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PROMOTING OF FIBROBLAST CELLS ATTACHMENT ON BARRIER BONE MEMBRANES USING ULTRAVIOLET LIGHT AND NON-THERMAL PLASMA

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Table of Contents

1. INTRODUCTION 4

2. AIM OF THE STUDY 10

3. MATERIALS AND METHODS 11

3.1. Bone Membranes and Sample Preparation 11

3.2. Cell Culture 13

3.3. UV Light and NTP Devices 14

3.4. Reference Material 15

3.5. Experimental Groups 15

3.6. Cells Attachment 16

3.7. Statistical Analysis 17

4. RESULTS 18

4.1. Cell Attachment 18

5. DISCUSSION 23

6. CONCLUSION 27

7. SUMMARY 28

8. ZUSAMMENFASSUNG 29

9. LIST OF ABBREVIATION 30

10. REFERENCES 31

11. ACKNOWLEDGE 36

12. CURRICULUM VITAE 37

13. EIDESSTATTLICHE VERSICHERUNG 38

List of Tables

table 1: distribution of the groups 15
table 2:mean ± standard deviation for numbers of cells attached to the surfaces. three areas were counted for each sample. highly significantly (t-test, p<0.001) increased numbers compared to the untreated one for each type of the membranes were in **bold and red** 22

List of Figures

figure 1: bone membrane act as a bariere. (clinical case; mkg department, uke - hamburg) 5
figure 2: ptfe membrane (opentex, purgo, dental biologics solution, germany). 11
figure 3: naturally cross linked collagen (boneprotect® guide, dentegris gmbh, germnay). 12
figure 4: collagen membrane (mucomatrix®, dentegris gmbh, germany). 12
figure 5: biopsy punch 6mm and 4 mm ø 12
figure 6: hemocytometer (hecht assistant, sondheim vor der rhon, germany). 13
figure 7: ultraviolet light device (therabeam® superosseo, ushio, tokyo, japan). 14
figure 8: non-thermal plasma device (diener electronic gmbh, ebhausen, germany). 14
figure 9: fluorescence microscopy 16
figure 10: cell attachment and morphology on different membranes. (a) non-treated,(b) uv-treated, (c) ntpo-treated. 20
figure 11: bar chart showing average values for numbers of cell attachment. for each sample, three areas were counted. average and standard deviation were then calculated. 21

1. INTRODUCTION

Bone is a living hard tissue, undergoing constant remodeling and self-renewal comprising the capacity to repair itself in response to injury throughout adult life. However, there are conditions in oral and maxillofacial surgery in which bone reformation is impaired and in which bone reformation is required in a huge amount above the normal potential for self-healing [1,2]. Additionally, systemic or periodontal diseases, tumors, posttraumatic or surgical defects, inflammation or congenital abnormalities can cause a lack of jawbone volume. Insufficient bone can negatively affect the patient's oral functions such as chewing, speaking as well as esthetic appearance. Bone is considered the second most transplanted tissue containing more than 2.2 million grafts carried out every year. Therefore, many techniques have been introduced in dentistry to repair and regenerate bone tissue such as bone block grafts, ridge splits, and guided bone regeneration (GBR) [3–8].

The guided bone regeneration concept is based on principles of guided tissue regeneration (GTR) which was first introduced by Nyman et al. by mid-1980s [9]. This method is based on the principle that specific cells participate in the regeneration of specific tissues. Guided bone regeneration is one of the most widely used methods for restoring local bone mass deficiencies in dentistry [10]. Guide bone regeneration can either be applied by using membranes alone or in combination with bone grafts (autologous, xenogeneic, allogeneic, or alloplastic) [1]. Moreover, rapid soft tissue sealing on a defect area may assist in reducing bacterial colonization and lower the risk of inflamation [11]. Accordingly, human gingival fibroblasts (HGF), which are concidered the most abundant cells in gingival tissue, need to attach to the GBR

membrane in order to form a cellular layer that cover the bone barrier surface and make it less obtainable for bacterial growth [11,12].

The ability of GBR membranes to provide space for an uneventful bone formation is challenging for the success of membranes [9]. Therefore, the main principle of GBR is to act as a barrier membrane for space maintenance over a defect area to enhance the growth of the osteogenic cells, prevent ingrowth of non-osseous cells and stimulate blood clot formation (Figure 1) [13–16].

Additionally, GBR membranes are divided into resorbable and non-resorbable membranes regarding their biological behaviour. Both types have been extensively investigated and used in dental clinics. Still, many efforts are made to develop the ideal GBR membrane. However, the membranes which are utilized for GBR have to fulfil several beneficial properties including biocompatibility, cell occlusion, space maintaining ability, membrane stability, tissue integration and clinical manageability [13,17,18].

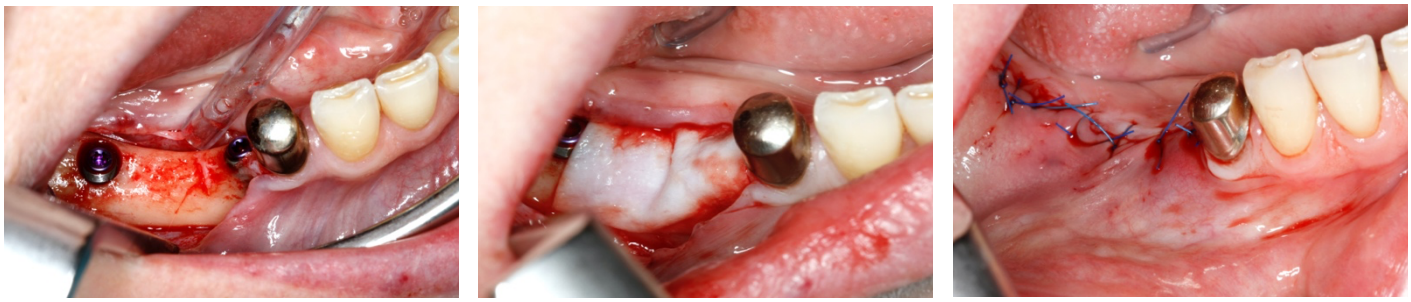


Figure 1: Membrane acting as a barrier. (clinical case; MKG department, UKE - Hamburg)

