Greenhouse-Geisser	,004	5,000	,001			
Visit * Side	Sphericity Assumed	,001	2	,000	,218	,808	,042
	Greenhouse-Geisser	,001	1,858	,000	,218	,793	,042
Error(Visit*Side)	Sphericity Assumed	,012	10	,001			
	Greenhouse-Geisser	,012	9,289	,001			

a. Parameter = R2, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,935	,541	2	,763	,939	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,102	2	,051	12,158	<,001	,575
	Greenhouse-Geisser	,102	1,877	,054	12,158	<,001	,575
Error(Visits)	Sphericity Assumed	,076	18	,004			
	Greenhouse-Geisser	,076	16,894	,004			

a. Parameter = R2, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,108*	,031	,022	-,200	-,016
	3	,027	,030	1,000	-,061	,115
2	1	,108*	,031	,022	,016	,200
	3	,135*	,025	,001	,061	,209
3	1	-,027	,030	1,000	-,115	,061
	2	-,135*	,025	,001	-,209	-,061

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,231	5,859	2	,053	,565	,618	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,047	2	,024	13,223	,002	,726

	Greenhouse-Geisser	,047	1,131	,042	13,223	,011	,726
Error(Visits)	Sphericity Assumed	,018	10	,002			
	Greenhouse-Geisser	,018	5,653	,003			

a. Parameter = R2, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,122*	,022	,008	-,199	-,045
	3	-,035	,015	,192	-,086	,017
2	1	,122*	,022	,008	,045	,199
	3	,087	,033	,139	-,030	,204
3	1	,035	,015	,192	-,017	,086
	2	-,087	,033	,139	-,204	,030

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,891	,927	2	,629	,901	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,091	2	,046	17,498	<,001	,660
	Greenhouse-Geisser	,091	1,803	,051	17,498	<,001	,660
Error(Visits)	Sphericity Assumed	,047	18	,003			
	Greenhouse-Geisser	,047	16,225	,003			

a. Parameter = R2, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,122*	,026	,003	-,197	-,047
	3	-,012	,023	1,000	-,081	,057
2	1	,122*	,026	,003	,047	,197
	3	,111*	,019	<,001	,055	,166
3	1	,012	,023	1,000	-,057	,081
	2	-,111*	,019	<,001	-,166	-,055

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,741	1,198	2	,549	,794	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,057	2	,029	10,820	,003	,684

	Greenhouse-Geisser	,057	1,589	,036	10,820	,007	,684
Error(Visits)	Sphericity Assumed	,026	10	,003			
	Greenhouse-Geisser	,026	7,944	,003			

a. Parameter = R2, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,130*	,023	,007	-,209	-,050
	3	-,024	,029	1,000	-,128	,079
2	1	,130*	,023	,007	,050	,209
	3	,105	,036	,096	-,021	,232
3	1	,024	,029	1,000	-,079	,128
	2	-,105	,036	,096	-,232	,021

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R2, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,738930	10	,1207962	,0381991
	ScreeningL4	,717530	10	,1078059	,0340912
Pair 2	Week12R4	,847120	10	,0551551	,0174416
	Week12L4	,839960	10	,0784073	,0247946
Pair 3	Week24R4	,712230	10	,1134267	,0358687
	Week24L4	,729360	10	,1073828	,0339574

a. Parameter = R2, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0214000	,0797811	,0252290	-,0356720	,0784720	,848	9	,209	,418
Pair 2	Week12R4 - Week12L4	,0071600	,0758813	,0239958	-,0471222	,0614422	,298	9	,386	,772
Pair 3	Week24R4 - Week24L4	-,0171300	,0885582	,0280046	-,0804807	,0462207	-,612	9	,278	,556

a. Parameter = R2, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,709317	6	,0770444	,0314533
	ScreeningL4	,702617	6	,0734229	,0299748
Pair 2	Week12R4	,831033	6	,0648259	,0264651
	Week12L4	,832400	6	,0629888	,0257151
Pair 3	Week24R4	,743933	6	,1016739	,0415082
	Week24L4	,726917	6	,0808035	,0329879

a. Parameter = R2, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0067000	,0600731	,0245247	-,0563429	,0697429	,273	5	,398	,796

Pair 2	Week12R4 - Week12L4	-,0013667	,0272447	,0111226	-,0299582	,0272249	-,123	5	,453	,907
Pair 3	Week24R4 - Week24L4	,0170167	,0425661	,0173775	-,0276537	,0616870	,979	5	,186	,372

a. Parameter = R2, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,738930	,1207962	,0381991
	ACS+HA	6	,709317	,0770444	,0314533
ScreeningL4	ACS	10	,717530	,1078059	,0340912
	ACS+HA	6	,702617	,0734229	,0299748
Week12R4	ACS	10	,847120	,0551551	,0174416
	ACS+HA	6	,831033	,0648259	,0264651
Week12L4	ACS	10	,839960	,0784073	,0247946
	ACS+HA	6	,832400	,0629888	,0257151
Week24R4	ACS	10	,712230	,1134267	,0358687
	ACS+HA	6	,743933	,1016739	,0415082
Week24L4	ACS	10	,729360	,1073828	,0339574
	ACS+HA	6	,726917	,0808035	,0329879

a. Parameter = R2

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	1,162	,299	,535	14	,301	,601	,0296133	,0553783	-,0891613	,1483880
	Equal variances not assumed			,598	13,867	,280	,559	,0296133	,0494821	-,0766107	,1358374
ScreeningL4	Equal variances assumed	,409	,533	,298	14	,385	,770	,0149133	,0500578	-,0924500	,1222767
	Equal variances not assumed			,329	13,631	,374	,748	,0149133	,0453949	-,0826972	,1125238
Week12R4	Equal variances assumed	,140	,714	,530	14	,302	,605	,0160867	,0303600	-,0490290	,0812023
	Equal variances not assumed			,508	9,311	,312	,624	,0160867	,0316955	-,0552505	,0874238
Week12L4	Equal variances assumed	,247	,627	,200	14	,422	,845	,0075600	,0378386	-,0735957	,0887157
	Equal variances not assumed			,212	12,579	,418	,836	,0075600	,0357216	-,0698756	,0849956
Week24R4	Equal variances assumed	,094	,764	-,561	14	,292	,583	-,0317033	,0564807	-,1528423	,0894356
	Equal variances not assumed			-,578	11,647	,287	,574	-,0317033	,0548588	-,1516332	,0882266
Week24L4	Equal variances assumed	,911	,356	,048	14	,481	,962	,0024433	,0509763	-,1068900	,1117766
	Equal variances not assumed			,052	13,062	,480	,960	,0024433	,0473424	-,0997842	,1046709

a. Parameter = R2

Skin firmness after repeated suction (R3, Uf_5)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	ACS	,825	10	,029
	ACS+HA	,866	6	,212
ScreeningL2	ACS	,941	10	,563
	ACS+HA	,801	6	,060
Week12R2	ACS	,931	10	,456
	ACS+HA	,869	6	,221
Week12L2	ACS	,978	10	,951
	ACS+HA	,830	6	,107
Week24R2	ACS	,874	10	,110
	ACS+HA	,975	6	,926
Week24L2	ACS	,817	10	,023
	ACS+HA	,995	6	,997

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,967	10	,857
	ACS+HA	,869	6	,221
ScreeningL4	ACS	,921	10	,367
	ACS+HA	,981	6	,957
Week12R4	ACS	,956	10	,742
	ACS+HA	,957	6	,798
Week12L4	ACS	,937	10	,517
	ACS+HA	,888	6	,309
Week24R4	ACS	,949	10	,660
	ACS+HA	,914	6	,467
Week24L4	ACS	,910	10	,284
	ACS+HA	,837	6	,124

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,753	2,268	2	,322	,802	,949	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,996	,028	2	,986	,996	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,127	2	,064	19,954	<,001
	Greenhouse-Geisser	,127	1,604	,079	19,954	<,001
Error(Visit)	Sphericity Assumed	,057	18	,003		
	Greenhouse-Geisser	,057	14,436	,004		
Side	Sphericity Assumed	,002	1	,002	1,328	,279
	Greenhouse-Geisser	,002	1,000	,002	1,328	,279
Error(Side)	Sphericity Assumed	,011	9	,001		
	Greenhouse-Geisser	,011	9,000	,001		
Visit * Side	Sphericity Assumed	,000	2	9,920E-5	,219	,805
	Greenhouse-Geisser	,000	1,993	9,955E-5	,219	,804
Error(Visit*Side)	Sphericity Assumed	,008	18	,000		
	Greenhouse-Geisser	,008	17,937	,000		

a. Parameter = R3, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,757	1,111	2	,574	,805	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,708	1,383	2	,501	,774	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,098	2	,049	8,193	,008
	Greenhouse-Geisser	,098	1,610	,061	8,193	,014
Error(Visit)	Sphericity Assumed	,060	10	,006		
	Greenhouse-Geisser	,060	8,048	,007		
Side	Sphericity Assumed	,001	1	,001	,267	,627
	Greenhouse-Geisser	,001	1,000	,001	,267	,627
Error(Side)	Sphericity Assumed	,012	5	,002		
	Greenhouse-Geisser	,012	5,000	,002		
Visit * Side	Sphericity Assumed	,001	2	,000	,647	,544
	Greenhouse-Geisser	,001	1,548	,001	,647	,512
Error(Visit*Side)	Sphericity Assumed	,007	10	,001		
	Greenhouse-Geisser	,007	7,738	,001		

a. Parameter = R3, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,723	2,595	2	,273	,783	,919	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,068	2	,034	17,123	<,001	,655
	Greenhouse-Geisser	,068	1,566	,043	17,123	<,001	,655
Error(Visits)	Sphericity Assumed	,036	18	,002			
	Greenhouse-Geisser	,036	14,096	,003			

a. Parameter = R3, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,107*	,024	,005	,036	,179
	3	,015	,019	1,000	-,041	,070
2	1	-,107*	,024	,005	-,179	-,036
	3	-,093*	,016	<,001	-,139	-,047
3	1	-,015	,019	1,000	-,070	,041
	2	,093*	,016	<,001	,047	,139

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,723	1,298	2	,523	,783	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,059	2	,029	9,087	,006	,645
	Greenhouse-Geisser	,059	1,566	,037	9,087	,011	,645
Error(Visits)	Sphericity Assumed	,032	10	,003			
	Greenhouse-Geisser	,032	7,830	,004			

a. Parameter = R3, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,113	,036	,078	-,015	,241
	3	-,015	,038	1,000	-,147	,118
2	1	-,113	,036	,078	-,241	,015
	3	-,128*	,023	,007	-,208	-,048
3	1	,015	,038	1,000	-,118	,147
	2	,128*	,023	,007	,048	,208

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,892	,912	2	,634	,903	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,060	2	,030	17,968	<,001	,666
	Greenhouse-Geisser	,060	1,805	,033	17,968	<,001	,666
Error(Visits)	Sphericity Assumed	,030	18	,002			
	Greenhouse-Geisser	,030	16,248	,002			

a. Parameter = R3, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,099*	,021	,003	,037	,160
	3	,009	,017	1,000	-,041	,059
2	1	-,099*	,021	,003	-,160	-,037
	3	-,090*	,016	,001	-,138	-,042
3	1	-,009	,017	1,000	-,059	,041
	2	,090*	,016	,001	,042	,138

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,879	,516	2	,773	,892	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,040	2	,020	5,830	,021	,538
	Greenhouse-Geisser	,040	1,784	,022	5,830	,026	,538
Error(Visits)	Sphericity Assumed	,034	10	,003			
	Greenhouse-Geisser	,034	8,920	,004			

a. Parameter = R3, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,092	,037	,167	-,039	,224
	3	-,014	,036	1,000	-,142	,114
2	1	-,092	,037	,167	-,224	,039
	3	-,106*	,027	,035	-,203	-,010
3	1	,014	,036	1,000	-,114	,142
	2	,106*	,027	,035	,010	,203

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,430100	10	,0903382	,0285674
	ScreeningL2	,414800	10	,0752740	,0238037
Pair 2	Week12R2	,322600	10	,0490537	,0155121
	Week12L2	,316100	10	,0395768	,0125153
Pair 3	Week24R2	,415600	10	,0642516	,0203181
	Week24L2	,405900	10	,0632867	,0200130

a. Parameter = R3, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	,0153000	,0480487	,0151943	-,0190720	,0496720	1,007	9	,170	,340
Pair 2	Week12R2 - Week12L2	,0065000	,0317744	,0100479	-,0162300	,0292300	,647	9	,267	,534
Pair 3	Week24R2 - Week24L2	,0097000	,0313122	,0099018	-,0126994	,0320994	,980	9	,176	,353

a. Parameter = R3, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,436833	6	,1039796	,0424495
	ScreeningL2	,438667	6	,0763274	,0311605
Pair 2	Week12R2	,323667	6	,0560167	,0228687
	Week12L2	,346333	6	,0390367	,0159367

Pair 3	Week24R2	,451500	6	,0366702	,0149705
	Week24L2	,452667	6	,0559881	,0228570

a. Parameter = R3, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0018333	,0457008	,0186573	-,0497934	,0461268	-,098	5	,463	,926
Pair 2	Week12R2 - Week12L2	-,0226667	,0339274	,0138508	-,0582713	,0129379	-1,636	5	,081	,163
Pair 3	Week24R2 - Week24L2	-,0011667	,0668533	,0272928	-,0713249	,0689916	-,043	5	,484	,968

a. Parameter = R3, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,430100	,0903382	,0285674
	ACS+HA	6	,436833	,1039796	,0424495
ScreeningL2	ACS	10	,414800	,0752740	,0238037
	ACS+HA	6	,438667	,0763274	,0311605
Week12R2	ACS	10	,322600	,0490537	,0155121
	ACS+HA	6	,323667	,0560167	,0228687
Week12L2	ACS	10	,316100	,0395768	,0125153
	ACS+HA	6	,346333	,0390367	,0159367
Week24R2	ACS	10	,415600	,0642516	,0203181
	ACS+HA	6	,451500	,0366702	,0149705
Week24L2	ACS	10	,405900	,0632867	,0200130
	ACS+HA	6	,452667	,0559881	,0228570

a. Parameter = R3

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR2	Equal variances assumed	,076	,787	-,137	14	,447	,893	-,0067333	,0492820	-,1124328	,0989661
	Equal variances not assumed			-,132	9,475	,449	,898	-,0067333	,0511670	-,1216028	,1081362
ScreeningL2	Equal variances assumed	,263	,616	-,611	14	,276	,551	-,0238667	,0390665	-,1076559	,0599226
	Equal variances not assumed			-,609	10,544	,278	,556	-,0238667	,0392122	-,1106302	,0628969
Week12R2	Equal variances assumed	,359	,559	-,040	14	,484	,969	-,0010667	,0266711	-,0582705	,0561371
	Equal variances not assumed			-,039	9,538	,485	,970	-,0010667	,0276334	-,0630445	,0609112
Week12L2	Equal variances assumed	,016	,900	-1,487	14	,080	,159	-,0302333	,0203382	-,0738544	,0133878

	Equal variances not assumed			-1,492	10,789	,082	,164	-,0302333	,0202635	-,0749396	,0144729
Week24R2	Equal variances assumed	1,906	,189	-1,242	14	,117	,235	-,0359000	,0289097	-,0979051	,0261051
	Equal variances not assumed			-1,422	13,998	,088	,177	-,0359000	,0252377	-,0900302	,0182302
Week24L2	Equal variances assumed	,087	,772	-1,490	14	,079	,158	-,0467667	,0313871	-,1140852	,0205519
	Equal variances not assumed			-1,539	11,764	,075	,150	-,0467667	,0303803	-,1131074	,0195741

a. Parameter = R3

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,810	1,681	2	,431	,841	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,979	,168	2	,919	,980	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,746	2	,373	40,425	<,001	,818
	Greenhouse-Geisser	,746	1,681	,444	40,425	<,001	,818
Error(Visit)	Sphericity Assumed	,166	18	,009			
	Greenhouse-Geisser	,166	15,132	,011			
Side	Sphericity Assumed	,001	1	,001	,093	,767	,010
	Greenhouse-Geisser	,001	1,000	,001	,093	,767	,010
Error(Side)	Sphericity Assumed	,062	9	,007			
	Greenhouse-Geisser	,062	9,000	,007			
Visit * Side	Sphericity Assumed	,005	2	,002	,709	,505	,073
	Greenhouse-Geisser	,005	1,959	,002	,709	,503	,073
Error(Visit*Side)	Sphericity Assumed	,058	18	,003			
	Greenhouse-Geisser	,058	17,633	,003			

a. Parameter = R3, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,911	,375	2	,829	,918	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,470	3,020	2	,221	,654	,791	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,303	2	,151	11,426	,003	,696
	Greenhouse-Geisser	,303	1,836	,165	11,426	,004	,696
Error(Visit)	Sphericity Assumed	,132	10	,013			
	Greenhouse-Geisser	,132	9,179	,014			
Side	Sphericity Assumed	,001	1	,001	,045	,840	,009
	Greenhouse-Geisser	,001	1,000	,001	,045	,840	,009
Error(Side)	Sphericity Assumed	,094	5	,019			
	Greenhouse-Geisser	,094	5,000	,019			

Visit * Side	Sphericity Assumed	,002	2	,001	,103	,903	,020
	Greenhouse-Geisser	,002	1,307	,001	,103	,821	,020
Error(Visit*Side)	Sphericity Assumed	,084	10	,008			
	Greenhouse-Geisser	,084	6,536	,013			

a. Parameter = R3, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,368	8,007	2	,018	,613	,659	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,338	2	,169	24,904	<,001	,735
	Greenhouse-Geisser	,338	1,225	,276	24,904	<,001	,735
Error(Visits)	Sphericity Assumed	,122	18	,007			
	Greenhouse-Geisser	,122	11,026	,011			

a. Parameter = R3, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,206*	,033	<,001	,108	,304
	3	-,035	,024	,529	-,105	,035
2	1	-,206*	,033	<,001	-,304	-,108
	3	-,241*	,049	,002	-,384	-,097
3	1	,035	,024	,529	-,035	,105
	2	,241*	,049	,002	,097	,384

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,767	1,063	2	,588	,811	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,175	2	,088	10,318	,004	,674
	Greenhouse-Geisser	,175	1,622	,108	10,318	,007	,674
Error(Visits)	Sphericity Assumed	,085	10	,008			
	Greenhouse-Geisser	,085	8,108	,010			

a. Parameter = R3, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,241*	,046	,010	,078	,405
	3	,107	,065	,475	-,122	,336
2	1	-,241*	,046	,010	-,405	-,078
	3	-,134	,046	,103	-,298	,030

3	1	-,107	,065	,475	-,336	,122
	2	,134	,046	,103	-,030	,298

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,837	1,420	2	,492	,860	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,413	2	,206	36,297	<,001	,801
	Greenhouse-Geisser	,413	1,720	,240	36,297	<,001	,801
Error(Visits)	Sphericity Assumed	,102	18	,006			
	Greenhouse-Geisser	,102	15,482	,007			

a. Parameter = R3, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,247*	,039	<,001	,133	,361
	3	-,004	,034	1,000	-,105	,097
2	1	-,247*	,039	<,001	-,361	-,133
	3	-,251*	,027	<,001	-,330	-,172
3	1	,004	,034	1,000	-,097	,105
	2	,251*	,027	<,001	,172	,330

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,820	,791	2	,673	,848	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,129	2	,065	4,912	,033	,496
	Greenhouse-Geisser	,129	1,696	,076	4,912	,042	,496
Error(Visits)	Sphericity Assumed	,132	10	,013			
	Greenhouse-Geisser	,132	8,478	,016			

a. Parameter = R3, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,207	,077	,130	-,065	,479
	3	,092	,052	,418	-,093	,276
2	1	-,207	,077	,130	-,479	,065
	3	-,115	,067	,438	-,353	,122

3	1	-,092	,052	,418	-,276	,093
	2	,115	,067	,438	-,122	,353

Based on estimated marginal means
a. Parameter = R3, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,785500	10	,1326250	,0419397
	ScreeningL4	,816100	10	,1312169	,0414944
Pair 2	Week12R4	,579900	10	,1342712	,0424603
	Week12L4	,569300	10	,1195018	,0377898
Pair 3	Week24R4	,820400	10	,1375752	,0435051
	Week24L4	,820100	10	,0947306	,0299564

a. Parameter = R3, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
			Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
		Mean			Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,0306000	,0662792	,0209593	-,0780133	,0168133	-1,460	9	,089	,178
Pair 2	Week12R4 - Week12L4	,0106000	,1130302	,0357433	-,0702569	,0914569	,297	9	,387	,774
Pair 3	Week24R4 - Week24L4	,0003000	,0982820	,0310795	-,0700067	,0706067	,010	9	,496	,993

a. Parameter = R3, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,916833	6	,2095867	,0855634
	ScreeningL4	,910000	6	,1175500	,0479896
Pair 2	Week12R4	,675667	6	,1121350	,0457789
	Week12L4	,702833	6	,0968533	,0395402
Pair 3	Week24R4	,809500	6	,1375685	,0561621
	Week24L4	,818333	6	,1067364	,0435750

a. Parameter = R3, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
			Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
		Mean			Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0068333	,2317770	,0946226	-,2364017	,2500684	,072	5	,473	,945
Pair 2	Week12R4 - Week12L4	-,0271667	,0911711	,0372204	-,1228449	,0685115	-,730	5	,249	,498
Pair 3	Week24R4 - Week24L4	-,0088333	,0962859	,0393085	-,1098791	,0922125	-,225	5	,416	,831

a. Parameter = R3, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,785500	,1326250	,0419397
	ACS+HA	6	,916833	,2095867	,0855634
ScreeningL4	ACS	10	,816100	,1312169	,0414944
	ACS+HA	6	,910000	,1175500	,0479896

Week12R4	ACS	10	,579900	,1342712	,0424603
	ACS+HA	6	,675667	,1121350	,0457789
Week12L4	ACS	10	,569300	,1195018	,0377898
	ACS+HA	6	,702833	,0968533	,0395402
Week24R4	ACS	10	,820400	,1375752	,0435051
	ACS+HA	6	,809500	,1375685	,0561621
Week24L4	ACS	10	,820100	,0947306	,0299564
	ACS+HA	6	,818333	,1067364	,0435750

a. Parameter = R3

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	1,490	,242	-1,548	14	,072	,144	-,1313333	,0848458	-,3133094	,0506428
	Equal variances not assumed			-1,378	7,452	,104	,208	-,1313333	,0952892	-,3539142	,0912476
ScreeningL4	Equal variances assumed	,541	,474	-1,437	14	,086	,173	-,0939000	,0653271	-,2340128	,0462128
	Equal variances not assumed			-1,480	11,653	,083	,165	-,0939000	,0634412	-,2325850	,0447850
Week12R4	Equal variances assumed	,475	,502	-1,462	14	,083	,166	-,0957667	,0654843	-,2362165	,0446832
	Equal variances not assumed			-1,534	12,262	,075	,150	-,0957667	,0624387	-,2314875	,0399542
Week12L4	Equal variances assumed	,354	,561	-2,310	14	,018	,037	-,1335333	,0578057	-,2575143	-,0095524
	Equal variances not assumed			-2,441	12,508	,015	,030	-,1335333	,0546946	-,2521678	-,0148988
Week24R4	Equal variances assumed	,339	,570	,153	14	,440	,880	,0109000	,0710423	-,1414706	,1632706
	Equal variances not assumed			,153	10,667	,440	,881	,0109000	,0710414	-,1460584	,1678584
Week24L4	Equal variances assumed	,072	,792	,034	14	,486	,973	,0017667	,0512191	-,1080874	,1116207
	Equal variances not assumed			,033	9,646	,487	,974	,0017667	,0528788	-,1166434	,1201767

a. Parameter = R3

Skin net elasticity (R5, Ur/Ue)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk		
		Statistic	df	Sig.

ScreeningR2	ACS	,966	10	,851
	ACS+HA	,894	6	,342
ScreeningL2	ACS	,891	10	,173
	ACS+HA	,865	6	,208
Week12R2	ACS	,884	10	,144
	ACS+HA	,915	6	,469
Week12L2	ACS	,880	10	,131
	ACS+HA	,935	6	,616
Week24R2	ACS	,899	10	,212
	ACS+HA	,978	6	,944
Week24L2	ACS	,941	10	,566
	ACS+HA	,944	6	,689

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,969	10	,882
	ACS+HA	,997	6	1,000
ScreeningL4	ACS	,984	10	,984
	ACS+HA	,840	6	,130
Week12R4	ACS	,942	10	,571
	ACS+HA	,955	6	,780
Week12L4	ACS	,926	10	,413
	ACS+HA	,892	6	,328
Week24R4	ACS	,891	10	,173
	ACS+HA	,842	6	,136
Week24L4	ACS	,842	10	,047
	ACS+HA	,977	6	,935

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,550	4,788	2	,091	,689	,774	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,896	,882	2	,643	,905	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,155	2	,078	10,758	<,001
	Greenhouse-Geisser	,155	1,379	,112	10,758	,004
Error(Visit)	Sphericity Assumed	,130	18	,007		
	Greenhouse-Geisser	,130	12,411	,010		
Side	Sphericity Assumed	,015	1	,015	5,853	,039
	Greenhouse-Geisser	,015	1,000	,015	5,853	,039
Error(Side)	Sphericity Assumed	,023	9	,003		
	Greenhouse-Geisser	,023	9,000	,003		
Visit * Side	Sphericity Assumed	,000	2	,000	,024	,977
	Greenhouse-Geisser	,000	1,811	,000	,024	,969
Error(Visit*Side)	Sphericity Assumed	,134	18	,007		
	Greenhouse-Geisser	,134	16,299	,008		

a. Parameter = R5, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,854	,631	2	,729	,873	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000

Visit * Side	,744	1,182	2	,554	,796	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,019	2	,010	1,517	,266
	Greenhouse-Geisser	,019	1,745	,011	1,517	,269
Error(Visit)	Sphericity Assumed	,063	10	,006		
	Greenhouse-Geisser	,063	8,726	,007		
Side	Sphericity Assumed	,001	1	,001	,353	,578
	Greenhouse-Geisser	,001	1,000	,001	,353	,578
Error(Side)	Sphericity Assumed	,008	5	,002		
	Greenhouse-Geisser	,008	5,000	,002		
Visit * Side	Sphericity Assumed	,002	2	,001	,243	,789
	Greenhouse-Geisser	,002	1,593	,001	,243	,742
Error(Visit*Side)	Sphericity Assumed	,044	10	,004		
	Greenhouse-Geisser	,044	7,963	,005		

a. Parameter = R5, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,715	2,687	2	,261	,778	,911	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,081	2	,041	5,017	,019	,358
	Greenhouse-Geisser	,081	1,556	,052	5,017	,029	,358
Error(Visits)	Sphericity Assumed	,145	18	,008			
	Greenhouse-Geisser	,145	14,005	,010			

a. Parameter = R5, Treatment = ACS

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-,105	,046	,148	-,241	,031
	3	,010	,028	1,000	-,071	,091
2	1	,105	,046	,148	-,031	,241
	3	,115	,044	,085	-,014	,244
3	1	-,010	,028	1,000	-,091	,071
	2	-,115	,044	,085	-,244	,014

Based on estimated marginal means
a. Parameter = R5, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,792	,933	2	,627	,828	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,008	2	,004	,523	,608	,095
	Greenhouse-Geisser	,008	1,656	,005	,523	,578	,095
Error(Visits)	Sphericity Assumed	,073	10	,007			

	Greenhouse-Geisser	,073	8,278	,009				

a. Parameter = R5, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,041	,060	1,000	-,252	,170
	3	,005	,045	1,000	-,153	,164
2	1	,041	,060	1,000	-,170	,252
	3	,046	,042	,968	-,103	,195
3	1	-,005	,045	1,000	-,164	,153
	2	-,046	,042	,968	-,195	,103

Based on estimated marginal means
a. Parameter = R5, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,940	,493	2	,782	,944	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,074	2	,037	5,638	,013	,385
	Greenhouse-Geisser	,074	1,887	,039	5,638	,014	,385
Error(Visits)	Sphericity Assumed	,119	18	,007			
	Greenhouse-Geisser	,119	16,985	,007			

a. Parameter = R5, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,106	,038	,065	-,218	,006
	3	-,001	,032	1,000	-,093	,092
2	1	,106	,038	,065	-,006	,218
	3	,105	,039	,071	-,008	,218
3	1	,001	,032	1,000	-,092	,093
	2	-,105	,039	,071	-,218	,008

Based on estimated marginal means
a. Parameter = R5, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,741	1,198	2	,549	,794	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,014	2	,007	2,030	,182	,289
	Greenhouse-Geisser	,014	1,589	,009	2,030	,195	,289
Error(Visits)	Sphericity Assumed	,034	10	,003			
	Greenhouse-Geisser	,034	7,944	,004			

a. Parameter = R5, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,023	,038	1,000	-,159	,112
	3	,043	,024	,383	-,040	,126
2	1	,023	,038	1,000	-,112	,159
	3	,066	,036	,384	-,062	,195
3	1	-,043	,024	,383	-,126	,040
	2	-,066	,036	,384	-,195	,062

