In vitro investigation of an intracranial flow diverter with a fibrin-based, hemostasis mimicking, nanocoating

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Abstract
Flow diversion aims at treatment of intracranial aneurysms via vessel remodeling mechanisms, avoiding the implantation of foreign materials into the aneurysm sack. However, complex implantation procedure, high metal surface and hemodynamic disturbance still pose a risk for thromboembolic complications in the clinical praxis. A novel fibrin and heparin based nano coating considered as a hemocompatible scaffold for neointimal formation was investigated regarding thrombogenicity and endothelialization. The fibrin-heparin coating was compared to a bare metal as well as fibrin- or heparin-coated flow diverters. The implants were tested separately in regard to inflammation and coagulation markers in two different in vitro hemocompatibility models conducted with human whole blood (n = 5). Endothelialization was investigated through a novel dynamic in vitro cell seeding model containing primary human cells with subsequent viability assay. It was demonstrated that platelet loss and platelet activation triggered by presence of a bare metal stent could be significantly reduced by applying the fibrin-heparin, fibrin and heparin coating. Viability of endothelial cells after proliferation was similar in fibrin-heparin compared to bare metal implants, with a slight, non-significant improvement observed in the fibrin-heparin group. The results suggest that the presented nanocoating has the potential to reduce thromboembolic complications in a clinical setting. Though the new model allowed for endothelial cell proliferation under flow conditions, a higher number of samples is required to assess a possible effect of the coating.

1. Introduction
Intracranial aneurysms are defined as a sagging in the wall of brain arteries and entail a certain risk of rupture [1], depending among others on morphological and hemodynamical factors. The consequences of a bleeding into the surrounding tissue include severe disability and death. The lethality of these incidents still remains at 35% in Europe and only one third of the survivors can return to their previous quality of life [2, 3]. Thus, the early detection and therapy of intracranial aneurysms is of utmost importance. Treatment of intracranial aneurysm with flow diverters has become established in the last 20 years [4], representing a paradigm shift compared to intraaneurysmal embolization techniques, where foreign material—e.g. in the form of metal coils—is inserted into the aneurysm to trigger clot formation [5, 6]. By means of a tightly woven fine meshed structure with a high metal density, flow diverters direct the blood flow mostly past the aneurysm. Intraaneurysmal blood flow deceleration as well as contact with foreign material favors coagulation in the aneurysm sack and thus a gradual development of an intraaneurysmal thrombus which prevents further bleeding [7]. At the same time flow diverters serve as a scaffold for endothelial cell proliferation and neointimal formation [8]. Both intraaneurysmal thrombus formation and primarily endothelialization of the scaffold [9] result in high occlusion rates within
vent platelet activation and coagulation. Reliable, well-tested and widely used technique to prepare the surface of the blood-contacting material is a network in a first step and, to further reduce thrombogenicity, covalently attached to heparin molecules. The resulting fibrin strands reinforce the platelet plug after vessel injury, stopping the bleeding and marking the final step of hemostasis. The fibrin network remains at the wound side later and assists the cells repairing the vessel endothelium by functioning as a temporary scaffold. Since the building of a fibrin network is presumably reduced or missing at strongly antithrombogenic surfaces, we hereby present a coating which mimics the structure of a naturally occurring vessel injury with the aim of an unimpaired or even promoted neointima formation and thus vessel healing and aneurysmal neck reconstruction.

For this study we investigated the passive behavior of the coated flow diverter in comparison to the bare implant surface in terms of coagulation, platelet activation and adhesion using two different mock circulatory loops perfused with heparinized human whole blood. The chandler loop as well as the flow loop model belong to the class of shear flow models, which stand out due to their ability to mimic vascular blood flow. The first mentioned requires the presence of air within the closed tube to enable blood circulation which comes with the risk of triggering aggregation of leukocytes and platelets as well as protein denaturation. The second model overcomes these drawbacks since it is pump controlled. However, this can at the same time be a disadvantage due to the mechanical stress that is applied to the blood through the pump. It may induce destruction of erythrocytes and reduce the models sensitivity. In order to avoid that certain effects are overlooked due to the disadvantages of one or the other model, we have used both models for our evaluation.

