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Novel Synthetic Clot Analogs for In-Vitro Stroke Modelling

Dissertation

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1. Publication

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RESEARCH ARTICLE

Novel synthetic clot analogs for in-vitro stroke modelling

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Abstract

Purpose

The increased demand for training of mechanical thrombectomy in ischemic stroke and development of new recanalization devices urges the creation of new simulation models both for training and device assessment. Clots properties have shown to play a role in procedural planning and thrombectomy device effectiveness. In this study, we analyzed the characteristics and applicability of completely synthetic, animal-free clots in the setting of an in-vitro model of mechanical thrombectomy for training and device assessment.

Methods

Synthetic clots based on agarose (n = 12) and silicone (n = 11) were evaluated in an in-vitro neurointervention simulation of mechanical thrombectomy with clot extraction devices. Calcified clots of mixed nature were simulated with addition of 3D printed structures. 9 clots were excluded due to insufficient vessel occlusion and failure to integrate with clot extraction device. Synthetic thrombi were characterized and compared using a categorical score-system on vessel occlusion, elasticity, fragmentation, adherence and device integration.

Results

Both agarose-based and silicone-based clots demonstrated relevant flow arrest and a good integration with the clot extraction device. Silicone-based clots scored higher on adherence to the vessel wall and elasticity.

Conclusion

Selected synthetic clots can successfully be implemented in an in-vitro training environment of mechanical thrombectomy. The clots' different properties might serve to mimic fibrin-rich and red blood cell-rich human thrombi.

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Introduction

Clinical evidence has shown in the past years the undeniable value of mechanical thrombectomy (MT) in the treatment of acute large vessel occlusion (LVO) [1]. This led to a global increase in the demand for interventionalists with technical proficiency in endovascular stroke treatment [2]. Training of neurointerventionalists may be challenging due to the delicate nature of neurovascular interventions and the scarce availability of dedicated stoke simulation models representing realistic stroke-specific interventional challenges. Animal models used for medical training or device testing are associated not only with an ethical dilemma but also with high costs and a poor reproducibility of human anatomy [3]. In this study we use a fully animal-free experimental setting, comprised of a previously described neurointervention simulation model—HAmburg Anatomical NEurointerventional Simulator (HANNES) [4] and custom-designed 3D-printed models of intracranial vasculature as a realistic and cost-effective training environment for mechanical thrombectomy (MT).

Clot composition has been shown to play a role in the etiology and characterization of ischemic stroke in large vessel occlusions [5]. This may play a role in the development and research of new therapy concepts and thrombectomy devices. One previous study states the importance of artificially made thrombi from human or pig´s blood in the pre-evaluation of thrombus extraction devices and as training material [6]. In this study we analyzed the feasibility of completely animal-free, synthetically made clots in the setting of neurovascular simulation of MT and their interaction with extraction devices.

Our hypothesis was that synthetic clots allow for sufficient vessel occlusion and device interaction in an experimental simulation model of mechanical thrombectomy when evaluated by experienced neurointerventionalists, and that different clot compositions may show different mechanical properties which may mimic characteristics found in human clots.

Material & methods

Synthetic clot development and selection

Synthetically produced clots (n = 23) mainly composed of agarose (n = 12, A1-12) or silicone (n = 11, S1-11) were tested in a previously described neurovascular simulation model [4]. Different concentrations of these compounds were mixed with a 3:1 mixture of methylchloroisothiazolinone (MCI) and methylisothiazolinone (MI) (C. Kreul GmbH & Co. KG, Hallerndorf, Germany) or/and micro-glass beads (MGB) (Carl Roth GmbH + Co KG, Karlsruhe, Germany) in different proportions in order to simulate different degrees of stiffness, elasticity, adhesion and fragmentation (Table 1). 3D-printed supporting structures, spiral- or barbed-shaped, were found to be most suitable to be integrated in agarose clots (n = 3) in order to simulate the irregularly shaped calcifications found in-vivo (Flexible Resin, Formlabs 2, MA, USA). Failure to cause a sufficient vessel occlusion (e.g., due to distinctive fragility) or interact with the clot extraction device (and thus, affecting sufficient assessment of stent-retrieval suitability) was considered an exclusion criterion. From the 23 clots that were intended for in-vitro testing, 9 clots were excluded: 4 out of 12 agarose-based clots due to insufficient vessel occlusion and failure to integrate with the stent retriever and 1 out of 12 due to failure to integrate with the stent retriever. 4 out of 11 silicone-based clots were excluded due to failure to interact with the stent retriever. Tubular shaped synthetic clots were prepared with a length of 9–11 mm and a diameter of 2.5 mm. Process of clot production is thoroughly described by Wortmann N. et al. [7].

Neurovascular simulation environment

Synthetic clot testing was performed in a neurovascular simulation environment (HANNES) [4, 8] integrated on a monoplane angiography system (AlluraClarity FD 20, Philips Healthcare, Best,

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Table 1. Summary of selected synthetic clots, their general composition and possible applicability.

Clot	Composition	Potential Application
A2	Agarose 2%, MCI/MI 10%	white clot
A3	Agarose 2%, MCI/MI 10%, spiral supporting structures	mixed calcified clot
A4	Agarose 2%, MCI/MI 10%, barbed supporting structures	white calcified clot
A5	Agarose 2%, MCI/MI 20%	white clot
A7	Agarose 2%, MCI/MI 5%	white clot
A9	Agarose 5%, MCI/MI 10%, supportive structures	white calcified clot
A12	Agarose 5%, MCI/MI 5%	white clot, fragile
S1	Silicone 30%, MCI/MI 30%	red clot
S3	Silicone 30%, MCI/MI 30%, preserved in oil	red clot
S4	Silicone 30%, MCI/MI 40%, MGB 10%	red clot
S5	Silicone 30%, MCI/MI 40%, MGB 20%	mixed clot
S6	Silicone 30%, MCI/MI 40%, MGB 30%	red clot
S9	Silicone 40%, MCI/MI 20%	white clot
S10	Silicone 40%, MCI/MI 30%	white clot

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The Netherlands). Commercially available standardized models of femoral vessels and thoracoabdominal aorta (Neuro Testing Model NTM00V02, United Biologics, Santa Ana, CA) were integrated with 3D-printed, patient specific common carotid and internal carotid arteries attached to a skull base prototype as previously described [4, 8]. Intracranial circulation was simulated using a realistic 3D-printed whole brain vascular model based on patient anatomy including anterior and posterior circulation with a right-sided posterior communicating artery. Physiological flow rate, pulse and body-temperature were simulated by an integrated fluid pump, equipped with a pulsatile valve and a heating system. The standard system configuration produced a flow rate of 0.4L/min, a pulse rate of 70 bpm and a system temperature of 37°C. Blood was substituted with a solution of water and small amount of commercially available soap for friction reduction.