Based on estimated marginal means
a. Parameter = R5, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,356900	10	,1315707	,0416063
	ScreeningL2	,384580	10	,0697450	,0220553
Pair 2	Week12R2	,461920	10	,1184983	,0374725
	Week12L2	,490590	10	,1249217	,0395037
Pair 3	Week24R2	,347070	10	,0867091	,0274198
	Week24L2	,385470	10	,1056475	,0334087

a. Parameter = R5, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0276800	,1250182	,0395342	-,1171127	,0617527	-,700	9	,251	,502
Pair 2	Week12R2 - Week12L2	-,0286700	,1098838	,0347483	-,1072761	,0499361	-,825	9	,215	,431
Pair 3	Week24R2 - Week24L2	-,0384000	,0852005	,0269428	-,0993488	,0225488	-1,425	9	,094	,188

a. Parameter = R5, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,311000	6	,0523755	,0213822
	ScreeningL2	,337383	6	,0638604	,0260709
Pair 2	Week12R2	,351900	6	,1181418	,0482312
	Week12L2	,360817	6	,0786963	,0321276
Pair 3	Week24R2	,305667	6	,0729318	,0297743
	Week24L2	,294433	6	,0322326	,0131589

a. Parameter = R5, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0263833	,0738108	,0301331	-,1038430	,0510763	-,876	5	,211	,421
Pair 2	Week12R2 - Week12L2	-,0089167	,1119595	,0457073	-,1264109	,1085776	-,195	5	,427	,853
Pair 3	Week24R2 - Week24L2	,0112333	,0527515	,0215357	-,0441260	,0665926	,522	5	,312	,624

a. Parameter = R5, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,356900	,1315707	,0416063
	ACS+HA	6	,311000	,0523755	,0213822
ScreeningL2	ACS	10	,384580	,0697450	,0220553
	ACS+HA	6	,337383	,0638604	,0260709
Week12R2	ACS	10	,461920	,1184983	,0374725
	ACS+HA	6	,351900	,1181418	,0482312
Week12L2	ACS	10	,490590	,1249217	,0395037
	ACS+HA	6	,360817	,0786963	,0321276
Week24R2	ACS	10	,347070	,0867091	,0274198
	ACS+HA	6	,305667	,0729318	,0297743
Week24L2	ACS	10	,385470	,1056475	,0334087
	ACS+HA	6	,294433	,0322326	,0131589

a. Parameter = R5

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR2	Equal variances assumed	2,637	,127	,808	14	,216	,433	,0459000	,0568228	-,0759728	,1677728
	Equal variances not assumed			,981	12,777	,172	,345	,0459000	,0467791	-,0553393	,1471393
ScreeningL2	Equal variances assumed	,670	,427	1,350	14	,099	,198	,0471967	,0349612	-,0277877	,1221810
	Equal variances not assumed			1,382	11,457	,097	,193	,0471967	,0341486	-,0275994	,1219928
Week12R2	Equal variances assumed	,071	,794	1,800	14	,047	,093	,1100200	,0611266	-,0210835	,2411235
	Equal variances not assumed			1,801	10,693	,050	,100	,1100200	,0610773	-,0248818	,2449218
Week12L2	Equal variances assumed	2,156	,164	2,271	14	,020	,039	,1297733	,0571405	,0072191	,2523275
	Equal variances not assumed			2,549	13,898	,012	,023	,1297733	,0509188	,0204884	,2390583
Week24R2	Equal variances assumed	,016	,902	,977	14	,173	,345	,0414033	,0423728	-,0494773	,1322840
	Equal variances not assumed			1,023	12,202	,163	,326	,0414033	,0404766	-,0466262	,1294329
Week24L2	Equal variances assumed	3,648	,077	2,029	14	,031	,062	,0910367	,0448590	-,0051763	,1872496
	Equal variances not assumed			2,535	11,510	,013	,027	,0910367	,0359068	,0124319	,1696414

a. Parameter = R5

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,480	5,879	2	,053	,658	,726	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,939	,506	2	,777	,942	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1,598	2	,799	71,341	<,001	,888
	Greenhouse-Geisser	1,598	1,315	1,215	71,341	<,001	,888
Error(Visit)	Sphericity Assumed	,202	18	,011			
	Greenhouse-Geisser	,202	11,839	,017			
Side	Sphericity Assumed	,012	1	,012	5,834	,039	,393
	Greenhouse-Geisser	,012	1,000	,012	5,834	,039	,393
Error(Side)	Sphericity Assumed	,018	9	,002			
	Greenhouse-Geisser	,018	9,000	,002			
Visit * Side	Sphericity Assumed	,017	2	,009	3,697	,045	,291
	Greenhouse-Geisser	,017	1,885	,009	3,697	,049	,291
Error(Visit*Side)	Sphericity Assumed	,043	18	,002			
	Greenhouse-Geisser	,043	16,961	,003			

a. Parameter = R5, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,651	1,714	2	,424	,742	,981	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,671	1,594	2	,451	,753	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,516	2	,258	16,441	<,001	,767
	Greenhouse-Geisser	,516	1,483	,348	16,441	,003	,767
Error(Visit)	Sphericity Assumed	,157	10	,016			
	Greenhouse-Geisser	,157	7,415	,021			
Side	Sphericity Assumed	,001	1	,001	,294	,611	,056
	Greenhouse-Geisser	,001	1,000	,001	,294	,611	,056
Error(Side)	Sphericity Assumed	,012	5	,002			
	Greenhouse-Geisser	,012	5,000	,002			
Visit * Side	Sphericity Assumed	,002	2	,001	,148	,864	,029
	Greenhouse-Geisser	,002	1,505	,001	,148	,807	,029
Error(Visit*Side)	Sphericity Assumed	,059	10	,006			
	Greenhouse-Geisser	,059	7,526	,008			

a. Parameter = R5, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,466	6,106	2	,047	,652	,717	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,705	2	,353	45,879	<,001	,836
	Greenhouse-Geisser	,705	1,304	,541	45,879	<,001	,836
Error(Visits)	Sphericity Assumed	,138	18	,008			
	Greenhouse-Geisser	,138	11,735	,012			

a. Parameter = R5, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,299*	,050	<,001	-,447	-,151
	3	,047	,025	,255	-,025	,119
2	1	,299*	,050	<,001	,151	,447
	3	,346*	,038	<,001	,234	,459
3	1	-,047	,025	,255	-,119	,025
	2	-,346*	,038	<,001	-,459	-,234

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,631	1,843	2	,398	,730	,956	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,285	2	,142	24,800	<,001	,832
	Greenhouse-Geisser	,285	1,461	,195	24,800	<,001	,832
Error(Visits)	Sphericity Assumed	,057	10	,006			
	Greenhouse-Geisser	,057	7,303	,008			

a. Parameter = R5, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,298*	,043	,003	-,451	-,145
	3	-,081	,031	,135	-,189	,027
2	1	,298*	,043	,003	,145	,451
	3	,217*	,054	,031	,025	,409
3	1	,081	,031	,135	-,027	,189
	2	-,217*	,054	,031	-,409	-,025

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visits	,590	4,219	2	,121	,709	,804	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,910	2	,455	77,410	<,001	,896
	Greenhouse-Geisser	,910	1,419	,642	77,410	<,001	,896
Error(Visits)	Sphericity Assumed	,106	18	,006			
	Greenhouse-Geisser	,106	12,767	,008			

a. Parameter = R5, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,378*	,037	<,001	-,487	-,268
	3	-,017	,021	1,000	-,078	,045
2	1	,378*	,037	<,001	,268	,487
	3	,361*	,041	<,001	,240	,482
3	1	,017	,021	1,000	-,045	,078
	2	-,361*	,041	<,001	-,482	-,240

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,718	1,326	2	,515	,780	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,233	2	,117	7,371	,011	,596
	Greenhouse-Geisser	,233	1,560	,149	7,371	,019	,596
Error(Visits)	Sphericity Assumed	,158	10	,016			
	Greenhouse-Geisser	,158	7,799	,020			

a. Parameter = R5, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,265	,077	,056	-,538	,008
	3	-,058	,051	,922	-,237	,121
2	1	,265	,077	,056	-,008	,538
	3	,207	,085	,177	-,094	,508
3	1	,058	,051	,922	-,121	,237
	2	-,207	,085	,177	-,508	,094

Based on estimated marginal means
a. Parameter = R5, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,563630	10	,1266751	,0400582

	ScreeningL4	,544190	10	,1075388	,0340067
Pair 2	Week12R4	,862550	10	,1207248	,0381765
	Week12L4	,921740	10	,1150232	,0363735
Pair 3	Week24R4	,516160	10	,0851970	,0269416
	Week24L4	,560740	10	,1176243	,0371961

a. Parameter = R5, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0194400	,0537089	,0169842	-,0189810	,0578610	1,145	9	,141	,282
Pair 2	Week12R4 - Week12L4	-,0591900	,0853521	,0269907	-,1202472	,0018672	-2,193	9	,028	,056
Pair 3	Week24R4 - Week24L4	-,0445800	,0579325	,0183199	-,0860224	-,0031376	-2,433	9	,019	,038

a. Parameter = R5, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,471800	6	,0767753	,0313434
	ScreeningL4	,499333	6	,1178627	,0481172
Pair 2	Week12R4	,769817	6	,1203895	,0491488
	Week12L4	,764283	6	,1702190	,0694916
Pair 3	Week24R4	,552850	6	,1199528	,0489705
	Week24L4	,556933	6	,1096828	,0447778

a. Parameter = R5, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,0275333	,0845741	,0345272	-,1162884	,0612217	-,797	5	,231	,461
Pair 2	Week12R4 - Week12L4	,0055333	,1385477	,0565618	-,1398635	,1509302	,098	5	,463	,926
Pair 3	Week24R4 - Week24L4	-,0040833	,0410460	,0167570	-,0471585	,0389918	-,244	5	,409	,817

a. Parameter = R5, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,563630	,1266751	,0400582
	ACS+HA	6	,471800	,0767753	,0313434
ScreeningL4	ACS	10	,544190	,1075388	,0340067
	ACS+HA	6	,499333	,1178627	,0481172
Week12R4	ACS	10	,862550	,1207248	,0381765
	ACS+HA	6	,769817	,1203895	,0491488
Week12L4	ACS	10	,921740	,1150232	,0363735
	ACS+HA	6	,764283	,1702190	,0694916
Week24R4	ACS	10	,516160	,0851970	,0269416
	ACS+HA	6	,552850	,1199528	,0489705
Week24L4	ACS	10	,560740	,1176243	,0371961
	ACS+HA	6	,556933	,1096828	,0447778

a. Parameter = R5

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	,846	,373	1,596	14	,066	,133	,0918300	,0575519	-,0316065	,2152665
	Equal variances not assumed			1,805	13,969	,046	,093	,0918300	,0508632	-,0172835	,2009435
ScreeningL4	Equal variances assumed	,512	,486	,780	14	,224	,448	,0448567	,0574936	-,0784548	,1681681
	Equal variances not assumed			,761	9,874	,232	,464	,0448567	,0589214	-,0866561	,1763694
Week12R4	Equal variances assumed	,033	,859	1,489	14	,079	,159	,0927333	,0622803	-,0408445	,2263112
	Equal variances not assumed			1,490	10,691	,083	,165	,0927333	,0622339	-,0447264	,2301931
Week12L4	Equal variances assumed	3,717	,074	2,221	14	,022	,043	,1574567	,0709051	,0053803	,3095330
	Equal variances not assumed			2,007	7,790	,040	,081	,1574567	,0784355	-,0242673	,3391807
Week24R4	Equal variances assumed	3,052	,103	-,718	14	,242	,485	-,0366900	,0511338	-,1463611	,0729811
	Equal variances not assumed			-,656	8,074	,265	,530	-,0366900	,0558924	-,1653731	,0919931
Week24L4	Equal variances assumed	,193	,667	,064	14	,475	,950	,0038067	,0593089	-,1233982	,1310115
	Equal variances not assumed			,065	11,294	,475	,949	,0038067	,0582117	-,1239112	,1315245

a. Parameter = R5

Ratio of viscoelastic to elastic extension (R6, Uv/Ue)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	ACS	,878	10	,122
	ACS+HA	,777	6	,036
ScreeningL2	ACS	,935	10	,503
	ACS+HA	,808	6	,069
Week12R2	ACS	,884	10	,143
	ACS+HA	,927	6	,556
Week12L2	ACS	,934	10	,483
	ACS+HA	,943	6	,687
Week24R2	ACS	,883	10	,141

	ACS+HA	,845	6	,143
Week24L2	ACS	,865	10	,088
	ACS+HA	,943	6	,680

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,915	10	,319
	ACS+HA	,798	6	,056
ScreeningL4	ACS	,901	10	,223
	ACS+HA	,982	6	,960
Week12R4	ACS	,934	10	,489
	ACS+HA	,891	6	,325
Week12L4	ACS	,946	10	,618
	ACS+HA	,919	6	,497
Week24R4	ACS	,943	10	,587
	ACS+HA	,737	6	,015
Week24L4	ACS	,761	10	,005
	ACS+HA	,689	6	,005

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,753	2,273	2	,321	,802	,949	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,570	4,493	2	,106	,699	,789	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,166	2	,083	6,240	,009
	Greenhouse-Geisser	,166	1,603	,103	6,240	,015
Error(Visit)	Sphericity Assumed	,239	18	,013		
	Greenhouse-Geisser	,239	14,431	,017		
Side	Sphericity Assumed	,005	1	,005	2,544	,145
	Greenhouse-Geisser	,005	1,000	,005	2,544	,145
Error(Side)	Sphericity Assumed	,017	9	,002		
	Greenhouse-Geisser	,017	9,000	,002		
Visit * Side	Sphericity Assumed	,000	2	,000	,035	,966
	Greenhouse-Geisser	,000	1,399	,000	,035	,920
Error(Visit*Side)	Sphericity Assumed	,058	18	,003		
	Greenhouse-Geisser	,058	12,590	,005		

a. Parameter = R6, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,396	3,703	2	,157	,624	,730	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,638	1,797	2	,407	,734	,964	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,049	2	,024	1,122	,363
	Greenhouse-Geisser	,049	1,247	,039	1,122	,348

Error(Visit)	Sphericity Assumed	,217	10	,022		
	Greenhouse-Geisser	,217	6,235	,035		
Side	Sphericity Assumed	,001	1	,001	,152	,713
	Greenhouse-Geisser	,001	1,000	,001	,152	,713
Error(Side)	Sphericity Assumed	,022	5	,004		
	Greenhouse-Geisser	,022	5,000	,004		
Visit * Side	Sphericity Assumed	,005	2	,002	1,811	,213
	Greenhouse-Geisser	,005	1,469	,003	1,811	,227
Error(Visit*Side)	Sphericity Assumed	,013	10	,001		
	Greenhouse-Geisser	,013	7,343	,002		

a. Parameter = R6, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,443	6,511	2	,039	,642	,703	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,077	2	,038	3,979	,037	,307
	Greenhouse-Geisser	,077	1,285	,060	3,979	,063	,307
Error(Visits)	Sphericity Assumed	,174	18	,010			
	Greenhouse-Geisser	,174	11,562	,015			

a. Parameter = R6, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,117	,054	,182	-,276	,043
	3	-,022	,023	1,000	-,090	,047
2	1	,117	,054	,182	-,043	,276
	3	,095	,048	,237	-,046	,235
3	1	,022	,023	1,000	-,047	,090
	2	-,095	,048	,237	-,235	,046

Based on estimated marginal means
a. Parameter = R6, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,510	2,694	2	,260	,671	,827	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,037	2	,018	1,767	,220	,261
	Greenhouse-Geisser	,037	1,342	,028	1,767	,235	,261
Error(Visits)	Sphericity Assumed	,105	10	,010			
	Greenhouse-Geisser	,105	6,711	,016			

a. Parameter = R6, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb

					Lower Bound	Upper Bound
1	2	-,041	,066	1,000	-,276	,194
	3	,069	,071	1,000	-,181	,318
2	1	,041	,066	1,000	-,194	,276
	3	,110	,033	,060	-,006	,225
3	1	-,069	,071	1,000	-,318	,181
	2	-,110	,033	,060	-,225	,006

Based on estimated marginal means
a. Parameter = R6, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,872	1,092	2	,579	,887	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,089	2	,044	6,536	,007	,421
	Greenhouse-Geisser	,089	1,774	,050	6,536	,010	,421
Error(Visits)	Sphericity Assumed	,122	18	,007			
	Greenhouse-Geisser	,122	15,963	,008			

a. Parameter = R6, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,125*	,033	,013	-,222	-,028
	3	-,022	,034	1,000	-,122	,077
2	1	,125*	,033	,013	,028	,222
	3	,103	,043	,121	-,023	,229
3	1	,022	,034	1,000	-,077	,122
	2	-,103	,043	,121	-,229	,023

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,354	4,158	2	,125	,607	,699	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,016	2	,008	,652	,542	,115
	Greenhouse-Geisser	,016	1,215	,013	,652	,480	,115
Error(Visits)	Sphericity Assumed	,125	10	,012			
	Greenhouse-Geisser	,125	6,074	,021			

a. Parameter = R6, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound

1	2	-,055	,071	1,000	-,307	,198
	3	,016	,081	1,000	-,269	,300
2	1	,055	,071	1,000	-,198	,307
	3	,070	,030	,203	-,037	,177
3	1	-,016	,081	1,000	-,300	,269
	2	-,070	,030	,203	-,177	,037

Based on estimated marginal means
a. Parameter = R6, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,386440	10	,0792303	,0250548
	ScreeningL2	,401200	10	,0675321	,0213555
Pair 2	Week12R2	,503040	10	,1588453	,0502313
	Week12L2	,526160	10	,0922803	,0291816
Pair 3	Week24R2	,408200	10	,1285651	,0406559
	Week24L2	,423320	10	,1216693	,0384752

a. Parameter = R6, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0147600	,0734281	,0232200	-,0672873	,0377673	-,636	9	,270	,541
Pair 2	Week12R2 - Week12L2	-,0231200	,0920295	,0291023	-,0889540	,0427140	-,794	9	,224	,447
Pair 3	Week24R2 - Week24L2	-,0151200	,0509872	,0161236	-,0515940	,0213540	-,938	9	,186	,373

a. Parameter = R6, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,432917	6	,1604977	,0655229
	ScreeningL2	,419333	6	,1618257	,0660651
Pair 2	Week12R2	,474067	6	,0305175	,0124587
	Week12L2	,473933	6	,0447975	,0182885
Pair 3	Week24R2	,364267	6	,0754601	,0308064
	Week24L2	,403750	6	,0965720	,0394254

a. Parameter = R6, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	,0135833	,0378699	,0154603	-,0261586	,0533253	,879	5	,210	,420
Pair 2	Week12R2 - Week12L2	,0001333	,0687838	,0280809	-,0720508	,0723175	,005	5	,498	,996
Pair 3	Week24R2 - Week24L2	-,0394833	,0872196	,0356073	-,1310147	,0520480	-1,109	5	,159	,318

a. Parameter = R6, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,386440	,0792303	,0250548
	ACS+HA	6	,432917	,1604977	,0655229
ScreeningL2	ACS	10	,401200	,0675321	,0213555
	ACS+HA	6	,419333	,1618257	,0660651
Week12R2	ACS	10	,503040	,1588453	,0502313
	ACS+HA	6	,474067	,0305175	,0124587
Week12L2	ACS	10	,526160	,0922803	,0291816
	ACS+HA	6	,473933	,0447975	,0182885
Week24R2	ACS	10	,408200	,1285651	,0406559
	ACS+HA	6	,364267	,0754601	,0308064
Week24L2	ACS	10	,423320	,1216693	,0384752
	ACS+HA	6	,403750	,0965720	,0394254

a. Parameter = R6

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR2	Equal variances assumed	1,031	,327	-,782	14	,224	,447	-,0464767	,0594089	-,1738961	,0809428
	Equal variances not assumed			-,663	6,492	,265	,530	-,0464767	,0701498	-,2150228	,1220694
ScreeningL2	Equal variances assumed	3,905	,068	-,317	14	,378	,756	-,0181333	,0572352	-,1408906	,1046239
	Equal variances not assumed			-,261	6,063	,401	,803	-,0181333	,0694309	-,1875995	,1513329
Week12R2	Equal variances assumed	3,880	,069	,436	14	,335	,669	,0289733	,0664391	-,1135243	,1714710
	Equal variances not assumed			,560	10,073	,294	,588	,0289733	,0517533	-,0862274	,1441740
Week12L2	Equal variances assumed	2,753	,119	1,285	14	,110	,220	,0522267	,0406319	-,0349201	,1393734
	Equal variances not assumed			1,517	13,664	,076	,152	,0522267	,0344388	-,0218081	,1262614
Week24R2	Equal variances assumed	2,776	,118	,756	14	,231	,462	,0439333	,0581020	-,0806832	,1685498
	Equal variances not assumed			,861	13,996	,202	,404	,0439333	,0510092	-,0654730	,1533397
Week24L2	Equal variances assumed	,531	,478	,334	14	,372	,743	,0195700	,0585315	-,1059676	,1451076
	Equal variances not assumed			,355	12,673	,364	,728	,0195700	,0550881	-,0997536	,1388936

a. Parameter = R6

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,627	3,730	2	,155	,729	,833	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,379	7,765	2	,021	,617	,665	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	1,134	2	,567	48,897	<,001	,845
	Greenhouse-Geisser	1,134	1,457	,778	48,897	<,001	,845
Error(Visit)	Sphericity Assumed	,209	18	,012			
	Greenhouse-Geisser	,209	13,113	,016			
Side	Sphericity Assumed	4,735E-5	1	4,735E-5	,012	,914	,001
	Greenhouse-Geisser	4,735E-5	1,000	4,735E-5	,012	,914	,001
Error(Side)	Sphericity Assumed	,035	9	,004			
	Greenhouse-Geisser	,035	9,000	,004			
Visit * Side	Sphericity Assumed	,013	2	,007	2,034	,160	,184
	Greenhouse-Geisser	,013	1,234	,011	2,034	,182	,184
Error(Visit*Side)	Sphericity Assumed	,058	18	,003			
	Greenhouse-Geisser	,058	11,103	,005			

a. Parameter = R6, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,740	1,205	2	,547	,794	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,922	,324	2	,851	,928	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,427	2	,213	8,747	,006	,636
	Greenhouse-Geisser	,427	1,587	,269	8,747	,012	,636
Error(Visit)	Sphericity Assumed	,244	10	,024			
	Greenhouse-Geisser	,244	7,935	,031			
Side	Sphericity Assumed	,005	1	,005	1,001	,363	,167
	Greenhouse-Geisser	,005	1,000	,005	1,001	,363	,167
Error(Side)	Sphericity Assumed	,024	5	,005			
	Greenhouse-Geisser	,024	5,000	,005			
Visit * Side	Sphericity Assumed	,001	2	,000	,534	,602	,096
	Greenhouse-Geisser	,001	1,856	,001	,534	,590	,096
Error(Visit*Side)	Sphericity Assumed	,009	10	,001			
	Greenhouse-Geisser	,009	9,279	,001			

a. Parameter = R6, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,654	3,399	2	,183	,743	,856	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,470	2	,235	30,810	<,001	,774
	Greenhouse-Geisser	,470	1,486	,316	30,810	<,001	,774
Error(Visits)	Sphericity Assumed	,137	18	,008			
	Greenhouse-Geisser	,137	13,372	,010			

a. Parameter = R6, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,252*	,044	<,001	-,380	-,124
	3	,025	,025	1,000	-,048	,099
2	1	,252*	,044	<,001	,124	,380
	3	,277*	,045	<,001	,145	,410
3	1	-,025	,025	1,000	-,099	,048
	2	-,277*	,045	<,001	-,410	-,145

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,866	,576	2	,750	,882	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,206	2	,103	8,262	,008	,623
	Greenhouse-Geisser	,206	1,763	,117	8,262	,011	,623
Error(Visits)	Sphericity Assumed	,125	10	,012			
	Greenhouse-Geisser	,125	8,817	,014			

a. Parameter = R6, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	-,258*	,071	,045	-,508	-,007
	3	-,089	,051	,435	-,270	,093
2	1	,258*	,071	,045	,007	,508
	3	,169	,069	,175	-,075	,414
3	1	,089	,051	,435	-,093	,270
	2	-,169	,069	,175	-,414	,075

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,487	5,753	2	,056	,661	,731	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,677	2	,339	46,958	<,001	,839
	Greenhouse-Geisser	,677	1,322	,512	46,958	<,001	,839
Error(Visits)	Sphericity Assumed	,130	18	,007			
	Greenhouse-Geisser	,130	11,898	,011			

a. Parameter = R6, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,324*	,040	<,001	-,442	-,207
	3	-,012	,022	1,000	-,076	,052
2	1	,324*	,040	<,001	,207	,442
	3	,313*	,047	<,001	,174	,452
3	1	,012	,022	1,000	-,052	,076
	2	-,313*	,047	<,001	-,452	-,174

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,592	2,100	2	,350	,710	,911	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,222	2	,111	8,648	,007	,634
	Greenhouse-Geisser	,222	1,420	,156	8,648	,016	,634
Error(Visits)	Sphericity Assumed	,128	10	,013			
	Greenhouse-Geisser	,128	7,100	,018			

a. Parameter = R6, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,263	,080	,067	-,546	,021
	3	-,070	,042	,472	-,217	,078
2	1	,263	,080	,067	-,021	,546
	3	,193	,068	,110	-,048	,434
3	1	,070	,042	,472	-,078	,217
	2	-,193	,068	,110	-,434	,048

Based on estimated marginal means
a. Parameter = R6, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,464570	10	,1038109	,0328279
	ScreeningL4	,429790	10	,0910853	,0288037
Pair 2	Week12R4	,716350	10	,1758376	,0556047

	Week12L4	,754170	10	,1868541	,0590885
Pair 3	Week24R4	,439160	10	,0812968	,0257083
	Week24L4	,441450	10	,0667213	,0210991

a. Parameter = R6, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0347800	,0414256	,0130999	,0051459	,0644141	2,655	9	,013	,026
Pair 2	Week12R4 - Week12L4	-,0378200	,1214165	,0383953	-,1246761	,0490361	-,985	9	,175	,350
Pair 3	Week24R4 - Week24L4	-,0022900	,0648815	,0205173	-,0487034	,0441234	-,112	9	,457	,914

a. Parameter = R6, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,412050	6	,0464701	,0189713
	ScreeningL4	,439883	6	,0689162	,0281349
Pair 2	Week12R4	,669817	6	,2169476	,0885685
	Week12L4	,702383	6	,2262602	,0923703
Pair 3	Week24R4	,500633	6	,1472704	,0601229
	Week24L4	,509433	6	,1540133	,0628757

a. Parameter = R6, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,0278333	,0706219	,0288313	-,1019465	,0462798	-,965	5	,189	,379
Pair 2	Week12R4 - Week12L4	-,0325667	,0797100	,0325415	-,1162172	,0510838	-1,001	5	,181	,363
Pair 3	Week24R4 - Week24L4	-,0088000	,0422439	,0172460	-,0531323	,0355323	-,510	5	,316	,632

a. Parameter = R6, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,464570	,1038109	,0328279
	ACS+HA	6	,412050	,0464701	,0189713
ScreeningL4	ACS	10	,429790	,0910853	,0288037
	ACS+HA	6	,439883	,0689162	,0281349
Week12R4	ACS	10	,716350	,1758376	,0556047
	ACS+HA	6	,669817	,2169476	,0885685
Week12L4	ACS	10	,754170	,1868541	,0590885
	ACS+HA	6	,702383	,2262602	,0923703
Week24R4	ACS	10	,439160	,0812968	,0257083
	ACS+HA	6	,500633	,1472704	,0601229
Week24L4	ACS	10	,441450	,0667213	,0210991
	ACS+HA	6	,509433	,1540133	,0628757

a. Parameter = R6

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	5,143	,040	1,159	14	,133	,266	,0525200	,0453111	-,0446627	,1497027
	Equal variances not assumed			1,385	13,338	,094	,189	,0525200	,0379155	-,0291811	,1342211
ScreeningL4	Equal variances assumed	2,195	,161	-,233	14	,410	,819	-,0100933	,0432965	-,1029552	,0827685
	Equal variances not assumed			-,251	13,025	,403	,806	-,0100933	,0402645	-,0970626	,0768759
Week12R4	Equal variances assumed	,523	,481	,470	14	,323	,645	,0465333	,0989084	-,1656041	,2586708
	Equal variances not assumed			,445	8,946	,333	,667	,0465333	,1045766	-,1902525	,2833192
Week12L4	Equal variances assumed	,756	,399	,497	14	,313	,627	,0517867	,1042158	-,1717339	,2753072
	Equal variances not assumed			,472	9,084	,324	,648	,0517867	,1096527	-,1959150	,2994883
Week24R4	Equal variances assumed	,824	,379	-1,087	14	,148	,295	-,0614733	,0565560	-,1827738	,0598272
	Equal variances not assumed			-,940	6,868	,190	,379	-,0614733	,0653887	-,2166975	,0937509
Week24L4	Equal variances assumed	2,597	,129	-1,237	14	,118	,237	-,0679833	,0549746	-,1858922	,0499256
	Equal variances not assumed			-1,025	6,146	,172	,344	-,0679833	,0663214	-,2293349	,0933683

a. Parameter = R6

Ratio of elastic recovery to total extension (R7, Ur/Uf)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	ACS	,942	10	,581
	ACS+HA	,966	6	,862
ScreeningL2	ACS	,929	10	,440
	ACS+HA	,843	6	,137
Week12R2	ACS	,935	10	,503
	ACS+HA	,900	6	,371
Week12L2	ACS	,867	10	,093
	ACS+HA	,930	6	,579
Week24R2	ACS	,965	10	,840
	ACS+HA	,979	6	,949
Week24L2	ACS	,944	10	,602
	ACS+HA	,911	6	,445

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,970	10	,895
	ACS+HA	,954	6	,776
ScreeningL4	ACS	,896	10	,197
	ACS+HA	,892	6	,331
Week12R4	ACS	,949	10	,653
	ACS+HA	,916	6	,478
Week12L4	ACS	,921	10	,363
	ACS+HA	,870	6	,225
Week24R4	ACS	,871	10	,103
	ACS+HA	,880	6	,270
Week24L4	ACS	,864	10	,086
	ACS+HA	,957	6	,800

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,763	2,163	2	,339	,808	,960	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,944	,462	2	,794	,947	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,036	2	,018	10,723	<,001
	Greenhouse-Geisser	,036	1,617	,022	10,723	,002
Error(Visit)	Sphericity Assumed	,030	18	,002		
	Greenhouse-Geisser	,030	14,553	,002		
Side	Sphericity Assumed	,005	1	,005	4,893	,054
	Greenhouse-Geisser	,005	1,000	,005	4,893	,054
Error(Side)	Sphericity Assumed	,009	9	,001		
	Greenhouse-Geisser	,009	9,000	,001		
Visit * Side	Sphericity Assumed	,000	2	,000	,040	,961
	Greenhouse-Geisser	,000	1,894	,000	,040	,956
Error(Visit*Side)	Sphericity Assumed	,047	18	,003		
	Greenhouse-Geisser	,047	17,044	,003		

a. Parameter = R7, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,676	1,565	2	,457	,755	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,823	,780	2	,677	,849	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,004	2	,002	1,009	,399
	Greenhouse-Geisser	,004	1,511	,002	1,009	,385
Error(Visit)	Sphericity Assumed	,018	10	,002		
	Greenhouse-Geisser	,018	7,554	,002		
Side	Sphericity Assumed	,000	1	,000	,569	,484

	Greenhouse-Geisser	,000	1,000	,000	,569	,484
Error(Side)	Sphericity Assumed	,003	5	,001		
	Greenhouse-Geisser	,003	5,000	,001		
Visit * Side	Sphericity Assumed	,002	2	,001	,483	,631
	Greenhouse-Geisser	,002	1,699	,001	,483	,603
Error(Visit*Side)	Sphericity Assumed	,023	10	,002		
	Greenhouse-Geisser	,023	8,495	,003		

a. Parameter = R7, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,929	,590	2	,744	,934	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,021	2	,010	4,124	,034	,314
	Greenhouse-Geisser	,021	1,867	,011	4,124	,037	,314
Error(Visits)	Sphericity Assumed	,045	18	,003			
	Greenhouse-Geisser	,045	16,804	,003			

a. Parameter = R7, Treatment = ACS

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-,051	,023	,159	-,118	,016
	3	,008	,019	1,000	-,048	,065
2	1	,051	,023	,159	-,016	,118
	3	,059	,025	,116	-,013	,131
3	1	-,008	,019	1,000	-,065	,048
	2	-,059	,025	,116	-,131	,013