Furthermore, non-resorbable membranes such as polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (e-PTFE), and titanium meshes are supposed to remain on the defect site, covered by soft tissue for a while, until the bone regeneration process is completed [19,20]. In 1984, PTFE membranes were first introduced into dentistry. It proved to be biocompatible and it preserves solidity during the bone formation process [21–23]. Korzinsks et al. studied the biocompatibility and macrophage response on PTFE membranes and found that PTFE may optimally support bone tissue healing [24]. According to the structure, PTFE can be divided into expanded polytetrafluoroethylene (e-PTFE) and high-density polytetrafluoroethylene (d-PTFE) [25].

Several studies demonstrated that e-PTFE is considered to be the most popular non-resorbable membrane in the dental field, due to its satisfying clinical outcome [26,27]. The e-PTFE membranes prevent fibroblasts and connective tissue from invading into the area allowing the osteogenic cells to reform new bone [25]. However, e-PTFE membranes may cause soft tissue dehiscence, which leads to premature exposure of the membrane, compromise of bone regeneration, and progression of infection [28,29].

Hence the associated complications of the e-PTFE membranes, d-PTFE membranes were introduced in the 1990s to reduce the risk of bacterial infection correlated to e-PTFE membranes [25,30]. These membranes do not require soft-tissue closure due to their small pore sizes and high density. Therefore, they prevent the travel of bacteria at the same time permitting oxygen diffusion [31]. However, a second surgical interfere is required to remove non-resorbable membranes after bone regeneration process is complete. A second surgery consumes more time and energy, it can also increase the patient's psychological and financial burden [32].

In the early 1990s the utilize of biodegradable membranes were developed to avoid some of the non-resorbable membranes complications. Previous researches reported that resorbable membranes undergo a physiological process and degrade after a certain period of time varied from 2 weeks up to 24 months, thus reducing patient discomfort and decreasing clinical complications compared to non-resorbable membranes [32–34]. However, the main challenge of bioresorbable membranes is the unpredictable resorption time, which can negatively affect bone formation [35].

Resorbable membranes can be manufactured from various types of materials (natural or synthetic) including collagen, pericardium, acellular dermal matrix, and platelet-rich fibrin [36]. Although collagen membranes degradation rate can be higher than the healing process, they have several benefits such as one-step surgical procedure, which increases patient comfort and the risk of newly regenerated tissues, enhances tissue integration with less chance of membrane exposure. The natural membranes made of collagen have low mechanical strength, possess a rapid degradation rate [37]. Therefore, to prevent the premature degradation of collagen membranes, various methods have been introduced to improve their durability by cross-linking the existing collagen fibers and creating resorbable cross-linked collagen membranes [38,39].

Several approaches have been developed throughout the years to improve bone membrane properties. Dogan et al. [40] reported that using low-level laser therapy (LLLT) in combination with GBR membranes can increase their effectiveness. Moreover, Angele et al. and Nimni et al. [41,42] had successfully improved the mechanical properties of collagen membranes and reduced the biodegradation rate by applying various chemical agents.

Ultraviolet (UV) light is defined as a portion of the electromagnetic spectrum between x-rays and visible light. The UV spectrum is divided into Vacuum UV (40-190 nm), Far UV (190-220 nm), UVC (220-290 nm) UVB (290-320 nm), UVA (320-400 nm). The sun is a primary natural source of UV radiation. Artificial sources include curing lamps, tanning booths, germicidal lamps, black lights, halogen lights, mercury vapour lamps, high-intensity discharge lamps, fluorescent and incandescent sources, and some types of lasers [43].

UV light has been frequently utilized as a tool in calculus detection in periodontal disease management, disinfection of root canal walls, and modification of implant surfaces to improve their clinical outcomes [43]. In addition, UV light can indirectly affect osteoblast functions by activation of GBR membranes. A study by Hong et al. [44] demonstrated that the application of ultraviolet (UV) on collagen membrane surface increased its resistance to degradation and improved the level of new bone formation. Acevedo et al. [45] observed that UV irradiation has a promising influence on biodegradable membranes in terms of biocompatibility and osteoconductive properties.

Plasma is considered the fourth state of matter, in addition to liquid, gas, and solid. In 1897, Sir William Crookes discovered plasma and described it as radiant matter. The name plasma was introduced by Irving Langmuir in 1929 [46]. Plasmas are classified into thermal and non-thermal plasma (NTP). Thermal plasmas have been used in industry for surface treatment. Whereas, NTP, that is generated at low pressure, has been newly developed and used in medical, dental, and biological fields [47,48]. The latter technique has gained much attention during the last decade in dentistry. It has been utilized in the decontamination of tooth cavities before restoration, drilling, in root canal treatment and in the modification of surface

characteristics of biomaterials. Tanaka et al. [49] reported that non-thermal plasma induced physiological outcomes in cells and tissues. Chengzan et al. [50] used non-thermal Aragon-oxygen plasma (NTAOP) to promote the activity of osteogenic proliferation and differentiation. Several studies have investigated UV light and NTP and demonstrated that both methods are able to enhance physicochemical properties of biomaterial surface by increasing the wettability, cell adhesion, and proliferation [51,52].

2. AIM OF THE STUDY

This experiment was carried out to evaluate the effectiveness of UV irradiation and NTP functionalization on interactions between GBR membranes (PTFE, naturally cross-linked collagen and collagen tissue matrix derived of animal dermis) and soft tissue cells (murine fibroblast cell line L929 and Human gingival fibroblast HGF), regarding cell attachment.