Mechanical recanalization experimental setup

A standard short 8F sheath was placed in the right femoral artery of the experimental model. An 8F-FlowGate guiding catheter (Stryker, MI, USA) was introduced into the internal carotid artery. Clots were directly administered into the common carotid artery of the experimental model via an additional, for this purpose constructed cervical arterial branch. The resulting clot position was evaluated with 10 ml of iodinated contrast medium (Imeron 150, Bracco, Milan, IT) in a single angiographic run (Fig 1). Each clot of the same type was extracted using three different techniques (one recanalization approach per clot): first with aspiration only, using a Sofia 5F or 6F aspiration catheter (Microvention, CA, USA), secondly with a Trevo 6/25 stent retriever (Stryker, MI, USA) and lastly the retrieval maneuver was repeated with a combined approach using the stent retriever and aspiration simultaneously [9]. Finally, selected clots were further evaluated for defined characteristics using a simple silicone tube (5 mm inner diameter; Roth, Karlsruhe, Germany) in order to observe clot interactions without other system factors, such as catheter manipulation, vessel model curvature or inner surface. The clots were positioned within the tube and the interaction with the stent retriever was closely observed and documented.

Data acquisition and analysis

Clots were qualitatively evaluated in the HANNES model by three neuroradiologists with an average of 5 years-experience in angiography. Evaluation was performed using an
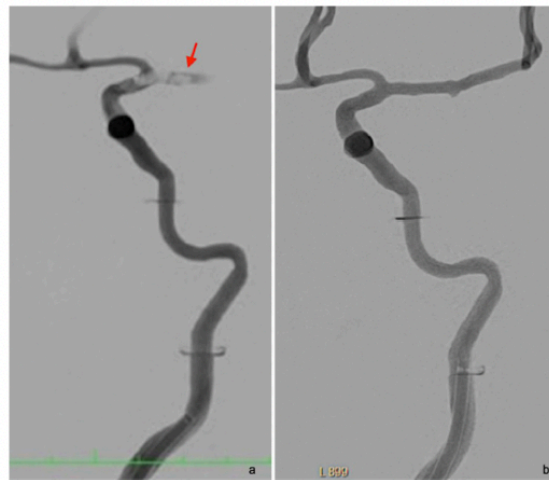
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Fig 1. Subtraction angiography of a custom made whole-brain vascular model showing, upon injection of iodinated contrast, a carotid-T occlusion created by an agarose-based synthetic clot (arrow) before (a) and after (b) mechanical recanalization. Note the guide catheter placed in the cervical ICA.

ordinal classification scale with a 5-point system (0 to 4) based on qualitative thrombi characteristics with five items: vessel occlusion, elasticity, fragmentation, adherence and device integration (Table 2). This was based on both clinical practice with human thrombi in MT as well as comparatively with other synthetic clots in the study (e.g. elasticity). Data documentation and scoring was performed on Microsoft Excel for Mac (Redmond, WA, USA). Standard descriptive statistics were performed using Prism 9 for macOS Version 9.1.0 (GraphPad Software, LA Jolla, CA, USA). Non-parametric qualitative scale ranks were analyzed using a Mann-Whitney Test performed on MedCalc (MedCalc Software, Ostend, Belgium). Results were displayed in terms of medians and p values. A p-value < 0.05 was considered significant.

Results

Thrombus design

Agarose-based thrombi were primarily soft and fragile, tendentially breaking before installation in the vascular model. Addition of MCI/MI showed to significantly reduce their breakability and helped producing compacter structures. Addition of other solid elements such as resins developed and printed in different shapes using 3D-printing techniques showed to be a feasible solution in the production of synthetic models mimicking the behavior of calcified clots of mixed nature. Fragmentation showed to be the main feature of agarose-based clots.

Pure silicone clots presented as too stiff and failed to show the adherence expected form fibrin clots. Realistic slightly more malleable and more adherent clots could be achieved by adding different concentrations of MCI/MI. Added micro-glass beads (MGB) lead to a further increase in fragmentation and adhesion to vascular model walls. Material "stickiness" was effectively reduced through preservation of clot material in an oily substance. Silicone-based clots showed variable degrees of adherence and elasticity as well as reduced fragmentation.

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Table 2. Classification scale for the qualitative evaluation of synthetic clot characteristics in an in-vitro experimental setting.

Criteria	Scoring
Vessel occlusion	0 = none
	1 = negligible flow arrest
Degree of flow arrest on the vessel occluded with a synthetic clot	2 = mild flow arrest
	3 = relevant flow arrest
	4 = complete flow arrest
Elasticity	0 = not elastic
	1 = minimally elastic
	2 = mildly elastic
Elastic deformation of synthetic clots upon interaction with wires and retrieval devices	3 = elastic
	4 = very elastic
Fragmentation	0 = not fragmented
	1 = minimally fragmented
	2 = moderately fragmented
Clot ability to fragment. Associated with higher rate of intraprocedural peripheral embolism	3 = easily fragmented
	4 = very fragmented
Adherence	0 = none
	1 = minimally adherent
	2 = moderately adherent
Measure of the clot's grip on the vessel wall. It is associated with a decreased probability of clot migration	3 = adherent
	4 = very adherent
Device integration	0 = no integration
	1 = minimal integration
Describes interaction of the clot with the retrieval device. A better device integration is associated with higher therapeutical success rate.	2 = moderate integration
	3 = good integration
	4 = very good integration

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Thrombus evaluation in an in-vitro setup

7 agarose-based clots and 7 silicone-based clots were included in the analysis. Both types of clots caused relevant flow arrest and demonstrated a good integration with the clot extraction device (Fig 2). A moderate tendency to fragmentation was observed in both types of clots. Silicone-based clots showed significant higher elasticity (median 2.5 vs 0.5, $p = 0.0058$) and adherence to the vessel wall (median 3.5 vs 0, $p = 0.0048$) upon removal with the retrieval device. The majority of clots could be retrieved by aspiration alone (n = 9, 64.3%), 4 out of 7 silicone-based and 5 out of 7 agarose-based clots.