Based on estimated marginal means

a. Parameter = R7, Treatment = ACS

b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,762	1,086	2	,581	,808	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,001	2	,001	,324	,730	,061
	Greenhouse-Geisser	,001	1,616	,001	,324	,688	,061
Error(Visits)	Sphericity Assumed	,023	10	,002			
	Greenhouse-Geisser	,023	8,080	,003			

a. Parameter = R7, Treatment = ACS+HA

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-,022	,033	1,000	-,140	,096
	3	-,007	,022	1,000	-,085	,071

2	1	,022	,033	1,000	-,096	,140
	3	,015	,026	1,000	-,078	,107
3	1	,007	,022	1,000	-,071	,085
	2	-,015	,026	1,000	-,107	,078

Based on estimated marginal means
a. Parameter = R7, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,984	,131	2	,937	,984	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,016	2	,008	4,304	,030	,324
	Greenhouse-Geisser	,016	1,968	,008	4,304	,030	,324
Error(Visits)	Sphericity Assumed	,033	18	,002			
	Greenhouse-Geisser	,033	17,713	,002			

a. Parameter = R7, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,046	,020	,142	-,106	,013
	3	,004	,018	1,000	-,050	,058
2	1	,046	,020	,142	-,013	,106
	3	,050	,018	,071	-,004	,104
3	1	-,004	,018	1,000	-,058	,050
	2	-,050	,018	,071	-,104	,004

Based on estimated marginal means
a. Parameter = R7, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,895	,446	2	,800	,905	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,004	2	,002	1,220	,336	,196
	Greenhouse-Geisser	,004	1,809	,002	1,220	,334	,196
Error(Visits)	Sphericity Assumed	,018	10	,002			
	Greenhouse-Geisser	,018	9,046	,002			

a. Parameter = R7, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,003	,026	1,000	-,094	,088
	3	,031	,020	,538	-,039	,102

2	1	,003	,026	1,000	-,088	,094
	3	,034	,026	,753	-,059	,127
3	1	-,031	,020	,538	-,102	,039
	2	-,034	,026	,753	-,127	,059

Based on estimated marginal means
a. Parameter = R7, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,256260	10	,0897280	,0283745
	ScreeningL2	,274410	10	,0480934	,0152085
Pair 2	Week12R2	,307270	10	,0679099	,0214750
	Week12L2	,320900	10	,0765179	,0241971
Pair 3	Week24R2	,247970	10	,0665594	,0210479
	Week24L2	,270730	10	,0706185	,0223315

a. Parameter = R7, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0181500	,0787408	,0249000	-,0744778	,0381778	-,729	9	,242	,485
Pair 2	Week12R2 - Week12L2	-,0136300	,0578181	,0182837	-,0549906	,0277306	-,745	9	,238	,475
Pair 3	Week24R2 - Week24L2	-,0227600	,0550312	,0174024	-,0621269	,0166069	-1,308	9	,112	,223

a. Parameter = R7, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,216833	6	,0246658	,0100698
	ScreeningL2	,241550	6	,0620271	,0253224
Pair 2	Week12R2	,238700	6	,0793238	,0323838
	Week12L2	,244683	6	,0513352	,0209575
Pair 3	Week24R2	,224067	6	,0530338	,0216510
	Week24L2	,210450	6	,0265699	,0108471

a. Parameter = R7, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0247167	,0526520	,0214951	-,0799715	,0305382	-1,150	5	,151	,302
Pair 2	Week12R2 - Week12L2	-,0059833	,0756931	,0309016	-,0854184	,0734517	-,194	5	,427	,854
Pair 3	Week24R2 - Week24L2	,0136167	,0406117	,0165797	-,0290027	,0562360	,821	5	,224	,449

a. Parameter = R7, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,256260	,0897280	,0283745

	ACS+HA	6	,216833	,0246658	,0100698
ScreeningL2	ACS	10	,274410	,0480934	,0152085
	ACS+HA	6	,241550	,0620271	,0253224
Week12R2	ACS	10	,307270	,0679099	,0214750
	ACS+HA	6	,238700	,0793238	,0323838
Week12L2	ACS	10	,320900	,0765179	,0241971
	ACS+HA	6	,244683	,0513352	,0209575
Week24R2	ACS	10	,247970	,0665594	,0210479
	ACS+HA	6	,224067	,0530338	,0216510
Week24L2	ACS	10	,270730	,0706185	,0223315
	ACS+HA	6	,210450	,0265699	,0108471

a. Parameter = R7

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR2	Equal variances assumed	5,554	,034	1,040	14	,158	,316	,0394267	,0379227	-,0419095	,1207628
	Equal variances not assumed			1,309	11,093	,108	,217	,0394267	,0301083	-,0267735	,1056269
ScreeningL2	Equal variances assumed	,356	,561	1,190	14	,127	,254	,0328600	,0276211	-,0263813	,0921013
	Equal variances not assumed			1,112	8,634	,148	,296	,0328600	,0295385	-,0343955	,1001155
Week12R2	Equal variances assumed	,619	,444	1,839	14	,044	,087	,0685700	,0372807	-,0113891	,1485291
	Equal variances not assumed			1,765	9,359	,055	,110	,0685700	,0388572	-,0188199	,1559599
Week12L2	Equal variances assumed	1,118	,308	2,152	14	,025	,049	,0762167	,0354217	,0002448	,1521886
	Equal variances not assumed			2,381	13,695	,016	,032	,0762167	,0320112	,0074159	,1450174
Week24R2	Equal variances assumed	,158	,697	,746	14	,234	,468	,0239033	,0320518	-,0448410	,0926477
	Equal variances not assumed			,792	12,643	,222	,443	,0239033	,0301957	-,0415182	,0893249
Week24L2	Equal variances assumed	5,322	,037	1,985	14	,034	,067	,0602800	,0303668	-,0048503	,1254103
	Equal variances not assumed			2,428	12,496	,016	,031	,0602800	,0248265	,0064247	,1141353

a. Parameter = R7

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,448	6,430	2	,040	,644	,706	,500

Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,979	,169	2	,919	,979	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,249	2	,125	69,314	<,001	,885
	Greenhouse-Geisser	,249	1,288	,193	69,314	<,001	,885
Error(Visit)	Sphericity Assumed	,032	18	,002			
	Greenhouse-Geisser	,032	11,595	,003			
Side	Sphericity Assumed	,005	1	,005	3,122	,111	,258
	Greenhouse-Geisser	,005	1,000	,005	3,122	,111	,258
Error(Side)	Sphericity Assumed	,013	9	,001			
	Greenhouse-Geisser	,013	9,000	,001			
Visit * Side	Sphericity Assumed	,003	2	,002	2,175	,143	,195
	Greenhouse-Geisser	,003	1,959	,002	2,175	,144	,195
Error(Visit*Side)	Sphericity Assumed	,014	18	,001			
	Greenhouse-Geisser	,014	17,631	,001			

a. Parameter = R7, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,535	2,499	2	,287	,683	,852	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,770	1,046	2	,593	,813	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,090	2	,045	18,756	<,001	,790
	Greenhouse-Geisser	,090	1,366	,066	18,756	,003	,790
Error(Visit)	Sphericity Assumed	,024	10	,002			
	Greenhouse-Geisser	,024	6,828	,004			
Side	Sphericity Assumed	2,890E-6	1	2,890E-6	,003	,957	,001
	Greenhouse-Geisser	2,890E-6	1,000	2,890E-6	,003	,957	,001
Error(Side)	Sphericity Assumed	,004	5	,001			
	Greenhouse-Geisser	,004	5,000	,001			
Visit * Side	Sphericity Assumed	,001	2	,000	,218	,808	,042
	Greenhouse-Geisser	,001	1,626	,001	,218	,765	,042
Error(Visit*Side)	Sphericity Assumed	,020	10	,002			
	Greenhouse-Geisser	,020	8,129	,003			

a. Parameter = R7, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,701	2,846	2	,241	,770	,898	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,116	2	,058	42,995	<,001	,827
	Greenhouse-Geisser	,116	1,539	,075	42,995	<,001	,827
Error(Visits)	Sphericity Assumed	,024	18	,001			
	Greenhouse-Geisser	,024	13,853	,002			

a. Parameter = R7, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,116*	,020	<,001	-,176	-,057
	3	,026	,014	,258	-,014	,067
2	1	,116*	,020	<,001	,057	,176
	3	,143*	,014	<,001	,101	,185
3	1	-,026	,014	,258	-,067	,014
	2	-,143*	,014	<,001	-,185	-,101

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,654	1,701	2	,427	,743	,983	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,054	2	,027	30,479	<,001	,859
	Greenhouse-Geisser	,054	1,485	,036	30,479	<,001	,859
Error(Visits)	Sphericity Assumed	,009	10	,001			
	Greenhouse-Geisser	,009	7,427	,001			

a. Parameter = R7, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,129*	,017	,002	-,188	-,070
	3	-,034	,012	,117	-,078	,009
2	1	,129*	,017	,002	,070	,188
	3	,095*	,021	,020	,020	,170
3	1	,034	,012	,117	-,009	,078
	2	-,095*	,021	,020	-,170	-,020

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,682	3,062	2	,216	,759	,880	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,137	2	,068	54,671	<,001	,859
	Greenhouse-Geisser	,137	1,517	,090	54,671	<,001	,859
Error(Visits)	Sphericity Assumed	,023	18	,001			
	Greenhouse-Geisser	,023	13,657	,002			

a. Parameter = R7, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,147*	,018	<,001	-,201	-,093
	3	-,008	,011	1,000	-,038	,023
2	1	,147*	,018	<,001	,093	,201
	3	,139*	,017	<,001	,089	,190
3	1	,008	,011	1,000	-,023	,038
	2	-,139*	,017	<,001	-,190	-,089

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,768	1,054	2	,590	,812	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,037	2	,019	5,213	,028	,510
	Greenhouse-Geisser	,037	1,624	,023	5,213	,040	,510
Error(Visits)	Sphericity Assumed	,036	10	,004			
	Greenhouse-Geisser	,036	8,119	,004			

a. Parameter = R7, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,105	,036	,104	-,234	,024
	3	-,020	,025	1,000	-,109	,069
2	1	,105	,036	,104	-,024	,234
	3	,085	,040	,263	-,056	,226
3	1	,020	,025	1,000	-,069	,109
	2	-,085	,040	,263	-,226	,056

Based on estimated marginal means
a. Parameter = R7, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,387590	10	,0917607	,0290173
	ScreeningL4	,383700	10	,0834006	,0263736
Pair 2	Week12R4	,504070	10	,0628928	,0198884
	Week12L4	,530590	10	,0826466	,0261352
Pair 3	Week24R4	,361170	10	,0704172	,0222679
	Week24L4	,391210	10	,0896398	,0283466

a. Parameter = R7, Treatment = ACS

Paired Samples Testa

	Paired Differences	t	df	Significance

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1 ScreeningR4 - ScreeningL4	,0038900	,0415351	,0131346	-,0258224	,0336024	,296	9	,387	,774
Pair 2 Week12R4 - Week12L4	-,0265200	,0526508	,0166496	-,0641841	,0111441	-1,593	9	,073	,146
Pair 3 Week24R4 - Week24L4	-,0300400	,0408092	,0129050	-,0592332	-,0008468	-2,328	9	,022	,045

a. Parameter = R7, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,334650	6	,0583312	,0238136
	ScreeningL4	,348033	6	,0872935	,0356374
Pair 2	Week12R4	,463700	6	,0676135	,0276031
	Week12L4	,452833	6	,1058625	,0432182
Pair 3	Week24R4	,369050	6	,0782093	,0319288
	Week24L4	,368233	6	,0578009	,0235971

a. Parameter = R7, Treatment = ACS+HA

Paired Samples Testa

	Paired Differences							Significance	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1 ScreeningR4 - ScreeningL4	-,0133833	,0603965	,0246568	-,0767656	,0499989	-,543	5	,305	,611
Pair 2 Week12R4 - Week12L4	,0108667	,0738506	,0301494	-,0666348	,0883681	,360	5	,367	,733
Pair 3 Week24R4 - Week24L4	,0008167	,0293896	,0119983	-,0300258	,0316592	,068	5	,474	,948

a. Parameter = R7, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,387590	,0917607	,0290173
	ACS+HA	6	,334650	,0583312	,0238136
ScreeningL4	ACS	10	,383700	,0834006	,0263736
	ACS+HA	6	,348033	,0872935	,0356374
Week12R4	ACS	10	,504070	,0628928	,0198884
	ACS+HA	6	,463700	,0676135	,0276031
Week12L4	ACS	10	,530590	,0826466	,0261352
	ACS+HA	6	,452833	,1058625	,0432182
Week24R4	ACS	10	,361170	,0704172	,0222679
	ACS+HA	6	,369050	,0782093	,0319288
Week24L4	ACS	10	,391210	,0896398	,0283466
	ACS+HA	6	,368233	,0578009	,0235971

a. Parameter = R7

Independent Samples Testa

Levene's Test for Equality of Variances		t-test for Equality of Means							
				Significance				95% Confidence Interval of the Difference	
F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper

ScreeningR4	Equal variances assumed	1,296	,274	1,259	14	,114	,229	,0529400	,0420414	-,0372299	,1431099
	Equal variances not assumed			1,410	13,876	,090	,180	,0529400	,0375379	-,0276383	,1335183
ScreeningL4	Equal variances assumed	,253	,623	,814	14	,215	,429	,0356667	,0437965	-,0582674	,1296007
	Equal variances not assumed			,804	10,266	,220	,439	,0356667	,0443350	-,0627722	,1341056
Week12R4	Equal variances assumed	,031	,862	1,210	14	,123	,246	,0403700	,0333688	-,0311989	,1119389
	Equal variances not assumed			1,187	10,036	,131	,263	,0403700	,0340218	-,0353981	,1161381
Week12L4	Equal variances assumed	1,876	,192	1,644	14	,061	,123	,0777567	,0473102	-,0237137	,1792270
	Equal variances not assumed			1,540	8,681	,080	,159	,0777567	,0505060	-,0371398	,1926532
Week24R4	Equal variances assumed	,148	,706	-,208	14	,419	,838	-,0078800	,0378495	-,0890591	,0732991
	Equal variances not assumed			-,202	9,764	,422	,844	-,0078800	,0389269	-,0949001	,0791401
Week24L4	Equal variances assumed	2,601	,129	,558	14	,293	,586	,0229767	,0411784	-,0653423	,1112956
	Equal variances not assumed			,623	13,836	,272	,543	,0229767	,0368830	-,0562175	,1021708

a. Parameter = R7

Skin recovery (R8, Ua)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	ACS	,831	10	,034
	ACS+HA	,968	6	,877
ScreeningL2	ACS	,924	10	,394
	ACS+HA	,951	6	,747
Week12R2	ACS	,971	10	,903
	ACS+HA	,959	6	,808
Week12L2	ACS	,977	10	,949
	ACS+HA	,917	6	,481
Week24R2	ACS	,885	10	,149
	ACS+HA	,974	6	,919
Week24L2	ACS	,946	10	,620
	ACS+HA	,941	6	,671

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,968	10	,877
	ACS+HA	,961	6	,828

ScreeningL4	ACS	,915	10	,319
	ACS+HA	,933	6	,603
Week12R4	ACS	,909	10	,271
	ACS+HA	,929	6	,576
Week12L4	ACS	,954	10	,720
	ACS+HA	,940	6	,663
Week24R4	ACS	,969	10	,879
	ACS+HA	,892	6	,327
Week24L4	ACS	,977	10	,950
	ACS+HA	,965	6	,857

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,898	,857	2	,652	,908	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,945	,451	2	,798	,948	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,052	2	,026	8,746	,002
	Greenhouse-Geisser	,052	1,816	,028	8,746	,003
Error(Visit)	Sphericity Assumed	,053	18	,003		
	Greenhouse-Geisser	,053	16,340	,003		
Side	Sphericity Assumed	1,667E-6	1	1,667E-6	,002	,968
	Greenhouse-Geisser	1,667E-6	1,000	1,667E-6	,002	,968
Error(Side)	Sphericity Assumed	,009	9	,001		
	Greenhouse-Geisser	,009	9,000	,001		
Visit * Side	Sphericity Assumed	,000	2	,000	,405	,673
	Greenhouse-Geisser	,000	1,896	,000	,405	,663
Error(Visit*Side)	Sphericity Assumed	,010	18	,001		
	Greenhouse-Geisser	,010	17,064	,001		

a. Parameter = R8, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,844	,679	2	,712	,865	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,868	,565	2	,754	,884	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,036	2	,018	3,013	,095
	Greenhouse-Geisser	,036	1,730	,021	3,013	,106
Error(Visit)	Sphericity Assumed	,059	10	,006		
	Greenhouse-Geisser	,059	8,650	,007		
Side	Sphericity Assumed	,001	1	,001	,987	,366
	Greenhouse-Geisser	,001	1,000	,001	,987	,366
Error(Side)	Sphericity Assumed	,007	5	,001		
	Greenhouse-Geisser	,007	5,000	,001		
Visit * Side	Sphericity Assumed	,003	2	,001	,793	,479
	Greenhouse-Geisser	,003	1,767	,002	,793	,467

Error(Visit*Side)	Sphericity Assumed	,017	10	,002		
	Greenhouse-Geisser	,017	8,836	,002		

a. Parameter = R8, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,787	1,920	2	,383	,824	,985	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,024	2	,012	5,118	,017	,363
	Greenhouse-Geisser	,024	1,648	,015	5,118	,025	,363
Error(Visits)	Sphericity Assumed	,043	18	,002			
	Greenhouse-Geisser	,043	14,835	,003			

a. Parameter = R8, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,069*	,021	,030	,007	,131
	3	,023	,026	1,000	-,054	,099
2	1	-,069*	,021	,030	-,131	-,007
	3	-,046	,017	,083	-,097	,005
3	1	-,023	,026	1,000	-,099	,054
	2	,046	,017	,083	-,005	,097

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,322	4,532	2	,104	,596	,676	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,025	2	,012	6,120	,018	,550
	Greenhouse-Geisser	,025	1,192	,021	6,120	,045	,550
Error(Visits)	Sphericity Assumed	,020	10	,002			
	Greenhouse-Geisser	,020	5,960	,003			

a. Parameter = R8, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,079	,034	,204	-,041	,199
	3	,000	,026	1,000	-,093	,093
2	1	-,079	,034	,204	-,199	,041
	3	-,079*	,014	,007	-,127	-,030
3	1	,000	,026	1,000	-,093	,093
	2	,079*	,014	,007	,030	,127

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8, Treatment = ACS+HA
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,985	,119	2	,942	,985	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,028	2	,014	12,532	<,001	,582
	Greenhouse-Geisser	,028	1,971	,014	12,532	<,001	,582
Error(Visits)	Sphericity Assumed	,020	18	,001			
	Greenhouse-Geisser	,020	17,739	,001			

a. Parameter = R8, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,070*	,016	,005	,024	,116
	3	,012	,014	1,000	-,029	,053
2	1	-,070*	,016	,005	-,116	-,024
	3	-,058*	,015	,011	-,101	-,014
3	1	-,012	,014	1,000	-,053	,029
	2	,058*	,015	,011	,014	,101

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,907	,390	2	,823	,915	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,014	2	,007	1,213	,338	,195
	Greenhouse-Geisser	,014	1,830	,007	1,213	,336	,195
Error(Visits)	Sphericity Assumed	,056	10	,006			
	Greenhouse-Geisser	,056	9,151	,006			

a. Parameter = R8, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,067	,042	,500	-,080	,214
	3	,030	,049	1,000	-,144	,204
2	1	-,067	,042	,500	-,214	,080
	3	-,038	,038	1,000	-,173	,098
3	1	-,030	,049	1,000	-,204	,144
	2	,038	,038	1,000	-,098	,173

Based on estimated marginal means
a. Parameter = R8, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,288400	10	,0737265	,0233144
	ScreeningL2	,285500	10	,0592288	,0187298
Pair 2	Week12R2	,219800	10	,0443416	,0140220
	Week12L2	,215800	10	,0389524	,0123178
Pair 3	Week24R2	,265500	10	,0689416	,0218012
	Week24L2	,273400	10	,0615236	,0194555

a. Parameter = R8, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	,0029000	,0433524	,0137092	-,0281125	,0339125	,212	9	,419	,837
Pair 2	Week12R2 - Week12L2	,0040000	,0281543	,0089032	-,0161404	,0241404	,449	9	,332	,664
Pair 3	Week24R2 - Week24L2	-,0079000	,0376901	,0119187	-,0348619	,0190619	-,663	9	,262	,524

a. Parameter = R8, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,271000	6	,0880909	,0359629
	ScreeningL2	,289667	6	,0921622	,0376251
Pair 2	Week12R2	,192167	6	,0333791	,0136270
	Week12L2	,222333	6	,0432697	,0176648
Pair 3	Week24R2	,271000	6	,0367478	,0150022
	Week24L2	,259833	6	,0721260	,0294453

a. Parameter = R8, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0186667	,0345871	,0141201	-,0549636	,0176303	-1,322	5	,122	,243
Pair 2	Week12R2 - Week12L2	-,0301667	,0421778	,0172190	-,0744296	,0140962	-1,752	5	,070	,140
Pair 3	Week24R2 - Week24L2	,0111667	,0823733	,0336288	-,0752788	,0976122	,332	5	,377	,753

a. Parameter = R8, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,288400	,0737265	,0233144
	ACS+HA	6	,271000	,0880909	,0359629
ScreeningL2	ACS	10	,285500	,0592288	,0187298
	ACS+HA	6	,289667	,0921622	,0376251
Week12R2	ACS	10	,219800	,0443416	,0140220
	ACS+HA	6	,192167	,0333791	,0136270

Week12L2	ACS	10	,215800	,0389524	,0123178
	ACS+HA	6	,222333	,0432697	,0176648
Week24R2	ACS	10	,265500	,0689416	,0218012
	ACS+HA	6	,271000	,0367478	,0150022
Week24L2	ACS	10	,273400	,0615236	,0194555
	ACS+HA	6	,259833	,0721260	,0294453

a. Parameter = R8

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR2	Equal variances assumed	,190	,670	,426	14	,338	,677	,0174000	,0408762	-,0702708	,1050708
	Equal variances not assumed			,406	9,185	,347	,694	,0174000	,0428590	-,0792574	,1140574
ScreeningL2	Equal variances assumed	,356	,560	-,111	14	,457	,913	-,0041667	,0375542	-,0847125	,0763792
	Equal variances not assumed			-,099	7,528	,462	,924	-,0041667	,0420292	-,1021534	,0938201
Week12R2	Equal variances assumed	,239	,632	1,313	14	,105	,210	,0276333	,0210516	-,0175179	,0727845
	Equal variances not assumed			1,413	13,060	,090	,181	,0276333	,0195528	-,0145883	,0698550
Week12L2	Equal variances assumed	,373	,551	-,312	14	,380	,760	-,0065333	,0209384	-,0514418	,0383751
	Equal variances not assumed			-,303	9,762	,384	,768	-,0065333	,0215354	-,0546760	,0416094
Week24R2	Equal variances assumed	2,402	,143	-,179	14	,430	,860	-,0055000	,0307148	-,0713767	,0603767
	Equal variances not assumed			-,208	13,922	,419	,838	-,0055000	,0264643	-,0622901	,0512901
Week24L2	Equal variances assumed	,317	,582	,401	14	,347	,694	,0135667	,0338279	-,0589870	,0861203
	Equal variances not assumed			,384	9,331	,355	,709	,0135667	,0352923	-,0658408	,0929741

a. Parameter = R8

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,942	,480	2	,787	,945	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,945	,455	2	,797	,948	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,179	2	,090	15,451	<,001	,632
	Greenhouse-Geisser	,179	1,890	,095	15,451	<,001	,632
Error(Visit)	Sphericity Assumed	,105	18	,006			
	Greenhouse-Geisser	,105	17,010	,006			
Side	Sphericity Assumed	2,282E-5	1	2,282E-5	,002	,964	,000
	Greenhouse-Geisser	2,282E-5	1,000	2,282E-5	,002	,964	,000
Error(Side)	Sphericity Assumed	,096	9	,011			
	Greenhouse-Geisser	,096	9,000	,011			
Visit * Side	Sphericity Assumed	,002	2	,001	,186	,832	,020
	Greenhouse-Geisser	,002	1,895	,001	,186	,821	,020
Error(Visit*Side)	Sphericity Assumed	,092	18	,005			
	Greenhouse-Geisser	,092	17,057	,005			

a. Parameter = R8, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,830	,748	2	,688	,854	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,409	3,574	2	,167	,629	,741	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,043	2	,022	4,175	,048	,455
	Greenhouse-Geisser	,043	1,709	,025	4,175	,059	,455
Error(Visit)	Sphericity Assumed	,052	10	,005			
	Greenhouse-Geisser	,052	8,544	,006			
Side	Sphericity Assumed	,000	1	,000	,017	,901	,003
	Greenhouse-Geisser	,000	1,000	,000	,017	,901	,003
Error(Side)	Sphericity Assumed	,055	5	,011			
	Greenhouse-Geisser	,055	5,000	,011			
Visit * Side	Sphericity Assumed	,002	2	,001	,147	,865	,029
	Greenhouse-Geisser	,002	1,257	,001	,147	,769	,029
Error(Visit*Side)	Sphericity Assumed	,053	10	,005			
	Greenhouse-Geisser	,053	6,286	,008			

a. Parameter = R8, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,738	2,435	2	,296	,792	,933	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,073	2	,037	6,256	,009	,410
	Greenhouse-Geisser	,073	1,584	,046	6,256	,015	,410
Error(Visits)	Sphericity Assumed	,105	18	,006			
	Greenhouse-Geisser	,105	14,258	,007			

a. Parameter = R8, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec

					Lower Bound	Upper Bound
1	2	,104*	,030	,023	,015	,194
	3	-,001	,028	1,000	-,084	,083
2	1	-,104*	,030	,023	-,194	-,015
	3	-,105	,042	,101	-,228	,018
3	1	,001	,028	1,000	-,083	,084
	2	,105	,042	,101	-,018	,228

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,989	,045	2	,978	,989	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,029	2	,014	2,701	,115	,351
	Greenhouse-Geisser	,029	1,978	,015	2,701	,116	,351
Error(Visits)	Sphericity Assumed	,053	10	,005			
	Greenhouse-Geisser	,053	9,890	,005			

a. Parameter = R8, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,097	,040	,181	-,045	,238
	3	,061	,044	,673	-,094	,216
2	1	-,097	,040	,181	-,238	,045
	3	-,036	,042	1,000	-,186	,114
3	1	-,061	,044	,673	-,216	,094
	2	,036	,042	1,000	-,114	,186

Based on estimated marginal means
a. Parameter = R8, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,423	6,883	2	,032	,634	,691	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,108	2	,054	10,693	<,001	,543
	Greenhouse-Geisser	,108	1,268	,085	10,693	,005	,543
Error(Visits)	Sphericity Assumed	,091	18	,005			
	Greenhouse-Geisser	,091	11,414	,008			

a. Parameter = R8, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,122*	,039	,036	,008	,237
	3	-,010	,035	1,000	-,113	,094
2	1	-,122*	,039	,036	-,237	-,008
	3	-,132*	,016	<,001	-,179	-,085
3	1	,010	,035	1,000	-,094	,113
	2	,132*	,016	<,001	,085	,179

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8, Treatment = ACS
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,539	2,469	2	,291	,685	,856	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,016	2	,008	1,562	,257	,238
	Greenhouse-Geisser	,016	1,369	,012	1,562	,265	,238
Error(Visits)	Sphericity Assumed	,052	10	,005			
	Greenhouse-Geisser	,052	6,847	,008			

a. Parameter = R8, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,068	,054	,789	-,122	,257
	3	,058	,035	,466	-,065	,181
2	1	-,068	,054	,789	-,257	,122
	3	-,010	,033	1,000	-,125	,106
3	1	-,058	,035	,466	-,181	,065
	2	,010	,033	1,000	-,106	,125

Based on estimated marginal means
a. Parameter = R8, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,534100	10	,1483460	,0469111
	ScreeningL4	,538400	10	,1475934	,0466731
Pair 2	Week12R4	,429800	10	,1182557	,0373957
	Week12L4	,416000	10	,1296919	,0410122
Pair 3	Week24R4	,534800	10	,1596564	,0504878
	Week24L4	,548000	10	,1384518	,0437823

a. Parameter = R8, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p

Pair 1	ScreeningR4 - ScreeningL4	-,0043000	,0922304	,0291658	-,0702777	,0616777	-,147	9	,443	,886
Pair 2	Week12R4 - Week12L4	,0138000	,1317294	,0416565	-,0804335	,1080335	,331	9	,374	,748
Pair 3	Week24R4 - Week24L4	-,0132000	,1255280	,0396955	-,1029974	,0765974	-,333	9	,374	,747

a. Parameter = R8, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,599667	6	,1757745	,0717596
	ScreeningL4	,584500	6	,1055400	,0430865
Pair 2	Week12R4	,502833	6	,1067622	,0435855
	Week12L4	,516833	6	,0872340	,0356131
Pair 3	Week24R4	,538833	6	,1243888	,0507815
	Week24L4	,526333	6	,0899126	,0367066

a. Parameter = R8, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences					t	df	Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	,0151667	,1917805	,0782941	-,1860946	,2164280	,194	5	,427	,854
Pair 2	Week12R4 - Week12L4	-,0140000	,0505134	,0206220	-,0670105	,0390105	-,679	5	,264	,527
Pair 3	Week24R4 - Week24L4	,0125000	,0604938	,0246965	-,0509844	,0759844	,506	5	,317	,634

a. Parameter = R8, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,534100	,1483460	,0469111
	ACS+HA	6	,599667	,1757745	,0717596
ScreeningL4	ACS	10	,538400	,1475934	,0466731
	ACS+HA	6	,584500	,1055400	,0430865
Week12R4	ACS	10	,429800	,1182557	,0373957
	ACS+HA	6	,502833	,1067622	,0435855
Week12L4	ACS	10	,416000	,1296919	,0410122
	ACS+HA	6	,516833	,0872340	,0356131
Week24R4	ACS	10	,534800	,1596564	,0504878
	ACS+HA	6	,538833	,1243888	,0507815
Week24L4	ACS	10	,548000	,1384518	,0437823
	ACS+HA	6	,526333	,0899126	,0367066

a. Parameter = R8

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	,065	,803	-,800	14	,219	,437	-,0655667	,0819457	-,2413226	,1101893
	Equal variances not assumed			-,765	9,248	,232	,463	-,0655667	,0857327	-,2587165	,1275832