We hypothesized that activating the GBR membranes with UV light or NTP oxygen could enhance the attachment of HGF and L929 cells more than non-activating ones.

3. MATERIALS AND METHODS

3.1. Bone Membranes and Sample Preparation

Three different membranes were used in the current study:

- Non-Resorbable polytetrafluoroethylene (PTFE) membrane (OpenTex, Purgo, Dental Biologics Solution, Germany; Figure 2).
- Collagen membrane which is a collagen tissue matrix derived of animal dermis (MucoMatrix®, Dentegris GmbH, Germany; Figure 4). It is a 3-dimensional stable matrix consisting of collagen and elastin.
- Naturally cross-linked collagen membrane (BoneProtect® Guide, Dentegris GmbH, Germnay; Figure 3).

Each membrane was cut using biopsy punches in 4 mm and 6 mm diameter (Figure 5), to fit in 96- and 24-well plat (SARSTEDT AG & Co. KG, Germany), respectively.

Figure 2: PTFE membrane (OpenTex, Purgo, Dental Biologics Solution, Germany).

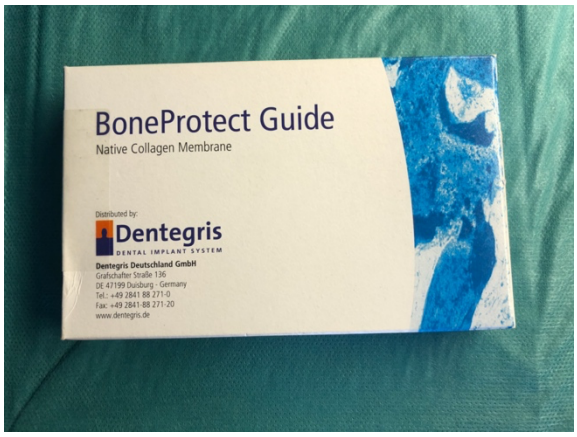


Figure 3: Naturally cross linked collagen (BoneProtect® Guide, Dentegris GmbH, Germnay).

Figure 4: Collagen membrane (MucoMatrix®, Dentegris GmbH, Germany).

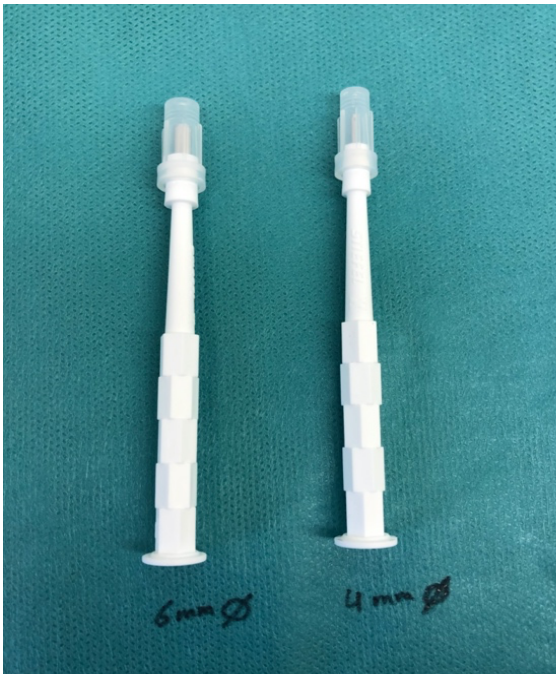


Figure 5: Biopsy punch 6mm and 4 mm Ø

3.2. Cell Culture

For all experiments, murine fibroblast (L929) (C57BL/6, Sigma–Aldrich, Munich, Germany) cells and primary human gingival fibroblast cells (HGF) were used. HGF cells were cultured from gingival tissue that was collected from a healthy patient after taking the informed consent, who underwent gingivectomy for esthetic reasons. L929 cells were cultured in minima essential medium (MEM, Gibco, Invitrogen, Paisley, UK), while HGF in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, Paisley, UK), both mediums were supplemented with 10% fetal bovine serum (FBS Gibco, Invitrogen, Paisley, UK) and 1 % penicillin/streptomycin (P/S, GibcoTM, InvitrogenTM, Paisley, UK). Cells were incubated in a humified atmosphere of 95% air and 5% CO_2 at 37 °C. The cell culture mediums were changed every 2-3 days and at 80% confluency, cells were detached using 0.05 % trypsin-EDTA (GibcoTM, InvitrogenTM, Paisley, UK) and counted using a hemocytometer (Hecht Assistant, Sondheim vor der Rhon, Germany; Figure 6). L929 and HGF were seeded onto the treated or non-treated membranes at a density of 0.5×10^5 /cm^2 assessing cell attachment and morphology, and 1×10^5 /cm^2 assessing viability and cytotoxicity.

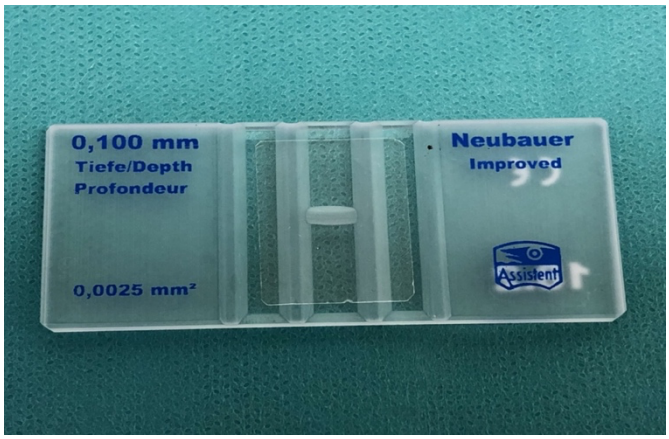


Figure 6: Hemocytometer (Hecht Assistant, Sondheim vor der Rhon, Germany).