Furthermore, we developed a new model for the perfusion of implants with a highly concentrated solution of primary human endothelial cells and evaluated their adherence and viability on the surfaces of both coated and bare implants. We discuss the potential impact of this new technology in the scope of in situ tissue regeneration as an alternative healing strategy to mere antithrombogenic or drug delivery based approaches.

2. Materials and methods

2.1. Coating procedure

For assessment of thrombogenicity, implants were coated with heparin-bounded fibrin or with fibrin alone in accordance with the procedure previously depicted by Kaplan et al. Briefly, fibrinogen was adsorbed on the surface and thrombin was attached to the binding site of the adsorbed molecule. Subsequent to the binding site of the adsorbed molecule. Subsequently, the surface was exposed to a solution containing more fibrinogen as well as antithrombin III and heparin. Due to the catalytic action of the
Figure 1. Schematic overview of the hemocompatibility evaluation of stents in a Chandler loop model (A) and the flow loop model (B). The table (C) indicates the different model parameters regarding the blood volume, tubing dimensions and the motion system.

2.2. Blood sampling

For hemocompatibility testing, blood of healthy donors was drawn by venipuncture. The collected blood samples were anticoagulated with 1.5 IU heparin ml\(^{-1}\) blood (LEO Pharma, Neu-Isenburg, Germany) and the intake of hemostasis-affecting drugs within the last 14 d were set as exclusion criteria to guarantee uninfluenced clotting behavior. This procedure is approved by the Ethics Committee of the medical faculty at the University of Tübingen (Project identification code: 270/2010BO1) and all subjects gave their written informed consent for inclusion before they participated in the study. This study was conducted in accordance with the Declaration of Helsinki.

2.3. Blood circulation models

Hemocompatibility investigation of the samples were conducted in two different models with different tube sizing and flow mechanisms.

The Chandler loop is a closed tube system that is partly filled with air and clamped into a rotating device resulting in blood circulation through the tubing \[39\]. Polyvinyl chloride (PVC) tubes with an inner diameter of 6.35 mm (Medtronic, Dublin, Ireland) and a length of 50 cm were loaded with the different test specimen (one specimen for each tube). Subsequently, the tubes were filled with 12 ml of heparinized (1.5 IU ml\(^{-1}\)) human whole blood (\(n = 5\)), closed with a silicon tubing and connected to the rotating device. The tubings were rotated vertically at 30 rotations per minute (rpm) in a water bath at 37 \(^{\circ}\)C for the next 60 min (figures 1(A) and (C)).

In the flow loop model blood is perfused through a heparinized tubing containing the test specimen by using a peristaltic pump at a specific rate as previously described extensively \[40\]. First, heparin-coated (Ension, Pittsburgh, USA) PVC tubes with an inner diameter of 3.175 mm (Saint-Gobain Performance Plastics, Courbevoie, France) and a length of 75 cm were loaded with a test specimen each. Subsequently, the tubings were connected to the pump head (Cole Parmer, Wertheim, Germany), and filled with a 0.9% sodium chloride (NaCl) solution (Fresenius-Kabi, Bad-Homburg, Germany) and the flow rate of the peristaltic pump was adjusted to 150 ml min\(^{-1}\). After removal of the NaCl solution tubings were filled with 6 ml heparinized human whole blood (\(n = 5\)) through a syringe (Henke-Sass-Wolf, Tuttingen, Germany) and tightly closed by using a silicon connection tubing. The tubings were placed in a 37 \(^{\circ}\)C water bath before the perfusion was started for 60 min (figures 1(B) and (C)). In each model, one empty tubing served as a control.

2.4. Blood count analysis and plasma collection

Several hemocompatibility markers were investigated from the freshly drawn blood (defined as

fibrin-bound thrombin, protected from inhibition through heparin-antithrombin III, the growth of a fibrin mesh on the surface was enabled up to a thickness of around 10 nm. For further heparin coating, heparin was activated and immediately covalently attached to the fibrin mesh. Reference implants were coated with (a) fibrin alone (following previous steps except for the covalent bonding of heparin), (b) heparin alone (performed as previously described \[25\]) or (c) without coating (bare surface). For endothelialization tests, only heparin-bounded fibrin and bare surface stents were evaluated.