ScreeningL4	Equal variances assumed	1,448	,249	-,666	14	,258	,516	-,0461000	,0692474	-,1946208	,1024208
	Equal variances not assumed			-,726	13,382	,240	,480	-,0461000	,0635203	-,1829301	,0907301
Week12R4	Equal variances assumed	,211	,653	-1,238	14	,118	,236	-,0730333	,0590158	-,1996096	,0535430
	Equal variances not assumed			-1,272	11,584	,114	,228	-,0730333	,0574294	-,1986619	,0525953
Week12L4	Equal variances assumed	,718	,411	-1,679	14	,058	,115	-,1008333	,0600680	-,2296664	,0279998
	Equal variances not assumed			-1,856	13,685	,043	,085	-,1008333	,0543166	-,2175833	,0159166
Week24R4	Equal variances assumed	,494	,494	-,053	14	,479	,959	-,0040333	,0764416	-,1679843	,1599177
	Equal variances not assumed			-,056	12,814	,478	,956	-,0040333	,0716085	-,1589624	,1508957
Week24L4	Equal variances assumed	1,347	,265	,340	14	,369	,739	,0216667	,0636869	-,1149282	,1582616
	Equal variances not assumed			,379	13,814	,355	,710	,0216667	,0571338	-,1010282	,1443615

a. Parameter = R8

Skin tiring (R9, Uf5 – Uf)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR2	ACS	,937	10	,516
	ACS+HA	,926	6	,553
ScreeningL2	ACS	,968	10	,875
	ACS+HA	,850	6	,157
Week12R2	ACS	,927	10	,418
	ACS+HA	,882	6	,279
Week12L2	ACS	,921	10	,365
	ACS+HA	,806	6	,066
Week24R2	ACS	,931	10	,455
	ACS+HA	,957	6	,798
Week24L2	ACS	,982	10	,977
	ACS+HA	,922	6	,523

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR4	ACS	,986	10	,989
	ACS+HA	,874	6	,245
ScreeningL4	ACS	,889	10	,165
	ACS+HA	,894	6	,337
Week12R4	ACS	,897	10	,205
	ACS+HA	,946	6	,706
Week12L4	ACS	,932	10	,470
	ACS+HA	,963	6	,844
Week24R4	ACS	,879	10	,127
	ACS+HA	,778	6	,037
Week24L4	ACS	,959	10	,772
	ACS+HA	,867	6	,214

General linear model 2 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,838	1,409	2	,494	,861	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,325	9,002	2	,011	,597	,636	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,000	2	,000	5,613	,013
	Greenhouse-Geisser	,000	1,722	,000	5,613	,018
Error(Visit)	Sphericity Assumed	,001	18	3,895E-5		
	Greenhouse-Geisser	,001	15,497	4,524E-5		
Side	Sphericity Assumed	1,215E-5	1	1,215E-5	,440	,524
	Greenhouse-Geisser	1,215E-5	1,000	1,215E-5	,440	,524
Error(Side)	Sphericity Assumed	,000	9	2,759E-5		
	Greenhouse-Geisser	,000	9,000	2,759E-5		
Visit * Side	Sphericity Assumed	1,710E-5	2	8,550E-6	,178	,838
	Greenhouse-Geisser	1,710E-5	1,194	1,433E-5	,178	,725
Error(Visit*Side)	Sphericity Assumed	,001	18	4,799E-5		
	Greenhouse-Geisser	,001	10,743	8,041E-5		

a. Parameter = R9, Treatment = ACS

General linear model 2 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,287	4,994	2	,082	,584	,653	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,525	2,581	2	,275	,678	,841	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,001	2	,000	7,344	,011
	Greenhouse-Geisser	,001	1,168	,001	7,344	,034
Error(Visit)	Sphericity Assumed	,001	10	5,043E-5		
	Greenhouse-Geisser	,001	5,838	8,638E-5		
Side	Sphericity Assumed	1,361E-6	1	1,361E-6	,016	,904
	Greenhouse-Geisser	1,361E-6	1,000	1,361E-6	,016	,904
Error(Side)	Sphericity Assumed	,000	5	8,436E-5		
	Greenhouse-Geisser	,000	5,000	8,436E-5		
Visit * Side	Sphericity Assumed	9,039E-5	2	4,519E-5	1,899	,200
	Greenhouse-Geisser	9,039E-5	1,356	6,668E-5	1,899	,218
Error(Visit*Side)	Sphericity Assumed	,000	10	2,379E-5		
	Greenhouse-Geisser	,000	6,778	3,511E-5		

a. Parameter = R9, Treatment = ACS+HA

General linear model 2 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,959	,334	2	,846	,961	1,000	,500

Tests of Within-Subjects Effects

Tests of Within-Subjects Effectsa
Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	2	8,703E-5	2,129	,148	,191
	Greenhouse-Geisser	,000	1,921	9,059E-5	2,129	,150	,191
Error(Visits)	Sphericity Assumed	,001	18	4,089E-5			
	Greenhouse-Geisser	,001	17,293	4,256E-5			

a. Parameter = R9, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,005	,003	,236	-,003	,014
	3	,001	,003	1,000	-,008	,010
2	1	-,005	,003	,236	-,014	,003
	3	-,005	,003	,352	-,012	,003
3	1	-,001	,003	1,000	-,010	,008
	2	,005	,003	,352	-,003	,012

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,223	6,004	2	,050	,563	,613	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	2	7,850E-5	1,977	,189	,283
	Greenhouse-Geisser	,000	1,125	,000	1,977	,215	,283
Error(Visits)	Sphericity Assumed	,000	10	3,970E-5			
	Greenhouse-Geisser	,000	5,627	7,055E-5			

a. Parameter = R9, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,006	,004	,667	-,009	,021
	3	,000	,001	1,000	-,005	,004
2	1	-,006	,004	,667	-,021	,009
	3	-,006	,004	,605	-,022	,009
3	1	,000	,001	1,000	-,004	,005
	2	,006	,004	,605	-,009	,022

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,686	3,017	2	,221	,761	,884	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	2	,000	3,042	,073	,253
	Greenhouse-Geisser	,000	1,522	,000	3,042	,091	,253
Error(Visits)	Sphericity Assumed	,001	18	4,606E-5			
	Greenhouse-Geisser	,001	13,697	6,053E-5			

a. Parameter = R9, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	,006	,002	,104	-,001	,013
	3	-,001	,004	1,000	-,012	,010
2	1	-,006	,002	,104	-,013	,001
	3	-,007	,003	,107	-,015	,001
3	1	,001	,004	1,000	-,010	,012
	2	,007	,003	,107	-,001	,015

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,519	2,624	2	,269	,675	,836	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,001	2	,000	9,763	,004	,661
	Greenhouse-Geisser	,001	1,350	,000	9,763	,014	,661
Error(Visits)	Sphericity Assumed	,000	10	3,452E-5			
	Greenhouse-Geisser	,000	6,752	5,113E-5			

a. Parameter = R9, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	,012	,004	,065	-,001	,025
	3	-,002	,002	1,000	-,008	,005
2	1	-,012	,004	,065	-,025	,001
	3	-,014	,004	,064	-,028	,001
3	1	,002	,002	1,000	-,005	,008
	2	,014	,004	,064	-,001	,028

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,045200	10	,0079554	,0025157
	ScreeningL2	,043700	10	,0061653	,0019496
Pair 2	Week12R2	,039700	10	,0065328	,0020659
	Week12L2	,037900	10	,0047246	,0014941
Pair 3	Week24R2	,044300	10	,0076456	,0024178

Week24L2	,044900	10	,0078238	,0024741

a. Parameter = R9, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	,0015000	,0098798	,0031243	-,0055676	,0085676	,480	9	,321	,643
Pair 2	Week12R2 - Week12L2	,0018000	,0060700	,0019195	-,0025422	,0061422	,938	9	,186	,373
Pair 3	Week24R2 - Week24L2	-,0006000	,0106165	,0033572	-,0081946	,0069946	-,179	9	,431	,862

a. Parameter = R9, Treatment = ACS

T-Test 2 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR2	,046500	6	,0074766	,0030523
	ScreeningL2	,047833	6	,0074677	,0030487
Pair 2	Week12R2	,040500	6	,0084558	,0034521
	Week12L2	,035667	6	,0112012	,0045729
Pair 3	Week24R2	,047000	6	,0093381	,0038123
	Week24L2	,049333	6	,0043665	,0017826

a. Parameter = R9, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR2 - ScreeningL2	-,0013333	,0096264	,0039299	-,0114356	,0087689	-,339	5	,374	,748
Pair 2	Week12R2 - Week12L2	,0048333	,0104960	,0042850	-,0061816	,0158482	1,128	5	,155	,311
Pair 3	Week24R2 - Week24L2	-,0023333	,0078145	,0031903	-,0105342	,0058675	-,731	5	,249	,497

a. Parameter = R9, Treatment = ACS+HA

T-Test 2 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR2	ACS	10	,045200	,0079554	,0025157
	ACS+HA	6	,046500	,0074766	,0030523
ScreeningL2	ACS	10	,043700	,0061653	,0019496
	ACS+HA	6	,047833	,0074677	,0030487
Week12R2	ACS	10	,039700	,0065328	,0020659
	ACS+HA	6	,040500	,0084558	,0034521
Week12L2	ACS	10	,037900	,0047246	,0014941
	ACS+HA	6	,035667	,0112012	,0045729
Week24R2	ACS	10	,044300	,0076456	,0024178
	ACS+HA	6	,047000	,0093381	,0038123
Week24L2	ACS	10	,044900	,0078238	,0024741
	ACS+HA	6	,049333	,0043665	,0017826

a. Parameter = R9

Independent Samples Testa

	Levene's Test for Equality of Variances	t-test for Equality of Means

		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
ScreeningR2	Equal variances assumed	,030	,865	-,323	14	,376	,751	-,0013000	,0040216	-,0099255	,0073255
	Equal variances not assumed			-,329	11,223	,374	,748	-,0013000	,0039554	-,0099848	,0073848
ScreeningL2	Equal variances assumed	,392	,541	-1,202	14	,125	,249	-,0041333	,0034391	-,0115094	,0032428
	Equal variances not assumed			-1,142	9,082	,141	,283	-,0041333	,0036188	-,0123083	,0040417
Week12R2	Equal variances assumed	,048	,830	-,213	14	,417	,835	-,0008000	,0037584	-,0088610	,0072610
	Equal variances not assumed			-,199	8,609	,423	,847	-,0008000	,0040230	-,0099640	,0083640
Week12L2	Equal variances assumed	8,020	,013	,562	14	,291	,583	,0022333	,0039719	-,0062855	,0107522
	Equal variances not assumed			,464	6,086	,329	,659	,0022333	,0048108	-,0094980	,0139646
Week24R2	Equal variances assumed	,306	,589	-,631	14	,269	,538	-,0027000	,0042809	-,0118815	,0064815
	Equal variances not assumed			-,598	9,020	,282	,564	-,0027000	,0045143	-,0129085	,0075085
Week24L2	Equal variances assumed	2,927	,109	-1,264	14	,114	,227	-,0044333	,0035084	-,0119582	,0030915
	Equal variances not assumed			-1,454	13,986	,084	,168	-,0044333	,0030494	-,0109743	,0021076

a. Parameter = R9

General linear model 4 mm RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,927	,607	2	,738	,932	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,853	1,276	2	,528	,871	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,001	2	,000	,686	,516	,071
	Greenhouse-Geisser	,001	1,864	,000	,686	,507	,071
Error(Visit)	Sphericity Assumed	,009	18	,001			
	Greenhouse-Geisser	,009	16,775	,001			
Side	Sphericity Assumed	7,707E-5	1	7,707E-5	,608	,456	,063
	Greenhouse-Geisser	7,707E-5	1,000	7,707E-5	,608	,456	,063
Error(Side)	Sphericity Assumed	,001	9	,000			
	Greenhouse-Geisser	,001	9,000	,000			
Visit * Side	Sphericity Assumed	,000	2	,000	2,415	,118	,212
	Greenhouse-Geisser	,000	1,743	,000	2,415	,127	,212
Error(Visit*Side)	Sphericity Assumed	,002	18	9,594E-5			

	Greenhouse-Geisser	,002	15,687	,000				

a. Parameter = R9, Treatment = ACS

General linear model 4 mm RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,490	2,850	2	,240	,662	,809	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,137	7,951	2	,019	,537	,566	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,001	2	,000	1,412	,288	,220
	Greenhouse-Geisser	,001	1,325	,001	1,412	,291	,220
Error(Visit)	Sphericity Assumed	,002	10	,000			
	Greenhouse-Geisser	,002	6,624	,000			
Side	Sphericity Assumed	,000	1	,000	1,322	,302	,209
	Greenhouse-Geisser	,000	1,000	,000	1,322	,302	,209
Error(Side)	Sphericity Assumed	,001	5	,000			
	Greenhouse-Geisser	,001	5,000	,000			
Visit * Side	Sphericity Assumed	,000	2	7,525E-5	,331	,726	,062
	Greenhouse-Geisser	,000	1,074	,000	,331	,604	,062
Error(Visit*Side)	Sphericity Assumed	,002	10	,000			
	Greenhouse-Geisser	,002	5,368	,000			

a. Parameter = R9, Treatment = ACS+HA

General linear model 4 mm R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,967	,270	2	,874	,968	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,001	2	,001	1,913	,177	,175
	Greenhouse-Geisser	,001	1,936	,001	1,913	,178	,175
Error(Visits)	Sphericity Assumed	,005	18	,000			
	Greenhouse-Geisser	,005	17,422	,000			

a. Parameter = R9, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,006	,007	1,000	-,027	,015
	3	-,015	,008	,322	-,039	,009
2	1	,006	,007	1,000	-,015	,027
	3	-,009	,007	,776	-,030	,013
3	1	,015	,008	,322	-,009	,039
	2	,009	,007	,776	-,013	,030

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,524	2,583	2	,275	,678	,841	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	2	,000	,936	,424	,158
	Greenhouse-Geisser	,000	1,355	,000	,936	,399	,158
Error(Visits)	Sphericity Assumed	,002	10	,000			
	Greenhouse-Geisser	,002	6,776	,000			

a. Parameter = R9, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,005	,011	1,000	-,033	,043
	3	-,007	,009	1,000	-,040	,026
2	1	-,005	,011	1,000	-,043	,033
	3	-,012	,005	,210	-,030	,006
3	1	,007	,009	1,000	-,026	,040
	2	,012	,005	,210	-,006	,030

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,859	1,219	2	,544	,876	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	7,227E-5	2	3,613E-5	,112	,895	,012
	Greenhouse-Geisser	7,227E-5	1,752	4,124E-5	,112	,870	,012
Error(Visits)	Sphericity Assumed	,006	18	,000			
	Greenhouse-Geisser	,006	15,770	,000			

a. Parameter = R9, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,004	,009	1,000	-,031	,023
	3	-,002	,008	1,000	-,026	,022
2	1	,004	,009	1,000	-,023	,031
	3	,002	,006	1,000	-,017	,021
3	1	,002	,008	1,000	-,022	,026
	2	-,002	,006	1,000	-,021	,017

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 4 mm L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc

					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,110	8,822	2	,012	,529	,552	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	2	,000	,841	,460	,144
	Greenhouse-Geisser	,000	1,058	,000	,841	,406	,144
Error(Visits)	Sphericity Assumed	,002	10	,000			
	Greenhouse-Geisser	,002	5,292	,000			

a. Parameter = R9, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,005	,012	1,000	-,047	,037
	3	-,011	,009	,788	-,044	,021
2	1	,005	,012	1,000	-,037	,047
	3	-,007	,004	,379	-,019	,006
3	1	,011	,009	,788	-,021	,044
	2	,007	,004	,379	-,006	,019

Based on estimated marginal means
a. Parameter = R9, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 4 mm R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,069200	10	,0158591	,0050151
	ScreeningL4	,076400	10	,0176081	,0055682
Pair 2	Week12R4	,075100	10	,0106296	,0033614
	Week12L4	,080200	10	,0175043	,0055353
Pair 3	Week24R4	,083900	10	,0196607	,0062173
	Week24L4	,078400	10	,0116256	,0036764

a. Parameter = R9, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,0072000	,0147784	,0046733	-,0177718	,0033718	-1,541	9	,079	,158
Pair 2	Week12R4 - Week12L4	-,0051000	,0155095	,0049045	-,0161948	,0059948	-1,040	9	,163	,326
Pair 3	Week24R4 - Week24L4	,0055000	,0133604	,0042249	-,0040574	,0150574	1,302	9	,113	,225

a. Parameter = R9, Treatment = ACS

T-Test 4 mm R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR4	,077833	6	,0264984	,0108179
	ScreeningL4	,078000	6	,0163095	,0066583
Pair 2	Week12R4	,072833	6	,0141904	,0057932
	Week12L4	,083000	6	,0230651	,0094163
Pair 3	Week24R4	,084833	6	,0227721	,0092967
	Week24L4	,089500	6	,0199474	,0081435

a. Parameter = R9, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR4 - ScreeningL4	-,0001667	,0225869	,0092211	-,0238701	,0235368	-,018	5	,493	,986
Pair 2	Week12R4 - Week12L4	-,0101667	,0214608	,0087613	-,0326884	,0123551	-1,160	5	,149	,298
Pair 3	Week24R4 - Week24L4	-,0046667	,0167292	,0068297	-,0222229	,0128896	-,683	5	,262	,525

a. Parameter = R9, Treatment = ACS+HA

T-Test 4 mm ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningR4	ACS	10	,069200	,0158591	,0050151
	ACS+HA	6	,077833	,0264984	,0108179
ScreeningL4	ACS	10	,076400	,0176081	,0055682
	ACS+HA	6	,078000	,0163095	,0066583
Week12R4	ACS	10	,075100	,0106296	,0033614
	ACS+HA	6	,072833	,0141904	,0057932
Week12L4	ACS	10	,080200	,0175043	,0055353
	ACS+HA	6	,083000	,0230651	,0094163
Week24R4	ACS	10	,083900	,0196607	,0062173
	ACS+HA	6	,084833	,0227721	,0092967
Week24L4	ACS	10	,078400	,0116256	,0036764
	ACS+HA	6	,089500	,0199474	,0081435

a. Parameter = R9

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningR4	Equal variances assumed	,436	,520	-,823	14	,212	,424	-,0086333	,0104876	-,0311269	,0138603
	Equal variances not assumed			-,724	7,195	,246	,492	-,0086333	,0119239	-,0366743	,0194076
ScreeningL4	Equal variances assumed	,078	,784	-,181	14	,430	,859	-,0016000	,0088591	-,0206009	,0174009
	Equal variances not assumed			-,184	11,354	,429	,857	-,0016000	,0086797	-,0206316	,0174316
Week12R4	Equal variances assumed	,430	,523	,365	14	,360	,721	,0022667	,0062086	-,0110496	,0155829
	Equal variances not assumed			,338	8,404	,372	,743	,0022667	,0066978	-,0130501	,0175834
Week12L4	Equal variances assumed	1,037	,326	-,276	14	,393	,787	-,0028000	,0101584	-,0245875	,0189875
	Equal variances not assumed			-,256	8,490	,402	,804	-,0028000	,0109228	-,0277374	,0221374
Week24R4	Equal variances assumed	,777	,393	-,087	14	,466	,932	-,0009333	,0107542	-,0239987	,0221321

	Equal variances not assumed			-,083	9,425	,468	,935	-,0009333	,0111840	-,0260604	,0241937
Week24L4	Equal variances assumed	2,701	,123	-1,420	14	,089	,177	-,0111000	,0078144	-,0278602	,0056602
	Equal variances not assumed			-1,242	7,082	,127	,254	-,0111000	,0089349	-,0321780	,0099780

a. Parameter = R9

Sonography –skin density and skin thickness

Means and SDs

density	n	ScreeningR	Week12R	Week24R	ScreeningL	Week12L	Week24L
mean							
ACS	10	31.30	25.97	27.66	32.81	28.83	29.94
ACS+HA	6	28.36	23.67	28.92	29.47	27.45	28.18
SD							
ACS	10	5.19	8.89	5.33	5.67	6.66	4.06
ACS+HA	6	7.18	4.79	3.86	7.37	3.66	5.02

thickness	n	ScreeningR	Week12R	Week24R	ScreeningL	Week12L	Week24L
mean							
ACS	10	1556.70	1575.50	1503.00	1530.60	1632.00	1510.20
ACS+HA	6	1589.67	1616.67	1592.50	1626.17	1645.83	1629.50
SD							
ACS	10	144.94	171.95	176.02	113.09	188.28	158.23
ACS+HA	6	167.80	103.92	154.64	121.46	177.92	137.13

Test of normality – Shapiro-Wilk

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR	ACS	,930	10	,447
	ACS+HA	,965	6	,858
Week12R	ACS	,838	10	,042
	ACS+HA	,814	6	,078
Week24R	ACS	,896	10	,196
	ACS+HA	,883	6	,283
ScreeningL	ACS	,952	10	,694
	ACS+HA	,910	6	,437
Week12L	ACS	,849	10	,057
	ACS+HA	,984	6	,971
Week24L	ACS	,959	10	,770
	ACS+HA	,913	6	,456

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR	ACS	,882	10	,137
	ACS+HA	,982	6	,963
Week12R	ACS	,833	10	,036

	ACS+HA	,835	6	,118
Week24R	ACS	,903	10	,236
	ACS+HA	,824	6	,096
ScreeningL	ACS	,966	10	,854
	ACS+HA	,756	6	,023
Week12L	ACS	,929	10	,438
	ACS+HA	,929	6	,575
Week24L	ACS	,873	10	,109
	ACS+HA	,757	6	,023

General linear model density RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,668	3,225	2	,199	,751	,868	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,900	,839	2	,657	,909	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	228,300	2	114,150	2,418	,117	,212
	Greenhouse-Geisser	228,300	1,502	152,019	2,418	,135	,212
Error(Visit)	Sphericity Assumed	849,715	18	47,206			
	Greenhouse-Geisser	849,715	13,516	62,867			
Side	Sphericity Assumed	73,726	1	73,726	2,276	,166	,202
	Greenhouse-Geisser	73,726	1,000	73,726	2,276	,166	,202
Error(Side)	Sphericity Assumed	291,535	9	32,393			
	Greenhouse-Geisser	291,535	9,000	32,393			
Visit * Side	Sphericity Assumed	4,527	2	2,264	,098	,907	,011
	Greenhouse-Geisser	4,527	1,819	2,489	,098	,891	,011
Error(Visit*Side)	Sphericity Assumed	415,953	18	23,108			
	Greenhouse-Geisser	415,953	16,370	25,409			

a. Treatment = ACS, Parameter = density

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	4,656	2,103	,162	-1,513	10,824
	3	3,259	1,590	,212	-1,406	7,924
2	1	-4,656	2,103	,162	-10,824	1,513
	3	-1,397	2,685	1,000	-9,273	6,480
3	1	-3,259	1,590	,212	-7,924	1,406
	2	1,397	2,685	1,000	-6,480	9,273

Based on estimated marginal means
a. Treatment = ACS, Parameter = density
b. Adjustment for multiple comparisons: Bonferroni.

General linear model density RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,487	2,882	2	,237	,661	,806	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,385	3,820	2	,148	,619	,722	,500

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared

Visit	Sphericity Assumed	81,392	2	40,696	2,553	,127	,338
	Greenhouse-Geisser	81,392	1,321	61,593	2,553	,156	,338
Error(Visit)	Sphericity Assumed	159,433	10	15,943			
	Greenhouse-Geisser	159,433	6,607	24,130			
Side	Sphericity Assumed	17,209	1	17,209	,638	,461	,113
	Greenhouse-Geisser	17,209	1,000	17,209	,638	,461	,113
Error(Side)	Sphericity Assumed	134,840	5	26,968			
	Greenhouse-Geisser	134,840	5,000	26,968			
Visit * Side	Sphericity Assumed	31,129	2	15,564	1,066	,380	,176
	Greenhouse-Geisser	31,129	1,238	25,139	1,066	,360	,176
Error(Visit*Side)	Sphericity Assumed	145,981	10	14,598			
	Greenhouse-Geisser	145,981	6,191	23,579			

a. Treatment = ACS+HA, Parameter = density

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	3,358	1,769	,349	-2,896	9,611
	3	,367	2,004	1,000	-6,716	7,451
2	1	-3,357	1,769	,349	-9,611	2,896
	3	-2,990	,907	,065	-6,196	,216
3	1	-,367	2,004	1,000	-7,451	6,716
	2	2,990	,907	,065	-,216	6,196

Based on estimated marginal means
a. Treatment = ACS+HA, Parameter = density
b. Adjustment for multiple comparisons: Bonferroni.

T-Test density ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Screening	ACS	10	32,0570	4,00296	1,26585
	ACS+HA	6	28,9158	5,61784	2,29347
Week12	ACS	10	27,4015	6,74312	2,13236
	ACS+HA	6	25,5583	3,60252	1,47072
Week24	ACS	10	28,7980	3,58458	1,13354
	ACS+HA	6	28,5483	4,30135	1,75602

a. Parameter = density

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Screening	Equal variances assumed	,316	,583	1,310	14	,106	,211	3,14117	2,39847	-2,00304	8,28537
	Equal variances not assumed			1,199	8,093	,132	,264	3,14117	2,61962	-2,88761	9,16994
Week12	Equal variances assumed	1,694	,214	,613	14	,275	,549	1,84317	3,00513	-4,60220	8,28853
	Equal variances not assumed			,712	13,927	,244	,488	1,84317	2,59037	-3,71536	7,40170

Week24	Equal variances assumed	1,207	,290	,125	14	,451	,902	,24967	1,99118	-4,02098	4,52031
	Equal variances not assumed			,119	9,152	,454	,907	,24967	2,09010	-4,46651	4,96584

a. Parameter = density

General linear model thickness RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,978	,179	2	,914	,978	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,618	3,854	2	,146	,723	,825	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	96152,233	2	48076,117	4,755	,022	,346
	Greenhouse-Geisser	96152,233	1,957	49139,748	4,755	,023	,346
Error(Visit)	Sphericity Assumed	181992,433	18	10110,691			
	Greenhouse-Geisser	181992,433	17,610	10334,379			
Side	Sphericity Assumed	2356,267	1	2356,267	,197	,668	,021
	Greenhouse-Geisser	2356,267	1,000	2356,267	,197	,668	,021
Error(Side)	Sphericity Assumed	107894,067	9	11988,230			
	Greenhouse-Geisser	107894,067	9,000	11988,230			
Visit * Side	Sphericity Assumed	17270,233	2	8635,117	1,061	,367	,105
	Greenhouse-Geisser	17270,233	1,447	11936,309	1,061	,351	,105
Error(Visit*Side)	Sphericity Assumed	146534,433	18	8140,802			
	Greenhouse-Geisser	146534,433	13,022	11253,018			

a. Treatment = ACS, Parameter = thickness

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-60,100	32,615	,295	-155,770	35,570
	3	37,050	33,268	,883	-60,535	134,635
2	1	60,100	32,615	,295	-35,570	155,770
	3	97,150*	29,373	,027	10,991	183,309
3	1	-37,050	33,268	,883	-134,635	60,535
	2	-97,150*	29,373	,027	-183,309	-10,991

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Treatment = ACS, Parameter = thickness
c. Adjustment for multiple comparisons: Bonferroni.

General linear model thickness RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,687	1,501	2	,472	,762	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,993	,027	2	,986	,993	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	3856,056	2	1928,028	,123	,886	,024

	Greenhouse-Geisser	3856,056	1,523	2531,331	,123	,834	,024
Error(Visit)	Sphericity Assumed	157076,944	10	15707,694			
	Greenhouse-Geisser	157076,944	7,617	20622,821			
Side	Sphericity Assumed	10540,444	1	10540,444	1,110	,340	,182
	Greenhouse-Geisser	10540,444	1,000	10540,444	1,110	,340	,182
Error(Side)	Sphericity Assumed	47475,889	5	9495,178			
	Greenhouse-Geisser	47475,889	5,000	9495,178			
Visit * Side	Sphericity Assumed	115,389	2	57,694	,018	,982	,004
	Greenhouse-Geisser	115,389	1,986	58,087	,018	,982	,004
Error(Visit*Side)	Sphericity Assumed	32338,278	10	3233,828			
	Greenhouse-Geisser	32338,278	9,932	3255,847			

a. Treatment = ACS+HA, Parameter = thickness

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-23,333	61,584	1,000	-240,979	194,312
	3	-3,083	35,683	1,000	-129,191	123,024
2	1	23,333	61,584	1,000	-194,312	240,979
	3	20,250	52,801	1,000	-166,355	206,855
3	1	3,083	35,683	1,000	-123,024	129,191
	2	-20,250	52,801	1,000	-206,855	166,355

Based on estimated marginal means
a. Treatment = ACS+HA, Parameter = thickness
b. Adjustment for multiple comparisons: Bonferroni.