3.3. UV Light and NTP Devices

In this study, a UV light oven (Figure 7) was used. It generates UV irradiation as a mixture of spectra with an intensity of 0.15 mW/cm^2 (λ = 253.7 nm). The non-thermal plasma apparatus (Diener Electronic GmbH, Ebhausen, Germany) was utilized for membrane activation (Figure 8). The generator frequency is 100 kHz. The vacuum chamber is made of borosilicate glass. Several treatment cycles are possible; the treatment conditions that were used in this study were 24 W, system pressure 1 mbar, gas flow rate 1.25 sccm, and gas purity > 99.5 %. Moreover, NTP is produced with peaks at λ = 240 nm using oxygen plasma. All samples in the experimental groups were treated for 12 minutes.

Figure 7: Ultraviolet light device.

Figure 8: Non-thermal plasma device (Diener Electronic GmbH, Ebhausen, Germany).

3.4. Reference Material

In the current study RMA, a polyurethane film containing 0.1% zincdiethyldithiocarbamate (Hatano Research Institute, Food, and Drug Safety Center, Hadano, Kanagawa, Japan) was used as the toxic control group.

3.5. Experimental Groups

Each membrane was randomly divided into 3 groups (Table 1). For each material, the membrane allocated to the control group were non-treated (NT). The membranes allocated to the experimental groups were treated either with UV light for 12 minutes (UV group) or with non-thermal plasma-oxygen for 12 minutes (NTPO group).

Membrane	**PTFE**		**Natural Collagen**		**Naturally cross-linked Collagen**	
Cell type	**L929**	**HGF**	**L929**	**HGF**	**L929**	**HGF**
Groups	NT	NT	NT	NT	NT	NT
	UV	UV	UV	UV	UV	UV
	NTPO	NTPO	NTPO	NTPO	NTPO	NTPO

Table 1: Distribution of non-treated (NT), ultraviolet treated (UV) and non-thermal plasma (Oxygen) (NTPO) treated groups.

3.6. Cells Attachment

All samples were seeded in 0.05 x 10^6 cells density in 0,5 ml medium in each well of 24-well plate. The assay was performed after 24 hours of incubation under standard cell culture conditions. The number of the attached living cells to the membrane surface was evaluated using live-dead staining (LDS), 30 µl per ml medium propidium iodide (PI) stock solution (50 µg/ml in PBS) and 250 µl per ml medium fresh fluorescein diacetate (FDA) working solution (5 mg/ml FDA in acetone stock solution, 20 µg/mL FDA stock in PBS) were added to each well.

After a short of incubation for 3 minutes at room temperature, samples were rinsed with PBS. The cells were immediately observed under fluorescence microscopy (Eclipse E200, Nikon, Tokyo, Japan) (Figure 9), where live cells were visible as green coloration and dead cells were visible as red coloration.

The number of living cells attached on membranes was evaluated using image J software (release 1.5 h, U.S. National Institutes of Health, Bethesda, MD, USA). Three images were to measure cells' number in each group.

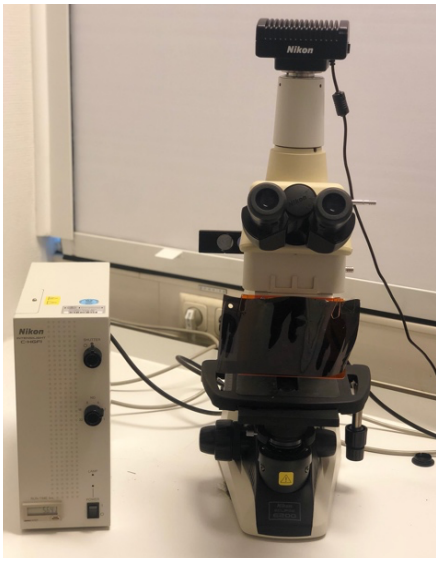


Figure 9: fluorescence microscopy

3.7. Statistical Analysis

Numerical data were explored for normality and variance homogeneity using Shapiro-Wilk and Leven's tests respectively. Data showed parametric distribution and homogeneity of variances across groups, so they were represented as mean and standard deviation (SD) values and were analyzed using t-test. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.0.3 for Windows [53].

4. RESULTS

4.1. Cell Attachment

Mean, standard deviation (SD) values and results of one-way ANOVA test for number of cell attachment are presented in table (2) and figure (11). A summary results of each group in (table 3).