Bare as well as coated implants were provided by Acandis GmbH (Pforzheim, Germany) based on the design of the Flow Diverter Derivo Embolization Device\( ^{\circledast}\), a self-expanding braided device with flared ends intended for the treatment if intracranial aneurysms of 2.5 mm to 6.0 mm vessel diameter. The device consists of 24 radiopaque Nitinol composite wires with electropolished surface finishing (BlueXide\( ^{\circledast}\)) looped at the distal end resulting in a 48-wired braid. The sample dimensions (diameter \(\times\) length) of the samples for the later described flow loop and Chandler loop model was 4.0 \(\times\) 40 mm and 6.0 \(\times\) 50 mm, respectively.

| Model parameters: | Chandler Loop Model | Flow Loop Model |
|-------------------|--------------------|-----------------|
| Blood volume      | 12 mL              | 6 mL            |
| Heparinization    | 1.5 IU / mL        | 1.5 IU / mL     |
| Tube length       | 50 cm              | 75 cm           |
| Tube diameter     | 6.35 mm            | 3.175 mm        |
| Motion system     | Rotating device: 30 rpm | Peristaltic pump: 150 mL / min |
| Incubation time   | 1 h                | 1 h             |
| Temperature       | 37 \(^{\circ}\)C    | 37 \(^{\circ}\)C  |
baseline) as well as after the tests in both flow models. Whole blood count analysis was performed for every sample using the blood analyzer (Axon Lab, Baden-Dättwil, Switzerland). Also, different plasmas were generated by incubating freshly drawn or perfused blood in monovettes containing either ethylenediaminetetraacetic acid (EDTA, Sarstedt, Nürnberg, Germany), citrate (Sarstedt, Nürnberg, Germany), or the citrate, theophylline, adenosine and dipyridamole mixture (CTAD; BD Biosciences, Heidelberg, Germany) as anticoagulatory agents. A comprehensive protocol for plasma generation was previously published [40].

2.5. Quantification of hematological markers from plasma samples
Specific enzyme-linked immunosorbent assays (ELISAs) were performed to quantify thrombin-antithrombin(TAT)-complex (Siemens Healthcare, Marburg, Germany) from citrate plasma, β-thromboglobulin (β-TG) (Stago, Düsseldorf, Germany) from CTAD plasma and the terminal complement complex (TCC = SC5b-9) (Tecomedical, Sissach, Switzerland) from EDTA plasma. All ELISAs were carried out in strict accordance with the manufacturer’s protocols.

2.6. Cell culture of human umbilical vein endothelial cells
For the investigation of the different stent surfaces interacting with endothelial cells, human umbilical cord vein endothelial cells (HUVECs) of five different donors were used. The cells were cultivated in VascuLife basal medium supplemented with the EnGS-Mv Microvascular Endothelial Kit except for hydrocortisone hemisuccinate (medium and supplements: Lifeline cell technologies, Frederick, USA) at 37 °C and 5% carbon dioxide (CO₂). Medium was changed twice a week and when confluent, HUVECs were trypsinized and centrifugated. After resuspension, HUVECs were counted with the CASY cell counter (Schräle System, Reutlingen, Germany) and seeded at the desired density.

2.7. Dynamic endothelialization model
To investigate the proliferation and viability of endothelial cells on the different substrates a novel, dynamic cell seeding model with subsequent vitality assay was established.

A PVC tubing with a length of 15 cm was filled with a gelatin solution (0.1% in ultrapure water; Merck, Darmstadt, Germany) and incubated for 15 min at room temperature. Subsequently, the gelatin solution was removed from the tubing and the uncoated or coated implant was inserted. A cell suspension containing 5.0 × 10⁵ HUVECs was poured into the tubing and its ends were sealed using sterile syringe filters (Sartorius, Goettingen, Germany). The loaded tubing was statically incubated at 37 °C and 5% CO₂ for 4 h. Afterwards for dynamic cell seeding the tube was attached to a rotating device (neoLab Migge, Heidelberg, Germany) with a rotation rate of 5 rpm and incubated for another 20 h. After a total of 24 h incubation the cell suspension was removed from the tube and replaced with fresh medium containing again 5.0 × 10⁵ HUVECs. The tubings containing the implants were incubated rotating for another 24 h until they were removed for subsequent analysis.

