Coating with Autologous Plasma Improves Biocompatibility of Mesh Grafts In Vitro: Development Stage of a Surgical Innovation

Holger Gerullis, 1,2,3 Evangelos Georgas, 1 Christoph Eimer, 1 Christian Arndt, 1 Dimitri Barski, 1 Bernhard Lammers, 4 Bernd Klosterhalfen, 5 Mihaly Borós, 6 and Thomas Otto 1,2,3

1 Department of Urology, Lukas Hospital, Preußenstraße 84, 41464 Neuss, Germany
2 West German Cancer Center (WTZ), University of Essen, Essen, Germany
3 German Centre for Assessment and Evaluation of Innovative Techniques in Medicine (DZITM), Germany
4 Department of Surgery, Lukas Hospital, Neuss, Germany
5 German Centre for Implant-Pathology, Dürren, Germany
6 Department of Experimental Surgery, University of Szeged, Hungary

Correspondence should be addressed to Holger Gerullis; holger.gerullis@gmx.net

1 Introduction

The use of alloplastic materials is widely spread in surgery for hernia, incontinence, and prolapse. The assessment of meshes prior to their clinical use remains reasonable to minimize complications. There are several models for assessing different meshes with regard to their biomechanic characteristics [1, 2]. Quality control for surgical meshes is an important issue. It is likely to get increasingly important in the future considering the ongoing discussion about new regulations for the approval of medical devices as well as intense pre- and postmarket surveillance [3].

Currently, predictive information on the biocompatibility and side effect probability is rare. Klinge and Klosterhalfen presented a very valuable classification of surgical meshes for hernia repair based on the analyses of 1,000 explanted meshes. [4]. The results of a recent in vitro approach [5] have been successfully validated in an animal long-term study following standardized recommendations for the assessment of surgical material and methods, IDEAL (Innovation, Development, Exploration, Assessment, and Long-term study) [6, 7]. This method warrants further development and evaluation as a possible manufacturer-independent tool for pre- and postmarket evaluation of meshes.
In current understanding, an optimal surgical mesh permits the transmigration and localisation of host cells and inhibits the adherence of visceral organs in order to avoid arrosion, foreign body induced pain, and so forth. Preoperative coating of meshes, with a protective layer on the visceral side of the mesh, has been previously investigated, mostly in in vivo approaches. These meshes reduced a foreign body reaction and improved biocompatibility. There are now, in fact, clear indications that surface modifications of meshes can help to influence a tissue reaction in vivo [8, 9]. However, in vitro approaches for native and modified mesh assessment are still scarce. The objective of this study was, therefore, to investigate the effect of coating of surgical meshes with autologous blood components using a recently established in vitro model for biocompatibility assessment.

### 2. Material and Methods

#### 2.1. General

The method has been reported previously and represents a semiquantitative approach, measuring the adherence of different tissues on the meshes' surface using a modification of the approach initially described by Melman and coworkers [5, 10].

#### 2.2. Meshes/Patients

Seven meshes currently used for various indications such as hernia repair, pelvic organ prolapse (POP), and stress urinary incontinence (SUI) were studied. Table 1 provides a short overview on important material characteristics. After receiving informed consent, we harvested tissue probes of muscle, fascia, and renal vein from 10 patients undergoing right side nephrectomy. Tissue processing was identical in all patients. All patients provided blood samples for further processing and isolation of blood components for subsequent mesh coating. Each mesh was tested with tissue and cells of each patient for comparison.

#### 2.3. Blood Sample Processing

Peripheral blood mononuclear cells (PBMCs), platelets, and plasma were used. Separation of PBMCs was accomplished through density gradient centrifugation using Ficoll [11]. For isolation of platelets and respective mediators the advanced tissue regeneration system (ATR by Curasan Inc.) was used (http://www.curasan.de/de/produkte/dental/atr/atr.php) [12]. Plasma preparation followed the classical method of Crowley [13].

#### 2.4. Mesh Coating

After isolating the three different blood components of each patient, we incubated the meshes (2 × 2 cm) with 10 mL of the respective suspension and incubated them over 12 hrs prior to testing with tissue. Successful plasma coating is exemplarily shown in Figure 1.