Discussion

In this experimental study, we developed and tested a novel concept of animal-free synthetic clots for training and research on mechanical thrombectomy, in an in-vitro setting, using a previously well-established neurovascular simulation model [4]. Clots which were unable to provide minimal vessel occlusion or sufficient interaction with retrieval devices were classified as unsuitable for training proposes and were therefore excluded from the analysis. We found that selected agarose-based and silicone-based clots were able to achieve sufficient vessel occlusion and integration with the clot extraction device. Silicone-based clots were more adherent to the vessel wall and more elastic than agarose-based clots.
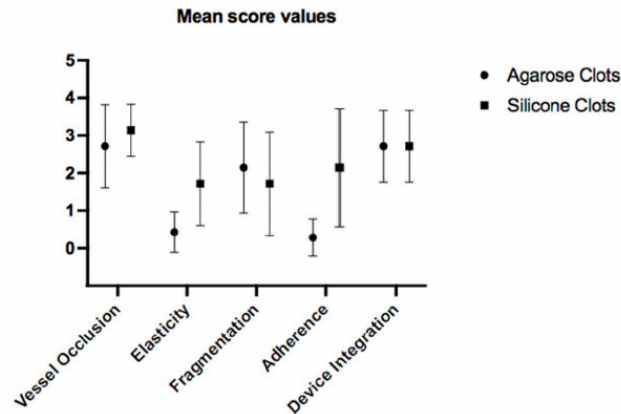
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Mean score values

Fig 2. Error bar graph comparing mean score values for agarose- and silicone-based artificial clots. Note specially the different mechanical behaviors concerning clot elasticity and adherence. Both clot groups presented a good to very good vessel occlusion and device integration.

Berndt et al. proposed a collection of embolus analogs based on human and pig's blood for device evaluation and training [6]. Other studies described embolus analogs based on a variety of animal and human blood [10] or through addition of synthetic components to human blood [11, 12]. To our knowledge, this study describes the first fully synthetic clot replacement model, free from animal or human blood. An animal-free experimentation setting is increasingly important due to not only the ethical concerns but also in order to establish a simplified and cost-effective solution for regular MT training and device research.

Previous studies have stated the importance of clot histological composition on its mechanical properties and secondarily, on the effectiveness of revascularization techniques in ischemic stroke [13]. Clots can be classified as red/soft clots, if the concentration of red blood cells (RBC) is at least 15% higher than platelets or fibrin, as white/hard clots, if concentration of fibrin is at least 15% higher than RBC and as mixed, if the RBC/fibrin concentration is present in any other proportion [14]. Several studies reported an association of clot composition with the pathophysiology of vascular occlusion: whereas cardioembolic thrombi present with higher mean proportions of fibrin, less RBCs and more WBCs, clots of non-cardioembolic etiologies, such as large artery atherosclerosis or cryptogenic stroke, are mainly associated with RBC-rich clots [15, 16]. However, clot composition remains highly variable, with other factors such as clot age and presence of calcifications, playing a role in their behavior [5].

Development of artificial thrombi with different properties is important for a realistic simulation of MT. In this study we describe artificial clots that potentially mimic the natural behaviors found in red, white, mixed or calcified clots. Red or RBC-rich clots present predominantly with a viscoelastic soft texture [17] and higher friability [18]. Clot permeability or perviousness is defined by the degree to which blood is able to flow through a vessel occlusion. RBC-rich thrombi are associated with higher perviousness and clot attenuation in CT [19, 20], which showed to correlate with better technical and clinical outcomes of patients undergoing MT [21]. Further studies have demonstrated that red clots are associated with reduced number of recanalization maneuvers and overall reduced procedural time [15].

White or fibrin-rich clots have been described as stiffer, presenting increased friction or adherence on the vessel wall [22]. Aged thromboemboli are especially compact structures,

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lacking elasticity and increasingly prone to fragmentation [10]. White clots present with lower attenuation on non-contrast-enhanced CT and increased resistance to both intravenous lysis and mechanical thrombectomy [23]. Technical aspects of MT such as speed of retrieval [24] or device selection [15] may be improved based on clot composition [20, 23, 25]. Namely utilization of stent retriever in MT seems to be less effective in the case of calcified thromboemboli [26].

The agarose-based clots presented in this study may mimic white, mostly aged clots, showing reduced elasticity and fairly high breakability. However, the expected adherence of such clot materials fails to be met. On the other hand, highly elastic silicone-based clots with variable degrees of breakability presented with high level of adherence. Nonetheless, in this study clot adherence did not affect the technical success of MT. Further developments on clot analogs composition are necessary to optimize artificially composed thrombi and to make them easily available to a higher number of neurovascular centers involved in the research and training of MT.

The exploratory study design with qualitative data interpretation is typically subject to researcher confirmation bias, which may influence the results and make the data less replicable. The neurointerventionalists were not blinded to clot type upon their evaluation. Interobserver differences could be overcome with further development of objective quantitative assessment methods in this in-vitro set up. Furthermore, an exact comparison between synthetic and human clots can only be achieved by comparing their mechanical properties.

Conclusion

Selected synthetically composed clot analogs achieved sufficient vessel occlusion and integration with the clot extraction device in an in-vitro stroke thrombectomy model. The characteristic properties of agarose-based and silicone-based clots may be used to mimic different types of human thrombi. Synthetic clots could effectively be used for device testing, as well as for training of endovascular treatment of ischemic stroke.

Supporting information

S1 Table. Qualitative scores obtained for each of the 23 evaluated clots. Clots that failed to interact with the clot removal device (Score = 0 for the category *device integration)* were classified as not suitable for an in-vitro experimental setting and thus excluded (marked in grey, n = 9).
(DOCX)

S1 Fig. (a-d). Silicone-based clots mixed with 30% MCI/MI with (c,d) and without microglass beads (a,b): before (a,c) and after (b,d) mechanical sheer stress showing different breakability and elasticity degrees.
(DOCX)

S2 Fig. (a-c). Retrieved clots with stent retriever. Agarose-based clots mixed with 10% MCI/ MI (a, c), with spiral (a) or barbed (c) supporting structures, and with 20% MCI/MI (b) were successfully retrieved with a clot extraction device (SolitaireTM 4mm x 20mm, Medtronic, Dublin, Ireland).
(DOCX)

Acknowledgments

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produced clots and their applicability in a neurovascular simulation situation were filed for patent with the German Patent Office under the reference DPMA, TU421/UKE366 on 12.05.2021.

