Autologous plasma coating - a new approach for improvement of the biocompatibility of mesh implants

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SUMMARY

Two recent warnings by the US Food and Drug Administration (FDA) relating to severe side-effects led to discussions concerning the biocompatibility requirements of surgical meshes. Our aim was to develop a standardized and manufacturer-independent *in vitro* test system for the adherence performance of tissue clusters (fibroblasts, endothelial cells and muscle-derived cells) as a marker for the biocompatibility of commercially available meshes. In this test system, we could establish a repeatable ranking of meshes with regard to their biocompatibility. The adherence behavior was independent of the individual patient features, suggesting that the biological behavior of a mesh is probably conditioned by the structure of the biomaterial or/and its chemical composition rather than by individual host characteristics/features. This *in vitro* test system has been shown to be a feasible pattern for the investigation of different mesh coating strategies. The coating of meshes prior to cultivation, *e.g.* with peripheral blood mononuclear cells (PBMCs), did not affect the adherence score, whereas coating with platelets and blood plasma increased the score, suggesting improved biocompatibility *in vitro*. Plasma coating exhibited the greatest potential to improve the *in vitro* adherence score. The previous ranking of native meshes remained consistent after coating, but established at a higher level.

In order to explore the predictive value and validity of the test system and also newly tested coating strategies, we translated the preliminary *in vitro* results into *in vivo* circumstances and conducted a large-animal experiment on sheep. The entire experimental approach followed the recently developed recommendations of the IDEAL (Innovation, Development Exploration, Assessment and Long-term) study for surgical innovations. In this long-term animal study, we demonstrated that our recently developed *in vitro* test system may predict the *in vivo* performances of the meshes. This effect was independent of the location of the mesh in the body, although its particular extent varies with the site of implantation. The coating of meshes with autologous plasma prior to implantation had positive effects on the biocompatibility of meshes *in vivo*. Investigation of the ultra-short-term determinants of the foreign body reaction (FBR) at the implant site *in vivo* revealed that the local inflammatory reaction is an early and susceptible event after mesh implantation. It cannot be influenced by prior plasma coating and does not depend on the localization of implantation. The project is continuing, the method currently being implemented in humans.
INTRODUCTION

Biocompatibility is defined as “the ability of a material to perform with an appropriate host response in a specific situation”, implying a symbiotic relationship of acceptance between the host and the respective implanted material. For a material to perform best, it needs to be integrated properly into the tissue, to generate an appropriate inflammatory response and to maintain mechanical integrity. As biocompatibility is described by the foreign body reaction (FBR) at the host-tissue/biomaterial interface, three crucial steps have been identified that describe the time course of the FBR: protein absorption, cell recruitment and, finally, fibrotic encapsulation and extracellular matrix formation. The dynamics of the FBR is greatly influenced by the biomaterial composition, and in particular the type of polymer, the material weight, the filament structure and the pore size. In accordance with early results, it is currently accepted that large porous meshes lead to the best tissue integration and, thanks to the reduced surface area, produce the least FBR, inflammation and fibrosis. Small-pore or microporous and foil- or layer-like mesh modifications, however, are associated with a significantly greater FBR and inflammation frequently related with the phenomenon of bridging, which finally may cause a significant contraction or shrinkage of the mesh.

Polypropylene (PP), polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE) are currently the most commonly used mesh materials, whereas PP seems to be the most frequently used polymer for the construction of surgical meshes. There appears to be a trend in current mesh development toward minimization of the mesh density and the use of macroporous weave patterns of monofilament PP. In a review analyzing the mechanical properties of and the tolerance to synthetic implants for stress urinary incontinence (SUI) and pelvic organ prolapsed (POP), Cosson et al. identified PP (known to offer durability and elasticity) as the most promising material for those indications.

Biocompatibility assessment of alloplastic materials through the use of appropriate cell cultures in vitro is a valid and accepted method which furnishes information about the toxicity of the investigated material, and the possible effects on the metabolism and growth of the cells. Although cells can be sensitively characterized with this method, and the conditions can easily be standardized, no complex tissue representative of the human body has yet been investigated.