#### 2.5. Tissue Preparation

The investigation of the adherence of specific cells has been previously described [14]. Our initial results with cell culture did not reveal sufficient cell growth; thus, we decided to use a minced tissue culture approach for the following investigations. Coincubation of implants with tissue clusters was also supported by the idea of reduced artificial modification processes due to shorter culture processing. In detail we proceeded as follows [5]: we extracted tissue probes originating from muscle, fascia, and renal vein at a length of 0.5–1.0 cm. Probes were incubated with Liforlab at room temperature. After crushing, we incubated DMEM/F12 plus 10% serum and incubated them over 12 hrs prior to testing with tissue. Successful plasma coating is exemplarily shown in Figure 1.

| Mesh                                      | Material                                      | Biomechanic characteristics                      |
|-------------------------------------------|-----------------------------------------------|--------------------------------------------------|
| Vitamesh, Proxy Biomedical                | Large pore monofilament Polypropylene         | Knit polypropylene, pore size 2410 μm, thickness (microns) 250, tear resistance \( F_{\text{max}} \) 33.7 |
| Dynamesh, FEG Textiltechnik               | Monofilament (PVDF) polyvinylidene fluoride   | Effective porosity: 58%, reactive surface: 1.97 m²/m², suture pull out strength: 31 N, tear propagation resistance: 28 N, pore size: 3000 μm |
| TFT Motif mesh, Proxy Biomedical          | Micromachined polytetrafluoroethylene         | Pore size 235 μm, thickness (microns) 150, tear resistance \( F_{\text{max}} \) 15.1 |
| TVT polypropylene                          | Polypropylene                                 | Nonabsorbable, permanent polypropylene suture, pore size of 164 × 96 μm |
| UltraPro Hernia System Medium (UHSM), Ethicon | Polypropylene reinforced with poliglecaprone fibers | Filament thickness 0.09 mm, mesh thickness 0.5 mm, \( F_{\text{max}} \) 69 N, pore size 300 μm |
| Proceed surgical mesh, Ethicon             | Monofilament polypropylene encapsulated with polydioxanone (PDS) | Closely knitted with small pores <1000 μm size, high tensile strength |
| Mersilene, Johnson and Johnson             | Multifilament mesh, polyethylene terephthalate | Density 0.19 g/cm³, pore size 120–85 μm |

Investigated meshes and their main characteristics [5].

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**Table 1: Meshes.**
the pelvic floor were used. Immunocytochemistry with specific cell markers was performed. The presence of myoblasts was confirmed by α-sarcomeric actin and desmin as markers of myogenic differentiation. Fibroblasts were stained with antibodies targeting vimentin, whereas antibodies against CD34 were used for endothelial cells. Data were generated for tissue samples of patients.

2.6. Morphological Study. The tissue cultures were maintained up to 4 months with frequent changes of medium, and assessment was repeated if possible. Meshes were investigated microscopically with regard to interstructural tissue connections and quantity of mesh adherent cells. The semiquantitative assessment scheme was based on the maximum number of adherent cells and size of tissue clusters per vision field. The adherence on the meshes was ranked none, fair, good, and excellent [10]. The respective scores of coated versus uncoated meshes were compared for each patient and each coating separately.

3. Results

Tissue growth was comparable in all approaches over 6 weeks. We did not observe macroscopic differences in the gross appearance of the meshes after tissue culture. No signs of infection were observed.

3.1. Microscopic Results. The testing of the biocompatibility of myoblasts, endothelial cells, and fibroblasts was observed under addition of BioGlue. According to the descriptive/semiquantitative approach described above, we revealed a ranking of the native meshes after 6 weeks [5]. The modified Melman score was subsequently used to the three different coating approaches for each patient. We observed comparable tissue ingrowth to the native mesh when analysing the PBMC-incubated meshes. Interestingly, the meshes previously incubated with ATR (Curasan Inc.) and the plasma coated meshes revealed a slightly better performance. Table 2 and Figure 2 show the ranking of the investigated native meshes and the different coating modifications. This trend was reproduced after 4 months of tissue culture. All individuals revealed comparable effects of tissue ingrowth in the native state and after coating with different blood components.