2.8. Cell viability assay
To determine the metabolic activity and viability of the adherent HUVECs on the surface of the uncoated or coated implants a colorimetric assay was used. The cell covered stents were removed from the tubing and washed three times with Roswell Park Memorial Institute (RPMI)-medium without phenol red (Life technologies, Carlsbad, USA) and incubated for 4 h at 37 °C with a 0.05% solution of 3-(4, 5-dimethyl-2-thiazoly)-2-, 5-diphenyl-2H-tetrazolium bromide (MTT; AppliChem, Darmstadt, Germany). Afterwards, the MTT solution was removed and 300 μl dimethyl sulfoxide (DMSO; Serva, Heidelberg, Germany) were added to dissolve the generated formazan crystals. Subsequently the absorbance of the formazan-DMSO solution was measured at 540 nm with the Mithras LB 940 Microplate Reader (Berthold technologies, Bad Wildbad, Germany).

2.9. Scanning electron microscopy
To be able to visualize the structure of adherent HUVECs on the implants surface, three samples of each group were prepared for scanning electron microscope (SEM) analysis. Subsequently to cell seeding, the implants were removed from the tubing and rinsed in phosphate buffered saline without Ca²⁺/Mg²⁺ (PBS-buffer; Life technologies, Carlsbad, USA) and incubated in 2% glutaraldehyde solution (SERVA electrophoresis, Heidelberg, Germany) overnight at 4 °C. To remove the remaining water, the samples were dehydrated in ethanol p.A. (Merck, Darmstadt, Germany) with increasing concentration of 40%–100%. Finally, the samples were dried by critical point drying then sputtered with gold palladium and subsequently analyzed with the SEM (Cambridge Instruments, Cambridge, UK).

2.10. Confocal laser scanning microscopy
To show the adherent HUVECs in their native state on the implants, two samples for each group were examined using confocal laser scanning microscopy (CLSM). The samples were removed from the tubing and cut into small pieces, which have been washed in a solution of PBS-buffer containing bovine serum albumin (BSA) and Tween-20 for 10 min. The fragments were incubated for 30 min in a PBS/BSA/Tween-20 solution containing 4′,6-diamidino-2-phenylindole (DAPI, dilution 1:1000; Sigma, St. Louis, USA)
and Phalloidin Alexa 546 (dilution 1:300; Molecular Probes, Eugene, USA) to stain the cells nuclei and the actin filaments. After another wash step the sample pieces were embedded with Mowiol 4–88 (Carl Roth, Karlsruhe, Germany) and imaged using the LSM 800 confocal laser scanning microscope (Zeiss, Oberkochen, Germany).

2.11. Statistics
All data obtained are given as mean with standard deviation. The ROUT method was applied to detect outliers. The data were tested for normal distribution with the Kolmogorov–Smirnov test. Normally distributed data were tested for differences between groups by a repeated measure ANOVA with Bonferroni’s multiple comparison test. Non-normally distributed data was compared by a non-parametric test (Friedman test with Dunn’s multiple comparison test). Statistical significance has been defined as \( p \leq 0.05 \). The entire statistical analysis was carried out with GraphPad Prism 6 (GraphPad Software, La Jolla, USA) (\( p < 0.05 \); \( **p < 0.01 \); \( ***p < 0.001 \); \( ****p < 0.0001 \)).

3. Results

3.1. Hemocompatibility analysis
The capability of the flow diverters to interact with blood plays an essential role for their possible use in clinical application. To analyze whether the differently coated materials influence hematological processes, the implants were subjected to two circulating loop systems for in vitro hemocompatibility evaluation. Each of these methods has its specific advantages and disadvantages, so for a comprehensive overview of blood-implant-interactions both tests were performed and separately interpreted.

3.2. In vitro Chandler loop model
The quantification of blood cells and blood parameters revealed that the incubation of the implants with blood did not lead to any alterations regarding the numbers of white blood cells (figure 2(A)), red blood cells (figure 2(B)) as well as hemoglobin values (figure 2(C)) and the hematocrit (figure 2(D)). However, the presence of an uncoated or fibrin-only coated implant led to a significantly decreased number of platelets (200 ± 60.5 \( \times \) 10 \(^3 \) \( \mu l^{-1} \) and 205 ± 49.7 \( \times \) 10 \(^3 \) \( \mu l^{-1} \) respectively) in comparison to the implants covered with a heparin (236 ± 65.1 \( \times \) 10 \(^3 \) \( \mu l^{-1} \), \( p = 0.0074 \)) or a fibrin-heparin coating (238 ± 63.3 \( \times \) 10 \(^3 \) \( \mu l^{-1} \), \( p = 0.0044 \)) as well as to the control (250 ± 63.4 \( \times \) 10 \(^3 \) \( \mu l^{-1} \), \( p = 0.0002 \)) (figure 2(E)).