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Writing – original draft: Helena Guerreiro, Anna A. Kyselyova.

Writing – review & editing: Fabian Flottmann.

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2. Project Description

2.2 . Procedural Simulation in Neurointervention

Training and research of neurovascular procedures were in the past mainly performed on live animals, e.g., pigs, rats, or rabbits[1]. These models have many disadvantages. Besides the ethical aspects, they are associated with high costs and unrealistic simulation of cerebral vascular anatomy and their pathologies[1]. Other negative aspect of animal models as a platform for neurovascular research and training is their limited reproducibility[2]. Thus, the need for the development of a complete simulation platform of neurovascular procedures to meet the continuously increasing needs for differentiated training in neurointervention[3].

2.2. HANNES
Hamburger ANatomical NEurointerventional Simulation Model

HANNES is a physical circulation model that enables realistic procedural simulation with real treatment devices. Its modular design (Fig. 1) using patient-specific 3D-printed vascular models enables the simulation and therapy of various neurovascular diseases, such as vascular occlusions, stenosis or aneurysms[4,5,6]. The core of the model is the blood vessel system, which is anatomically realistic representing the human vasculature from their access points at the inguinal and radial artery via the aorta to the intracranial arteries. The model is constituted by fixed parts, such as the base frame, the control unit, the fluid pump, and the skull base as well as, removable, easily replaceable segments. With the help of defined interfaces and specially developed adapters, it is possible to easily exchange vascular segments such as the aortic arch, cervical and cerebral arteries. The adapters are characterized by an edge-free transition of the internal structure of the vascular models for a more realistic user experience. HANNES is integrated in a dedicated angiography unit in the Hermann-Zeumer Simulation Lab (Fig. 2) in the University Medical Center Hamburg-Eppendorf (UKE). The project is based on the results of a German Federal Ministry of Education and Research (BMBF)-funded research collaboration between the Technical University Hamburg and the UKE such as ELBE-NTM - *Development and Evaluation of a Patient-Based Neurointerventional Training Model*, funded within the framework of the BMBF funding program: "Alternative Methods to Animal Experiments" (Grant Nr.

031L0068A)[4]. The simulation lab is currently an important platform for procedural training in the form of workshops and courses for physicians and medical assistants. Furthermore, it plays an important role also as a realistic environment for the testing of new devices before their application in the clinical setting.
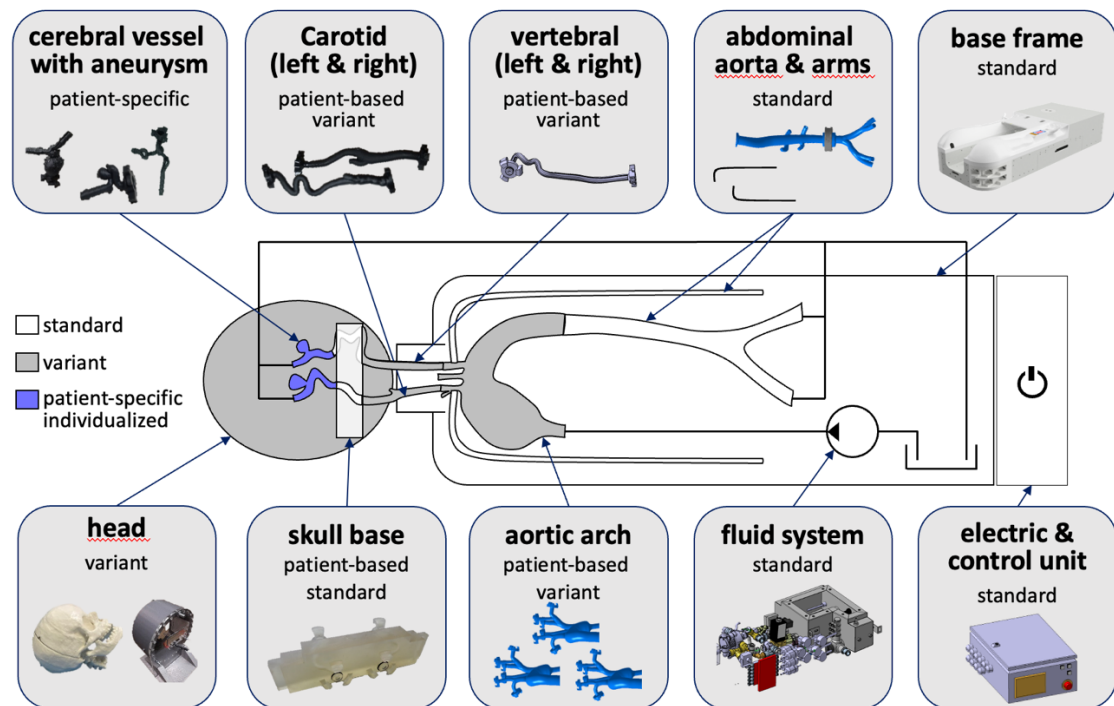

Fig. 1: Schematic diagram of HANNES showing its modular design with fixed supporting structures (white) and modular, interchangeable patient-specific units (grey and blue). (Adapted from Spallek et al.[4])

2.3. COSY SMILE

COmpletely SYnthetic Stroke Model for InterventionaL Development and Education

The BMBF-funded project COSY SMILE (Grant Nr. 161L0154B) aimed to develop a completely animal-free endovascular simulation model of ischemic stroke treatment for training and research purposes. The stroke model is a further development of HANNES. The hereby developed models enable the learning and practicing of mechanical thrombectomy under different levels of difficulty and easily replicable, standardized conditions. Mechanical thrombectomy is a catheter-based procedure, which involves the navigation of a catheter through the vasculature to reach the