A. PTFE membrane:

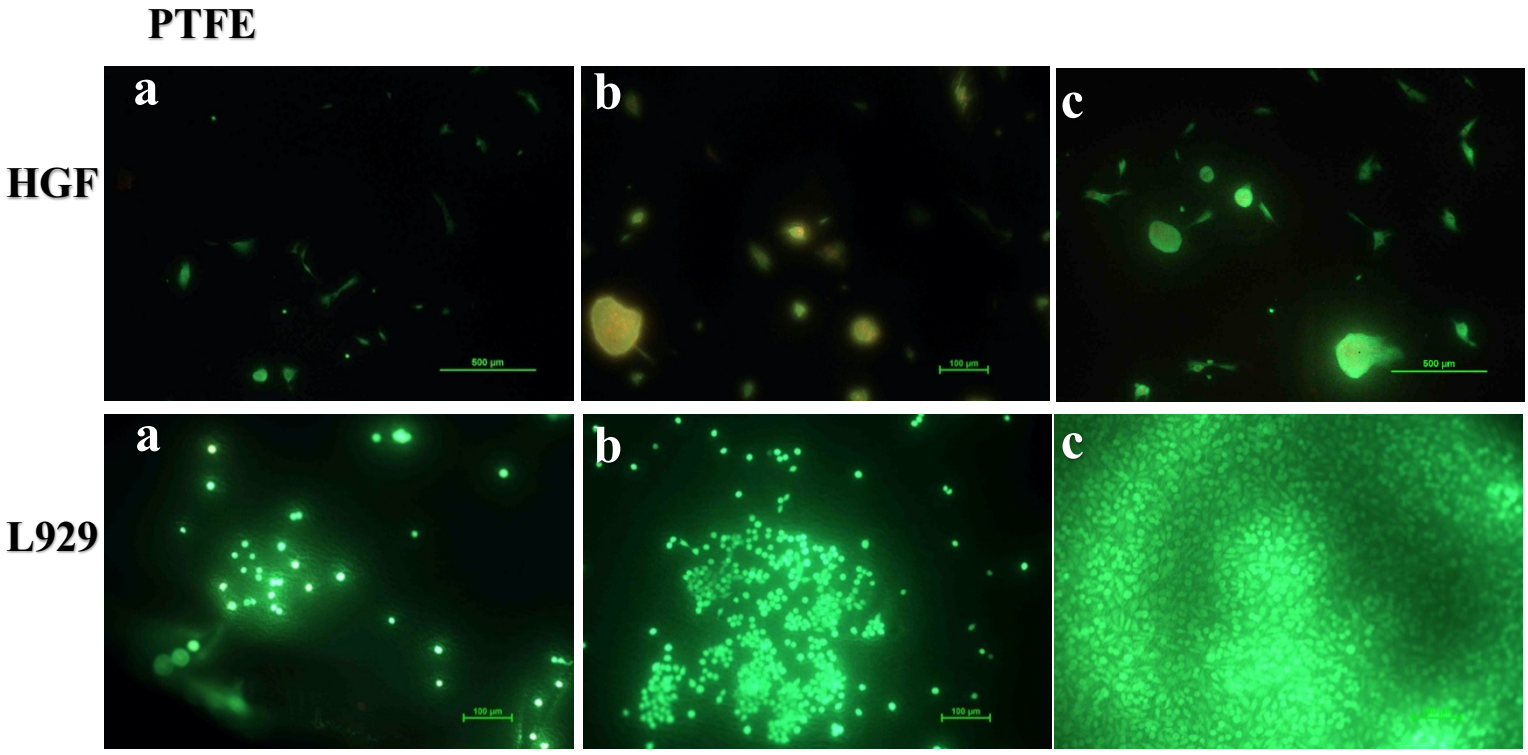
Generally, attachment of HGF and L929 cells on non-treated PTFE membrane was poor. Both UV light and NTPO treatments hardly increased the HGF cell attachment in comparison to non-treated one. On the other hand, L929 cell attachment was improved upon UV irradiation and dramatically increased by NTPO treatment (figure 10). However non-thermal plasma oxygen treated groups displayed the highest mean value in both cell groups followed by UV treated groups. Whereas non-treated samples displayed the lowest mean value. Pairwise comparisons showed that all groups were significantly different from each other ($p<0.001$).

B. Collagen membrane:

For both HGF and L929 cells, the effect of modified collagen membrane groups was statistically significant ($p<0.001$) compared to non-treated group. non-thermal plasma oxygen treated groups showed the largest mean value followed by UV treated, at the same time, non-treated samples demonstrated the lowest mean value.

C. Naturally cross-linked membrane:

Regarding HGF cells, the UV treated group showed the highest mean value followed by NTPO group while non-treated samples demonstrated the lowest mean value. Pairwise comparisons showed no significant difference between the groups ($p<0.001$). While for L929 cells, NTPO treated samples showed the highest mean value followed by UV treated and non-treated showed the lowest mean value. Pairwise comparisons showed that samples treated with different treatments were not significantly different from each other ($p<0.001$).