When implanted for a particular indication, a mesh represents a foreign body which induces a FBR, which is an important surrogate for its biocompatibility performance. A considerable influence on the dynamics of the FBR is exerted by the biomaterial
composition, and in particular the type of polymer, the material weight, the filament structure and the pore size. The exact FBR mechanisms and respective time flow in vivo are not entirely understood, but the rapid accumulation of huge numbers of phagocytic cells, and especially blood monocytes and tissue-derived macrophages, play a crucial role in it.

Application of surgical meshes and current controversies

In a Public Health Notification issued in 2008, the FDA reported more than 1000 unexpected and severe adverse events, mostly associated with transvaginal placement of a surgical mesh. In 2011, a second FDA warning was announced on the basis of 2,874 newly identified Medical Device Reports: 1,503 associated with POP repairs, and 1,371 associated with SUI repairs. These two FDA warnings have led to several regulatory changes for surgical meshes, including the upgrading of risk classifications, requirements for clinical studies to address the risks and benefits of meshes used to treat POP and SUI and the expanded post-market monitoring of device performance. In the assessment of quality standards for surgical meshes, comparability with other meshes with regard to quality and stage of development should be possible. Despite the existence of several models for the assessment of different meshes with regard to their particular biomechanical characteristics, there are currently virtually no standardized tools for comparisons among meshes. Many alloplastic materials are still being used without proper trials and are recommended by manufacturers rather than on the basis of data arising from in vitro or in vivo experiences.

In 2009, the Lancet dedicated a series to the topic of ”Surgical Innovation and Evaluation” and its current status. A five-stage description of the surgical development process has been proposed, the IDEAL model which allows the assignment of every surgical innovation, e.g. surgical technique, alloplastic materials, etc., to its particular corresponding step of development. The IDEAL framework, has so far been used in retrospective studies, and in particular in the description of surgical procedures. However, this framework is highly recommended for the application in the development of surgical innovations different from surgeries.

MAIN GOALS

The initial purpose of our studies was to develop and standardize an in vitro test system with which to investigate biocompatibility features of surgical meshes. Once having developed this test system, we aimed to implement mesh-coating strategies and subsequently to investigate their influence on biocompatibility in in vitro performance. In order to explore the predictive value and validity of the test system and also newly tested coating strategies,
we conducted a large-animal experiment in sheep. The entire approach followed the recently developed recommendations of IDEAL for surgical innovations in order to ensure comparability, visibility and confirmability

1. **Study I.** The main purpose was to investigate and develop an *in vitro* approach for an assessment of the biocompatibility features of surgical meshes. Seven different mesh types, currently used in various indications, were randomly selected and microscopically investigated after incubation for 6 weeks with regard to their adherence performance, using a tissue culture approach, with tissues representative of fibroblasts, muscle cells and endothelial cells, originating from 10 different patients.

2. **Study II.** We aimed to investigate different mesh coating modalities with autologous blood components and their impact on the biocompatibility performance of the meshes *in vitro*, using the previously developed test system. Seven different mesh types were therefore coated prior to cultivation with autologous PBMCs, platelets and blood plasma and subsequently incubated for 6 weeks in a minced tissue assay. The adherence performance of the tissues on the meshes was investigated microscopically, assessed semi-quantitatively and compared with the native counterparts, using a previously developed scoring system.

3. The goals of **Study III** and **Study IV** were to translate the preliminary *in vitro* results into an animal model in order to validate the *in vitro* test system and to explore its predictive value for *in vivo* surroundings. Three different meshes [TVT (Tension-free Vaginal Tape), UltraPro® and polyvinylidene fluoride PVDF] with different previous *in vitro* performance scores were implanted in female sheep in a native or a plasma-coated version. In the ultra-short-term study, meshes were explanted and investigated histochemically for inflammatory infiltrate, macrophage infiltration, vessel formation, myofibroblast invasion and connective tissue accumulation at the implant site at 5 min, 20 min, 60 min and 120 min. In the long-term study, meshes were explanted after 3, 6, 12 or 24 months and processed.