4. Discussion

Many scientists agree that the choice of the appropriate mesh is as important as the surgical technique when determining clinical outcomes after mesh applying surgeries independent of the particular indication [15, 16]. Currently, a plethora of commercially available meshes makes the decision of which mesh to apply very difficult. Two FDA warnings from 2008 and 2011 reported more than 3,500 severe adverse events after mesh application, mostly in POP and SIU patients. As a consequence the FDA recommended consideration of regulatory changes including an upgrading in risk classifications for meshes, clinical studies to address the risks
and benefits of meshes, and expanded postmarket monitoring of device performance [17]. It was suggested that data on adequate functional performance and material safety should get increasingly in the focus of premarket review for mesh devices. Thus, preclinical investigations in terms of bench and/or animal testing could represent a new standard requirement to confirm that engineering specifications are met and that the material and/or specific modification chosen for a mesh is sufficiently biocompatible. To date, there are numerous reports of mesh modification approaches in order to improve their biocompatibility. The permanent character of a foreign body implant may cause persistent and increased inflammation with ongoing collagen disposition leading to extensive fibrosis. Impaired host acceptance of the implanted mesh is likely to appear through chronic inflammation and extensive fibrosis [18]. In order to tackle the problem of extensive foreign body reaction (FBR) initiated by early local inflammation, several researchers have modified the chemical and physical properties of meshes by different coating approaches resulting on altered local reaction and tissue response, mostly using in vivo experiments. Various compounds have been tested so far for mesh coating purposes, however, the majority in in vivo models, mostly after setting a pathological defect being repaired by the investigated meshes [9,19–22]. Besides numerous in vivo experiments, Bryan and coworkers provide an in vitro model to facilitate mesh choice in uncomplicated hernia repair by quantitatively determining of neutrophil activation and degranulation in different mesh types [23]. Their approach represents one of the few in vitro assessment tools for meshes, currently available in the literature. In their experiments, reactive oxygen species (ROS), released by activated neutrophils leading to nonspecific host tissue damage and potential mechanical weakening, have been measured on the surface of 6 different meshes. The authors investigated native, nonmodified meshes. However, they concluded mesh structure being a greater determinant of ROS release than chemical composition. It seems likely that their sophisticated assay could be used for mesh assessment after different coating approaches as well. This would be a conclusive further development comparable to the approach presented here, which represents an advancement of the initially described in vitro assessment tool for native meshes [5]. The aim of this study was to implement and assess an easy mesh-coating procedure in vitro and to investigate if coating of meshes with autologous blood components shows different in vitro interaction characteristics with different tissues types compared to 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 humoral immune defence. This approach was based on the assumption that the extent to which an implanted alloplastic material elicits an acute local inflammatory response has impact on the long-term outcome when applied in vivo [24]. In order to investigate cellular and noncellular components, we separately investigated PBMC, plasma, and platelets with the respective mediators. Incubation with peripheral blood mononuclear cells did not result in modification of the adherence score for the investigated tissues. This may be explained by the reduced ability of those cells to maintain in permanent contact with the polymer surface of the meshes as previously shown [5]. In contrast, blood plasma and ATR resulted in better adherence performance and increased biocompatibility in all meshes. A main limitation of this study is that no inflammatory reaction as normally cascading in vivo was imitated as the in vitro approach was sterile. However, predictability for in vivo circumstances is currently being tested in an animal model in sheep for the coated and noncoated meshes. First results describe a predictive value of the in vitro system for mesh performance in in vivo surroundings [6].