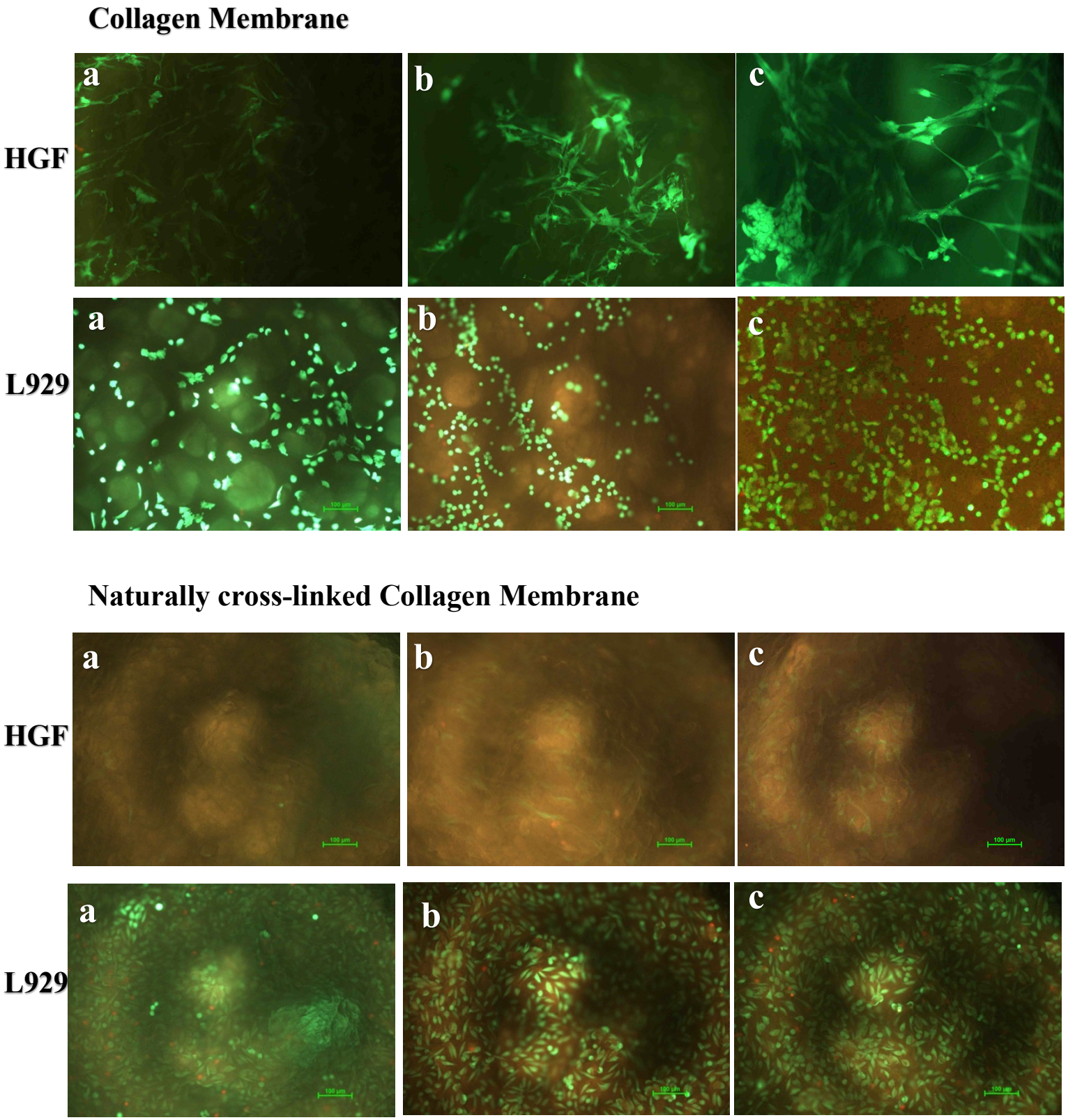


Figure 10: Cell attachment and morphology on different membranes. (a) non-treated,(b) UV-treated, (c) NTPO-treated.

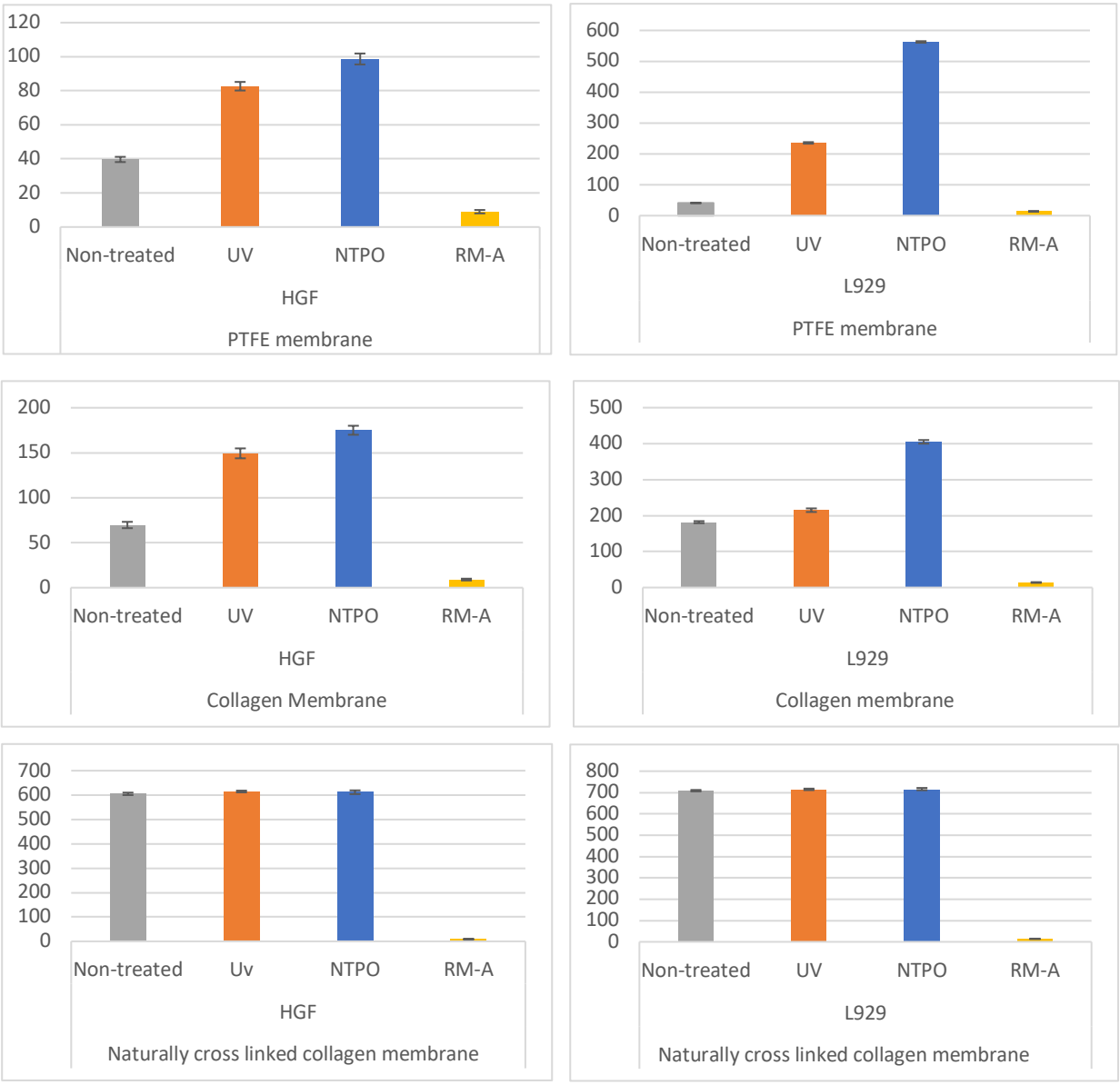


Figure 11: Bar chart showing average values for numbers of cell attachment. For each sample, three areas were counted. Average and standard deviation were then calculated. Please re-word this!