**MATERIAL AND METHODS**

**In vivo experiments, meshes/patients**

We randomly identified alloplastic materials currently applied as implants for different surgical indications covering hernia repair, POP and SUI. A total of 7 different meshes were investigated. The alloplastic materials were prepared in 2 x 2 cm fragments for further investigations. Additionally, we harvested tissue probes of muscle, fascia and renal vein from 10 patients undergoing right-side nephrectomy. All patients gave their informed
consent previously. The tissues and cells were processed identically in all patients. Each mesh was tested with the tissue and cells of each patient for comparison purposes.

**Tissue preparation and mesh incubation**

We extracted tissue probes originating from muscle, fascia and renal vein at a length of 0.5-1.0 cm each from 10 different patients. After crushing, we incubated the tissue with phosphate-buffered saline and, after 2 additional washing procedures, incubation was performed with DMEM/F12 plus 10% serum and 1% glutamine + 1% penicillin/streptomycin. After successful expansion and growth (80-90% adherent growth) of the tissue pellets, the different alloplastic materials were added. Thus, the prepared and expanded tissue probes consisting of myoblasts, endothelial cells and fibroblasts presenting relevant tissues of the pelvic floor were used in vitro in order to create a model for investigation of the integrity of the different mesh types. Myoblasts were detected by α-sarcomeric actin and desmin as markers of myogene differentiation. Fibroblasts were stained with antibodies targeting vimentin, whereas antibodies against cluster of differentiation 34 (CD34) were used for the verification of endothelial cells. We reproduced every single approach 10 times with tissue probes from the 10 different patients.

**Mesh coating**

PBMCs, platelets and plasma were used. PBMCs were separated through density gradient centrifugation using Ficoll. For the isolation of platelets and the respective mediators, the Advanced Tissue Regeneration System (ATR® by Curasan Inc) was used (http://www.curasan.de/de/produkte/dental/atr/atr.php). The plasma preparation procedure followed the classical method of Crowley. After isolation of the 3 different blood components of each patient, we incubated the meshes (2 x 2 cm) with 10 ml of the respective suspension and incubated them for 12 h prior to testing with tissue.

**Morphological study**

The adherence and the cell count (if possible) were assessed microscopically and through the use of immunohistochemistry after co-incubation of the cells with different types of alloplastic meshes. The test duration was 6 weeks. Meshes were investigated with regard to interstructural tissue connections and the quantity of mesh-adherent cells. Tissue cultures were maintained up to 4 months, with frequent changes of the medium, and assessment was repeated if possible. A descriptive/semiquantitative assessment pattern was used in order to describe the adherence of tissue to the investigated mesh materials. The assessment pattern was based on the maximum identifiable quantity of mesh-adherent cells within a tissue
cluster per vision field. Adherence performance was ranked after assessment of the quality and quantity of the tissue clusters/cells as none, fair, good or excellent.

*In vivo experiments*

The experimental protocol was approved by the Animal Welfare Committee at the University of Szeged (license/permission No. V01353/2010). Fourteen 6-month old, female sheep weighing 20-25 kg were housed and cared for at the University farm for experimental animal studies. We included 2 animals more than the 12 needed for safety calculations. All the animals had free access to food and water, and were cared for by an educated keeper and routinely inspected by a veterinarian. On the basis of the previously described test system and the resulting ranking, we selected 3 meshes representing good, intermediate and poor *in vitro* performance; PVDF, UltraPro® and TVT. We chose 3 different locations in the sheep to implant the meshes via open surgery. In order to represent different *in vivo* surroundings, 3 meshes were placed in the following localizations: a) interaperitoneally, b) as fascia onlay and c) as muscle onlay (fascia sublay). Then, 3 plasma-coated versions of the same mesh type were implanted in equivalent localizations on the contralateral side of the torso. Meshes had to be incubated with autologous plasma at least 12 h prior to implantation. This procedure was performed in 14 animals, resulting in 4 animals per mesh type (plus 2 animals with PP TVT/PVDF).