affected vascular segment, namely in the cerebral vasculature, and either by means of aspiration, stent retriever or combined approaches, removal of blood clot causing cerebral flow reduction[7]. Ischemic stroke is one of the leading causes of death and disability worldwide. Mechanical thrombectomy has shown irrefutable evidence in the treatment of ischemic stroke caused by large vessel occlusion[7]. Consequently, the need for medical specialists proficient in the technique as increased dramatically in the last years[8]. And with this, the need for realistic, robust and replicable training environments.
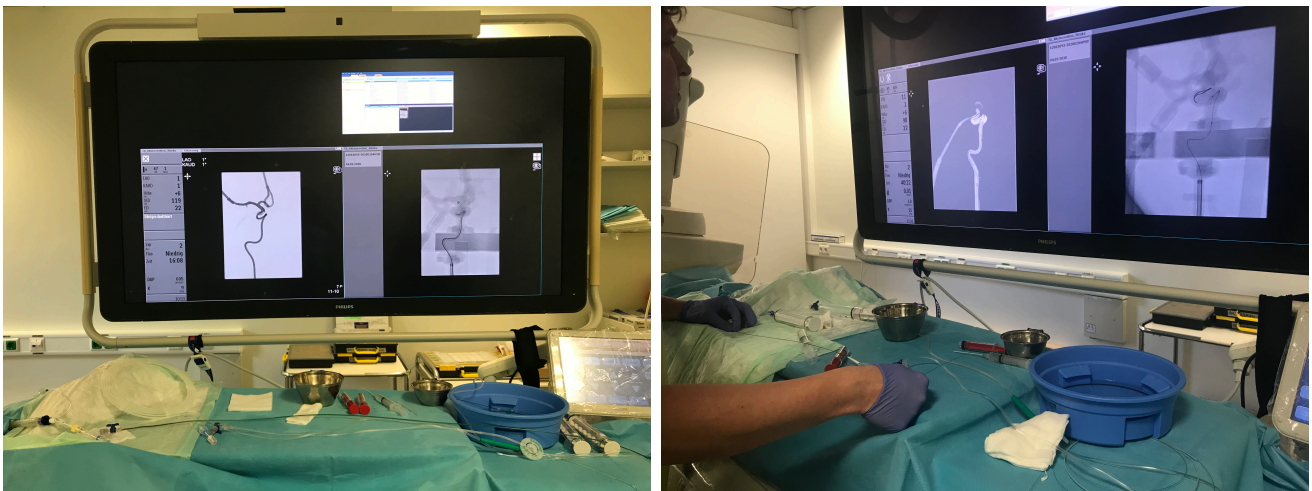

Fig. 2: Simulation of mechanical thrombectomy at the angiography in the Hermann-Zeumer Lab using HANNES simulation model in a realistic setup.

The development of the stroke model has been divided in different subgroups, in order to achieve various mechanical thrombectomy scenarios:

- *Development of vascular geometries:*

One of the biggest challenges of mechanical thrombectomy is the access to the occlusion site[9]. Ischemic stroke affects mainly the elderly population, often with comorbidities which commonly implies tortuous vascular configurations[10]. Thus, the aim here was to build a patient-based 3D printed portfolio of vascular anatomies (Fig. 3), comprising cervical vessels with regular, elongated, and kinked configurations, siphon anatomical variants and whole-brain vascular models.
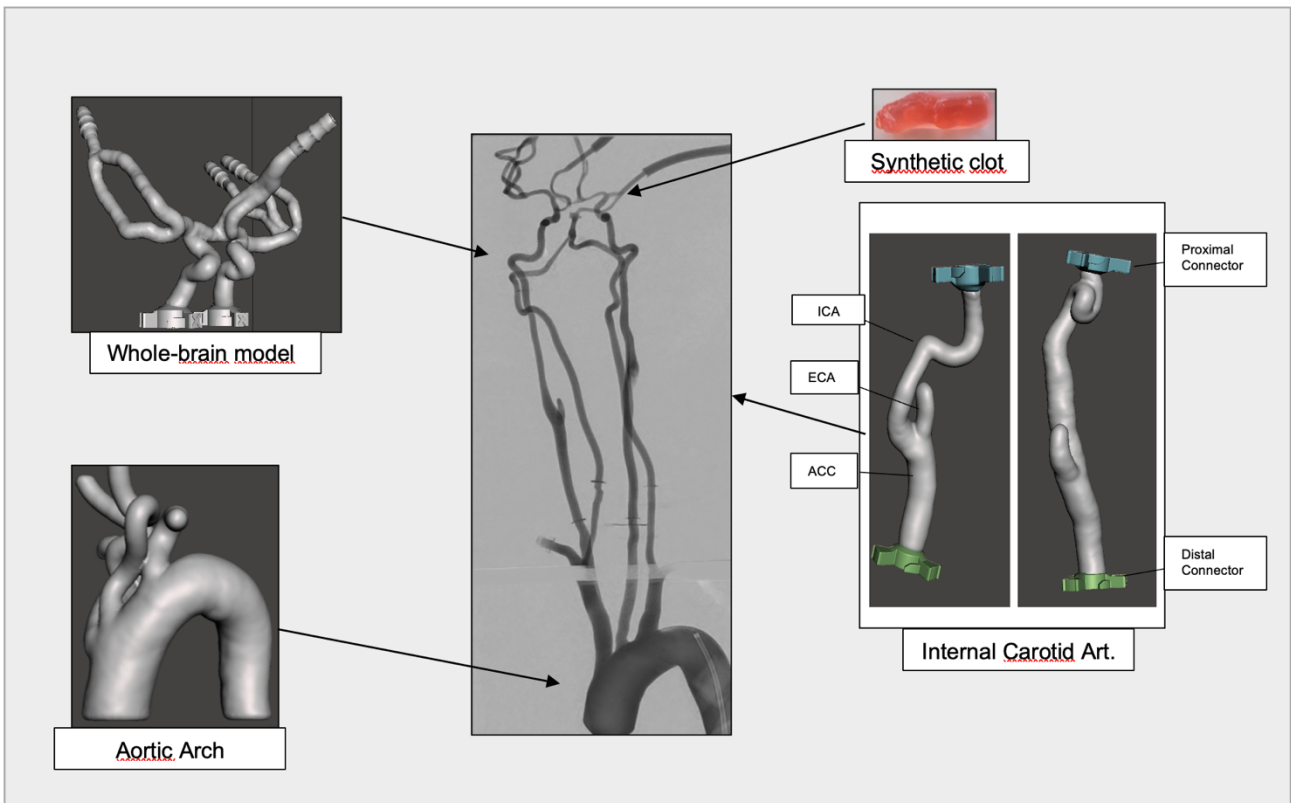
Fig. 3: Development of vessel geometries. Digital subtraction angiography of the vascular models adapted in HANNES with an occlusion in the left M1-Segment by artificial clot and illustration of patient-based segmentations before 3D printing in STL-Format. Note the absence of lumen irregularities at connecting sites.