T-Test thickness ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Screening	ACS	10	1543,6500	115,44649	36,50739
	ACS+HA	6	1607,9167	137,08735	55,96568
Week12	ACS	10	1603,7500	164,74176	52,09592
	ACS+HA	6	1631,2500	139,74146	57,04921
Week24	ACS	10	1506,6000	151,04208	47,76370
	ACS+HA	6	1611,0000	133,18746	54,37355

a. Parameter = thickness

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Screening	Equal variances assumed	,004	,948	-1,007	14	,166	,331	-64,26667	63,83248	-201,17372	72,64038
	Equal variances not assumed			-,962	9,232	,180	,361	-64,26667	66,82025	-214,84786	86,31453
Week12	Equal variances assumed	,050	,826	-,341	14	,369	,738	-27,50000	80,69897	-200,58208	145,58208
	Equal variances not assumed			-,356	12,130	,364	,728	-27,50000	77,25670	-195,62833	140,62833
Week24	Equal variances assumed	,223	,644	-1,395	14	,092	,185	-104,40000	74,83543	-264,90603	56,10603

	Equal variances not assumed			-1,443	11,793	,088	,175	-104,40000	72,37302	-262,39529	53,59529

a. Parameter = thickness

PRIMOS –skin topography

Means and SDs

Mean	PC		Ra		Rmax		Rp		Rz		Wt	
Treatment	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA	ACS	ACS+HA
n	10	6	10	6	10	6	10	6	10	6	10	6
ScreeningR	23.000	22.167	20.780	22.283	150.190	153.533	80.850	74.733	112.730	114.700	96.640	104.117
Week12R	22.900	22.667	21.220	23.633	151.450	171.017	81.530	83.467	114.260	123.617	93.470	111.667
Week24R	23.400	22.833	20.910	22.483	148.750	153.917	78.430	78.800	111.930	117.400	90.160	95.567
ScreeningL	22.900	22.333	22.250	20.617	173.010	151.883	97.650	87.667	122.680	111.767	98.150	88.317
Week12L	22.800	21.833	21.320	21.867	165.390	158.067	94.080	87.600	117.630	116.817	85.960	92.067
Week24L	23.200	23.000	21.620	21.433	166.830	160.183	94.570	86.650	118.510	115.700	87.170	87.400
SD												
ScreeningR	1.491	2.229	4.975	4.690	39.178	23.903	15.924	9.692	28.116	20.228	42.976	46.489
Week12R	1.595	2.160	5.105	4.550	38.623	35.606	19.156	10.074	27.685	22.115	34.339	41.203
Week24R	1.647	2.483	4.390	6.196	36.588	34.898	15.830	16.690	24.683	29.912	40.276	45.017
ScreeningL	1.792	1.033	4.842	2.978	50.874	31.467	32.097	24.627	29.592	18.927	52.553	14.803
Week12L	1.135	1.835	4.727	2.674	47.018	19.340	29.625	15.036	28.063	16.982	42.208	21.775
Week24L	1.033	1.897	4.414	1.258	30.517	11.958	17.618	15.308	23.337	11.350	39.506	12.657

Mean skin roughness (Ra)

Test of normality – Shapiro-Wilk

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR	ACS	,894	10	,187
	ACS+HA	,882	6	,278
ScreeningL	ACS	,896	10	,197
	ACS+HA	,901	6	,380
Week12R	ACS	,787	10	,010
	ACS+HA	,971	6	,901
Week12L	ACS	,757	10	,004
	ACS+HA	,973	6	,911
Week24R	ACS	,846	10	,052
	ACS+HA	,848	6	,151
Week24L	ACS	,893	10	,184
	ACS+HA	,904	6	,400

General linear model RL ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,916	,702	2	,704	,923	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,392	7,496	2	,024	,622	,673	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	,817	2	,408	,081	,923
	Greenhouse-Geisser	,817	1,845	,443	,081	,910
Error(Visit)	Sphericity Assumed	91,303	18	5,072		
	Greenhouse-Geisser	91,303	16,605	5,499		
Side	Sphericity Assumed	8,664	1	8,664	2,740	,132
	Greenhouse-Geisser	8,664	1,000	8,664	2,740	,132
Error(Side)	Sphericity Assumed	28,456	9	3,162		
	Greenhouse-Geisser	28,456	9,000	3,162		
Visit * Side	Sphericity Assumed	4,711	2	2,355	,544	,590
	Greenhouse-Geisser	4,711	1,244	3,788	,544	,514
Error(Visit*Side)	Sphericity Assumed	77,909	18	4,328		
	Greenhouse-Geisser	77,909	11,193	6,961		

a. Parameter = Ra, Treatment = ACS

General linear model RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,632	1,833	2	,400	,731	,957	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,779	1,000	2	,607	,819	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	10,301	2	5,150	,919	,430
	Greenhouse-Geisser	10,301	1,462	7,044	,919	,409
Error(Visit)	Sphericity Assumed	56,053	10	5,605		
	Greenhouse-Geisser	56,053	7,312	7,666		
Side	Sphericity Assumed	20,100	1	20,100	1,273	,310
	Greenhouse-Geisser	20,100	1,000	20,100	1,273	,310
Error(Side)	Sphericity Assumed	78,961	5	15,792		
	Greenhouse-Geisser	78,961	5,000	15,792		
Visit * Side	Sphericity Assumed	,904	2	,452	,038	,962
	Greenhouse-Geisser	,904	1,638	,552	,038	,939
Error(Visit*Side)	Sphericity Assumed	117,669	10	11,767		
	Greenhouse-Geisser	117,669	8,189	14,370		

a. Parameter = Ra, Treatment = ACS+HA

General linear model R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,447	6,441	2	,040	,644	,705	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	1,022	2	,511	,080	,924	,009
	Greenhouse-Geisser	1,022	1,288	,794	,080	,842	,009

Error(Visits)	Sphericity Assumed	115,151	18	6,397			
	Greenhouse-Geisser	115,151	11,591	9,935			

a. Parameter = Ra, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,440	1,392	1,000	-4,524	3,644
	3	-,130	,596	1,000	-1,879	1,619
2	1	,440	1,392	1,000	-3,644	4,524
	3	,310	1,243	1,000	-3,336	3,956
3	1	,130	,596	1,000	-1,619	1,879
	2	-,310	1,243	1,000	-3,956	3,336

Based on estimated marginal means
a. Parameter = Ra, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,703	1,409	2	,494	,771	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	6,370	2	3,185	,201	,821	,039
	Greenhouse-Geisser	6,370	1,542	4,131	,201	,768	,039
Error(Visits)	Sphericity Assumed	158,717	10	15,872			
	Greenhouse-Geisser	158,717	7,710	20,585			

a. Parameter = Ra, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-1,350	2,219	1,000	-9,193	6,493
	3	-,200	1,731	1,000	-6,318	5,918
2	1	1,350	2,219	1,000	-6,493	9,193
	3	1,150	2,820	1,000	-8,815	11,115
3	1	,200	1,731	1,000	-5,918	6,318
	2	-1,150	2,820	1,000	-11,115	8,815

Based on estimated marginal means
a. Parameter = Ra, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,961	,316	2	,854	,963	1,000	,500

Tests of Within-Subjects Effects

Source	Visits	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Linear	1,984	1	1,984	,583	,465	,061
	Quadratic	2,521	1	2,521	,970	,350	,097
Error(Visits)	Linear	30,661	9	3,407			

	Quadratic	23,400	9	2,600			

a. Parameter = Ra, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,930	,797	,820	-1,408	3,268
	3	,630	,825	1,000	-1,791	3,051
2	1	-,930	,797	,820	-3,268	1,408
	3	-,300	,697	1,000	-2,344	1,744
3	1	-,630	,825	1,000	-3,051	1,791
	2	,300	,697	1,000	-1,744	2,344

Based on estimated marginal means
a. Parameter = Ra, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,582	2,166	2	,339	,705	,900	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	4,834	2	2,417	1,611	,247	,244
	Greenhouse-Geisser	4,834	1,410	3,428	1,611	,257	,244
Error(Visits)	Sphericity Assumed	15,006	10	1,501			
	Greenhouse-Geisser	15,006	7,052	2,128			

a. Parameter = Ra, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-1,250	,422	,094	-2,740	,240
	3	-,817	,827	1,000	-3,740	2,107
2	1	1,250	,422	,094	-,240	2,740
	3	,433	,799	1,000	-2,391	3,257
3	1	,817	,827	1,000	-2,107	3,740
	2	-,433	,799	1,000	-3,257	2,391

Based on estimated marginal means
a. Parameter = Ra, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test Ra R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	20,780	10	4,9752	1,5733
	ScreeningL	22,250	10	4,8422	1,5312
Pair 2	Week12R	21,220	10	5,1049	1,6143
	Week12L	21,320	10	4,7269	1,4948
Pair 3	Week24R	20,910	10	4,3900	1,3882
	Week24L	21,620	10	4,4141	1,3959

a. Parameter = Ra, Treatment = ACS

Paired Samples Testa

Paired Differences	t	df	Significance

					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-1,4700	3,0093	,9516	-3,6227	,6827	-1,545	9	,078	,157
Pair 2	Week12R - Week12L	-,1000	2,9844	,9438	-2,2349	2,0349	-,106	9	,459	,918
Pair 3	Week24R - Week24L	-,7100	2,3821	,7533	-2,4140	,9940	-,943	9	,185	,371

a. Parameter = Ra, Treatment = ACS

T-Test Ra R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	22,283	6	4,6902	1,9148
	ScreeningL	20,617	6	2,9775	1,2156
Pair 2	Week12R	23,633	6	4,5505	1,8577
	Week12L	21,867	6	2,6741	1,0917
Pair 3	Week24R	22,483	6	6,1963	2,5296
	Week24L	21,433	6	1,2580	,5136

a. Parameter = Ra, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	1,6667	4,7790	1,9510	-3,3486	6,6819	,854	5	,216	,432
Pair 2	Week12R - Week12L	1,7667	4,8599	1,9840	-3,3335	6,8668	,890	5	,207	,414
Pair 3	Week24R - Week24L	1,0500	5,6741	2,3164	-4,9046	7,0046	,453	5	,335	,669

a. Parameter = Ra, Treatment = ACS+HA

T-Test Ra ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningL	ACS	10	22,250	4,8422	1,5312
	ACS+HA	6	20,617	2,9775	1,2156
ScreeningR	ACS	10	20,780	4,9752	1,5733
	ACS+HA	6	22,283	4,6902	1,9148
Week12L	ACS	10	21,320	4,7269	1,4948
	ACS+HA	6	21,867	2,6741	1,0917
Week12R	ACS	10	21,220	5,1049	1,6143
	ACS+HA	6	23,633	4,5505	1,8577
Week24L	ACS	10	21,620	4,4141	1,3959
	ACS+HA	6	21,433	1,2580	,5136
Week24R	ACS	10	20,910	4,3900	1,3882
	ACS+HA	6	22,483	6,1963	2,5296

a. Parameter = Ra

Independent Samples Testa

Levene's Test for Equality of Variances		t-test for Equality of Means							
				Significance				95% Confidence Interval of the Difference	
F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper

ScreeningL	Equal variances assumed	2,565	,132	,741	14	,236	,471	1,6333	2,2054	-3,0968	6,3635
	Equal variances not assumed			,835	13,947	,209	,418	1,6333	1,9551	-2,5614	5,8280
ScreeningR	Equal variances assumed	,005	,944	-,597	14	,280	,560	-1,5033	2,5176	-6,9031	3,8964
	Equal variances not assumed			-,607	11,196	,278	,556	-1,5033	2,4782	-6,9463	3,9396
Week12L	Equal variances assumed	1,256	,281	-,257	14	,400	,801	-,5467	2,1240	-5,1022	4,0089
	Equal variances not assumed			-,295	13,995	,386	,772	-,5467	1,8510	-4,5168	3,4235
Week12R	Equal variances assumed	,026	,874	-,951	14	,179	,358	-2,4133	2,5376	-7,8559	3,0293
	Equal variances not assumed			-,981	11,697	,173	,347	-2,4133	2,4611	-7,7911	2,9644
Week24L	Equal variances assumed	3,914	,068	,100	14	,461	,922	,1867	1,8684	-3,8206	4,1939
	Equal variances not assumed			,126	11,231	,451	,902	,1867	1,4873	-3,0787	3,4521
Week24R	Equal variances assumed	1,378	,260	-,596	14	,280	,560	-1,5733	2,6382	-7,2318	4,0851
	Equal variances not assumed			-,545	8,059	,300	,600	-1,5733	2,8855	-8,2188	5,0722

a. Parameter = Ra

Maximum roughness (Rmax)

Test of normality – Shapiro-Wilk

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR	ACS	,888	10	,160
	ACS+HA	,794	6	,052
ScreeningL	ACS	,896	10	,196
	ACS+HA	,795	6	,053
Week12R	ACS	,828	10	,032
	ACS+HA	,960	6	,818
Week12L	ACS	,812	10	,020
	ACS+HA	,958	6	,808
Week24R	ACS	,847	10	,053
	ACS+HA	,865	6	,207
Week24L	ACS	,876	10	,117
	ACS+HA	,817	6	,083

General linear model RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc

					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,942	,474	2	,789	,946	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,769	2,099	2	,350	,812	,966	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	166,836	2	83,418	,222	,803
	Greenhouse-Geisser	166,836	1,891	88,215	,222	,791
Error(Visit)	Sphericity Assumed	6753,644	18	375,202		
	Greenhouse-Geisser	6753,644	17,021	396,780		
Side	Sphericity Assumed	5012,376	1	5012,376	29,281	<,001
	Greenhouse-Geisser	5012,376	1,000	5012,376	29,281	<,001
Error(Side)	Sphericity Assumed	1540,641	9	171,182		
	Greenhouse-Geisser	1540,641	9,000	171,182		
Visit * Side	Sphericity Assumed	197,436	2	98,718	,237	,792
	Greenhouse-Geisser	197,436	1,625	121,500	,237	,747
Error(Visit*Side)	Sphericity Assumed	7513,317	18	417,407		
	Greenhouse-Geisser	7513,317	14,625	513,735		

a. Parameter = Rmax, Treatment = ACS

General linear model RL ACS + HA

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,677	1,562	2	,458	,756	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,918	,343	2	,842	,924	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	860,012	2	430,006	1,082	,376
	Greenhouse-Geisser	860,012	1,511	569,044	1,082	,365
Error(Visit)	Sphericity Assumed	3975,835	10	397,583		
	Greenhouse-Geisser	3975,835	7,557	526,138		
Side	Sphericity Assumed	69,444	1	69,444	,094	,771
	Greenhouse-Geisser	69,444	1,000	69,444	,094	,771
Error(Side)	Sphericity Assumed	3690,682	5	738,136		
	Greenhouse-Geisser	3690,682	5,000	738,136		
Visit * Side	Sphericity Assumed	559,644	2	279,822	,377	,696
	Greenhouse-Geisser	559,644	1,848	302,808	,377	,681
Error(Visit*Side)	Sphericity Assumed	7429,809	10	742,981		
	Greenhouse-Geisser	7429,809	9,241	804,012		

a. Parameter = Rmax, Treatment = ACS+HA

General linear model R ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,424	6,862	2	,032	,635	,691	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	36,504	2	18,252	,039	,962	,004
	Greenhouse-Geisser	36,504	1,269	28,763	,039	,897	,004

Error(Visits)	Sphericity Assumed	8507,156	18	472,620			
	Greenhouse-Geisser	8507,156	11,422	744,785			

a. Parameter = Rmax, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	-1,260	12,509	1,000	-37,953	35,433
	3	1,440	5,678	1,000	-15,214	18,094
2	1	1,260	12,509	1,000	-35,433	37,953
	3	2,700	9,740	1,000	-25,870	31,270
3	1	-1,440	5,678	1,000	-18,094	15,214
	2	-2,700	9,740	1,000	-31,270	25,870

Based on estimated marginal means
a. Parameter = Rmax, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,668	1,616	2	,446	,751	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	1196,448	2	598,224	,623	,556	,111
	Greenhouse-Geisser	1196,448	1,501	797,018	,623	,518	,111
Error(Visits)	Sphericity Assumed	9606,486	10	960,649			
	Greenhouse-Geisser	9606,486	7,506	1279,879			

a. Parameter = Rmax, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	-17,483	18,277	1,000	-82,076	47,109
	3	-,383	12,405	1,000	-44,225	43,458
2	1	17,483	18,277	1,000	-47,109	82,076
	3	17,100	21,742	1,000	-59,739	93,939
3	1	,383	12,405	1,000	-43,458	44,225
	2	-17,100	21,742	1,000	-93,939	59,739

Based on estimated marginal means
a. Parameter = Rmax, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,864	1,168	2	,558	,880	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	327,768	2	163,884	,512	,608	,054
	Greenhouse-Geisser	327,768	1,761	186,151	,512	,586	,054
Error(Visits)	Sphericity Assumed	5759,805	18	319,989			
	Greenhouse-Geisser	5759,805	15,847	363,466			

a. Parameter = Rmax, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	7,620	7,611	1,000	-14,705	29,945
	3	6,180	9,315	1,000	-21,145	33,505
2	1	-7,620	7,611	1,000	-29,945	14,705
	3	-1,440	6,877	1,000	-21,612	18,732
3	1	-6,180	9,315	1,000	-33,505	21,145
	2	1,440	6,877	1,000	-18,732	21,612

Based on estimated marginal means
a. Parameter = Rmax, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,415	3,520	2	,172	,631	,745	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	223,208	2	111,604	,620	,557	,110
	Greenhouse-Geisser	223,208	1,262	176,915	,620	,496	,110
Error(Visits)	Sphericity Assumed	1799,159	10	179,916			
	Greenhouse-Geisser	1799,159	6,308	285,203			

a. Parameter = Rmax, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-6,183	6,163	1,000	-27,964	15,597
	3	-8,300	10,288	1,000	-44,658	28,058
2	1	6,183	6,163	1,000	-15,597	27,964
	3	-2,117	6,008	1,000	-23,350	19,117
3	1	8,300	10,288	1,000	-28,058	44,658
	2	2,117	6,008	1,000	-19,117	23,350

Based on estimated marginal means
a. Parameter = Rmax, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test Rmax R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	150,190	10	39,1777	12,3891
	ScreeningL	173,010	10	50,8735	16,0876
Pair 2	Week12R	151,450	10	38,6229	12,2136
	Week12L	165,390	10	47,0178	14,8683
Pair 3	Week24R	148,750	10	36,5880	11,5701
	Week24L	166,830	10	30,5172	9,6504

a. Parameter = Rmax, Treatment = ACS

Paired Samples Testa

		Paired Differences Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Significance One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-22,8200	29,7159	9,3970	-44,0775	-1,5625	-2,428	9	,019	,038

Pair 2	Week12R - Week12L	-13,9400	30,7903	9,7368	-35,9661	8,0861	-1,432	9	,093	,186
Pair 3	Week24R - Week24L	-18,0800	13,4503	4,2534	-27,7018	-8,4582	-4,251	9	,001	,002

a. Parameter = Rmax, Treatment = ACS

T-Test Rmax R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	153,533	6	23,9034	9,7585
	ScreeningL	151,883	6	31,4670	12,8464
Pair 2	Week12R	171,017	6	35,6060	14,5361
	Week12L	158,067	6	19,3401	7,8956
Pair 3	Week24R	153,917	6	34,8975	14,2469
	Week24L	160,183	6	11,9582	4,8819

a. Parameter = Rmax, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	1,6500	36,2855	14,8135	-36,4293	39,7293	,111	5	,458	,916
Pair 2	Week12R - Week12L	12,9500	44,5155	18,1734	-33,7661	59,6661	,713	5	,254	,508
Pair 3	Week24R - Week24L	-6,2667	33,9106	13,8440	-41,8537	29,3204	-,453	5	,335	,670

a. Parameter = Rmax, Treatment = ACS+HA

T-Test Rmax ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningL	ACS	10	173,010	50,8735	16,0876
	ACS+HA	6	151,883	31,4670	12,8464
ScreeningR	ACS	10	150,190	39,1777	12,3891
	ACS+HA	6	153,533	23,9034	9,7585
Week12L	ACS	10	165,390	47,0178	14,8683
	ACS+HA	6	158,067	19,3401	7,8956
Week12R	ACS	10	151,450	38,6229	12,2136
	ACS+HA	6	171,017	35,6060	14,5361
Week24L	ACS	10	166,830	30,5172	9,6504
	ACS+HA	6	160,183	11,9582	4,8819
Week24R	ACS	10	148,750	36,5880	11,5701
	ACS+HA	6	153,917	34,8975	14,2469

a. Parameter = Rmax

Independent Samples Testa

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
ScreeningL	Equal variances assumed	1,304	,273	,911	14	,189	,378	21,1267	23,1944	-28,6203	70,8737

	Equal variances not assumed			1,026	13,937	,161	,322	21,1267	20,5874	-23,0476	65,3010
ScreeningR	Equal variances assumed	1,048	,323	-,188	14	,427	,854	-3,3433	17,8197	-41,5627	34,8761
	Equal variances not assumed			-,212	13,960	,418	,835	-3,3433	15,7708	-37,1775	30,4908
Week12L	Equal variances assumed	3,406	,086	,360	14	,362	,724	7,3233	20,3616	-36,3480	50,9947
	Equal variances not assumed			,435	12,939	,335	,671	7,3233	16,8347	-29,0631	43,7098
Week12R	Equal variances assumed	,007	,933	-1,008	14	,165	,330	-19,5667	19,4028	-61,1815	22,0481
	Equal variances not assumed			-1,031	11,396	,162	,324	-19,5667	18,9861	-61,1781	22,0448
Week24L	Equal variances assumed	2,527	,134	,505	14	,311	,621	6,6467	13,1632	-21,5856	34,8789
	Equal variances not assumed			,615	12,699	,275	,550	6,6467	10,8149	-16,7740	30,0674
Week24R	Equal variances assumed	,018	,896	-,278	14	,393	,785	-5,1667	18,5869	-45,0316	34,6983
	Equal variances not assumed			-,282	11,090	,392	,784	-5,1667	18,3532	-45,5218	35,1885

a. Parameter = Rmax

Mean depth of roughness (Rz)

Test of normality – Shapiro-Wilk

		Shapiro-Wilk		
	Treatment	Statistic	df	Sig.
ScreeningR	ACS	,910	10	,278
	ACS+HA	,835	6	,118
ScreeningL	ACS	,906	10	,254
	ACS+HA	,844	6	,142
Week12R	ACS	,758	10	,004
	ACS+HA	,911	6	,440
Week12L	ACS	,772	10	,007
	ACS+HA	,935	6	,621
Week24R	ACS	,843	10	,048
	ACS+HA	,896	6	,353
Week24L	ACS	,885	10	,148
	ACS+HA	,958	6	,805

General linear model RL ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,968	,264	2	,876	,969	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,410	7,140	2	,028	,629	,683	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	65,323	2	32,661	,200	,821
	Greenhouse-Geisser	65,323	1,937	33,723	,200	,814
Error(Visit)	Sphericity Assumed	2940,394	18	163,355		
	Greenhouse-Geisser	2940,394	17,434	168,662		
Side	Sphericity Assumed	660,017	1	660,017	10,741	,010
	Greenhouse-Geisser	660,017	1,000	660,017	10,741	,010
Error(Side)	Sphericity Assumed	553,043	9	61,449		
	Greenhouse-Geisser	553,043	9,000	61,449		
Visit * Side	Sphericity Assumed	108,262	2	54,131	,371	,695
	Greenhouse-Geisser	108,262	1,258	86,088	,371	,603
Error(Visit*Side)	Sphericity Assumed	2628,388	18	146,022		
	Greenhouse-Geisser	2628,388	11,318	232,226		

a. Parameter = Rz, Treatment = ACS

General linear model RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,785	,968	2	,616	,823	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,819	,796	2	,672	,847	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	292,847	2	146,423	,875	,446
	Greenhouse-Geisser	292,847	1,646	177,901	,875	,432
Error(Visit)	Sphericity Assumed	1673,263	10	167,326		
	Greenhouse-Geisser	1673,263	8,231	203,298		
Side	Sphericity Assumed	130,721	1	130,721	,403	,553
	Greenhouse-Geisser	130,721	1,000	130,721	,403	,553
Error(Side)	Sphericity Assumed	1621,169	5	324,234		
	Greenhouse-Geisser	1621,169	5,000	324,234		
Visit * Side	Sphericity Assumed	42,482	2	21,241	,075	,928
	Greenhouse-Geisser	42,482	1,694	25,076	,075	,902
Error(Visit*Side)	Sphericity Assumed	2817,388	10	281,739		
	Greenhouse-Geisser	2817,388	8,471	332,601		

a. Parameter = Rz, Treatment = ACS+HA

General linear model R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,459	6,236	2	,044	,649	,712	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	28,033	2	14,016	,070	,933	,008
	Greenhouse-Geisser	28,033	1,298	21,605	,070	,857	,008
	Huynh-Feldt	28,033	1,425	19,673	,070	,876	,008
	Lower-bound	28,033	1,000	28,033	,070	,798	,008
Error(Visits)	Sphericity Assumed	3616,834	18	200,935			
	Greenhouse-Geisser	3616,834	11,678	309,718			

	Huynh-Feldt	3616,834	12,824	282,031			
	Lower-bound	3616,834	9,000	401,870			

a. Parameter = Rz, Treatment = ACS

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-1,530	8,007	1,000	-25,017	21,957
	3	,800	3,630	1,000	-9,848	11,448
2	1	1,530	8,007	1,000	-21,957	25,017
	3	2,330	6,578	1,000	-16,966	21,626
3	1	-,800	3,630	1,000	-11,448	9,848
	2	-2,330	6,578	1,000	-21,626	16,966

Based on estimated marginal means
a. Parameter = Rz, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model R ACS + HA

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,773	1,030	2	,598	,815	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	250,888	2	125,444	,310	,741	,058
	Greenhouse-Geisser	250,888	1,630	153,919	,310	,700	,058
Error(Visits)	Sphericity Assumed	4052,626	10	405,263			
	Greenhouse-Geisser	4052,626	8,150	497,255			

a. Parameter = Rz, Treatment = ACS+HA

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-8,917	11,733	1,000	-50,382	32,549
	3	-2,700	8,838	1,000	-33,934	28,534
2	1	8,917	11,733	1,000	-32,549	50,382
	3	6,217	13,766	1,000	-42,433	54,866
3	1	2,700	8,838	1,000	-28,534	33,934
	2	-6,217	13,766	1,000	-54,866	42,433

Based on estimated marginal means
a. Parameter = Rz, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,899	,854	2	,653	,908	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	145,553	2	72,776	,671	,523	,069

	Greenhouse-Geisser	145,553	1,816	80,141	,671	,511	,069
Error(Visits)	Sphericity Assumed	1951,947	18	108,442			
	Greenhouse-Geisser	1951,947	16,346	119,415			

a. Parameter = Rz, Treatment = ACS

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	5,050	4,661	,920	-8,621	18,721
	3	4,170	5,258	1,000	-11,254	19,594
2	1	-5,050	4,661	,920	-18,721	8,621
	3	-,880	3,962	1,000	-12,502	10,742
3	1	-4,170	5,258	1,000	-19,594	11,254
	2	,880	3,962	1,000	-10,742	12,502

Based on estimated marginal means
a. Parameter = Rz, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS + HA

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,414	3,527	2	,171	,631	,744	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	84,441	2	42,221	,964	,414	,162
	Greenhouse-Geisser	84,441	1,261	66,961	,964	,387	,162
Error(Visits)	Sphericity Assumed	438,026	10	43,803			
	Greenhouse-Geisser	438,026	6,305	69,470			

a. Parameter = Rz, Treatment = ACS+HA

Pairwise Comparisons

					95% Confidence Interval for Differenceb	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-5,050	1,851	,124	-11,593	1,493
	3	-3,933	4,521	1,000	-19,912	12,046
2	1	5,050	1,851	,124	-1,493	11,593
	3	1,117	4,465	1,000	-14,662	16,895
3	1	3,933	4,521	1,000	-12,046	19,912
	2	-1,117	4,465	1,000	-16,895	14,662

Based on estimated marginal means
a. Parameter = Rz, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test Rz R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	112,730	10	28,1158	8,8910
	ScreeningL	122,680	10	29,5918	9,3578
Pair 2	Week12R	114,260	10	27,6848	8,7547
	Week12L	117,630	10	28,0629	8,8743
Pair 3	Week24R	111,930	10	24,6835	7,8056
	Week24L	118,510	10	23,3369	7,3798

a. Parameter = Rz, Treatment = ACS

Paired Samples Testa

	Paired Differences							Significance	
				95% Confidence Interval of the Difference					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	-9,9500	17,4659	5,5232	-22,4443	2,5443	-1,801	9	,053	,105
Pair 2 Week12R - Week12L	-3,3700	17,5904	5,5626	-15,9534	9,2134	-,606	9	,280	,560
Pair 3 Week24R - Week24L	-6,5800	9,6180	3,0415	-13,4603	,3003	-2,163	9	,029	,059

a. Parameter = Rz, Treatment = ACS

T-Test Rz R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	114,700	6	20,2284	8,2582
	ScreeningL	111,767	6	18,9273	7,7270
Pair 2	Week12R	123,617	6	22,1152	9,0285
	Week12L	116,817	6	16,9823	6,9330
Pair 3	Week24R	117,400	6	29,9119	12,2115
	Week24L	115,700	6	11,3504	4,6338

a. Parameter = Rz, Treatment = ACS+HA

Paired Samples Testa

	Paired Differences							Significance	
				95% Confidence Interval of the Difference					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	2,9333	22,8236	9,3177	-21,0186	26,8853	,315	5	,383	,766
Pair 2 Week12R - Week12L	6,8000	24,2761	9,9107	-18,6762	32,2762	,686	5	,262	,523
Pair 3 Week24R - Week24L	1,7000	25,7910	10,5291	-25,3660	28,7660	,161	5	,439	,878

a. Parameter = Rz, Treatment = ACS+HA

T-Test Rz ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningL	ACS	10	122,680	29,5918	9,3578
	ACS+HA	6	111,767	18,9273	7,7270
ScreeningR	ACS	10	112,730	28,1158	8,8910
	ACS+HA	6	114,700	20,2284	8,2582
Week12L	ACS	10	117,630	28,0629	8,8743
	ACS+HA	6	116,817	16,9823	6,9330
Week12R	ACS	10	114,260	27,6848	8,7547
	ACS+HA	6	123,617	22,1152	9,0285
Week24L	ACS	10	118,510	23,3369	7,3798
	ACS+HA	6	115,700	11,3504	4,6338
Week24R	ACS	10	111,930	24,6835	7,8056
	ACS+HA	6	117,400	29,9119	12,2115

a. Parameter = Rz

Independent Samples Testa

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	df	Significance	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference

						One-Sided p	Two-Sided p			Lower	Upper
ScreeningL	Equal variances assumed	1,733	,209	,804	14	,217	,435	10,9133	13,5733	-18,1985	40,0251
	Equal variances not assumed			,899	13,859	,192	,384	10,9133	12,1357	-15,1399	36,9666
ScreeningR	Equal variances assumed	,419	,528	-,149	14	,442	,884	-1,9700	13,2092	-30,3010	26,3610
	Equal variances not assumed			-,162	13,347	,437	,873	-1,9700	12,1346	-28,1161	24,1761
Week12L	Equal variances assumed	1,138	,304	,064	14	,475	,950	,8133	12,7464	-26,5250	28,1517
	Equal variances not assumed			,072	13,971	,472	,943	,8133	11,2614	-23,3447	24,9713
Week12R	Equal variances assumed	,076	,787	-,701	14	,247	,495	-9,3567	13,3406	-37,9693	19,2560
	Equal variances not assumed			-,744	12,623	,235	,470	-9,3567	12,5761	-36,6084	17,8951
Week24L	Equal variances assumed	3,008	,105	,273	14	,394	,789	2,8100	10,2777	-19,2335	24,8535
	Equal variances not assumed			,322	13,671	,376	,752	2,8100	8,7140	-15,9219	21,5419
Week24R	Equal variances assumed	,528	,479	-,397	14	,349	,697	-5,4700	13,7716	-35,0072	24,0672
	Equal variances not assumed			-,377	9,079	,357	,715	-5,4700	14,4930	-38,2123	27,2723

a. Parameter = Rz

Maximum profile peak (Rp)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	ACS	,945	10	,605
	ACS+HA	,848	6	,150
ScreeningL	ACS	,864	10	,085
	ACS+HA	,840	6	,129
Week12R	ACS	,803	10	,016
	ACS+HA	,962	6	,838
Week12L	ACS	,811	10	,020
	ACS+HA	,961	6	,831
Week24R	ACS	,912	10	,294
	ACS+HA	,903	6	,393
Week24L	ACS	,969	10	,877
	ACS+HA	,975	6	,926