An interesting observation in the current study is that all meshes which have previously been ranked with regard to their biocompatibility performance show an increased score after plasma coating and maintain their position in the ranking, compared to the other investigated meshes. This supports the thesis that coating with plasma may have an effect independent of the mesh; however, at least in vitro, all meshes could improve their performance but low ranked meshes could not increase their position compared to natively better positioned counterparts. The thesis of Bryan

| Mesh type                          | Native | PBMC | ATR  | Plasma |
|-----------------------------------|--------|------|------|--------|
| Dynamesh, FEG Textiltechnik       | 2,2    | 2,1  | 2,6  | 2,7    |
| TFT Motifmesh, Proxy Biomedical   | 2,0    | 2,0  | 2,3  | 2,7    |
| Vitamesh, Proxy Biomedical        | 1,6    | 1,4  | 1,7  | 1,9    |
| Ultrapro hernia system medium (UHSM), ethicon | 1,4    | 1,4  | 1,6  | 1,6    |
| Mersilene band, Johnson and Johnson | 1,2    | 1,2  | 1,4  | 1,5    |
| Proceed surgical mesh, ethicon    | 1,2    | 1,1  | 1,4  | 1,5    |
| TVT polypropylene                 | 1,0    | 1,1  | 1,4  | 1,6    |

For assessing the adherence score after 6 weeks, we evaluated each mesh with tissue of ten different patients. After semiquantitative determination, we conducted the frequency distribution value of the score results for each mesh. (points/10).

| Scoring                | Description                                                                 |
|------------------------|-----------------------------------------------------------------------------|
| None (0 point)         | no tissue ingrowth                                                          |
| Fair (1 point)         | thin bands of fibroblasts and small collagen deposits between mesh filaments |
| Good (2 points)        | moderately thick bands of fibroblasts and collagen deposits between mesh filaments |
| Excellent (3 points)   | nearly all spaces between mesh filaments occupied by fibroblasts, collagen deposits, and capillaries |
and coworkers can thereby be supported: mesh structure seems to be an important determinant of the in vitro performance in the native and coated configuration of a mesh [23]. Mesh related complications are known to be related to extensive local inflammation, representing the first step of a foreign body reaction [25]. This foreign body reaction after implantation of a mesh is assumed to be triggered by secretion of a variety of proteins attracting inflammatory cells to migrate to the site of injury, finally leading to extracellular matrix regulation and collagen deposition [26]. In a recent study, Brandt and colleagues investigated the effect of mesh coating (PVDF, polyvinylidene fluoride) with different substances affecting the cortisone metabolism. In their in vivo approach they found that hydrocortisone and spironolactone protected from inflammatory response ended up in smaller granuloma at the implant site of the mesh and decreased the collagen formation [27]. Their approach suggested that the respective coating approaches are a possible way to attenuate local inflammatory processes in order to reduce FBR. This is supported by other research groups who showed altered local cell activation and tissue responses after modifying the chemical and/or physical properties of meshes via coating attempts, thus, leading to the hypothesis that coating of polymer surfaces may represent an opportunity to improve mesh integration and biocompatibility [28]. Assuming the adherence performance of tissue on a mesh as possible marker for its biocompatibility seems logic independent from the respective clinical use of the implant. Although, an exaggerated foreign body reaction/tissue response is discussed to be related to clinical complications, a positive role in mesh incorporation at the implant site may be triggered by bioactive mediators like epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or transforming growth factor (TGF) and others produced by e.g., fibroblasts or smooth muscle cells. Thus, the cultivation and positive adherence of cell clusters consisting of those cell types and the respective assessment and comparison, as shown here, may be helpful for considering a mesh regarding its possible tissue ingrowth and capacity of formation of connective tissue. Coating of meshes with plasma and ATR seem to have a positive effect on those features.

5. Conclusion

Plasma coating of meshes leads to an improved biocompatibility score in a novel in vitro test system warranting in vivo assessment of the procedure.

Abbreviations

FBR: Foreign body reaction  
FDA: American food and drug administration  
POP: Pelvic organ prolapse  
SUI: Stress urinary incontinence  
EGF: Epidermal growth factor  
bFGF: Basic fibroblast growth factor  
TGF: Transforming growth factor  
PBMC: Peripheral blood mononuclear cell  
ATR: Advanced tissue regeneration system  
TVT: Tension free vaginal tape  
CD: Cluster of differentiation.

Conflict of Interests

The authors declare that they have no conflict of interests.

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