- *Development of synthetic clots*

Main topic of this work and described in detail under section 2.4.

- *Development of stenosis models*

Vascular stenosis can, on one hand, be the cause of thrombus formation and on the other hand, significantly complicate the path to the occlusion site. Therefore, the treatment of stenosis in the cervical and intracranial vessels by means of stent-protected percutaneous transluminal angioplasty (SPAC) should also be made possible in the training model. The stenosis simulation models for application in the internal carotid artery were conceptualized by the project partners at TUHH. These were successfully integrated and tested in the HANNES model.

- *Emboli detection chamber*

The quantification of treatment success was made possible by the development and integration of an emboli detection chamber. A software for the detection of thrombi was developed by the cooperation partners at TUHH. After integration of the measuring chamber in HANNES, tests were performed with two experienced neuroradiologists. Designed synthetic clots prone to fragmentation were used in the setting of mechanical thrombectomy simulation. The diameter and number of distally flowing particles could be detected and quantified during the thrombectomy simulation. An assignment of the particles to a corresponding vessel outflow was made possible by the integration of different tubes, passing through a source of light, connecting to a "venous" outflow returning to the pump. The results are displayed in real time and can be exported and saved after training.

2.4. Synthetic Clots

The main topic of this work, the development of synthetic clots, was an integrant part of the COSY SMILE Project. The synthetic clots described here were granted a patent under the following description: *synthetic clot models for the simulation of diverse vessel occlusion scenarios in a neurointerventional, mechanical thrombectomy training model* – Patent Nr. 10 2021 112 467. The developed agarose- and silicone-based synthetic clots aimed to substitute animal-based thrombi. These were designed to optimally simulate the different human clot properties. Human clots have been characterized as red or soft clots, if the concentration of red blood cells is at least 15% higher than platelets or fibrin, as white/hard clots, if concentration of fibrin is at least 15% higher than RBC and as mixed, if the RBC/fibrin concentration is present in different proportions[11]. Clot compositions have been shown to play a role in the etiology and characterization of ischemic stroke in large vessel occlusions[12]. Furthermore, this may be determinant for the success of mechanical thrombectomy[13], for interaction with

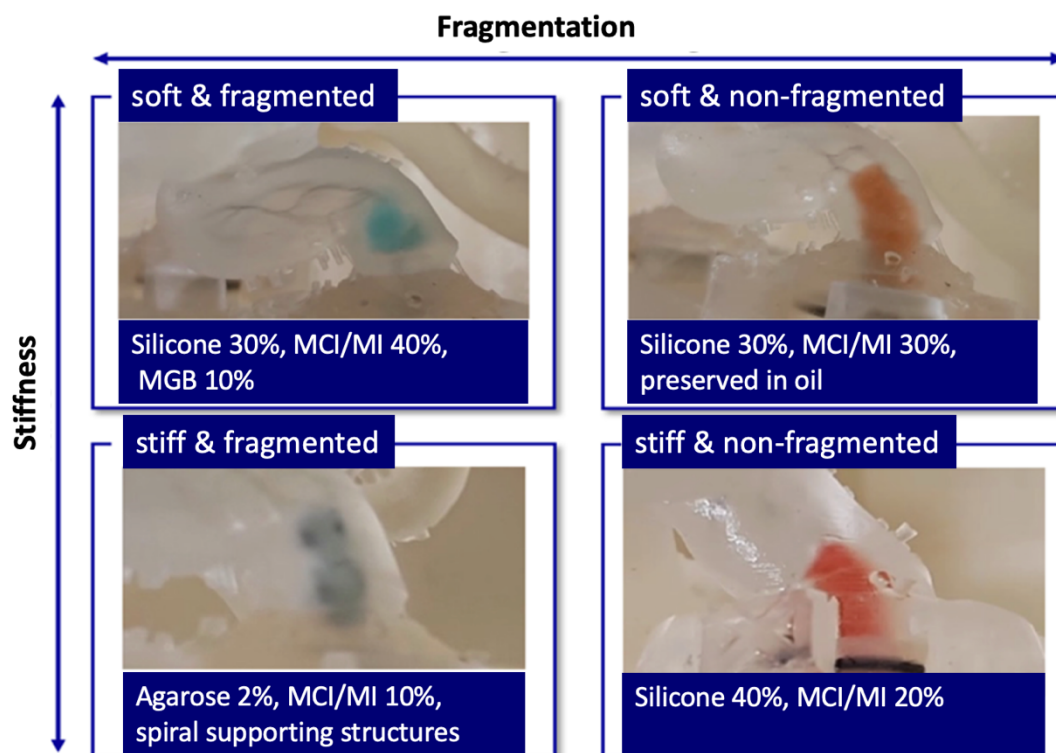clot removal devices[14], as well as for the need of adjuvant therapies such as intracranial stenting.

Fig. 4: 3D-printed vessel models integrated in HANNES with occlusions generated through the placement of synthetic clots. Clots with different compositions presented with distinct features, which were comparable to the features found in human clots.

A total of 23 clots were produced: 13 were mainly composed of agarose and 12 of silicone. These were mixed with methylchloroisothiazolinone (MIC), methylisothiazolinone (MI) and/or micro-glass beads to achieve different consistencies (Fig. 4). 9 clots were excluded due to failure to integrate with stent retriever and/or lack of occlusive capacities. Clots were tested and evaluated during internal interim assessments. There were three final tests comparing the different compositions. The tests were performed in the context of an in vitro simulation of mechanical thrombectomy using a standard technique with stent retrieval system in HANNES. The synthetic clots were classified using an ordinal 5-point classification scale based on the following qualitative features: Vessel occlusion, elasticity, fragmentation, adherence, and interaction with the retrieval system (stent integration, wire perforation and retraction resistance). A final test was performed in a simplified simulation environment in which the interaction of the stent retriever with the thrombus was

evaluated directly in a silicone tube (Fig. 5). Concomitant simulation factors potentially affecting the results could hence be minimized.
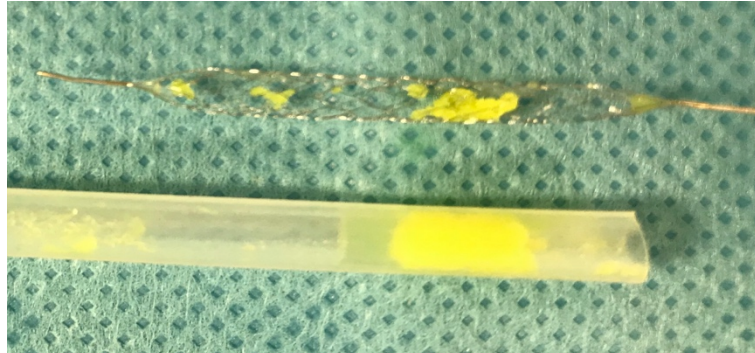
Fig. 5: Partially retrieved fragile agarose-based synthetic clot from a simple silicone tube integrated within the stent-retriever mesh (Trevo™, Stryker, Kalamazoo, MI, USA).