General linear model RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visit	,982	,149	2	,928	,982	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,910	,754	2	,686	,917	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	75,690	2	37,845	,148	,863
	Greenhouse-Geisser	75,690	1,964	38,542	,148	,860
Error(Visit)	Sphericity Assumed	4591,983	18	255,110		
	Greenhouse-Geisser	4591,983	17,675	259,805		
Side	Sphericity Assumed	3448,900	1	3448,900	14,820	,004
	Greenhouse-Geisser	3448,900	1,000	3448,900	14,820	,004
Error(Side)	Sphericity Assumed	2094,448	9	232,716		
	Greenhouse-Geisser	2094,448	9,000	232,716		
Visit * Side	Sphericity Assumed	52,310	2	26,155	,238	,791
	Greenhouse-Geisser	52,310	1,835	28,508	,238	,773
Error(Visit*Side)	Sphericity Assumed	1981,196	18	110,066		
	Greenhouse-Geisser	1981,196	16,514	119,967		

a. Parameter = Rp, Treatment = ACS

General linear model RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,695	1,453	2	,484	,767	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,555	2,357	2	,308	,692	,872	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	115,961	2	57,980	,689	,524
	Greenhouse-Geisser	115,961	1,533	75,639	,689	,493
Error(Visit)	Sphericity Assumed	841,486	10	84,149		
	Greenhouse-Geisser	841,486	7,665	109,777		
Side	Sphericity Assumed	620,840	1	620,840	1,468	,280
	Greenhouse-Geisser	620,840	1,000	620,840	1,468	,280
Error(Side)	Sphericity Assumed	2113,878	5	422,776		
	Greenhouse-Geisser	2113,878	5,000	422,776		
Visit * Side	Sphericity Assumed	117,094	2	58,547	,465	,641
	Greenhouse-Geisser	117,094	1,384	84,612	,465	,579
Error(Visit*Side)	Sphericity Assumed	1259,413	10	125,941		
	Greenhouse-Geisser	1259,413	6,919	182,010		

a. Parameter = Rp, Treatment = ACS+HA

General linear model R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,595	4,149	2	,126	,712	,808	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	53,096	2	26,548	,180	,836	,020
	Greenhouse-Geisser	53,096	1,424	37,292	,180	,764	,020

Error(Visits)	Sphericity Assumed	2649,344	18	147,186			
	Greenhouse-Geisser	2649,344	12,814	206,752			

a. Parameter = Rp, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,680	6,706	1,000	-20,349	18,989
	3	2,420	3,551	1,000	-7,995	12,835
2	1	,680	6,706	1,000	-18,989	20,349
	3	3,100	5,544	1,000	-13,164	19,364
3	1	-2,420	3,551	1,000	-12,835	7,995
	2	-3,100	5,544	1,000	-19,364	13,164

Based on estimated marginal means
a. Parameter = Rp, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,812	,833	2	,660	,842	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	229,173	2	114,587	1,402	,291	,219
	Greenhouse-Geisser	229,173	1,684	136,118	1,402	,292	,219
Error(Visits)	Sphericity Assumed	817,093	10	81,709			
	Greenhouse-Geisser	817,093	8,418	97,063			

a. Parameter = Rp, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-8,733	4,111	,261	-23,261	5,794
	3	-4,067	5,241	1,000	-22,589	14,456
2	1	8,733	4,111	,261	-5,794	23,261
	3	4,667	6,111	1,000	-16,930	26,263
3	1	4,067	5,241	1,000	-14,456	22,589
	2	-4,667	6,111	1,000	-26,263	16,930

Based on estimated marginal means
a. Parameter = Rp, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,843	1,370	2	,504	,864	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	74,905	2	37,452	,172	,844	,019
	Greenhouse-Geisser	74,905	1,728	43,346	,172	,813	,019

Error(Visits)	Sphericity Assumed	3923,835	18	217,991			
	Greenhouse-Geisser	3923,835	15,553	252,293			

a. Parameter = Rp, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	3,570	5,697	1,000	-13,142	20,282
	3	3,080	7,786	1,000	-19,760	25,920
2	1	-3,570	5,697	1,000	-20,282	13,142
	3	-,490	6,141	1,000	-18,503	17,523
3	1	-3,080	7,786	1,000	-25,920	19,760
	2	,490	6,141	1,000	-17,523	18,503

Based on estimated marginal means
a. Parameter = Rp, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,167	7,153	2	,028	,546	,582	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	3,881	2	1,941	,015	,985	,003
	Greenhouse-Geisser	3,881	1,091	3,557	,015	,922	,003
Error(Visits)	Sphericity Assumed	1283,806	10	128,381			
	Greenhouse-Geisser	1283,806	5,456	235,289			

a. Parameter = Rp, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,067	5,015	1,000	-17,656	17,789
	3	1,017	9,044	1,000	-30,944	32,978
2	1	-,067	5,015	1,000	-17,789	17,656
	3	,950	4,631	1,000	-15,417	17,317
3	1	-1,017	9,044	1,000	-32,978	30,944
	2	-,950	4,631	1,000	-17,317	15,417

Based on estimated marginal means
a. Parameter = Rp, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test Rp R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	80,850	10	15,9241	5,0357
	ScreeningL	97,650	10	32,0970	10,1500
Pair 2	Week12R	81,530	10	19,1563	6,0578
	Week12L	94,080	10	29,6253	9,3683
Pair 3	Week24R	78,430	10	15,8304	5,0060
	Week24L	94,570	10	17,6180	5,5713

a. Parameter = Rp, Treatment = ACS

Paired Samples Testa

Paired Differences	t	df	Significance

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	-16,8000	23,5491	7,4469	-33,6460	,0460	-2,256	9	,025	,051
Pair 2 Week12R - Week12L	-12,5500	17,5960	5,5644	-25,1374	,0374	-2,255	9	,025	,051
Pair 3 Week24R - Week24L	-16,1400	6,4436	2,0377	-20,7495	-11,5305	-7,921	9	<,001	<,001

a. Parameter = Rp, Treatment = ACS

T-Test Rp R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	74,733	6	9,6916	3,9566
	ScreeningL	87,667	6	24,6270	10,0539
Pair 2	Week12R	83,467	6	10,0743	4,1128
	Week12L	87,600	6	15,0357	6,1383
Pair 3	Week24R	78,800	6	16,6898	6,8136
	Week24L	86,650	6	15,3084	6,2496

a. Parameter = Rp, Treatment = ACS+HA

Paired Samples Testa

	Paired Differences							Significance	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	-12,9333	24,5335	10,0158	-38,6797	12,8130	-1,291	5	,127	,253
Pair 2 Week12R - Week12L	-4,1333	17,9130	7,3129	-22,9318	14,6652	-,565	5	,298	,596
Pair 3 Week24R - Week24L	-7,8500	20,6530	8,4316	-29,5240	13,8240	-,931	5	,197	,395

a. Parameter = Rp, Treatment = ACS+HA

T-Test Rp ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningL	ACS	10	97,650	32,0970	10,1500
	ACS+HA	6	87,667	24,6270	10,0539
ScreeningR	ACS	10	80,850	15,9241	5,0357
	ACS+HA	6	74,733	9,6916	3,9566
Week12L	ACS	10	94,080	29,6253	9,3683
	ACS+HA	6	87,600	15,0357	6,1383
Week12R	ACS	10	81,530	19,1563	6,0578
	ACS+HA	6	83,467	10,0743	4,1128
Week24L	ACS	10	94,570	17,6180	5,5713
	ACS+HA	6	86,650	15,3084	6,2496
Week24R	ACS	10	78,430	15,8304	5,0060
	ACS+HA	6	78,800	16,6898	6,8136

a. Parameter = Rp

Independent Samples Testa

Levene's Test for Equality of Variances		t-test for Equality of Means							
				Significance				95% Confidence Interval of the Difference	
F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper

ScreeningL	Equal variances assumed	,410	,532	,652	14	,262	,525	9,9833	15,3091	-22,8515	42,8182
	Equal variances not assumed			,699	12,926	,249	,497	9,9833	14,2865	-20,8986	40,8653
ScreeningR	Equal variances assumed	1,014	,331	,845	14	,206	,412	6,1167	7,2399	-9,4113	21,6447
	Equal variances not assumed			,955	13,963	,178	,356	6,1167	6,4041	-7,6221	19,8554
Week12L	Equal variances assumed	1,147	,302	,494	14	,314	,629	6,4800	13,1144	-21,6475	34,6075
	Equal variances not assumed			,579	13,806	,286	,572	6,4800	11,2002	-17,5737	30,5337
Week12R	Equal variances assumed	,892	,361	-,227	14	,412	,823	-1,9367	8,5190	-20,2082	16,3348
	Equal variances not assumed			-,264	13,895	,398	,795	-1,9367	7,3220	-17,6519	13,7786
Week24L	Equal variances assumed	,054	,820	,911	14	,189	,378	7,9200	8,6907	-10,7198	26,5598
	Equal variances not assumed			,946	11,922	,181	,363	7,9200	8,3724	-10,3352	26,1752
Week24R	Equal variances assumed	,097	,760	-,044	14	,483	,965	-,3700	8,3360	-18,2489	17,5089
	Equal variances not assumed			-,044	10,203	,483	,966	-,3700	8,4549	-19,1579	18,4179

a. Parameter = Rp

Waviness (Wt)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	ACS	,796	10	,013
	ACS+HA	,862	6	,195
ScreeningL	ACS	,678	10	<,001
	ACS+HA	,892	6	,328
Week12R	ACS	,881	10	,134
	ACS+HA	,727	6	,012
Week12L	ACS	,651	10	<,001
	ACS+HA	,907	6	,415
Week24R	ACS	,726	10	,002
	ACS+HA	,681	6	,004
Week24L	ACS	,703	10	<,001
	ACS+HA	,908	6	,421

General linear model RL ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visit	,856	1,248	2	,536	,874	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,691	2,960	2	,228	,764	,888	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	908,652	2	454,326	3,874	,040
	Greenhouse-Geisser	908,652	1,748	519,940	3,874	,048
Error(Visit)	Sphericity Assumed	2110,988	18	117,277		
	Greenhouse-Geisser	2110,988	15,728	134,214		
Side	Sphericity Assumed	134,700	1	134,700	,238	,637
	Greenhouse-Geisser	134,700	1,000	134,700	,238	,637
Error(Side)	Sphericity Assumed	5096,152	9	566,239		
	Greenhouse-Geisser	5096,152	9,000	566,239		
Visit * Side	Sphericity Assumed	203,401	2	101,701	,612	,553
	Greenhouse-Geisser	203,401	1,528	133,155	,612	,514
Error(Visit*Side)	Sphericity Assumed	2991,152	18	166,175		
	Greenhouse-Geisser	2991,152	13,748	217,570		

a. Parameter = Wt, Treatment = ACS

General linear model RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,301	4,797	2	,091	,589	,662	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,855	,625	2	,732	,874	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	648,562	2	324,281	1,444	,281
	Greenhouse-Geisser	648,562	1,177	550,809	1,444	,285
Error(Visit)	Sphericity Assumed	2245,758	10	224,576		
	Greenhouse-Geisser	2245,758	5,887	381,454		
Side	Sphericity Assumed	1898,054	1	1898,054	1,682	,251
	Greenhouse-Geisser	1898,054	1,000	1898,054	1,682	,251
Error(Side)	Sphericity Assumed	5641,316	5	1128,263		
	Greenhouse-Geisser	5641,316	5,000	1128,263		
Visit * Side	Sphericity Assumed	203,429	2	101,714	,287	,757
	Greenhouse-Geisser	203,429	1,747	116,430	,287	,729
Error(Visit*Side)	Sphericity Assumed	3544,911	10	354,491		
	Greenhouse-Geisser	3544,911	8,736	405,776		

a. Parameter = Wt, Treatment = ACS+HA

General linear model R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,636	3,621	2	,164	,733	,840	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	209,985	2	104,992	,654	,532	,068

	Greenhouse-Geisser	209,985	1,466	143,215	,654	,490	,068
Error(Visits)	Sphericity Assumed	2891,589	18	160,644			
	Greenhouse-Geisser	2891,589	13,196	219,127			

a. Parameter = Wt, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	3,170	6,301	1,000	-15,312	21,652
	3	6,480	3,590	,314	-4,051	17,011
2	1	-3,170	6,301	1,000	-21,652	15,312
	3	3,310	6,618	1,000	-16,103	22,723
3	1	-6,480	3,590	,314	-17,011	4,051
	2	-3,310	6,618	1,000	-22,723	16,103

Based on estimated marginal means
a. Parameter = Wt, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,558	2,334	2	,311	,693	,875	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	778,630	2	389,315	,805	,474	,139
	Greenhouse-Geisser	778,630	1,387	561,395	,805	,441	,139
Error(Visits)	Sphericity Assumed	4838,370	10	483,837			
	Greenhouse-Geisser	4838,370	6,935	697,697			

a. Parameter = Wt, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-7,550	11,420	1,000	-47,909	32,809
	3	8,550	9,369	1,000	-24,562	41,662
2	1	7,550	11,420	1,000	-32,809	47,909
	3	16,100	16,298	1,000	-41,501	73,701
3	1	-8,550	9,369	1,000	-41,662	24,562
	2	-16,100	16,298	1,000	-73,701	41,501

Based on estimated marginal means
a. Parameter = Wt, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,841	1,382	2	,501	,863	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	902,069	2	451,034	3,673	,046	,290
	Greenhouse-Geisser	902,069	1,726	522,607	3,673	,055	,290

Error(Visits)	Sphericity Assumed	2210,551	18	122,808			
	Greenhouse-Geisser	2210,551	15,535	142,296			

a. Parameter = Wt, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	12,190	5,837	,199	-4,932	29,312
	3	10,980	4,669	,130	-2,717	24,677
2	1	-12,190	5,837	,199	-29,312	4,932
	3	-1,210	4,220	1,000	-13,590	11,170
3	1	-10,980	4,669	,130	-24,677	2,717
	2	1,210	4,220	1,000	-11,170	13,590

Based on estimated marginal means
a. Parameter = Wt, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,977	,094	2	,954	,977	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	73,361	2	36,681	,385	,690	,072
	Greenhouse-Geisser	73,361	1,955	37,529	,385	,686	,072
Error(Visits)	Sphericity Assumed	952,299	10	95,230			
	Greenhouse-Geisser	952,299	9,774	97,433			

a. Parameter = Wt, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-3,750	5,937	1,000	-24,732	17,232
	3	,917	5,733	1,000	-19,344	21,177
2	1	3,750	5,937	1,000	-17,232	24,732
	3	4,667	5,207	1,000	-13,736	23,070
3	1	-,917	5,733	1,000	-21,177	19,344
	2	-4,667	5,207	1,000	-23,070	13,736

Based on estimated marginal means
a. Parameter = Wt, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test Wt R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	96,640	10	42,9758	13,5901
	ScreeningL	98,150	10	52,5531	16,6187
Pair 2	Week12R	93,470	10	34,3387	10,8589
	Week12L	85,960	10	42,2083	13,3474
Pair 3	Week24R	90,160	10	40,2761	12,7364
	Week24L	87,170	10	39,5064	12,4930

a. Parameter = Wt, Treatment = ACS

Paired Samples Testa

	Paired Differences					t	df	Significance	
				95% Confidence Interval of the Difference					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	-1,5100	26,7639	8,4635	-20,6558	17,6358	-,178	9	,431	,862
Pair 2 Week12R - Week12L	7,5100	24,7870	7,8383	-10,2215	25,2415	,958	9	,182	,363
Pair 3 Week24R - Week24L	2,9900	21,5981	6,8299	-12,4603	18,4403	,438	9	,336	,672

a. Parameter = Wt, Treatment = ACS

T-Test Wt R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	104,117	6	46,4888	18,9790
	ScreeningL	88,317	6	14,8026	6,0431
Pair 2	Week12R	111,667	6	41,2033	16,8212
	Week12L	92,067	6	21,7748	8,8895
Pair 3	Week24R	95,567	6	45,0166	18,3780
	Week24L	87,400	6	12,6575	5,1674

a. Parameter = Wt, Treatment = ACS+HA

Paired Samples Testa

	Paired Differences					t	df	Significance	
				95% Confidence Interval of the Difference					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	15,8000	41,8598	17,0892	-28,1291	59,7291	,925	5	,199	,398
Pair 2 Week12R - Week12L	19,6000	23,8639	9,7424	-5,4436	44,6436	2,012	5	,050	,100
Pair 3 Week24R - Week24L	8,1667	36,7800	15,0154	-30,4316	46,7649	,544	5	,305	,610

a. Parameter = Wt, Treatment = ACS+HA

T-Test Wt ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningL	ACS	10	98,150	52,5531	16,6187
	ACS+HA	6	88,317	14,8026	6,0431
ScreeningR	ACS	10	96,640	42,9758	13,5901
	ACS+HA	6	104,117	46,4888	18,9790
Week12L	ACS	10	85,960	42,2083	13,3474
	ACS+HA	6	92,067	21,7748	8,8895
Week12R	ACS	10	93,470	34,3387	10,8589
	ACS+HA	6	111,667	41,2033	16,8212
Week24L	ACS	10	87,170	39,5064	12,4930
	ACS+HA	6	87,400	12,6575	5,1674
Week24R	ACS	10	90,160	40,2761	12,7364
	ACS+HA	6	95,567	45,0166	18,3780

a. Parameter = Wt

Independent Samples Testa

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	df	Significance	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference

						One-Sided p	Two-Sided p			Lower	Upper
ScreeningL	Equal variances assumed	1,213	,289	,442	14	,333	,665	9,8333	22,2334	-37,8526	57,5193
	Equal variances not assumed			,556	11,185	,295	,589	9,8333	17,6834	-29,0090	48,6756
ScreeningR	Equal variances assumed	,001	,982	-,327	14	,374	,748	-7,4767	22,8570	-56,5001	41,5468
	Equal variances not assumed			-,320	9,984	,378	,755	-7,4767	23,3429	-59,4994	44,5461
Week12L	Equal variances assumed	,260	,618	-,326	14	,375	,749	-6,1067	18,7233	-46,2643	34,0509
	Equal variances not assumed			-,381	13,850	,355	,709	-6,1067	16,0368	-40,5371	28,3238
Week12R	Equal variances assumed	,866	,368	-,954	14	,178	,356	-18,1967	19,0742	-59,1068	22,7135
	Equal variances not assumed			-,909	9,153	,193	,387	-18,1967	20,0217	-63,3740	26,9806
Week24L	Equal variances assumed	,922	,353	-,014	14	,495	,989	-,2300	16,8171	-36,2992	35,8392
	Equal variances not assumed			-,017	11,725	,493	,987	-,2300	13,5195	-29,7632	29,3032
Week24R	Equal variances assumed	,002	,961	-,249	14	,403	,807	-5,4067	21,7045	-51,9582	41,1448
	Equal variances not assumed			-,242	9,712	,407	,814	-5,4067	22,3599	-55,4289	44,6155

a. Parameter = Wt

Number of peaks (PC)

Test of normality – Shapiro-Wilk

	Treatment	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	ACS	,918	10	,338
	ACS+HA	,958	6	,801
ScreeningL	ACS	,848	10	,055
	ACS+HA	,915	6	,473
Week12R	ACS	,929	10	,441
	ACS+HA	,840	6	,129
Week12L	ACS	,933	10	,479
	ACS+HA	,850	6	,158
Week24R	ACS	,841	10	,045
	ACS+HA	,957	6	,794
Week24L	ACS	,895	10	,191
	ACS+HA	,914	6	,466

General linear model RL ACS

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visit	,907	,777	2	,678	,915	1,000	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,639	3,578	2	,167	,735	,843	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	2,233	2	1,117	,968	,399
	Greenhouse-Geisser	2,233	1,831	1,220	,968	,393
Error(Visit)	Sphericity Assumed	20,767	18	1,154		
	Greenhouse-Geisser	20,767	16,475	1,261		
Side	Sphericity Assumed	,267	1	,267	,298	,599
	Greenhouse-Geisser	,267	1,000	,267	,298	,599
Error(Side)	Sphericity Assumed	8,067	9	,896		
	Greenhouse-Geisser	8,067	9,000	,896		
Visit * Side	Sphericity Assumed	,033	2	,017	,031	,969
	Greenhouse-Geisser	,033	1,470	,023	,031	,934
Error(Visit*Side)	Sphericity Assumed	9,633	18	,535		
	Greenhouse-Geisser	9,633	13,229	,728		

a. Parameter = PC, Treatment = ACS

General linear model RL ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,649	1,730	2	,421	,740	,978	,500
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,933	,278	2	,870	,937	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Visit	Sphericity Assumed	3,556	2	1,778	1,758	,222
	Greenhouse-Geisser	3,556	1,480	2,402	1,758	,234
Error(Visit)	Sphericity Assumed	10,111	10	1,011		
	Greenhouse-Geisser	10,111	7,401	1,366		
Side	Sphericity Assumed	,250	1	,250	,079	,791
	Greenhouse-Geisser	,250	1,000	,250	,079	,791
Error(Side)	Sphericity Assumed	15,917	5	3,183		
	Greenhouse-Geisser	15,917	5,000	3,183		
Visit * Side	Sphericity Assumed	2,000	2	1,000	,698	,520
	Greenhouse-Geisser	2,000	1,874	1,067	,698	,513
Error(Visit*Side)	Sphericity Assumed	14,333	10	1,433		
	Greenhouse-Geisser	14,333	9,372	1,529		

a. Parameter = PC, Treatment = ACS+HA

General linear model R ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,955	,371	2	,831	,957	1,000	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	1,400	2	,700	,904	,422	,091

	Greenhouse-Geisser	1,400	1,913	,732	,904	,419	,091
Error(Visits)	Sphericity Assumed	13,933	18	,774			
	Greenhouse-Geisser	13,933	17,219	,809			

a. Parameter = PC, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,100	,433	1,000	-1,171	1,371
	3	-,400	,371	,928	-1,489	,689
2	1	-,100	,433	1,000	-1,371	1,171
	3	-,500	,373	,638	-1,593	,593
3	1	,400	,371	,928	-,689	1,489
	2	,500	,373	,638	-,593	1,593

Based on estimated marginal means
a. Parameter = PC, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model R ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,850	,650	2	,723	,870	1,000	,500

Tests of Within-Subjects Effects

Tests of Within-Subjects Effectsa
Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	1,444	2	,722	,844	,458	,144
	Greenhouse-Geisser	1,444	1,739	,831	,844	,447	,144
Error(Visits)	Sphericity Assumed	8,556	10	,856			
	Greenhouse-Geisser	8,556	8,696	,984			

a. Parameter = PC, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,500	,563	1,000	-2,489	1,489
	3	-,667	,422	,524	-2,157	,823
2	1	,500	,563	1,000	-1,489	2,489
	3	-,167	,601	1,000	-2,290	1,957
3	1	,667	,422	,524	-,823	2,157
	2	,167	,601	1,000	-1,957	2,290

Based on estimated marginal means
a. Parameter = PC, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,522	5,197	2	,074	,677	,754	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,867	2	,433	,474	,630	,050
	Greenhouse-Geisser	,867	1,353	,640	,474	,561	,050
Error(Visits)	Sphericity Assumed	16,467	18	,915			
	Greenhouse-Geisser	16,467	12,180	1,352			

a. Parameter = PC, Treatment = ACS

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,100	,433	1,000	-1,171	1,371
	3	-,300	,539	1,000	-1,880	1,280
2	1	-,100	,433	1,000	-1,371	1,171
	3	-,400	,267	,504	-1,182	,382
3	1	,300	,539	1,000	-1,280	1,880
	2	,400	,267	,504	-,382	1,182

Based on estimated marginal means
a. Parameter = PC, Treatment = ACS
b. Adjustment for multiple comparisons: Bonferroni.

General linear model L ACS + HA

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,630	1,847	2	,397	,730	,955	,500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	4,111	2	2,056	1,294	,316	,206
	Greenhouse-Geisser	4,111	1,460	2,816	1,294	,314	,206
Error(Visits)	Sphericity Assumed	15,889	10	1,589			
	Greenhouse-Geisser	15,889	7,301	2,176			

a. Parameter = PC, Treatment = ACS+HA

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,500	,764	1,000	-2,199	3,199
	3	-,667	,882	1,000	-3,783	2,450
2	1	-,500	,764	1,000	-3,199	2,199
	3	-1,167	,477	,175	-2,853	,520
3	1	,667	,882	1,000	-2,450	3,783
	2	1,167	,477	,175	-,520	2,853

Based on estimated marginal means
a. Parameter = PC, Treatment = ACS+HA
b. Adjustment for multiple comparisons: Bonferroni.

T-Test PC R vs L ACS

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	23,000	10	1,4907	,4714
	ScreeningL	22,900	10	1,7920	,5667
Pair 2	Week12R	22,900	10	1,5951	,5044
	Week12L	22,800	10	1,1353	,3590
Pair 3	Week24R	23,400	10	1,6465	,5207
	Week24L	23,200	10	1,0328	,3266

a. Parameter = PC, Treatment = ACS

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	,1000	1,4491	,4583	-,9367	1,1367	,218	9	,416	,832
Pair 2	Week12R - Week12L	,1000	,7379	,2333	-,4278	,6278	,429	9	,339	,678
Pair 3	Week24R - Week24L	,2000	1,1353	,3590	-,6121	1,0121	,557	9	,296	,591

a. Parameter = PC, Treatment = ACS

T-Test PC R vs L ACS + HA

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	22,167	6	2,2286	,9098
	ScreeningL	22,333	6	1,0328	,4216
Pair 2	Week12R	22,667	6	2,1602	,8819
	Week12L	21,833	6	1,8348	,7491
Pair 3	Week24R	22,833	6	2,4833	1,0138
	Week24L	23,000	6	1,8974	,7746

a. Parameter = PC, Treatment = ACS+HA

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,1667	2,1370	,8724	-2,4093	2,0760	-,191	5	,428	,856
Pair 2	Week12R - Week12L	,8333	1,7224	,7032	-,9742	2,6409	1,185	5	,145	,289
Pair 3	Week24R - Week24L	-,1667	2,1370	,8724	-2,4093	2,0760	-,191	5	,428	,856

a. Parameter = PC, Treatment = ACS+HA

T-Test PC ACS vs ACS + HA

Group Statisticsa

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
ScreeningL	ACS	10	22,900	1,7920	,5667
	ACS+HA	6	22,333	1,0328	,4216
ScreeningR	ACS	10	23,000	1,4907	,4714
	ACS+HA	6	22,167	2,2286	,9098
Week12L	ACS	10	22,800	1,1353	,3590
	ACS+HA	6	21,833	1,8348	,7491
Week12R	ACS	10	22,900	1,5951	,5044
	ACS+HA	6	22,667	2,1602	,8819
Week24L	ACS	10	23,200	1,0328	,3266
	ACS+HA	6	23,000	1,8974	,7746
Week24R	ACS	10	23,400	1,6465	,5207
	ACS+HA	6	22,833	2,4833	1,0138

a. Parameter = PC

Independent Samples Testa

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	df	Significance		Std. Error Difference	95% Confidence Interval of the Difference

						One-Sided p	Two-Sided p	Mean Difference		Lower	Upper
ScreeningL	Equal variances assumed	2,976	,107	,702	14	,247	,494	,5667	,8075	-1,1653	2,2986
	Equal variances not assumed			,802	14,000	,218	,436	,5667	,7063	-,9482	2,0816
ScreeningR	Equal variances assumed	2,486	,137	,902	14	,191	,382	,8333	,9241	-1,1487	2,8153
	Equal variances not assumed			,813	7,735	,220	,440	,8333	1,0247	-1,5438	3,2104
Week12L	Equal variances assumed	2,903	,110	1,314	14	,105	,210	,9667	,7359	-,6117	2,5451
	Equal variances not assumed			1,164	7,346	,140	,281	,9667	,8307	-,9790	2,9123
Week12R	Equal variances assumed	1,496	,241	,249	14	,404	,807	,2333	,9384	-1,7794	2,2460
	Equal variances not assumed			,230	8,312	,412	,824	,2333	1,0160	-2,0943	2,5610
Week24L	Equal variances assumed	1,294	,274	,276	14	,393	,787	,2000	,7251	-1,3551	1,7551
	Equal variances not assumed			,238	6,816	,409	,819	,2000	,8406	-1,7987	2,1987
Week24R	Equal variances assumed	,860	,369	,552	14	,295	,589	,5667	1,0257	-1,6332	2,7666
	Equal variances not assumed			,497	7,689	,316	,633	,5667	1,1397	-2,0801	3,2134

a. Parameter = PC

ACS in vivo study III data and statistical analyses

Patient data and skin condition

Pateint-number	Age	Skin condition				
		normal	dry	oily	not sensitive	sensitive
1	45	x			x	
2	50	x			x	
3	57	x			x	
4	45	x			x	
5	55	x			x	
6	46	x				x
7	41		x		x	
8	49	x			x	
9	53	x			x	
10	48	x			x	
11	64		x		x	
12	35	x				x
13	64	x			x	
14	44	x			x	
15	41	x			x	
16	30	x			x	
17	38	x				x
18	40		x		x	
19	53	x			x	
20	52	x			x	

Corneometry – skin hydration

Corneometer data, mean of three measurements

Patient	ScreeningR1	ScreeningL1	Week0R1	Week0L1	Week12R1	Week12L1	Week24R1	Week24L1	Week36R1	Week36L1	Week48R1	Week48L1
1	46.000	41.600	38.833	45.400	34.367	32.767	42.167	35.467	47.233	32.533	44.733	41.933
2	25.567	21.933	21.433	23.033	26.933	24.067	18.167	22.833	30.167	39.133	22.367	23.233
3	48.500	49.200	46.800	45.167	41.933	33.400	42.600	42.600	44.533	44.700	57.033	48.333
4	46.333	60.867	40.067	36.500	43.800	28.867	46.133	46.900	43.067	47.233	39.767	43.700
5	64.867	75.533	36.967	35.200	48.567	38.233	56.100	54.567	54.600	62.733	52.667	46.700
6	36.633	37.200	33.567	39.267	31.833	34.800	44.467	38.633	47.967	48.100	61.200	49.067
7	62.467	49.900	55.433	55.867	37.567	46.633	35.400	34.633	47.400	60.200	51.900	45.900
8	42.400	42.033	32.733	47.100	28.400	26.100	36.567	28.700	33.833	27.600	56.300	53.733
9	48.933	49.633	36.400	46.833	40.933	36.933	32.767	30.300	52.767	50.133	35.800	33.567
10	38.233	30.433	21.167	23.500	44.600	41.833	20.600	15.133	35.300	24.233	23.633	23.267
11	32.600	31.100	24.067	22.367	25.333	21.367	30.233	28.467	28.433	25.367	34.900	28.233
12	67.800	30.567	44.900	43.900	40.767	46.633	49.900	34.333	50.100	34.167	52.633	45.867
13	45.933	42.500	38.467	47.367	31.467	45.867	35.733	38.633	43.033	56.067	56.433	52.733
14	53.000	54.233	36.667	54.900	44.800	43.533	35.200	41.133	54.133	64.533	48.567	47.633
15	48.667	57.200	51.467	50.467	37.800	44.700	28.900	36.000	62.233	64.000	43.200	45.600
16	43.533	62.933	43.533	41.367	43.167	49.100	40.900	55.933	37.667	44.967	30.333	38.067
17	44.633	34.300	34.200	31.033	33.633	39.533	39.700	37.333	31.500	34.900	43.000	37.133
18	26.533	28.767	25.467	29.833	31.267	23.400	27.067	36.433	19.900	25.367	26.367	44.733
19	36.300	36.400	31.833	40.100	41.367	28.467	29.633	27.533	34.233	28.767	21.267	39.900
20	37.633	33.567	30.700	30.067	25.267	24.467	29.133	35.467	30.200	20.067	29.733	35.467
Mean	**44.828**	**43.495**	**36.235**	**39.463**	**36.690**	**35.535**	**36.068**	**36.052**	**41.415**	**41.740**	**41.592**	**41.240**
SD	**11.367**	**13.714**	**9.323**	**10.237**	**7.131**	**9.027**	**9.484**	**9.665**	**10.866**	**14.579**	**12.895**	**8.852**

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,955	20	,458
ScreeningL	,957	20	,484
Week0R	,974	20	,828
Week0L	,951	20	,377
Week12R	,943	20	,271
Week12L	,933	20	,179
Week24R	,988	20	,994

Week24L	,961	20	,571
Week36R	,976	20	,869
Week36L	,931	20	,163
Week48R	,936	20	,199
Week48L	,922	20	,106

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonb Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,339	18,519	14	,188	,741	,943	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,123	35,869	14	,001	,627	,765	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	2229,855	5	445,971	5,612	<,001	,228
	Greenhouse-Geisser	2229,855	3,707	601,570	5,612	<,001	,228
Error(Visit)	Sphericity Assumed	7549,548	95	79,469			
	Greenhouse-Geisser	7549,548	70,428	107,196			
Side	Sphericity Assumed	,809	1	,809	,013	,910	,001
	Greenhouse-Geisser	,809	1,000	,809	,013	,910	,001
Error(Side)	Sphericity Assumed	1162,474	19	61,183			
	Greenhouse-Geisser	1162,474	19,000	61,183			
Visit * Side	Sphericity Assumed	136,826	5	27,365	,916	,474	,046
	Greenhouse-Geisser	136,826	3,134	43,665	,916	,442	,046
Error(Visit*Side)	Sphericity Assumed	2837,151	95	29,865			
	Greenhouse-Geisser	2837,151	59,537	47,653			

Pairwise Comparisons

(I) Visit	(J) Visit	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	6,312*	1,761	,030	,406	12,219
	3	8,049*	1,709	,002	2,317	13,781
	4	8,102*	1,667	,002	2,509	13,694
	5	2,584	1,693	1,000	-3,093	8,261
	6	2,746	2,286	1,000	-4,920	10,412
2	1	-6,312*	1,761	,030	-12,219	-,406
	3	1,737	1,719	1,000	-4,030	7,504
	4	1,789	2,083	1,000	-5,197	8,775
	5	-3,728	1,838	,851	-9,892	2,436
	6	-3,567	1,747	,830	-9,427	2,294
3	1	-8,049*	1,709	,002	-13,781	-2,317
	2	-1,737	1,719	1,000	-7,504	4,030
	4	,052	1,950	1,000	-6,489	6,594
	5	-5,465	1,986	,190	-12,126	1,196
	6	-5,303	2,414	,609	-13,399	2,793
4	1	-8,102*	1,667	,002	-13,694	-2,509
	2	-1,789	2,083	1,000	-8,775	5,197
	3	-,052	1,950	1,000	-6,594	6,489
	5	-5,518	2,468	,564	-13,797	2,762
	6	-5,356	1,885	,157	-11,680	,968
5	1	-2,584	1,693	1,000	-8,261	3,093
	2	3,728	1,838	,851	-2,436	9,892
	3	5,465	1,986	,190	-1,196	12,126
	4	5,518	2,468	,564	-2,762	13,797
	6	,162	2,401	1,000	-7,891	8,215
6	1	-2,746	2,286	1,000	-10,412	4,920
	2	3,567	1,747	,830	-2,294	9,427
	3	5,303	2,414	,609	-2,793	13,399

	4	5,356	1,885	,157	-,968	11,680
	5	-,162	2,401	1,000	-8,215	7,891

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
b. Adjustment for multiple comparisons: Bonferroni.