For a short-term study, 3 additional female sheep weighing from 20 - 25 kg and at least 6 months old were included. The protocol for housing and veterinary maintenance was as in the long-term study. For every native mesh implant, a respective plasma-coated version was implanted in an equivalent localization on the contralateral side of the torso. The length of incubation prior to surgery was at least 12 h. We selected 1 sheep per investigated mesh, *i.e.* resulting in 3 animals. The investigated meshes were again PVDF, UltraPro® and TVT.

After 3, 6, 12 and 24 months, three animals, respectively, underwent surgery for mesh explantation. The meshes were identified and then harvested, extent of local reactions was described macroscopically. The animals were sacrificed directly after mesh explantation and harvesting of probes of parenchymatous organs (liver, intestine, kidney, lung, heart). The harvested material was then assessed for foreign body reaction, scar formation and inflammatory reaction. For the short term study, explantation time points were 5 min, 20 min, 60 min, 120 min, respectively.

All mesh specimens were studied by light microscopy which was controlled by immunohistochemistry. The antibodies included polyclonal rabbit anti-human CD3, 1:50, as pan-marker for T-lymphocytes (DAKO, Hamburg, Germany), polyclonal rabbit anti-human
CD138, 1:50 as pan-marker for plasma cells (DAKO, Hamburg, Germany), monoclonal mouse anti-porcine CD68, 1:50 (DAKO, Hamburg, Germany), as pan-marker for macrophages, monoclonal anti-human CD15, 1:10 (Becton Dickinson, Heidelberg, Germany) as marker for polymorphonuclear granulocytes, polyclonal rabbit anti-actin protein, 1:200 (DAKO, Hamburg, Germany) and monoclonal anti-CD34 1:200 (BIOMOL, Hamburg, Germany), as markers for fibromyocytes, and monoclonal porcine CD31, 1:10 (DIANOVA, Hamburg, Germany), as marker for endothelial cells. The morphometric evaluation consisted of a quantitative cell analysis of the inflammatory reaction and soft-tissue reaction. The parameters measured were the inflammatory infiltrate (µm), connective tissue (µm), vessels (%), macrophages (%), leukocytes (%), polymorphonuclear granulocytes (%), and fibroblasts (%), and TUNEL, Ki67 and HSP 70 expressing cells (%).

RESULTS

Tissue culturing was successful in 100% of the probes. After 6 weeks, the investigated meshes were ranked according to the descriptive/semiquantitative Melman score. Interestingly, after 4 months of tissue culturing the adhesion performance was comparable for all the meshes. The entire experiment was reproduced as described and a modified Melman score was subsequently used for the 3 different coating approaches for each patient. Analysis of the PBMC-incubated meshes indicated tissue ingrowth comparable to that for the native mesh. Interestingly, the meshes previously incubated with ATR® (Curasan Inc) and the plasma-coated meshes exhibited a slightly better performance. This trend was reproduced after 4 months of tissue culturing. All individuals displayed comparable effects of tissue ingrowth in the native state and after coating with the different blood components.

In vivo experiments

After the surgical implantation procedure we did not see major complications in the animals during the long-term study during 24 months. In the microscopic investigation of the different mesh reactions after explantation, the main focus was on the parameters measured for inflammatory infiltrate, connective tissue and macrophages (CD68). High levels of connective tissue reaction and inflammatory reaction were assumed as indicative of a reduced biocompatibility. The ranking originating from the in vitro test system was reproducible, characterizing PVDF as the mesh (among the 3 meshes investigated) with the least FBR, scar formation and inflammatory reaction at every individual time point. Reinforced PP (UltraPro®) was in second and PP (TVT) in third position. This constant ranking was repeated throughout the entire experiment. Moreover, the modified coated versions of the 3
meshes revealed the same result at a lower level of the respective reactions, the entire experiment suggested a beneficial effect of plasma coating prior to implantation.