The analysis of the hematologic plasma markers in the test groups after blood incubation showed a similar picture (figure 3(A)). The TAT complex concentration, which is an indicator of the activation status of the coagulation system, was significantly increased in the bare metal stent group (42.8 ± 9.3 \( \mu g l^{-1} \)) (versus control: 20.2 ± 10.2 \( \mu g l^{-1} \), \( p = 0.0014 \); versus heparin:...
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Figure 3. Quantification of plasmatic hemocompatibility markers before and after incubation of blood in the Chandler loop. The activation of the clotting cascade (TAT) was significantly increased in the bare stent group (A), whereas the activation of the complement system (SC5b-9) (B) as well as platelet activation (β-thromboglobulin) (C) was uninfluenced by the implants. ($n = 5$; $**p < 0.01; ***p < 0.001; ****p < 0.0001$).

Figure 4. Hemocompatibility testing of implants perfused in the Flow Loop model. The uncoated stent led to significant differences in the number of white blood cells (A) but did not influence the red blood cell count (B). It also led to a significant decrease in hemoglobin values (C) whereas hematocrit remained uninfluenced (D). The number of platelets was significantly decreased too, compared to other test groups (E). ($n = 5$; $*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001$).

14.6 ± 5.7 µg l⁻¹, $p < 0.0001$; versus fibrin: 15.4 ± 6.4 µg l⁻¹, $p = 0.0003$; versus fibrin-heparin: 16.1 ± 4.2 µg l⁻¹, $p = 0.0002$), indicating a significant increase regarding the clotting activity. The heparin, as well as the fibrin and the fibrin-heparin coated stents prevented the activation of the coagulation system, since no increased TAT levels compared to the control tubing were determined.

No significant differences of SC5b-9 levels, representative for the complement cascade, and β-thromboglobulin, a marker for platelet activation, were observed between the test groups (figures 3(B) and (C)).

3.3. In vitro flow loop model

The analysis of the blood count showed that the presence of different implants led to significant changes regarding the number of white blood cells (figure 4(A)). Perfusion of a bare metal implant resulted in a significantly decreased number of white blood cells (5.3 ± 1.4 × 10³ µl⁻¹) compared to the control (6.0 ± 1.9 × 10³ µl⁻¹, $p = 0.0293$) and the incubation of blood with a fibrin coated implant resulted in a significant decrease of leukocytes (5.1 ± 1.4 × 10³ µl⁻¹) in comparison to the heparin coated implant (5.7 ± 1.3 × 10³ µl⁻¹, $p = 0.0231$). Regarding the number of red blood cells, the different test groups did not distinguish
significantly (figure 4(B)). The measured values for hemoglobin were almost equal, solely the hemoglobin content in the blood incubated with the bare metal stent was significantly lower (11.6 ± 0.8 g dl⁻¹) than in the blood incubated with the fibrin coated implant (12.2 ± 1.1 g dl⁻¹, \( p = 0.0148 \)) (figure 4(C)). The hematocrit of the blood samples did not show significant differences (figure 4(D)). However, also in this test the number of platelets was strongly affected by the incubation of blood with a bare metal implant (figure 4(E)). A significant decrease in platelet number was observed when comparing the uncoated stent (105.6 ± 26.5 \( \times \) 10³ \( \mu l^{-1} \)) to any other test group (versus control: 195.4 ± 40.0 \( \times \) 10³ \( \mu l^{-1} \), \( p = 0.0002 \); versus fibrin: 168.6 ± 28.7 \( \times \) 10³ \( \mu l^{-1} \), \( p = 0.0079 \); heparin: 196.0 ± 57.9 \( \times \) 10³ \( \mu l^{-1} \), \( p = 0.0002 \); versus fibrin-heparin: 202.8 ± 32.0 \( \times \) 10³ \( \mu l^{-1} \), \( p < 0.0001 \)).

When assessing the TAT complex levels, a tendency towards increased values for incubation with the bare metal implant were observed although there was no significant evidence (figure 5(A)).