Cutometry – mechanical properties of the skin

Means and SDs

Mean								
Parameter	**R0**	**R2**	**R3**	**R5**	**R6**	**R7**	**R8**	**R9**
n	20	20	20	20	20	20	20	20
ScreeningR	0.420	0.691	0.468	0.473	0.396	0.340	0.291	0.048
Week0R	0.426	0.701	0.476	0.466	0.375	0.340	0.299	0.050
Week12R	0.451	0.709	0.502	0.437	0.350	0.324	0.320	0.051
Week24R	0.461	0.707	0.512	0.428	0.344	0.319	0.325	0.051
Week36R	0.456	0.700	0.508	0.407	0.337	0.305	0.319	0.051
Week48R	0.476	0.709	0.526	0.410	0.318	0.311	0.338	0.050
ScreeningL	0.455	0.712	0.510	0.487	0.374	0.355	0.323	0.055
Week0L	0.447	0.688	0.500	0.455	0.363	0.333	0.306	0.054
Week12L	0.484	0.700	0.540	0.436	0.337	0.327	0.337	0.055
Week24L	0.469	0.722	0.526	0.440	0.345	0.329	0.337	0.057
Week36L	0.496	0.718	0.551	0.433	0.321	0.327	0.353	0.055
Week48L	0.493	0.689	0.546	0.402	0.315	0.307	0.339	0.053
SD	**R0**	**R2**	**R3**	**R5**	**R6**	**R7**	**R8**	**R9**
ScreeningR	0.044	0.071	0.049	0.086	0.060	0.066	0.048	0.009
Week0R	0.064	0.064	0.066	0.105	0.068	0.082	0.055	0.011
Week12R	0.058	0.060	0.058	0.084	0.068	0.064	0.051	0.009
Week24R	0.052	0.069	0.056	0.095	0.042	0.072	0.045	0.009
Week36R	0.067	0.081	0.070	0.093	0.061	0.073	0.058	0.010
Week48R	0.054	0.095	0.059	0.094	0.047	0.073	0.064	0.010
ScreeningL	0.070	0.075	0.075	0.098	0.062	0.072	0.056	0.007
Week0L	0.075	0.081	0.076	0.119	0.060	0.083	0.057	0.008
Week12L	0.087	0.106	0.091	0.108	0.064	0.087	0.074	0.009
Week24L	0.071	0.069	0.077	0.093	0.056	0.075	0.052	0.010
Week36L	0.076	0.110	0.079	0.128	0.058	0.094	0.064	0.009
Week48L	0.069	0.099	0.071	0.101	0.076	0.080	0.065	0.009

Skin firmness (R0, Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,969	20	,743
ScreeningL	,956	20	,464
Week0R	,989	20	,997
Week0L	,944	20	,283
Week12R	,901	20	,043
Week12L	,923	20	,115
Week24R	,977	20	,885
Week24L	,967	20	,691
Week36R	,947	20	,319
Week36L	,972	20	,804

Week48R	,974	20	,830
Week48L	,932	20	,172

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,440	14,035	14	,452	,757	,969	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,641	7,615	14	,910	,856	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,080	5	,016	10,753	<,001	,361
	Greenhouse-Geisser	,080	3,787	,021	10,753	<,001	,361
Error(Visit)	Sphericity Assumed	,141	95	,001			
	Greenhouse-Geisser	,141	71,948	,002			
Side	Sphericity Assumed	,039	1	,039	7,288	,014	,277
	Greenhouse-Geisser	,039	1,000	,039	7,288	,014	,277
Error(Side)	Sphericity Assumed	,102	19	,005			
	Greenhouse-Geisser	,102	19,000	,005			
Visit * Side	Sphericity Assumed	,007	5	,001	1,839	,113	,088
	Greenhouse-Geisser	,007	4,282	,002	1,839	,125	,088
Error(Visit*Side)	Sphericity Assumed	,077	95	,001			
	Greenhouse-Geisser	,077	81,363	,001			

a. Parameter = R0

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,559	9,955	14	,768	,835	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,045	5	,009	9,420	<,001	,331
	Greenhouse-Geisser	,045	4,173	,011	9,420	<,001	,331
Error(Visits)	Sphericity Assumed	,092	95	,001			
	Greenhouse-Geisser	,092	79,280	,001			

a. Parameter = R0

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,006	,010	1,000	-,040	,027
	3	-,031*	,009	,042	-,062	-,001
	4	-,041*	,009	,004	-,072	-,010
	5	-,037*	,011	,040	-,072	-,001
	6	-,056*	,007	<,001	-,079	-,033
2	1	,006	,010	1,000	-,027	,040
	3	-,025	,009	,152	-,054	,004
	4	-,035	,010	,051	-,069	,000

	5	-,030	,011	,251	-,068	,008
	6	-,049*	,009	<,001	-,081	-,018
3	1	,031*	,009	,042	,001	,062
	2	,025	,009	,152	-,004	,054
	4	-,010	,011	1,000	-,047	,028
	5	-,005	,011	1,000	-,043	,033
	6	-,025	,010	,367	-,059	,009
4	1	,041*	,009	,004	,010	,072
	2	,035	,010	,051	,000	,069
	3	,010	,011	1,000	-,028	,047
	5	,004	,010	1,000	-,030	,039
	6	-,015	,008	1,000	-,042	,012
5	1	,037*	,011	,040	,001	,072
	2	,030	,011	,251	-,008	,068
	3	,005	,011	1,000	-,033	,043
	4	-,004	,010	1,000	-,039	,030
	6	-,019	,010	,892	-,052	,013
6	1	,056*	,007	<,001	,033	,079
	2	,049*	,009	<,001	,018	,081
	3	,025	,010	,367	-,009	,059
	4	,015	,008	1,000	-,012	,042
	5	,019	,010	,892	-,013	,052

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,415	15,023	14	,381	,755	,966	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,042	5	,008	6,290	<,001	,249
	Greenhouse-Geisser	,042	3,775	,011	6,290	<,001	,249
Error(Visits)	Sphericity Assumed	,126	95	,001			
	Greenhouse-Geisser	,126	71,733	,002			

a. Parameter = R0

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,008	,009	1,000	-,024	,040
	3	-,030	,012	,389	-,071	,012
	4	-,014	,008	1,000	-,040	,011
	5	-,041*	,011	,018	-,077	-,005
	6	-,038	,012	,057	-,077	,001
2	1	-,008	,009	1,000	-,040	,024
	3	-,038*	,011	,035	-,074	-,002
	4	-,022	,008	,176	-,049	,005
	5	-,049*	,009	<,001	-,081	-,018
	6	-,046*	,014	,048	-,092	,000
3	1	,030	,012	,389	-,012	,071
	2	,038*	,011	,035	,002	,074
	4	,015	,013	1,000	-,027	,057

	5	-,011	,011	1,000	-,050	,027
	6	-,008	,014	1,000	-,055	,039
4	1	,014	,008	1,000	-,011	,040
	2	,022	,008	,176	-,005	,049
	3	-,015	,013	1,000	-,057	,027
	5	-,027	,010	,279	-,062	,008
	6	-,024	,013	1,000	-,068	,020
5	1	,041*	,011	,018	,005	,077
	2	,049*	,009	<,001	,018	,081
	3	,011	,011	1,000	-,027	,050
	4	,027	,010	,279	-,008	,062
	6	,003	,015	1,000	-,046	,052
6	1	,038	,012	,057	-,001	,077
	2	,046*	,014	,048	,000	,092
	3	,008	,014	1,000	-,039	,055
	4	,024	,013	1,000	-,020	,068
	5	-,003	,015	1,000	-,052	,046

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R0
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R0

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,419800	20	,0439169	,0098201
	ScreeningL	,454600	20	,0701070	,0156764
Pair 2	Week0R	,426250	20	,0640089	,0143128
	Week0L	,446550	20	,0750105	,0167729
Pair 3	Week12R	,450950	20	,0578514	,0129360
	Week12L	,484400	20	,0868043	,0194100
Pair 4	Week24R	,460800	20	,0524782	,0117345
	Week24L	,469000	20	,0710085	,0158780
Pair 5	Week36R	,456300	20	,0669816	,0149775
	Week36L	,495750	20	,0757377	,0169355
Pair 6	Week48R	,475650	20	,0541502	,0121083
	Week48L	,492650	20	,0693582	,0155090

a. Parameter = R0

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Devia-tion	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,0348000	,0565198	,0126382	-,0612521	-,0083479	-2,754	19	,006	,013
Pair 2	Week0R - Week0L	-,0203000	,0429444	,0096027	-,0403986	-,0002014	-2,114	19	,024	,048
Pair 3	Week12R - Week12L	-,0334500	,0574525	,0128468	-,0603386	-,0065614	-2,604	19	,009	,017
Pair 4	Week24R - Week24L	-,0082000	,0579679	,0129620	-,0353298	,0189298	-,633	19	,267	,535
Pair 5	Week36R - Week36L	-,0394500	,0636193	,0142257	-,0692248	-,0096752	-2,773	19	,006	,012
Pair 6	Week48R - Week48L	-,0170000	,0553762	,0123825	-,0429169	,0089169	-1,373	19	,093	,186

a. Parameter = R0

Skin gross elasticity (R2, Ua/Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,962	20	,576
ScreeningL	,953	20	,408
Week0R	,986	20	,986
Week0L	,935	20	,195
Week12R	,966	20	,679
Week12L	,860	20	,008
Week24R	,941	20	,255
Week24L	,851	20	,005
Week36R	,969	20	,737
Week36L	,945	20	,296
Week48R	,974	20	,832
Week48L	,942	20	,265

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,464	13,149	14	,519	,780	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,447	13,755	14	,473	,759	,972	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,010	5	,002	,706	,620	,036
	Greenhouse-Geisser	,010	3,902	,003	,706	,587	,036
Error(Visit)	Sphericity Assumed	,267	95	,003			
	Greenhouse-Geisser	,267	74,138	,004			
Side	Sphericity Assumed	,000	1	,000	,067	,799	,004
	Greenhouse-Geisser	,000	1,000	,000	,067	,799	,004
Error(Side)	Sphericity Assumed	,065	19	,003			
	Greenhouse-Geisser	,065	19,000	,003			
Visit * Side	Sphericity Assumed	,016	5	,003	1,591	,170	,077
	Greenhouse-Geisser	,016	3,796	,004	1,591	,189	,077
Error(Visit*Side)	Sphericity Assumed	,196	95	,002			
	Greenhouse-Geisser	,196	72,127	,003			

a. Parameter = R2

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,310	20,044	14	,132	,708	,891	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,005	5	,001	,437	,822	,022
	Greenhouse-Geisser	,005	3,542	,001	,437	,759	,022

Error(Visits)	Sphericity Assumed	,214	95	,002			
	Greenhouse-Geisser	,214	67,307	,003			

a. Parameter = R2

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	-,010	,017	1,000	-,067	,047
	3	-,018	,012	1,000	-,059	,022
	4	-,016	,014	1,000	-,062	,031
	5	-,009	,018	1,000	-,070	,053
	6	-,018	,018	1,000	-,078	,042
2	1	,010	,017	1,000	-,047	,067
	3	-,008	,014	1,000	-,055	,039
	4	-,005	,010	1,000	-,039	,028
	5	,002	,016	1,000	-,052	,055
	6	-,008	,016	1,000	-,062	,046
3	1	,018	,012	1,000	-,022	,059
	2	,008	,014	1,000	-,039	,055
	4	,002	,013	1,000	-,041	,046
	5	,009	,014	1,000	-,036	,055
	6	,000	,014	1,000	-,048	,047
4	1	,016	,014	1,000	-,031	,062
	2	,005	,010	1,000	-,028	,039
	3	-,002	,013	1,000	-,046	,041
	5	,007	,018	1,000	-,052	,066
	6	-,003	,016	1,000	-,056	,050
5	1	,009	,018	1,000	-,053	,070
	2	-,002	,016	1,000	-,055	,052
	3	-,009	,014	1,000	-,055	,036
	4	-,007	,018	1,000	-,066	,052
	6	-,010	,013	1,000	-,052	,033
6	1	,018	,018	1,000	-,042	,078
	2	,008	,016	1,000	-,046	,062
	3	,000	,014	1,000	-,047	,048
	4	,003	,016	1,000	-,050	,056
	5	,010	,013	1,000	-,033	,052

Based on estimated marginal means
a. Parameter = R2
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,463	13,157	14	,519	,761	,976	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,021	5	,004	1,634	,158	,079
	Greenhouse-Geisser	,021	3,806	,006	1,634	,178	,079
Error(Visits)	Sphericity Assumed	,249	95	,003			
	Greenhouse-Geisser	,249	72,316	,003			

a. Parameter = R2

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,024	,013	1,000	-,018	,066
	3	,012	,016	1,000	-,041	,065
	4	-,010	,013	1,000	-,053	,032
	5	-,006	,019	1,000	-,070	,058
	6	,023	,016	1,000	-,030	,075
2	1	-,024	,013	1,000	-,066	,018
	3	-,012	,017	1,000	-,067	,044
	4	-,034	,012	,121	-,073	,005
	5	-,030	,019	1,000	-,094	,034
	6	-,001	,014	1,000	-,048	,045
3	1	-,012	,016	1,000	-,065	,041
	2	,012	,017	1,000	-,044	,067
	4	-,023	,013	1,000	-,067	,021
	5	-,018	,016	1,000	-,073	,037
	6	,010	,019	1,000	-,053	,074
4	1	,010	,013	1,000	-,032	,053
	2	,034	,012	,121	-,005	,073
	3	,023	,013	1,000	-,021	,067
	5	,005	,017	1,000	-,053	,062
	6	,033	,017	,927	-,023	,089
5	1	,006	,019	1,000	-,058	,070
	2	,030	,019	1,000	-,034	,094
	3	,018	,016	1,000	-,037	,073
	4	-,005	,017	1,000	-,062	,053
	6	,028	,020	1,000	-,040	,097
6	1	-,023	,016	1,000	-,075	,030
	2	,001	,014	1,000	-,045	,048
	3	-,010	,019	1,000	-,074	,053
	4	-,033	,017	,927	-,089	,023
	5	-,028	,020	1,000	-,097	,040

Based on estimated marginal means
a. Parameter = R2
b. Adjustment for multiple comparisons: Bonferroni.

T-Test R2 2 mm

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,691020	20	,0705497	,0157754
	ScreeningL	,711820	20	,0748902	,0167460
Pair 2	Week0R	,701215	20	,0642799	,0143734
	Week0L	,687935	20	,0805243	,0180058
Pair 3	Week12R	,709100	20	,0602163	,0134648
	Week12L	,699560	20	,1061644	,0237391
Pair 4	Week24R	,706610	20	,0693660	,0155107
	Week24L	,722260	20	,0686872	,0153589
Pair 5	Week36R	,699655	20	,0812562	,0181694
	Week36L	,717615	20	,1096704	,0245231
Pair 6	Week48R	,709205	20	,0954844	,0213510
	Week48L	,689295	20	,0991152	,0221628

a. Parameter = R2

Paired Samples Testa

	Paired Differences							Significance	
				95% Confidence Interval of the Difference					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1 ScreeningR - ScreeningL	-,0208000	,0555920	,0124308	-,0468179	,0052179	-1,673	19	,055	,111

Pair 2	Week0R - Week0L	,0132800	,0598139	,0133748	-,0147138	,0412738	,993	19	,167	,333
Pair 3	Week12R - Week12L	,0095400	,0718459	,0160652	-,0240849	,0431649	,594	19	,280	,560
Pair 4	Week24R - Week24L	-,0156500	,0535886	,0119828	-,0407302	,0094302	-1,306	19	,104	,207
Pair 5	Week36R - Week36L	-,0179600	,0907995	,0203034	-,0604555	,0245355	-,885	19	,194	,387
Pair 6	Week48R - Week48L	,0199100	,0668937	,0149579	-,0113972	,0512172	1,331	19	,099	,199

a. Parameter = R2

Skin firmness after repeated suction (R3, Uf_5)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,979	20	,921
ScreeningL	,960	20	,553
Week0R	,981	20	,950
Week0L	,936	20	,202
Week12R	,925	20	,125
Week12L	,912	20	,070
Week24R	,964	20	,635
Week24L	,975	20	,847
Week36R	,948	20	,345
Week36L	,964	20	,634
Week48R	,983	20	,968
Week48L	,940	20	,237

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,520	11,171	14	,676	,801	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,635	7,775	14	,902	,851	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,083	5	,017	11,309	<,001	,373
	Greenhouse-Geisser	,083	4,003	,021	11,309	<,001	,373
Error(Visit)	Sphericity Assumed	,139	95	,001			
	Greenhouse-Geisser	,139	76,058	,002			
Side	Sphericity Assumed	,054	1	,054	8,356	,009	,305
	Greenhouse-Geisser	,054	1,000	,054	8,356	,009	,305
Error(Side)	Sphericity Assumed	,123	19	,006			
	Greenhouse-Geisser	,123	19,000	,006			
Visit * Side	Sphericity Assumed	,008	5	,002	1,697	,143	,082
	Greenhouse-Geisser	,008	4,256	,002	1,697	,155	,082
Error(Visit*Side)	Sphericity Assumed	,085	95	,001			
	Greenhouse-Geisser	,085	80,864	,001			

a. Parameter = R3

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,621	8,152	14	,883	,870	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,049	5	,010	9,668	<,001	,337
	Greenhouse-Geisser	,049	4,351	,011	9,668	<,001	,337
Error(Visits)	Sphericity Assumed	,095	95	,001			
	Greenhouse-Geisser	,095	82,676	,001			

a. Parameter = R3

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,008	,010	1,000	-,043	,026
	3	-,034*	,009	,013	-,063	-,005
	4	-,044*	,010	,005	-,077	-,010
	5	-,040*	,011	,021	-,075	-,004
	6	-,058*	,007	<,001	-,083	-,033
2	1	,008	,010	1,000	-,026	,043
	3	-,026	,010	,218	-,058	,006
	4	-,035*	,010	,037	-,070	-,001
	5	-,031	,011	,170	-,069	,006
	6	-,049*	,010	,001	-,082	-,017
3	1	,034*	,009	,013	,005	,063
	2	,026	,010	,218	-,006	,058
	4	-,010	,011	1,000	-,047	,028
	5	-,006	,012	1,000	-,045	,033
	6	-,024	,010	,524	-,059	,011
4	1	,044*	,010	,005	,010	,077
	2	,035*	,010	,037	,001	,070
	3	,010	,011	1,000	-,028	,047
	5	,004	,011	1,000	-,032	,041
	6	-,014	,009	1,000	-,043	,015
5	1	,040*	,011	,021	,004	,075
	2	,031	,011	,170	-,006	,069
	3	,006	,012	1,000	-,033	,045
	4	-,004	,011	1,000	-,041	,032
	6	-,018	,009	1,000	-,049	,013
6	1	,058*	,007	<,001	,033	,083
	2	,049*	,010	,001	,017	,082
	3	,024	,010	,524	-,011	,059
	4	,014	,009	1,000	-,015	,043
	5	,018	,009	1,000	-,013	,049

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound

Visits	,437	14,161	14	,442	,766	,984	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,042	5	,008	6,185	<,001	,246
	Greenhouse-Geisser	,042	3,832	,011	6,185	<,001	,246
Error(Visits)	Sphericity Assumed	,128	95	,001			
	Greenhouse-Geisser	,128	72,806	,002			

a. Parameter = R3

Pairwise Comparisons

					95% Confidence Interval for Differencec	
(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	Lower Bound	Upper Bound
1	2	,010	,010	1,000	-,024	,043
	3	-,030	,013	,432	-,072	,012
	4	-,016	,009	1,000	-,045	,013
	5	-,041*	,010	,013	-,076	-,006
	6	-,036	,011	,053	-,073	,000
2	1	-,010	,010	1,000	-,043	,024
	3	-,040*	,012	,044	-,078	-,001
	4	-,026	,008	,086	-,053	,002
	5	-,051*	,009	<,001	-,082	-,019
	6	-,046*	,014	,046	-,091	-,001
3	1	,030	,013	,432	-,012	,072
	2	,040*	,012	,044	,001	,078
	4	,014	,014	1,000	-,032	,059
	5	-,011	,012	1,000	-,051	,029
	6	-,006	,013	1,000	-,051	,039
4	1	,016	,009	1,000	-,013	,045
	2	,026	,008	,086	-,002	,053
	3	-,014	,014	1,000	-,059	,032
	5	-,025	,010	,404	-,060	,010
	6	-,020	,013	1,000	-,065	,024
5	1	,041*	,010	,013	,006	,076
	2	,051*	,009	<,001	,019	,082
	3	,011	,012	1,000	-,029	,051
	4	,025	,010	,404	-,010	,060
	6	,005	,014	1,000	-,043	,052
6	1	,036	,011	,053	,000	,073
	2	,046*	,014	,046	,001	,091
	3	,006	,013	1,000	-,039	,051
	4	,020	,013	1,000	-,024	,065
	5	-,005	,014	1,000	-,052	,043

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R3
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R3

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,468100	20	,0489789	,0109520
	ScreeningL	,509800	20	,0748933	,0167467
Pair 2	Week0R	,476300	20	,0663627	,0148392
	Week0L	,500050	20	,0763499	,0170724
Pair 3	Week12R	,502000	20	,0584997	,0130809
	Week12L	,539650	20	,0912667	,0204079
Pair 4	Week24R	,511800	20	,0560475	,0125326

	Week24L	,525750	20	,0765725	,0171221
Pair 5	Week36R	,507650	20	,0695226	,0155457
	Week36L	,550650	20	,0788685	,0176355
Pair 6	Week48R	,525700	20	,0588988	,0131702
	Week48L	,545950	20	,0709028	,0158543

a. Parameter = R3

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,0417000	,0598622	,0133856	-,0697164	-,0136836	-3,115	19	,003	,006
Pair 2	Week0R - Week0L	-,0237500	,0431666	,0096523	-,0439526	-,0035474	-2,461	19	,012	,024
Pair 3	Week12R - Week12L	-,0376500	,0664128	,0148504	-,0687321	-,0065679	-2,535	19	,010	,020
Pair 4	Week24R - Week24L	-,0139500	,0621098	,0138882	-,0430183	,0151183	-1,004	19	,164	,328
Pair 5	Week36R - Week36L	-,0430000	,0679597	,0151963	-,0748061	-,0111939	-2,830	19	,005	,011
Pair 6	Week48R - Week48L	-,0202500	,0595199	,0133091	-,0481062	,0076062	-1,522	19	,072	,145

a. Parameter = R3

Skin net elasticity (R5, Ur/Ue)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,976	20	,867
ScreeningL	,942	20	,264
Week0R	,928	20	,141
Week0L	,945	20	,293
Week12R	,985	20	,983
Week12L	,962	20	,577
Week24R	,941	20	,245
Week24L	,990	20	,999
Week36R	,961	20	,573
Week36L	,975	20	,858
Week48R	,968	20	,707
Week48L	,959	20	,518

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,630	7,890	14	,896	,835	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,486	12,336	14	,584	,812	1,000	,200

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared

Visit	Sphericity Assumed	,145	5	,029	8,404	<,001	,307
	Greenhouse-Geisser	,145	4,175	,035	8,404	<,001	,307
Error(Visit)	Sphericity Assumed	,327	95	,003			
	Greenhouse-Geisser	,327	79,334	,004			
Side	Sphericity Assumed	,002	1	,002	,467	,503	,024
	Greenhouse-Geisser	,002	1,000	,002	,467	,503	,024
Error(Side)	Sphericity Assumed	,072	19	,004			
	Greenhouse-Geisser	,072	19,000	,004			
Visit * Side	Sphericity Assumed	,010	5	,002	,820	,538	,041
	Greenhouse-Geisser	,010	4,060	,003	,820	,518	,041
Error(Visit*Side)	Sphericity Assumed	,240	95	,003			
	Greenhouse-Geisser	,240	77,148	,003			

a. Parameter = R5

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,605	8,594	14	,858	,842	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,077	5	,015	5,193	<,001	,215
	Greenhouse-Geisser	,077	4,211	,018	5,193	<,001	,215
Error(Visits)	Sphericity Assumed	,281	95	,003			
	Greenhouse-Geisser	,281	80,006	,004			

a. Parameter = R5

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,007	,021	1,000	-,063	,076
	3	,035	,016	,659	-,019	,090
	4	,045	,015	,132	-,007	,096
	5	,066*	,018	,021	,007	,124
	6	,063*	,015	,008	,012	,114
2	1	-,007	,021	1,000	-,076	,063
	3	,029	,018	1,000	-,032	,090
	4	,038	,019	,879	-,026	,102
	5	,059*	,017	,036	,002	,115
	6	,056	,020	,151	-,010	,123
3	1	-,035	,016	,659	-,090	,019
	2	-,029	,018	1,000	-,090	,032
	4	,010	,017	1,000	-,049	,068
	5	,030	,017	1,000	-,025	,086
	6	,028	,014	,979	-,020	,076
4	1	-,045	,015	,132	-,096	,007
	2	-,038	,019	,879	-,102	,026
	3	-,010	,017	1,000	-,068	,049
	5	,021	,019	1,000	-,043	,085
	6	,018	,016	1,000	-,035	,071
5	1	-,066*	,018	,021	-,124	-,007
	2	-,059*	,017	,036	-,115	-,002
	3	-,030	,017	1,000	-,086	,025
	4	-,021	,019	1,000	-,085	,043
	6	-,002	,014	1,000	-,050	,045
6	1	-,063*	,015	,008	-,114	-,012

	2	-,056	,020	,151	-,123	,010
	3	-,028	,014	,979	-,076	,020
	4	-,018	,016	1,000	-,071	,035
	5	,002	,014	1,000	-,045	,050