During the ultra-short-term study, high levels of connective tissue reaction and inflammatory reaction were assumed to be indicative of a reduced biocompatibility. Within the first 2 h after implantation, an early invasion of macrophages at a comparable extent was observed in all meshes, culminating after 120 min. The induced inflammatory reaction expressed by the extent of inflammatory infiltrate revealed the same trend, but increased slowly. Macrophage invasion was detectable after 20 min at a relatively high level of about 50%, and increased slightly up to 70%. Interestingly, the macrophage invasion was highest in the PVDF meshes, which in the long-term approach performed best with lowest chronic inflammation. The respective early inflammatory infiltrate continuously increased within the first 60 min in all the investigated meshes. However, after 120 min this trend was reversed in the PVDF meshes. In contrast for TVT and UltraPro® the inflammatory infiltrate was still increasing up to 120 min. Not surprisingly, no connective tissue was observed after 120 min. Additionally measured endothelial cells representative of vessel integration and myofibroblasts were all negative during the initial 120 min after implantation. Two markers, representative of early FBR signs, did show relevant activity within the first 2 h after mesh implantation. For those markers, therefore, inflammatory tissue and macrophage invasion, a comparison of the coated versus the uncoated version of the respective meshes was possible, but did not indicate relevant differences.

Each mesh (coated and uncoated) was placed and investigated in 3 different positions of the torso. Differences regarding the reaction of the FBR determinants on the meshes were not observed either in the short-term or in the long-term approach when the different implant locations were compared. Further, the plasma coating did not influence the mesh performance in the different regions of the body.

DISCUSSION

The aim of Study I was to investigate whether different tissues of the pelvic floor demonstrate different in vitro interaction characteristics with alloplastic materials currently used as meshes in different clinical indications, i.e. biocompatibility features rather than mechanical characteristics. We searched for a feasible and reproducible test system that allows the assessment and comparison of meshes with regard to their in vitro adherence scores to different tissues, as markers of their biocompatibility.
The biocompatibility assessment of alloplastic materials through the use of appropriate cell cultures in vitro is a valid and accepted method which yields information about the toxicity of the investigated material, and possible effects on the metabolism and growth of the cells. Although investigation of the adherence of specific cells is useful, we consider that the co-incubation of implants with tissue clusters is comparable to in vivo processes. Besides the presence of fibroblasts, we investigated muscle-derived and endothelial cells presenting relevant tissues of the pelvic floor. These observations resulted in a ranking of the investigated meshes as concerns their affinity for the co-incubated tissues/cells. Our ranking is in good accordance with the suggestions of Amid. However, material features other than pore size seem to play a role for the in vitro performance, explaining the different scores. Moreover, it emerged reproducibly that the adherence behavior was independent of the individual patient features, thereby supporting the idea that the biological behavior of a mesh in contact with host tissues, is mostly conditioned by the structure of the biomaterial or/and its chemical composition rather than by individual host characteristics/features. PVDF, a polymer with good textile and biological properties, displayed the best adherence performance in our test system. This polymer is currently applied as PVDF-coated PP mesh for intraperitoneal-only repair. The main characteristics of this mesh are its macroporosity, a decreased adhesion rate to the bowel and a favorable biocompatibility in vivo, with low rates of inflammation and fibrosis.

The cultivation and positive adherence of cell clusters consisting of these cell types and the respective assessment and comparison, as shown here, may be helpful in the consideration of a mesh as regards its possible tissue ingrowth and capacity to form connective tissue. However, the in vivo behavior of a particular alloplastic material cannot be reliably extrapolated from in vitro studies, and appropriate in vivo approaches are therefore needed. The possible predictive value of these in vitro results with respect to cell and tissue adherence in vivo was the target in the following animal investigations.