The results of this study enabled not only a successful patent application but also in the practical setting, the complete replacement of animal-based thrombi in the training of mechanical thrombectomy at the Hermann-Zeumer Lab. Their different characteristics are now routinely used for the simulation of different scenarios of intracranial vessel occlusion.

3. Summary

This work was an important part of the development of a complete simulation set-up for the training and research of mechanical thrombectomy. An animal-free experimentation setting is increasingly important due to not only the ethical concerns but also to establish a replicable, simplified, and cost-effective solution for differentiated training.

The agarose-based synthetic clots presented in this study may mimic white, rigid clots with reduced breakability. However, the expected stickiness of white clots failed to be met. The silicone-based clots with their high level of elasticity may mimic red clots. Addition of solid resins developed and printed in different shapes using 3D-printing techniques showed to be a feasible solution for the replication of calcified clots of mixed nature. However, further optimization of clot adherence capability is needed and is planned, as part of the subsequent project COSY SMILE 2 (BMBF Grant Nr. 16LW0165K).

In summary, the developed synthetic clot analogs showed to successfully mimic the main natural mechanical properties of human clots and can effectively be used in the procedural simulation setting for training of endovascular treatment of ischemic stroke as well as for device tests. They provide a readily available, cost-effective alternative to animal- or human-based clots.

3. Zusammenfassung

Diese Arbeit war ein wichtiger Bestandteil der Entwicklung eines vollständigen Simulationsaufbaus für das Training und Forschung im Bereich der mechanischen Thrombektomie. Eine tierfreie Versuchsumgebung wird nicht nur aus ethischen Gründen immer wichtiger, sondern auch, um eine reproduzierbare, vereinfachte und kosteneffiziente Lösung für eine differenzierte Ausbildung zu schaffen.

Die in dieser Studie vorgestellten synthetischen Thromben auf Agarosebasis können weiße, rigidere Thromben mit geringer Fragmentierung imitieren. Die erwartete Adhärenz der weißen Thromben wurde jedoch nicht erreicht. Die künstlichen Thromben auf Silikonbasis mit ihrer hohen Elastizität könnten rote Thromben nachbilden. Die Zugabe von festen Harzen, die mit Hilfe von 3D-Drucktechniken entwickelt und in verschiedenen Formen gedruckt wurden, erwies sich als praktikable Lösung für die Nachbildung von kalzifizierten Thromben gemischter Natur. Die Klebrigkeit der synthetischen Thromben muss jedoch noch weiter optimiert werden. Diese Optimierung ist im Rahmen des Folgeprojekts COSY SMILE 2 (BMBF-Förderkennzeichen: 16LW0165K) geplant.

Zusammenfassend hat sich hier gezeigt, dass die entwickelten synthetischen Thrombenanaloga die wichtigsten natürlichen mechanischen Eigenschaften menschlicher Thromben erfolgreich simulieren und in der Verfahrenssimulation für das Training der endovaskulären Behandlung des ischämischen Schlaganfalls sowie für Device-Tests effektiv eingesetzt werden können. Sie stellen eine gut verfügbare, kostengünstige Alternative zu Thromben aus Tieren oder Menschen dar.

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5. Author Contribution

I integrate the research team of the Neurointerventional Simulation Lab (Herman-Zeumer Labor) since October 2019. I started being a part of the precursor project COSY SMILE 1, led by the at the time senior consultant PD Dr. Andreas Frölich. I took an active part since the beginning on product development and evaluation as well as with project management. An important part of this was the successful BMBF grant application for the successor project COSY SMILE 2, in which I had the opportunity to assume team leadership. Within the COSY SMILE 2 I have been responsible for project organization, experimental design, product development and testing as well as for its scientific evaluation. In the project subdivision for development of artificial clots I worked in close contact with the cooperation partners at TUHH in the development and conceptualization of artificial clots. I managed the investigation, the data curation and analysis as well completion of the manuscript.

A detailed description of the authors contribution can be found on the page 8 of the publication.

6. Acknowledgements

7. Curriculum Vitae

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Education

Charles University in Prague, Czech Republic   Oct. 2004 – Jan. 2011

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Professional Affiliations

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European Society of Neuroradiology (ESNR)	since 2019
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Clinical Experience

Diagnostic and Interventional Neuroradiologist   October 2019 - Present

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General Practitioner   Nov. 2012 – Dez. 2012

Medical Flight Evacuations – *Medline*; Luanda, Angola

General Practitioner and Emergency Doctor   Jun. 2011 – Oct. 2012

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Research

Current Projects:

-   Project coordinator: COSY SMILE 1 & 2 – Completely Synthetic Stroke Model for Interventional Development and Education; Project funded by the Federal Ministry of Education and Research (BMBF, 161L0154A & 16LW0165K)

-   Procedural simulation and device testing in a neurovascular simulation lab (Hermann-Zeumer Simulation Lab)

-   Organizing Committee – eFellowship Program of the European Society of Minimally Invasive Neurological Therapy (ESMINT) 2021-2023

Previous Affiliations:

- **Neuroimaging Research Group,** Second Faculty of Medicine, Prague   2008 – 2011

- **International Clinical Research Center (ICRC),** St. Anne's University Brno   2011 – 2014

- **MARA –** Marburg University Research Academy   Jan. 2012 – Mar. 2014

- **Guest Researcher,** Neurosurgery Research Group   Jan. 2012 – Mar. 2014
  Philipps Universität Marburg

Publications:

- Brabec J, Rulseh A, Horinek D, Pala A, **Guerreiro H**, Buskova J, Petrovicky P, Nemcova V, Krasensky J, Seidl Z, Nimsky C, Sonka K.; *Volume of the amygdala is reduced in patients with narcolepsy - a structural MRI study.*; Neuro Endocrinol Lett.2011; 32(5):652-6.