Membrane	Cell	Number of cell attachment (mean±SD)		
		Non-treated	UV	NTPO
PTFE membrane	HGF	40±2	**83±3**	**99±3**
	L929	41±1	**236±3**	**563±3**
Collagen membrane	HGF	70±4	**149±6**	**175±5**
	L929	182±3	**215±5**	**405±5**
Naturally cross-linked collagen membrane	HGF	605±5	615±4	612±8
	L929	709±3	715±4	716±5

Table 2: Mean ± standard deviation for numbers of cells attached to the surfaces. Three areas were counted for each sample. Highly significantly (T-test, P<0.001) increased numbers compared to the untreated one for each type of the membranes were in **bold and red**

Bone membrane	Cell	Cell attachment		
		Non-treated	UV	NTPO
PTFE membrane	HGF	Extremely poor	Slightly improved	Slightly improved
	L929	Poor	Drastically improved	Drastically improved
Collagen membrane	HGF	Poor	Significantly improved	Significantly improved
	L929	Good	Slightly improved	Significantly improved
Naturally cross-linked collagen membrane	HGF	Excellent	No more change	No more change
	L929	Excellent	No more change	No more change

Table 3: Summary of the results

5. DISCUSSION

GBR membranes are introduced for separating the growth of soft tissue that proliferates rapidly from bone tissue which grows slowly. They may also enhance clot stabilization and stimulate the secretion of growth factors and osteogenic cells [10,54]. However, most of the GBR membranes do not fulfil all the requirements for the ideal membrane for the GBR method [21], therefore, different methods for membrane modifications have been introduced to improve their outcomes. UV light and non-thermal plasma irradiation have been proven to boost the behaviour of soft tissue cells by increasing the hydrophilic ability of biomaterial surfaces [45,51].

Therefore, this study aimed to examine the effectiveness of PTFE, collagen membranes and naturally cross-linked collagen on soft tissue attachment after being treated with UV light and NTP oxygen and compared them to non-treated controls. Treatment duration was 12 minutes which may an adequate time to achieve satisfying results [55,56] because functionalization of more than 12 minutes may be hardly practicable under clinical conditions.

Fibroblasts, the fiber forming cells are the most abundant connective tissue cells with a significant role in wound healing [11]. Therefore, HGF cells were utilized to examine attachment on GBR membranes specimens, whereas the L929 cell line was utilized to strengthen the reliability of the results.

The outcomes of the current research indicated that treatment with NTP oxygen and UV light on PTFE and collagen membrane can increase the early attachment of fibroblast cells.

Previous studies demonstrated that PTFE membranes have high biocompatibility and space making capacity [22,23]. However, a major complication related to PTFE clinically is the exposure of the membrane into the oral cavity as a result of soft tissue shortage, leading to bacterial penetration through the membrane and migration into the regeneration area [29,57]. In the present study, we activated PTFE membrane using NTP oxygen and demonstrated a dramatically increase in attachment rate of L929 cells which may indicate an increased level of cell proliferation. These findings suggest that oxygen plasma irradiation may enhance the attachment of fibroblasts cells and may improve the early sealing of soft tissue, consequently, may reduce the risk of exposition.

Other studies may support these results, Guo et al. [56] treated abutment surfaces using NTP oxygen for 12 minutes and demonstrated improvement of soft-tissue cell attachment. Similarly, Kwon et al [58] found that NTP increased mRNA expression of growth factors in human gingival fibroblast and suggested that this method could be useful in gingival wound healing. A study by Kieft et al [55], reported that NTP oxygen can modify the extracellular matrix of fibroblast cells, culminating in improved attachment and proliferation. Besides, Khorasani et al. [60] displayed that, the cell-material interactions were attributed to the increase in the wettability of biomaterial surfaces during NTP oxygen treatment.

In the current study, after 12 minutes of UV irradiation, the attachment of L929 cells was increased on the surface of the PTFE membrane in comparison to non-treated group after 24 hours of incubation. These results can be explained by increasing the wettability and oxygen content of the membrane's surfaces after UV light exposure. Moreover, UV light can transform the electrostatic state of materials'

surface into a positive charge, which can improve protein adsorption and cellular adhesion [61]. However, HGF cells hardly attached to non-treated PTFE membrane. Both NTPO and UV treatments poorly increased the cell numbers on PTFE surfaces.

Blumenthal et al [62]. and Pitaru et al.[63,64] found that collagen membranes could be used for GTR and their application is comparable to those of PTFE membranes [65]. Moreover, collagen membranes are widely used due to their biocompatibility and lower risk of exposure. However, the early absorption of natural collagen membranes is an obstacle in successful GBR procedures.

Therefore, modifications of collagen membranes have been introduced to promote the mechanical properties and slower biodegradation rate. Various chemical agents such as glutaraldehyde and carbodiimide have enhanced cross-linkage between collagen molecules. However, these agents reduced membrane biocompatibility due to their toxicity as chemical agents [66]. Consequently, several studies investigated the outcome of UV light on collagen membranes. Davidenko et al.[67] reported that UV-treated collagen membranes are more resistant to degradation than non-treated ones, with favorable mechanical properties and maintained biological functionality. This occurs when UV irradiation is absorbed by aromatic rings, it elevates their energy level and results in the production of radicals the create crosslinking between collagen molecules.

Naturally cross linked collagen membranes are collagen membranes that have been treated to improve the resistance to degradation and enhance the biocompatibility of the membrane [68]. Consequently, in the current study, HGF and L929 cell attachment rate was improved on non-treated naturally cross-linked collagen

group and the number of live cells covered almost all the membrane surface. Accordingly, UV irradiation and NTPO treatment did not increase the number of the cells, hence no room available for a better attachment.