**In vitro plasma coating**

The aim of our study was to implement and assess a facile mesh-coating procedure in vitro and to investigate whether the coating of meshes with autologous blood components shows different in vitro interaction characteristics with different tissues types as compared with native meshes. We used autologous blood components as they are relatively easy to obtain from the respective patients and contain relevant cells and substances involved in the humoral immune defense. This approach was based on the assumption that the extent to which an implanted alloplastic material elicits an acute local inflammatory response has an
impact on the long-term outcome when applied \textit{in vivo}. In order to investigate cellular and non-cellular components, we separately investigated PBMC, plasma and platelets with the respective mediators. Incubation with PBMCs did not result in modification of the adherence score for the investigated tissues. This may be explained by the reduced ability of these cells to maintain permanent contact with the polymer surface of the meshes, as previously shown. In contrast, blood plasma and ATR resulted in a better adherence performance and increased biocompatibility in all meshes. An interesting observation in the current study is that all meshes previously ranked with regard to their biocompatibility performance displayed an increased score after plasma coating and maintained their position in the ranking relative compared to the other investigated meshes. This supports the thesis that coating with plasma may have an effect independent of the mesh, and, at least \textit{in vitro}, all meshes could improve their performance, but low-ranked meshes could not increase their position relative to natively better-positioned counterparts. The assumption of the adherence performance of tissues on a mesh as a possible marker of its biocompatibility seems logical, independently of the respective clinical use of the implant. Thus, the cultivation and positive adherence of cell clusters consisting of these cell types and the respective assessment and comparison, as shown here, may be helpful for considering the possible tissue ingrowth and capacity of a mesh to form connective tissue. The coating of meshes with plasma and ATR appears to have a positive effect on those features.

A main limitation of this study was that no inflammatory reaction as normally cascading \textit{in vivo} was imitated as the \textit{in vitro} approach was sterile. The next step in the current approach was to translate the previous \textit{in vitro} results into \textit{in vivo} surroundings. In our \textit{in vivo} study, the histologic investigations for inflammatory infiltrates indicated a slight reaction associated with PVDF, which was increased in reinforced PP (UltraPro®) and even more so in PP (TVT). This reduced inflammatory reaction can be considered an expression of good biocompatibility. However, this observed postoperative sign of an inflammatory reaction was non-infectious, as counts for cells involved in the infectious immune defense, \textit{e.g.} CD3, remained unaltered at low levels. In addition, when the connective tissue investigated, the same trend was observed: PVDF exhibited the thinnest layer of connective tissue, followed by reinforced PP (UltraPro®) and PP (TVT). There was a macrophage decrease in all meshes during the postoperative follow-up, but the highest number of macrophages was seen in the TVT meshes and the \textit{in vitro} ranking was consistent as regards this marker. Macrophages are key mediators involved in the foreign body immune reaction, suggesting that this reaction was stronger in PP (TVT) than in the other two applied meshes.
As concerns the investigated parameters, macrophage invasion, inflammatory tissue and connective tissue formation, this study confirmed the previously established in vitro ranking of the 3 investigated meshes repeatedly throughout the entire animal experiment, after 3, 6, 12 and 24 months. Moreover, when the meshes were modified by pre-implant coating with autologous plasma, the ranking remained constant. This supports the assumption that the recently developed tissue culture in vitro test system for meshes is able to predict the in vivo performance of the meshes. Practically, the test system helps to distinguish between meshes with good and poorer healing performances. The previously described in vitro test system was sterile, and thus no physiological in vivo reaction such as FBR or inflammation could be imitated. This indicates that the adherence ability of a mesh is crucial for subsequent FBRs or inflammatory processes which determine the in vivo performance of the meshes. Moreover, as in the in vitro approach, we did not see individual recipient features that influenced the performance of the meshes. Besides material quality issues, we assume that the process which caused FBR to the meshes must have occurred in the early period, before 3 months after implantation, since there was no further tendency to change during the following explantations. Our results indicate that an early process is responsible for the extent of a FBR and the mid- and long-term performance of an implanted mesh. This tendency is independent of the location of the mesh in the body, although its particular extent varies, depending on the site of implantation.