The quantification of the platelet activation marker \( \beta \)-TG (figure 5(C)) showed that the activation in blood incubated with the uncoated implant (3280 ± 189.2 IU ml⁻¹), but not with the coated ones, was significantly increased in comparison to the control (1238 ± 333.7 IU ml⁻¹, \( p = 0.0014 \)).

3.4. Dynamic cell seeding and endothelialization
The dynamic cell seeding (figure 6(A)) with subsequent viability assay (figure 6(B)) revealed a non-significant difference (\( p = 0.2793 \)) with a tendency towards increased cell viability on the surface of the fibrin heparin coated implant compared to the uncoated implant (figure 6(C): 98.55% versus 89.28%).

Visualization of the stent surface was performed using SEM (figure 7(A)) and CLSM (figure 7(B)). The results agree with the cell viability assay: HUVECs have adhered to both implants and grow with their typical outstretched morphology.

4. Discussion
Endoluminal implants for intracranial vessel treatments face high challenges in term of anatomical,
Figure 7. Microscopic analyses of the implants after HUVEC seeding. The SEM (A) and the CLSM (B) images show evenly distributed cell growth on the uncoated stent as well as the fibrin heparin coated implant (A: 1000-fold; B: magnification 50-fold, white: DAPI, red: actin).

physiological and biological compatibility. Small vessel dimensions ranging between 5 mm in the internal carotid artery and 2 mm or less in vessels distally to the circle of Willis and a moderate flow regime ranging between 250 and 150 ml min$^{-1}$ [41] represent critical conditions in terms of blood coagulation. Particularly in flow diverters, several micro wires crossing each other at hundreds of intersection points lead to fluid dynamical changes possibly resulting in blood stagnation or shear stress modulation effects [42, 43] that are potentially responsible for activation of coagulatory pathways and platelet activation. Moreover, despite a highly flexible implant geometry and manipulation efforts during the implantation, inadequate vessel apposition is reported in clinical cases with the potential implication of blood flow disturbance such as blood stagnation between the implant structure and the vessel wall [44]. Radial expansion force and possibly a repetitive implant elongation and axial contraction affected by the pulsatile blood pressure curve can potentially stress the endothelium. Finally, a foreign surface is known to be a potential trigger for thrombotic reactions and can intensify the fluid dynamic effects and resulting endothelium damage. Taken together, the mentioned factors are probably the cause for the observed clinical complications after flow diverter implantation, like in-stent thrombosis, side branch occlusion or distal embolism, during the procedure as well as in the short-middle term [45]. These circumstances bear the risk that the prevention of aneurysm rupture can quickly turn into a device-related ischemic stroke, a dramatic scenario for patients who undergo the treatment in part without having become symptomatic. Reflecting this background, it appears reasonable to design an implant surface that can prevent platelet activation and coagulation.

Moreover, the main requirements for the coating of implants for blood vessels that run through the head and neck are crucially different from the ones used for coronary stents. Whereas usually, for the latter kind of implants prevention of restenosis is the main requirement to coating performance and is mostly achieved by using cytostatic drugs to impede cell proliferation, this approach could be counterproductive in intracranial vessel treatment. Indeed, the focus is shifted here to the reconstruction of the vessels wall at the aneurysm neck, depicting the opening between the vessel and the aneurysm, by controlled proliferation of endothelial cells and thus neointima formation. These considerations result in two main requirements for the implant surface. First, a fast and controlled neointima reconstruction at the aneurysm neck and along the whole implant structure should assure the complete separation of the implant from the blood flow for a long-term prevention of the thrombus formation. Second, the implant should be antithrombogenic, avoiding acute thromboembolic complications before completed endothelialization, possibly leading to an ischemic stroke. As soon as the complete vessel regeneration has occurred, an
antiplatelet therapy is obsolete, bearing the advantage of reduced bleeding complications.

Considering the physiology of wound healing, the thrombus formation and the related thromboinflammatory process is the main mechanism which triggers cell proliferation and thus the regeneration of the injured tissue [46]. With other words, the fibrin network forming the scaffold for the thrombus is the natural, biological substrate where endothelial cells, among other cells like fibroblasts and leukocytes, infiltrate, proliferate and finally build a new vessel wall, while at the same time providing blood supply into the new formed tissue [47]. Since formation of a fibrin strain network is impaired on the surface of strictly antithrombogenic stents, we conceived and developed a novel coating technology with the aim of an unhindered or even intensified neointima formation despite an antithrombogenic behavior. The artificial fibrin network in the coating is conceived to mimic the final step of hemostasis, the phase in which further thrombus formation is inhibited and endothelial cells proliferate and support tissue regeneration. At the same time, integration of covalent bonded heparin aiming at a reduction of coagulation.