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R5
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,755	4,797	14	,989	,904	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,078	5	,016	5,204	<,001	,215
	Greenhouse-Geisser	,078	4,518	,017	5,204	<,001	,215
Error(Visits)	Sphericity Assumed	,286	95	,003			
	Greenhouse-Geisser	,286	85,836	,003			

a. Parameter = R5

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,033	,016	,848	-,021	,087
	3	,052	,018	,134	-,008	,111
	4	,047	,018	,232	-,012	,107
	5	,055	,019	,162	-,010	,120
	6	,085*	,015	<,001	,035	,135
2	1	-,033	,016	,848	-,087	,021
	3	,019	,017	1,000	-,039	,077
	4	,014	,019	1,000	-,050	,079
	5	,022	,017	1,000	-,034	,078
	6	,052	,017	,089	-,004	,108
3	1	-,052	,018	,134	-,111	,008
	2	-,019	,017	1,000	-,077	,039
	4	-,004	,018	1,000	-,065	,056
	5	,003	,018	1,000	-,056	,063
	6	,033	,016	,800	-,021	,088
4	1	-,047	,018	,232	-,107	,012
	2	-,014	,019	1,000	-,079	,050
	3	,004	,018	1,000	-,056	,065
	5	,008	,019	1,000	-,057	,072
	6	,038	,014	,226	-,010	,085
5	1	-,055	,019	,162	-,120	,010
	2	-,022	,017	1,000	-,078	,034
	3	-,003	,018	1,000	-,063	,056
	4	-,008	,019	1,000	-,072	,057
	6	,030	,018	1,000	-,029	,090
6	1	-,085*	,015	<,001	-,135	-,035
	2	-,052	,017	,089	-,108	,004
	3	-,033	,016	,800	-,088	,021
	4	-,038	,014	,226	-,085	,010
	5	-,030	,018	1,000	-,090	,029

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.

a. Parameter = R5
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R5

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,472665	20	,0863946	,0193184
	ScreeningL	,487465	20	,0978346	,0218765
Pair 2	Week0R	,466055	20	,1047402	,0234206
	Week0L	,454570	20	,1187202	,0265466
Pair 3	Week12R	,437480	20	,0838459	,0187485
	Week12L	,435920	20	,1082342	,0242019
Pair 4	Week24R	,427780	20	,0947089	,0211775
	Week24L	,440230	20	,0932308	,0208470
Pair 5	Week36R	,407160	20	,0932084	,0208420
	Week36L	,432690	20	,1276655	,0285469
Pair 6	Week48R	,409620	20	,0940116	,0210216
	Week48L	,402495	20	,1011049	,0226077

a. Parameter = R5

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Devia-tion	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,0148000	,0775606	,0173431	-,0510995	,0214995	-,853	19	,202	,404
Pair 2	Week0R - Week0L	,0114850	,0676113	,0151183	-,0201581	,0431281	,760	19	,228	,457
Pair 3	Week12R - Week12L	,0015600	,0626966	,0140194	-,0277829	,0309029	,111	19	,456	,913
Pair 4	Week24R - Week24L	-,0124500	,0663627	,0148391	-,0435087	,0186087	-,839	19	,206	,412
Pair 5	Week36R - Week36L	-,0255300	,0970254	,0216955	-,0709393	,0198793	-1,177	19	,127	,254
Pair 6	Week48R - Week48L	,0071250	,0668538	,0149490	-,0241635	,0384135	,477	19	,320	,639

a. Parameter = R5

Ratio of viscoelastic to elastic extension (R6, Uv/Ue)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,957	20	,486
ScreeningL	,968	20	,717
Week0R	,940	20	,242
Week0L	,965	20	,656
Week12R	,950	20	,370
Week12L	,945	20	,302
Week24R	,934	20	,187
Week24L	,881	20	,019
Week36R	,935	20	,191
Week36L	,921	20	,104
Week48R	,920	20	,098
Week48L	,759	20	<,001

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,635	7,774	14	,902	,852	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,504	11,709	14	,633	,809	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,126	5	,025	8,899	<,001	,319
	Greenhouse-Geisser	,126	4,259	,030	8,899	<,001	,319
Error(Visit)	Sphericity Assumed	,269	95	,003			
	Greenhouse-Geisser	,269	80,924	,003			
Side	Sphericity Assumed	,007	1	,007	1,606	,220	,078
	Greenhouse-Geisser	,007	1,000	,007	1,606	,220	,078
Error(Side)	Sphericity Assumed	,080	19	,004			
	Greenhouse-Geisser	,080	19,000	,004			
Visit * Side	Sphericity Assumed	,004	5	,001	,408	,842	,021
	Greenhouse-Geisser	,004	4,047	,001	,408	,805	,021
Error(Visit*Side)	Sphericity Assumed	,169	95	,002			
	Greenhouse-Geisser	,169	76,902	,002			

a. Parameter = R6

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,482	12,487	14	,571	,828	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,077	5	,015	8,728	<,001	,315
	Greenhouse-Geisser	,077	4,142	,019	8,728	<,001	,315
Error(Visits)	Sphericity Assumed	,168	95	,002			
	Greenhouse-Geisser	,168	78,699	,002			

a. Parameter = R6

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,021	,014	1,000	-,025	,067
	3	,046	,015	,108	-,005	,098
	4	,052*	,012	,007	,011	,093
	5	,059*	,016	,020	,006	,111
	6	,078*	,010	<,001	,044	,112
2	1	-,021	,014	1,000	-,067	,025
	3	,025	,015	1,000	-,026	,075
	4	,030	,014	,673	-,017	,078
	5	,037	,013	,154	-,007	,081
	6	,056*	,012	,002	,017	,096
3	1	-,046	,015	,108	-,098	,005

	2	-,025	,015	1,000	-,075	,026
	4	,006	,015	1,000	-,044	,055
	5	,013	,015	1,000	-,039	,064
	6	,032	,012	,258	-,009	,072
4	1	-,052*	,012	,007	-,093	-,011
	2	-,030	,014	,673	-,078	,017
	3	-,006	,015	1,000	-,055	,044
	5	,007	,012	1,000	-,035	,049
	6	,026	,009	,128	-,004	,056
5	1	-,059*	,016	,020	-,111	-,006
	2	-,037	,013	,154	-,081	,007
	3	-,013	,015	1,000	-,064	,039
	4	-,007	,012	1,000	-,049	,035
	6	,019	,013	1,000	-,024	,062
6	1	-,078*	,010	<,001	-,112	-,044
	2	-,056*	,012	,002	-,096	-,017
	3	-,032	,012	,258	-,072	,009
	4	-,026	,009	,128	-,056	,004
	5	-,019	,013	1,000	-,062	,024

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6
c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,246	23,950	14	,048	,684	,852	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,053	5	,011	3,694	,004	,163
	Greenhouse-Geisser	,053	3,420	,015	3,694	,013	,163
Error(Visits)	Sphericity Assumed	,270	95	,003			
	Greenhouse-Geisser	,270	64,982	,004			

a. Parameter = R6

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,011	,013	1,000	-,032	,054
	3	,037	,016	,443	-,016	,089
	4	,029	,011	,248	-,008	,065
	5	,053*	,015	,039	,002	,104
	6	,058	,021	,189	-,013	,130
2	1	-,011	,013	1,000	-,054	,032
	3	,026	,015	1,000	-,025	,076
	4	,018	,014	1,000	-,028	,064
	5	,042*	,012	,041	,001	,083
	6	,048	,022	,716	-,028	,123
3	1	-,037	,016	,443	-,089	,016
	2	-,026	,015	1,000	-,076	,025
	4	-,008	,014	1,000	-,054	,038
	5	,016	,017	1,000	-,040	,073
	6	,022	,021	1,000	-,049	,092
4	1	-,029	,011	,248	-,065	,008
	2	-,018	,014	1,000	-,064	,028

	3	,008	,014	1,000	-,038	,054
	5	,024	,018	1,000	-,036	,084
	6	,030	,019	1,000	-,034	,093
5	1	-,053*	,015	,039	-,104	-,002
	2	-,042*	,012	,041	-,083	-,001
	3	-,016	,017	1,000	-,073	,040
	4	-,024	,018	1,000	-,084	,036
	6	,006	,020	1,000	-,062	,073
6	1	-,058	,021	,189	-,130	,013
	2	-,048	,022	,716	-,123	,028
	3	-,022	,021	1,000	-,092	,049
	4	-,030	,019	1,000	-,093	,034
	5	-,006	,020	1,000	-,073	,062

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R6
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R6

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,395910	20	,0601505	,0134501
	ScreeningL	,373845	20	,0624624	,0139670
Pair 2	Week0R	,374560	20	,0677155	,0151416
	Week0L	,363000	20	,0595662	,0133194
Pair 3	Week12R	,349715	20	,0676902	,0151360
	Week12L	,337295	20	,0644998	,0144226
Pair 4	Week24R	,344165	20	,0423624	,0094725
	Week24L	,345165	20	,0558617	,0124911
Pair 5	Week36R	,337160	20	,0605257	,0135340
	Week36L	,321030	20	,0576301	,0128865
Pair 6	Week48R	,318150	20	,0466589	,0104332
	Week48L	,315450	20	,0758368	,0169576

a. Parameter = R6

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	,0220650	,0490862	,0109760	-,0009080	,0450380	2,010	19	,029	,059
Pair 2	Week0R - Week0L	,0115600	,0590276	,0131990	-,0160658	,0391858	,876	19	,196	,392
Pair 3	Week12R - Week12L	,0124200	,0688341	,0153918	-,0197954	,0446354	,807	19	,215	,430
Pair 4	Week24R - Week24L	-,0010000	,0503905	,0112677	-,0245835	,0225835	-,089	19	,465	,930
Pair 5	Week36R - Week36L	,0161300	,0786017	,0175759	-,0206567	,0529167	,918	19	,185	,370
Pair 6	Week48R - Week48L	,0027000	,0832334	,0186116	-,0362544	,0416544	,145	19	,443	,886

a. Parameter = R6

Ratio of elastic recovery to total extension (R7, Ur/Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk		
	Statistic	df	Sig.
ScreeningR	,987	20	,990

ScreeningL	,967	20	,700
Week0R	,851	20	,006
Week0L	,953	20	,412
Week12R	,963	20	,603
Week12L	,953	20	,413
Week24R	,947	20	,319
Week24L	,990	20	,998
Week36R	,951	20	,379
Week36L	,971	20	,769
Week48R	,967	20	,683
Week48L	,966	20	,663

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,631	7,868	14	,897	,854	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,530	10,843	14	,702	,827	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,038	5	,008	4,508	<,001	,192
	Greenhouse-Geisser	,038	4,269	,009	4,508	,002	,192
Error(Visit)	Sphericity Assumed	,160	95	,002			
	Greenhouse-Geisser	,160	81,118	,002			
Side	Sphericity Assumed	,003	1	,003	1,314	,266	,065
	Greenhouse-Geisser	,003	1,000	,003	1,314	,266	,065
Error(Side)	Sphericity Assumed	,038	19	,002			
	Greenhouse-Geisser	,038	19,000	,002			
Visit * Side	Sphericity Assumed	,006	5	,001	,993	,427	,050
	Greenhouse-Geisser	,006	4,135	,002	,993	,418	,050
Error(Visit*Side)	Sphericity Assumed	,121	95	,001			
	Greenhouse-Geisser	,121	78,560	,002			

a. Parameter = R7

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,457	13,380	14	,501	,756	,968	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,021	5	,004	2,603	,030	,120
	Greenhouse-Geisser	,021	3,781	,005	2,603	,046	,120
Error(Visits)	Sphericity Assumed	,150	95	,002			
	Greenhouse-Geisser	,150	71,845	,002			

a. Parameter = R7

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,000	,016	1,000	-,053	,052
	3	,015	,011	1,000	-,022	,052

	4	,021	,010	,770	-,013	,054
	5	,034	,013	,262	-,010	,078
	6	,028	,010	,199	-,006	,063
2	1	,000	,016	1,000	-,052	,053
	3	,015	,015	1,000	-,035	,066
	4	,021	,014	1,000	-,026	,068
	5	,035	,012	,150	-,006	,075
	6	,029	,015	,984	-,021	,078
3	1	-,015	,011	1,000	-,052	,022
	2	-,015	,015	1,000	-,066	,035
	4	,006	,012	1,000	-,033	,045
	5	,019	,013	1,000	-,023	,061
	6	,013	,011	1,000	-,022	,049
4	1	-,021	,010	,770	-,054	,013
	2	-,021	,014	1,000	-,068	,026
	3	-,006	,012	1,000	-,045	,033
	5	,013	,014	1,000	-,034	,061
	6	,008	,012	1,000	-,031	,046
5	1	-,034	,013	,262	-,078	,010
	2	-,035	,012	,150	-,075	,006
	3	-,019	,013	1,000	-,061	,023
	4	-,013	,014	1,000	-,061	,034
	6	-,006	,010	1,000	-,040	,029
6	1	-,028	,010	,199	-,063	,006
	2	-,029	,015	,984	-,078	,021
	3	-,013	,011	1,000	-,049	,022
	4	-,008	,012	1,000	-,046	,031
	5	,006	,010	1,000	-,029	,040

Based on estimated marginal means
a. Parameter = R7
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,683	6,523	14	,952	,881	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,024	5	,005	3,439	,007	,153
	Greenhouse-Geisser	,024	4,407	,005	3,439	,010	,153
Error(Visits)	Sphericity Assumed	,131	95	,001			
	Greenhouse-Geisser	,131	83,726	,002			

a. Parameter = R7

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec	
					Lower Bound	Upper Bound
1	2	,022	,010	,611	-,012	,056
	3	,028	,012	,492	-,013	,068
	4	,026	,013	,849	-,017	,070
	5	,028	,014	,807	-,018	,073
	6	,048*	,010	,002	,014	,083
2	1	-,022	,010	,611	-,056	,012
	3	,006	,011	1,000	-,030	,041
	4	,004	,013	1,000	-,038	,047

	5	,006	,012	1,000	-,034	,046
	6	,026	,010	,252	-,007	,059
3	1	-,028	,012	,492	-,068	,013
	2	-,006	,011	1,000	-,041	,030
	4	-,001	,013	1,000	-,044	,041
	5	9,500E-5	,012	1,000	-,041	,041
	6	,020	,012	1,000	-,020	,060
4	1	-,026	,013	,849	-,070	,017
	2	-,004	,013	1,000	-,047	,038
	3	,001	,013	1,000	-,041	,044
	5	,001	,013	1,000	-,042	,045
	6	,022	,010	,587	-,011	,055
5	1	-,028	,014	,807	-,073	,018
	2	-,006	,012	1,000	-,046	,034
	3	-9,500E-5	,012	1,000	-,041	,041
	4	-,001	,013	1,000	-,045	,042
	6	,020	,012	1,000	-,019	,059
6	1	-,048*	,010	,002	-,083	-,014
	2	-,026	,010	,252	-,059	,007
	3	-,020	,012	1,000	-,060	,020
	4	-,022	,010	,587	-,055	,011
	5	-,020	,012	1,000	-,059	,019

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R7
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R7

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,339510	20	,0663441	,0148350
	ScreeningL	,355135	20	,0723292	,0161733
Pair 2	Week0R	,339865	20	,0815970	,0182457
	Week0L	,333065	20	,0829865	,0185563
Pair 3	Week12R	,324425	20	,0636771	,0142386
	Week12L	,327325	20	,0870813	,0194720
Pair 4	Week24R	,318700	20	,0720490	,0161106
	Week24L	,328685	20	,0746288	,0166875
Pair 5	Week36R	,305345	20	,0728247	,0162841
	Week36L	,327230	20	,0940791	,0210367
Pair 6	Week48R	,311100	20	,0733900	,0164105
	Week48L	,306985	20	,0799405	,0178752

a. Parameter = R7

Paired Samples Testa

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,0156250	,0568363	,0127090	-,0422252	,0109752	-1,229	19	,117	,234
Pair 2	Week0R - Week0L	,0068000	,0490910	,0109771	-,0161753	,0297753	,619	19	,271	,543
Pair 3	Week12R - Week12L	-,0029000	,0507261	,0113427	-,0266405	,0208405	-,256	19	,400	,801
Pair 4	Week24R - Week24L	-,0099850	,0454188	,0101560	-,0312416	,0112716	-,983	19	,169	,338
Pair 5	Week36R - Week36L	-,0218850	,0679787	,0152005	-,0537000	,0099300	-1,440	19	,083	,166
Pair 6	Week48R - Week48L	,0041150	,0429187	,0095969	-,0159716	,0242016	,429	19	,336	,673

a. Parameter = R7

Skin recovery (R8, Ua)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,980	20	,933
ScreeningL	,949	20	,358
Week0R	,882	20	,019
Week0L	,977	20	,893
Week12R	,893	20	,030
Week12L	,878	20	,016
Week24R	,914	20	,075
Week24L	,969	20	,729
Week36R	,975	20	,859
Week36L	,951	20	,390
Week48R	,967	20	,693
Week48L	,994	20	1,000

General linear model 2 mm RL

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,382	16,438	14	,292	,740	,941	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,360	17,492	14	,235	,771	,992	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,047	5	,009	7,128	<,001	,273
	Greenhouse-Geisser	,047	3,699	,013	7,128	<,001	,273
Error(Visit)	Sphericity Assumed	,125	95	,001			
	Greenhouse-Geisser	,125	70,279	,002			
Side	Sphericity Assumed	,018	1	,018	8,980	,007	,321
	Greenhouse-Geisser	,018	1,000	,018	8,980	,007	,321
Error(Side)	Sphericity Assumed	,038	19	,002			
	Greenhouse-Geisser	,038	19,000	,002			
Visit * Side	Sphericity Assumed	,009	5	,002	1,670	,149	,081
	Greenhouse-Geisser	,009	3,854	,002	1,670	,168	,081
Error(Visit*Side)	Sphericity Assumed	,102	95	,001			
	Greenhouse-Geisser	,102	73,234	,001			

a. Parameter = R8

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,544	10,399	14	,736	,813	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,030	5	,006	6,435	<,001	,253
	Greenhouse-Geisser	,030	4,063	,007	6,435	<,001	,253

Error(Visits)	Sphericity Assumed	,089	95	,001			
	Greenhouse-Geisser	,089	77,201	,001			

a. Parameter = R8

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	-,008	,011	1,000	-,046	,030
	3	-,029*	,008	,030	-,056	-,002
	4	-,034*	,008	,004	-,060	-,008
	5	-,028	,010	,219	-,062	,007
	6	-,047*	,009	<,001	-,078	-,016
2	1	,008	,011	1,000	-,030	,046
	3	-,021	,010	,672	-,054	,012
	4	-,026	,010	,307	-,061	,009
	5	-,020	,010	,921	-,053	,014
	6	-,039	,012	,064	-,080	,001
3	1	,029*	,008	,030	,002	,056
	2	,021	,010	,672	-,012	,054
	4	-,005	,008	1,000	-,032	,022
	5	,001	,009	1,000	-,029	,031
	6	-,018	,011	1,000	-,054	,017
4	1	,034*	,008	,004	,008	,060
	2	,026	,010	,307	-,009	,061
	3	,005	,008	1,000	-,022	,032
	5	,006	,009	1,000	-,025	,038
	6	-,013	,010	1,000	-,045	,019
5	1	,028	,010	,219	-,007	,062
	2	,020	,010	,921	-,014	,053
	3	-,001	,009	1,000	-,031	,029
	4	-,006	,009	1,000	-,038	,025
	6	-,019	,008	,499	-,048	,009
6	1	,047*	,009	<,001	,016	,078
	2	,039	,012	,064	-,001	,080
	3	,018	,011	1,000	-,017	,054
	4	,013	,010	1,000	-,019	,045
	5	,019	,008	,499	-,009	,048

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

a. Parameter = R8

c. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,314	19,812	14	,140	,695	,869	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,026	5	,005	3,546	,006	,157
	Greenhouse-Geisser	,026	3,474	,007	3,546	,015	,157
Error(Visits)	Sphericity Assumed	,138	95	,001			
	Greenhouse-Geisser	,138	66,006	,002			

a. Parameter = R8

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.c	95% Confidence Interval for Differencec Lower Bound	Upper Bound
1	2	,017	,009	1,000	-,013	,046
	3	-,014	,011	1,000	-,052	,024
	4	-,015	,007	,970	-,040	,010
	5	-,030	,012	,340	-,071	,011
	6	-,016	,014	1,000	-,061	,029
2	1	-,017	,009	1,000	-,046	,013
	3	-,031	,011	,143	-,066	,005
	4	-,031*	,008	,013	-,058	-,005
	5	-,047*	,012	,020	-,089	-,005
	6	-,033	,014	,400	-,079	,013
3	1	,014	,011	1,000	-,024	,052
	2	,031	,011	,143	-,005	,066
	4	-,001	,011	1,000	-,039	,037
	5	-,016	,010	1,000	-,049	,016
	6	-,002	,014	1,000	-,051	,046
4	1	,015	,007	,970	-,010	,040
	2	,031*	,008	,013	,005	,058
	3	,001	,011	1,000	-,037	,039
	5	-,015	,012	1,000	-,056	,025
	6	-,002	,015	1,000	-,051	,048
5	1	,030	,012	,340	-,011	,071
	2	,047*	,012	,020	,005	,089
	3	,016	,010	1,000	-,016	,049
	4	,015	,012	1,000	-,025	,056
	6	,014	,017	1,000	-,041	,069
6	1	,016	,014	1,000	-,029	,061
	2	,033	,014	,400	-,013	,079
	3	,002	,014	1,000	-,046	,051
	4	,002	,015	1,000	-,048	,051
	5	-,014	,017	1,000	-,069	,041

Based on estimated marginal means
*. The mean difference is significant at the ,05 level.
a. Parameter = R8
c. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R8

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,290850	20	,0482027	,0107785
	ScreeningL	,322750	20	,0560234	,0125272
Pair 2	Week0R	,298800	20	,0550489	,0123093
	Week0L	,306050	20	,0568706	,0127166
Pair 3	Week12R	,319750	20	,0507583	,0113499
	Week12L	,336700	20	,0737350	,0164877
Pair 4	Week24R	,324850	20	,0445861	,0099698
	Week24L	,337350	20	,0519162	,0116088
Pair 5	Week36R	,318500	20	,0576856	,0128989
	Week36L	,352850	20	,0639846	,0143074
Pair 6	Week48R	,337950	20	,0642655	,0143702
	Week48L	,338850	20	,0651090	,0145588

a. Parameter = R8

Paired Samples Testa

Paired Differences						Significance	
Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	t	df	One-Sided p	Two-Sided p

					Lower	Upper				
Pair 1	ScreeningR - ScreeningL	-,0319000	,0493952	,0110451	-,0550177	-,0087823	-2,888	19	,005	,009
Pair 2	Week0R - Week0L	-,0072500	,0380123	,0084998	-,0250403	,0105403	-,853	19	,202	,404
Pair 3	Week12R - Week12L	-,0169500	,0540229	,0120799	-,0422335	,0083335	-1,403	19	,088	,177
Pair 4	Week24R - Week24L	-,0125000	,0401582	,0089797	-,0312946	,0062946	-1,392	19	,090	,180
Pair 5	Week36R - Week36L	-,0343500	,0618311	,0138258	-,0632878	-,0054122	-2,484	19	,011	,022
Pair 6	Week48R - Week48L	-,0009000	,0500441	,0111902	-,0243214	,0225214	-,080	19	,468	,937

a. Parameter = R8

Skin tiring (R9, Uf5 – Uf)

Test of normality – Shapiro-Wilk

	Shapiro-Wilk Statistic	df	Sig.
ScreeningR	,978	20	,909
ScreeningL	,965	20	,644
Week0R	,945	20	,295
Week0L	,963	20	,605
Week12R	,945	20	,303
Week12L	,941	20	,247
Week24R	,926	20	,130
Week24L	,961	20	,566
Week36R	,973	20	,826
Week36L	,933	20	,177
Week48R	,913	20	,073
Week48L	,892	20	,029

General linear model 2 mm RL

Mauchly's Test of Sphericity

					Epsilonc		
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visit	,422	14,733	14	,401	,780	1,000	,200
Side	1,000	,000	0	.	1,000	1,000	1,000
Visit * Side	,453	13,555	14	,488	,781	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visit	Sphericity Assumed	,000	5	3,562E-5	,595	,704	,030
	Greenhouse-Geisser	,000	3,902	4,564E-5	,595	,663	,030
Error(Visit)	Sphericity Assumed	,006	95	5,983E-5			
	Greenhouse-Geisser	,006	74,138	7,667E-5			
Side	Sphericity Assumed	,001	1	,001	9,275	,007	,328
	Greenhouse-Geisser	,001	1,000	,001	9,275	,007	,328
Error(Side)	Sphericity Assumed	,003	19	,000			
	Greenhouse-Geisser	,003	19,000	,000			
Visit * Side	Sphericity Assumed	,000	5	2,196E-5	,444	,816	,023
	Greenhouse-Geisser	,000	3,904	2,812E-5	,444	,772	,023
Error(Visit*Side)	Sphericity Assumed	,005	95	4,942E-5			
	Greenhouse-Geisser	,005	74,176	6,329E-5			

a. Parameter = R9

General linear model 2 mm R

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,461	13,260	14	,511	,774	,997	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	5	2,512E-5	,436	,822	,022
	Greenhouse-Geisser	,000	3,870	3,246E-5	,436	,776	,022
Error(Visits)	Sphericity Assumed	,005	95	5,761E-5			
	Greenhouse-Geisser	,005	73,527	7,444E-5			

a. Parameter = R9

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb	
					Lower Bound	Upper Bound
1	2	-,002	,003	1,000	-,012	,008
	3	-,003	,002	1,000	-,010	,005
	4	-,003	,002	1,000	-,010	,004
	5	-,003	,003	1,000	-,012	,006
	6	-,002	,002	1,000	-,009	,006
2	1	,002	,003	1,000	-,008	,012
	3	-,001	,002	1,000	-,009	,007
	4	-,001	,003	1,000	-,011	,009
	5	-,001	,002	1,000	-,010	,007
	6	,000	,002	1,000	-,007	,007
3	1	,003	,002	1,000	-,005	,010
	2	,001	,002	1,000	-,007	,009
	4	5,000E-5	,002	1,000	-,008	,008
	5	,000	,002	1,000	-,008	,008
	6	,001	,002	1,000	-,005	,007
4	1	,003	,002	1,000	-,004	,010
	2	,001	,003	1,000	-,009	,011
	3	-5,000E-5	,002	1,000	-,008	,008
	5	,000	,002	1,000	-,008	,008
	6	,001	,003	1,000	-,008	,010
5	1	,003	,003	1,000	-,006	,012
	2	,001	,002	1,000	-,007	,010
	3	,000	,002	1,000	-,008	,008
	4	,000	,002	1,000	-,008	,008
	6	,001	,002	1,000	-,007	,010
6	1	,002	,002	1,000	-,006	,009
	2	,000	,002	1,000	-,007	,007
	3	-,001	,002	1,000	-,007	,005
	4	-,001	,003	1,000	-,010	,008
	5	-,001	,002	1,000	-,010	,007

Based on estimated marginal means
a. Parameter = R9
b. Adjustment for multiple comparisons: Bonferroni.

General linear model 2 mm L

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilonc		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Visits	,492	12,140	14	,599	,796	1,000	,200

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Visits	Sphericity Assumed	,000	5	3,245E-5	,628	,678	,032
	Greenhouse-Geisser	,000	3,980	4,077E-5	,628	,643	,032
Error(Visits)	Sphericity Assumed	,005	95	5,164E-5			
	Greenhouse-Geisser	,005	75,625	6,487E-5			

a. Parameter = R9

Pairwise Comparisons

(I) Visits	(J) Visits	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Differenceb Lower Bound	Upper Bound
1	2	,002	,003	1,000	-,007	,011
	3	-5,000E-5	,002	1,000	-,005	,005
	4	-,002	,002	1,000	-,009	,006
	5	,000	,002	1,000	-,007	,008
	6	,002	,002	1,000	-,005	,008
2	1	-,002	,003	1,000	-,011	,007
	3	-,002	,003	1,000	-,011	,008
	4	-,003	,003	1,000	-,012	,005
	5	-,001	,002	1,000	-,010	,007
	6	,000	,002	1,000	-,008	,008
3	1	5,000E-5	,002	1,000	-,005	,005
	2	,002	,003	1,000	-,008	,011
	4	-,002	,002	1,000	-,010	,007
	5	,000	,002	1,000	-,007	,008
	6	,002	,002	1,000	-,006	,009
4	1	,002	,002	1,000	-,006	,009
	2	,003	,003	1,000	-,005	,012
	3	,002	,002	1,000	-,007	,010
	5	,002	,002	1,000	-,006	,010
	6	,003	,002	1,000	-,003	,010
5	1	,000	,002	1,000	-,008	,007
	2	,001	,002	1,000	-,007	,010
	3	,000	,002	1,000	-,008	,007
	4	-,002	,002	1,000	-,010	,006
	6	,002	,002	1,000	-,004	,008
6	1	-,002	,002	1,000	-,008	,005
	2	,000	,002	1,000	-,008	,008
	3	-,002	,002	1,000	-,009	,006
	4	-,003	,002	1,000	-,010	,003
	5	-,002	,002	1,000	-,008	,004

Based on estimated marginal means
a. Parameter = R9
b. Adjustment for multiple comparisons: Bonferroni.

T-Test 2 mm R9

Paired Samples Statisticsa

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ScreeningR	,048300	20	,0092001	,0020572
	ScreeningL	,055200	20	,0069555	,0015553
Pair 2	Week0R	,050050	20	,0111046	,0024831
	Week0L	,053500	20	,0080426	,0017984
Pair 3	Week12R	,051050	20	,0088584	,0019808
	Week12L	,055250	20	,0091241	,0020402
Pair 4	Week24R	,051000	20	,0091364	,0020430
	Week24L	,056750	20	,0096074	,0021483
Pair 5	Week36R	,051350	20	,0095602	,0021377
	Week36L	,054900	20	,0090780	,0020299
Pair 6	Week48R	,050050	20	,0098434	,0022010

Week48L	,053300	20	,0089507	,0020014

a. Parameter = R9

Paired Samples Testa

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	ScreeningR - ScreeningL	-,0069000	,0089319	,0019972	-,0110803	-,0027197	-3,455	19	,001	,003
Pair 2	Week0R - Week0L	-,0034500	,0108117	,0024176	-,0085100	,0016100	-1,427	19	,085	,170
Pair 3	Week12R - Week12L	-,0042000	,0133559	,0029865	-,0104507	,0020507	-1,406	19	,088	,176
Pair 4	Week24R - Week24L	-,0057500	,0103562	,0023157	-,0105968	-,0009032	-2,483	19	,011	,023
Pair 5	Week36R - Week36L	-,0035500	,0117718	,0026323	-,0090594	,0019594	-1,349	19	,097	,193
Pair 6	Week48R - Week48L	-,0032500	,0117154	,0026196	-,0087330	,0022330	-1,241	19	,115	,230

a. Parameter = R9

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Finally, I hope I can pass the passion and determination that are necessary for the realization and completion of such a project to my daughter, Paula.

Thank you.

Declaration on Oath

"I hereby declare upon oath that I have written the present dissertation independently and have not used further resources and aids than those stated in this dissertation.

I, the undersigned, declare that the submitted written bound copy of the dissertation is identical to the submitted electronic version.

I hereby declare that the dissertation has never been submitted in the present form or similar to any other university or board of examiners."

Date: 26.03.2023 Signature: D. Hertz-Kleptow

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