Several animal studies have been proposed and reported for the investigation of local reactions after the implantation of a mesh graft. The present study is the first experimental study conducted in sheep, with a 2-year observation period, and we could demonstrate the predictive value of our recently developed in vitro cell culture approach for the biocompatibility assessment of meshes when translated to in vivo circumstances. In a second attempt, we investigated coating approaches for meshes in order to improve their biocompatibility. In preliminary experiments, mesh coating with autologous plasma was shown to reduce FBRs both in vitro and in vivo. A plasma coating seems to have a consistent improving effect on the performance of the mesh as regards connective tissue development and inflammatory local reactions at the implant site, suggesting an improved biocompatibility.

Another purpose of our large-animal study was to investigate in vivo biocompatibility predictors for 3 different meshes by measuring early and long-term signs of a FBR such as macrophage invasion, and inflammatory reaction and connective tissue determination at the implant site of the meshes. By relating the ultra-short-term data to the long-term data in the
same species (i.e. sheep), we could show that the process of determination of a FBR is defined early in the course after implantation for markers of local acute inflammation. In contrast, myofibroblast invasion, vascularization and connective tissue adhesion are not relevantly presented in the ultra-short-term course. The extent of macrophage invasion and inflammatory tissue does not relevantly increase after 120 min as compared with the values for 3 months after explantation or later. Our previously described method to improve the biocompatibility performance of meshes in vivo and in vitro by autologous plasma coating before implantation did not have an effect on the early inflammatory events, as the respective values for inflammatory infiltrate and macrophage invasion did not differ from coated to native meshes. However, markers such as connective tissue organization, myofibroblast invasion and endothelial cells, characteristic of vascularization, are detectable after 3 months post-implantation and show different extents in the 3 investigated meshes.

To the best of our knowledge, our results reflect the longest combined short and long-term in vivo approach to the investigation of biocompatibility issues on meshes. In addition, no ultra-short-term investigations in vivo have ever been reported so far, as most of the currently available studies investigated effects on meshes at the earliest after 7-21 days. In an investigation of prolene and a porcine dermal collagen implant Zheng et al. identified a first acute-phase reaction after 48 h, peaking on day 7-14. Our data add ultra-short-term information, suggesting that this reaction starts even earlier in the course, after a matter of minutes.

7. SUMMARY OF FINDINGS

The steps of the presented experiments and the development of this surgical innovation (the plasma coating of meshes prior to implantation) strictly followed the IDEAL recommendations at every step in order to ensure comparability and transparency.

1. We have developed a manufacturer-independent in vitro test system for the adherence performance of tissue clusters (fibroblasts, endothelial cells and muscle-derived cells) as a marker for the biocompatibility of commercially available meshes. In this test system, we established a repeatable ranking of meshes with regard to their biocompatibility. The adherence behavior was independent of the individual patient features, suggesting that the biological behavior of a mesh is probably conditioned by the structure of the biomaterial or/and its chemical composition rather than by individual host characteristics/features.
2. The *in vitro* test system is a feasible pattern for the investigation of different coating strategies of meshes. The coating of meshes prior to cultivation with PBMCs, for instance, did not affect the adherence score, whereas coating with platelets and blood plasma increased the score, suggesting an improved biocompatibility *in vitro*. The plasma coating displayed the greatest potential to improve the *in vitro* adherence score. The previous ranking of the native meshes remained consistent after coating, but was established at a higher level.

3. In a long-term animal study in sheep, we demonstrated that the developed *in vitro* test system for the biocompatibility of meshes may predict the *in vivo* performance of the meshes. This effect is independent of the location of the mesh in the body, although its particular extent varies depending on the site of implantation. The coating of meshes with autologous plasma prior to implantation seems to have a positive effect on the biocompatibility of meshes *in vivo*.

4. Investigations of the ultra-short-term determinants of the FBR at the implant site *in vivo* revealed that the local inflammatory reaction is an early and susceptible event after mesh implantation. It cannot be influenced by prior plasma coating and does not depend on the localization of implantation.
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