Referring thereto, extensive analysis about the interaction of human whole blood with the novel as well as control coatings was performed in this study. The implants consisted on a mesh of microwires made of nitinol, an alloy well known for its outstandingly biocompatibility [48] were tested separately with two different in vitro hemocompatibility models. Both models independently of each other showed a significant reduction of platelet adhesion in fibrin-heparin and heparin coatings compared to bare implants, while formation of the TAT complex was reduced for all coated implants in Chandler Loop model. The anticoagulatory character of the fibrin heparin coating is especially reflected in the increased number of platelets compared to a fibrin only coating on the stent surface in the Chandler Loop model: it is a well-known fact that heparin prevents blood clotting [33, 49]. Moreover, fibrin alone reduced platelet adhesion compared to bare implants in the Flow Loop model, confirming a certain passive behavior of the fibrin network also in absence of fibrin.

To evaluate and compare the interaction of coated and bare surfaces with endothelial cells, a novel dynamic cell seeding model was developed and for the first time presented in this study. Implants were perfused for 48 h by an endothelial cell containing solution. Due to the fixation of the implant containing a tube to a rotation device, an alternate flow was produced within the tube lumen.

The results, including viability assay, SEM and confocal microscopy, indicate that the coating is slightly though not-significant superior in term of endothelial cell proliferation then the bare electropolished surface of the Nitinol implant, whose good cell compatibility was proved in a previous study [50].

The findings make in our view the novel approach potentially superior to a complete inhibition of hemostasis by means of a mere antithrombogenic stent surface, since this could potentially interact with the crucial mechanisms of vessel regeneration and compromise, delay or in the worst case inhibit the building of a neointima layer.

Surface optimization is a very central area of research regarding the clinical application of biomedical progresses since the body is a harsh environment for materials and, at the same time, materials can provoke an adverse effect on the biological environment [51]. Whereas the initial idea was to hide potentially corrosive surfaces under an inert coating [52] the focus today is put on biomimicking coating strategies, which prevent the biomaterial from being recognized as foreign bodies. As mentioned before multiple approaches are used to address this such as the immobilization of biomolecules like heparin [53].

Another type of coating, besides heparin based, is the use of phosphorylcholine, which has been applied as flow diverter coating mimicking the nonreactive surfaces of red blood cells [34] and resulting in improved thrombogenicity of the implant [30, 55]. Moreover, use of hydrophilic coatings have been shown to reduce the adhesion of platelets on the implant surfaces and hence provide anti-thrombogenic properties [56].

Further research is needed to evaluate long-term efficacy and safety of surface technology. Though in vitro tests allow reproducible and standardized conditions, in vivo studies are needed to account for the complex inflammatory and coagulatory pathways occurring in a living organism. Further limitations of our study relate to the tube material, which implies an inherent thrombogenicity and thus makes the use of heparinized blood unavoidable. Moreover, standardized tube geometry does not account for mechanical properties in complex vessel geometries. Coating of surface could potentially influence wire friction in the phase of the implantation and thus wall apposition and influence on blood flow.

Last, we performed the study with five samples per group. At this size, no significant differences were detected for different parameters, though a trend was detectable. A higher number of samples would be favorable for future experiments, particularly if small differences are expected because of a relatively small implant surface compared to the tube setting.

5. Conclusion

In an in vitro setup, we investigated and proved the cell as well as blood compatibility of a novel, fibrin-based coating technology compared to different control surfaces. A novel mock loop allowed
for the evaluation of endothelial cell proliferation under dynamic conditions. It could be shown that the implant with the fibrin-heparin coating has a significantly lower tendency to trigger platelet adhesion on the stent surface as well as to cause the formation of TAT after incubation with blood when compared to the bare metal implant. Furthermore, there is a reduced tendency towards the secretion of \( \beta \)-TG indicating that the activation of platelet is can be reduced with applying the fibrin-heparin coating to the implant.

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