Furthermore, the attachment and adhesion of HGF and L929 cells displayed significant improvement on collagen membranes which lead to an enhanced fibroblast proliferation rate following a short UV light treatment of 12 minutes when compared with the non-treated group. This results can be related to UV functionalization that remove organic contamination, increase the wettability, and convert the electrostatic state of the material's surfaces into a positive charge, which improves protein absorption and cell growth [61]. Brezavšček et al, reported that UV treatment can increase the osteoconductive ability of biomaterials [69]

Moreover, for the present study, modified collagen membrane using NTPO significantly enhanced HGF and L929 attachment when compared with non-treated groups (Table 3). The current results are in correspondence with Catillo-Dali et al [70] where the properties of Poly(lactic-co-glycolic) acid membrane were examined after being exposed to NTP and showed an increase in membrane surface roughness and consequent improvement in cellular adhesion. It also enhanced the biodegradation capability of the resorption and elevated the osteosynthetic activity. Moreover, these results enhance the suggestion that; short time UV irradiation treatment can be a novel technique to boost the compatibility of soft tissue cells.

Irradiation of dental implants with UV or treatment with NTP to improve their physical, topographical, biological as well as chemical surface conditions have been reported by previous studies. Guo et al. [56], Henningsen et al. [51,] and Smeets et al. [71]

emphasized the positive influence of both methods on implant surfaces. UV irradiation and NTP treatment have reduced the carbonization level, increased oxide layer thickness, removed the organic contamination, and induced hydrophilization [72]. The significant improve in HGF and L929 proliferation on GBR membranes, after NTPO is a further proof that NTPO can enhance the biocompatibility of GBR membranes. As can be seen, the number of attached HGF and L929 cells on PTFE and collagen membranes were higher in NTPO group than in UV light group, however both were improved in comparison to control group.

The outcomes of the current study indicate that modifying of GBR membranes using UV light or non-thermal plasma oxygen may promote fibroblast cells attachment which consequently improve their proliferation and enhance the premature closure of soft tissue wounds. However, the experiments carried were at in vitro level. Therefore, further clinical studies are necessary for more detailed assessment for the effects of UV light and NTPO on PTFE, natural collagen and naturally cross-linked collagen membranes.

6. CONCLUSION

Within the limitation of the current study, the results showed that non-thermal plasma oxygen and UV light treatment can enhance the attachment of HGF and L929 cells which consequently improve their proliferation, on PTFE and collagen membranes. Furthermore, naturally cross-linked collagen membrane is a modified collagen membrane, therefore UV irradiation and NTPO treatment did not improve the attachment rate of both fibroblast cell types denoting the lack of space for cells to attach.

7. SUMMARY

The purpose of this study was to evaluate the effectiveness of ultraviolet irradiation and non-thermal plasma oxygen functionalization on interactions between guided bone regeneration membranes (polytetrafluoroethylene, naturally cross-linked collagen and collagen tissue matrix derived of animal dermis) and soft tissue cells (murine fibroblast cell line L929 and human gingival fibroblast cells), regarding cell attachment. Three types of guided bone regeneration membranes were used. Each membrane was allocated into 3 groups; a control group (non-treated membrane) and two experimental groups treated for 12 minutes; one using ultraviolet light and the other treated using non-thermal plasma oxygen. *Results:* Both ultraviolet light and non-thermal plasma oxygen groups showed significant difference in human gingival cells and murine fibroblast cell attachment on polytetrafluoroethylene and collagen membranes compared to non-treated groups. At the same time, ultraviolet light irradiation and non-thermal plasma oxygen treatment did not improve the attachment rate of both fibroblast cells on naturally cross-linked collagen membrane. Ultraviolet light and non-thermal plasma oxygen activations are promising methods to improve attachment of soft tissue cells on barrier membranes for guided bone regeneration.

8. ZUSAMMENFASSUNG

Zweck dieser Studie ist die Bewertung der Wirksamkeit der Ultraviolettes - Bestrahlung sowie der Kaltes-Plasma-Sauerstofffunktionalisierung (non-thermal plasma) auf Wechselwirkungen zwischen Knochenregenerationsmembranen (Polytetrafluorethylen, natürliche Kollagenvernetzung und Kollagengewebematrix aus tierischer Dermis) und Weichgewebezellen (Fibroblastenzelllinie L929 der Maus und menschlicher Gingivale Fibroblastenzellen) hinsichtlich der Zellanhaftung. Drei Arten von Knochenregenerationsmembranen kamen zum Einsatz. Jede Membran wurde dabei in drei Gruppen eingeteilt: eine Kontrollgruppe (unbehandelte Membran) und zwei Versuchsgruppen, die jeweils zwölf Minuten lang behandelt wurden - eine hiervon unter Verwendung ultravioletten Lichts, die andere unter Einsatz von Kaltes Plasma-Sauerstoff. Folgend Ergebnisse liegen vor: Sowohl die Ultraviolettes -Licht- als auch die Kaltes Plasma-Sauerstoffgruppen zeigten einen signifikanten Unterschied zwischen menschlichen Gingivazellen und der Anlagerung von Maus-Fibroblastenzellen an Polytetrafluorethylen- und Kollagenmembranen im Vergleich zu nicht behandelten Gruppen. Gleichzeitig verbesserte die Bestrahlung mit ultraviolettem Licht und die Behandlung mit Kaltes-Plasma-Sauerstoff die Anlagerungsrate beider Fibroblastenzellen auf einer natürlich vernetzten Kollagenmembran nicht. Ultraviolettes Licht und Kaltes-Plasma-Sauerstoffaktivierungen sind vielversprechende Methoden zur Verbesserung der Anhaftung von Weichgewebezellen an Barrieremembranen zur Knochenregeneration.

9. LIST OF ABBREVIATION

Guided Bone Regeneration	GBR
Guided Tissue Regeneration	GTR
Polytetrafluoroethylene	PTFE
Expanded Polytetrafluoroethylene	e-PTFE
Low Level Laser Therapy	LLLT
Ultraviolet	UV
Non-Thermal-Plasma	NTP
Non-Thermal- Aragon Oxygen Plasma	NTPAO
Human Gingival Fibroblast	HGF
Propidium Iodide	IP
Fluorescein Diacetate	FDA

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12. CURRICULUM VITAE

„Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt“.

13. EIDESSTATTLICHE VERSICHERUNG

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