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- Kresbach C, Bronsema A, **Guerreiro H**, Rutkowski S, Schüller U, Winkler B; *Long-term survival of an adolescent glioblastoma patient under treatment with vinblastine and valproic acid illustrates*

importance of methylation profiling; Child's Nervous System; 2021 Jun; https://doi.org/10.1007/s00381-021-05278-6

- Wortmann N, **Guerreiro H**, Kyselyova A, Frölich AM, Fiehler J, Krause D; *Development and Manufacturing of Cervical Stenosis Models for the Integration into a Neurointerventional Simulation Model;* Proceedings of the ASME 2021 – International Mechanical Engineering Congress and Exposition IMECE 2021- 71030

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- **Guerreiro H**, Flottmann F, Kyselyova AA, Wagner M, Brekenfeld C, Eckert B, Illies T, Wodarg F, Fiehler J, Bester M; *First Experience with Walrus Balloon Guide Catheter in a Whole-Body Flow Model;* - submitted, under review

Funding

Presentations:

Guerreiro H., Dinnies, S., Sehner S., Wenzel U., Adam G., Regier M., *Efficiency of Percutaneos Angioplasty in Renal Artery Stenosis – a 15 Year single centre experience in a large tertiaty care centre* – CIRSE 2017

Guerreiro H, Adam G, Regier M; *Transcatheter embolisation of iatrogenic arteriovenous fistula in renal allografts* - CIRSE 2018 Book of Abstracts (P-664)

Guerreiro H, Busch JD; *Implantation of totally implanted venous access devices in the upper arm – technical considerations* - CIRSE 2018 Book of Abstracts (P-180)

H. Guerreiro, H. Schröder, F.D. Busch, J. Spretke, K. Sellenschloh, G. Huber, G. Adam, H. Ittrich, J.D. Busch; *Quantification of mechanical properties of long-term in vivo used silicone catheter lines according to DIN 10555-3* – CIRSE 2019 Book of Abstracts (P- 901)

Guerreiro H, Nielsen M, Sentker T, Schmidt E, Kniep H, Fiehler J, Werner R; *Deep learning-based automated device detection for assessment standardisation in mechanical thrombectomy;* ePoster – 14th ESMINT Congress, 7-9th September 2022

Guerreiro H, Wortmann N, Andersek T, Ngo TN, Frölich AM, Krause D, Fiehler J, Kyselyova AA, Flottmann F; *Novel synthetic clot analogs for in-vitro stroke modelling;* ePoster – 14th ESMINT Congress, 7-9th September 2022

Guerreiro H, Flottmann F, Kyselyova AA, Wagner M, Brekenfeld C, Eckert B, Illies T, Wodarg F, Fiehler J, Bester M; *First Experience with Walrus Balloon Guide Catheter in a Whole-Body Flow Model;* 54. Jahrestagung der Deutschen Gesellschaft für Neuroradiologie e.V.

Courses and Qualifications

NExT Coiling Workshop   Mar. 2023

Microvention Headquarters Düsseldorf

Workshop Contour Device   Jun 2022

Department of diagnostic and interventional Radiology and Neuroradiology, Kiel

Workshop & Hands-on-Training Extra- und intraaneurysmal Flow-Diversion   Sep. 2021

Department of Neuroradiology University Hospital Erlangen, Germany

eFellowship Interventional Neuroradiology   May – Oct. 2021

Deutsche Gesellschaft für Neuroradiologie

European Course in Minimally Invasive Neurological Therapy (ECMINT 4)   May 2021- Dec 2022

European Society of Minimally Invasive Neurological Therapy (ESMINT)

8th Stroke Winter School   Jan/Feb. 2021

ESO-ESMINT-ESNR

European Course in Pediatric Neuroradiology 10th Cycle Module 1&2   2020/2021

European Society of Pediatric Neuroradiology

Interventional Radiology Workshop – Part 1 and 2   Nov. 2018, Apr. 2019

(Herbst-Fortbildung Interventionelle Radiologie)

Städtisches Klinikum Karlsruhe, Zentralinstitut für Bildgebende Diagnostik

Alumni in the Program *Researchers for the Future (Forscher für die Zukunft, FFZ)*   Year 2016/2017

German Radiological Society

Intensive Course of Musculoskeletal Radiology   Sept. 2017

German Radiological Society

Research with Mouse Models   Sept. 2015

University Medical Centre Hamburg – Eppendorf (2-Day Course)

German Certificate for Radiation Protection   May, Dez. 2015

(X-Ray, CT and Interventional Radiology)

North German Institute for Radiation Protection of the Christian-Albrechts University in Kiel and Berlin Medical Board, Germany

Medical Car of Emergency and Ressuscitation   June 2012

ALS, ATLS, PLS, Catastrophe Management, paediatric and obstetric emergencies (6 Weeks)

National Institute of Medical Emergencies (*INEM* – Portugal).

Advanced Life Support (ALS)   June 2012

Alento – Organization for Training in Reanimation

Certification: Portuguese Resuscitation Council und European Resuscitation Council

Undergraduate Training

Internal Medicine Internship (1 Month)   Jun. 2008 – Jul. 2008

Department of Internal Medicine, Hospital Universitario Calixto Garcia, La Havana, Cuba

Paediatrics Internship (1 Month)   Jul. 2008 – Aug. 2008

Department of Paediatrics, Hospital Universitario Calixto Garcia, La Havana, Cuba

Clinical Rotation in Paediatrics (6 Weeks)   Mar.2008 – Apr.2008

Department of Paediatrics, Santa Maria Teaching Hospital, Lisboa, Portugal

Personal Skills and Competences

Languages

Portuguese (native language)

English, German and Spanish (proficient)

French and Czech (basic)

Medical Software

Medical imaging/Processing: Scanview, MRI Processor, FreeSurfer, Osirix, MevisLab, PACS

Centricity (GE Healthcare)
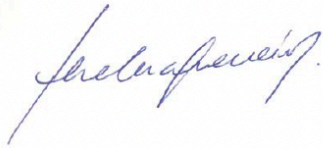
Glintt Healthcare Solutions Software

Mint Lesion Medical Software (RECIST-Evaluation)

3D Printing for research purposes: Seg32; Meshmixer; PreForm

Hobbies

Analogical Photography, Yoga, Cinema, Literature and Music

Hamburg 31th March 2023

(Helena Guerreiro)

8. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: ....