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Evaluation of potential biomaterials for temporary undescended testicle protection

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Abstract: The clinical picture of crytorchidism describes a deviation in the position of the testicles outside the scrotum and is one of the most common anomalies in male newborns. The production of customized anti-adhesion membranes as artificial biomaterial pouches seems to be a key element in the temporary isolation of the testicles during two-stage orchiopexy. In this work, we produced electrospun PLGA scaffolds with and without lecithin to compare this highly porous fibrous membrane with commerically available ePTFE and biological matrices. First, the surface morphology of all specimens was assessed by SEM. In addition, their wettability, mechanical behaviour, and biological response were considered in an initial screening. Cell tests over 3 days using a human fibroblast cell line showed the potential functionality of lecithin within electrospun fiber nonwovens and promising results for the tissue matrix, which still need to be confirmed in future studies. The next step will focus on further reducing adhesion of specific cells for testicular pouch application.

Keywords: anti-adhesive, membrane, ePTFE, tissue, electrospun nonwoven.

1 Introduction

The clinical picture of undescended testicles describes a deviation in the position of the testicles outside the scrotum and is one of the most common anomalies in male newborns. Undescended testicles are observed most frequently in premature babies, with a prevalence of up to 30%, while the incidence in term infants ranges from 1 to 3% [1]. If left untreated, cryptorchidism can lead to a significant reduction or even loss of male fertility and increase the risk of testicular cancer later in life [2]. The treatment of this malposition is typically surgical, and in some cases, so-called orchiopexy is performed in two stages to ensure complete dislocation of the testicles [3,4]. However, a prevalent complication is the development of postoperative adhesion after the first stage, which can interfere with subsequent surgery aimed at correcting the position of the testicle.

Over time, methodes using artificial biomaterial membranes have been initiated to minimize the risks and ensure testicular protection during surgical procedures. The investigations focused on silastic film, oxidized regenerated cellulose (ORC) membrane, and bioinert expanded polytetra-fluoroethylene (ePTFE) membrane [3–5]. For example, the *Gore Preclude* membrane and the *Interceed* barrier, have already been tested in connection with undescended testicles [3,4].

According to recent literature [6], several biomaterials are already being used as physical adhesion barriers to separate the injured from the surrounding tissue, e.g. synthetic films, hydrogels, and micro- and nanoparticles [7] or biological tissue replacement materials [8]. Especially, tissue-like (non-) resorbable nanofiber nonwovens present a barrier alternative to ePTFE [9]. Even medical meshes or membrane patches protecting against or reducing postoperative adhesion, encapsulation, or scarring are now commercially available.

In light of the aforementioned background, the present study investigates the development of an artificial testicular antiadhesive pouch. The objective of this investigation is to substantiate preliminary biomaterials by opening a broad material basis (synthetic, biological, bioinert) for evaluating their efficacy. The characterization encompasses the analysis of their morphology, wettability, mechanical behaviour, and biological response. In this regard, an artificial nonwoven of poly(lactid-co-glycolid) (PLGA) functionalized with or without lecithin was compared with three different clinically approved ePTFE membranes and a tissue matrix, providing an extensive evaluation of entirely different materials and surface textures in the context of the clinical application of the artificial testicular pouch.

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2 Materials and Methods

To study special electrospun nonwovens in comparison with biological tissue and ePTFE matrices, two fiber matrices with random arrangement were prepared via one-step electrospinning from corresponding polymer solutions using a 4SPIN device (Contipro a.s., Czech Republic). The polymer PLGA 8515 (Resomer LG 857 S, Evonik Industries AG, Germany) and the phospholipid lecithin (Carl Roth, Germany) with a content of 10 wt% were used, similar to a reported PCL study [10]. All solvents are used as received.

The Strattice RTM (Reconstructive Tissue Matrix, AbbVie Inc., USA) membrane — derived from porcine skin — is a non-crosslinked acellular dermal matrix and also being used as a commercially available biological material with good anti-adhesive properties [11].

Expanded polytetrafluoroethylene (ePTFE) membranes, such as Gore Preclude, Gore Tex and Gore Dualmesh (W. L. Gore & Associates Inc., USA), are already in use as effective not resorbable, bioinert adhesion barriers in different application areas, e.g. pericardial space [12]. The Gore Preclude membrane has shown positive results in a two-stage orchidopexy procedure, making it a potential candidate for testicular pouches. Two additional ePTFE membranes are used as reference materials to confirm the effectiveness of ePTFE and identify any product-specific differences. Table 1 lists the key features of test specimens.

Table 1: Investigated biomaterials with key properties and thickness (n = 5).

material		key features	thickness [µm]
PLGA nonwoven		dry, bioresorbable (> 6 months),	280
PLGA/Lec		specially electrospun nonwoven	290
Strattice RTM		wet, non-crosslinked acellular matrix, medically approved	1430
	Preclude	dry, non-resorbable/ bioinert	100
Gore	Tex	ePTFE, medically approved,	400
	Dualmesh	side specific features	950

Surface characterization: Scanning electron microscopy (SEM) images were taken at various magnifications on Au sputter coated samples using a Quanta FEG 250 (Thermo Fisher Scientific, USA).

Mechanical properties: Tensile specimens (n = 2) were punched out of the membranes according to the standard test geometry 1BB (DIN EN ISO 527-2). Uniaxial tensile tests were carried out with a Zwick/Roell Z 2.5/TN (Zwick GmbH & Co. KG, Germany) using a 50 kN load cell and a crosshead speed of 25 mm/min. Tensile force was measured as a function of sample elongation.

Wettability: Contact angle measurements of sessile drops were carried out to determine the wettability of the electrospun meshes using a DSA 25 - Drop Shape Analyzer (KRÜSS GmbH, Germany). Measurements were performed with 2 µl drop volume and 1 to 10 s equilibration time under constant conditions. Deionized water and diiodomethane served as test liquids with different polarities to calculate the surface free energy (SFE). Contact angles were determined for each sample (n = 2) by averaging the values of both drop sides. SFE as well as polar and dispersive components were calculated using Krüss Advance software (V.1.13).

Cell biological testing: Initial in vitro cell culture studies (direct contact) were carried out in an incubator Memmert ICO240med (Memmert GmbH, Germany) under simulated in vivo conditions at 5% CO_2, 90% RH, and 37 °C with human HT-1080 fibroblasts and THP-1 monocyte cell line. Tissue culture polystyrene (TCPS) served as a common surface for growth control (NC, negative control). A positive control group (PC) was treated with a cytotoxic concentration of 10^{-4} M disulfiram/ tetraethylthiuramdisulfid (TETD). The metabolic activity of cells was determined via CellQuanti Blue Assay (Biotrend Chemicals GmbH, Germany) after 72 h (n = 1 with four replicates).

3 Results and Discussion

3.1 Fiber matrix surface

To compare surfaces of both membrane sides SEM imaging was performed (Figure 1), showing indeed different fiber matrices regarding the three ePTFE membranes, the nonwovens and the tissue surface.

Gore Preclude has many tiny island-shaped areas (≤ 2 µm) on both sides, which have a dense, non-fibrous structure. These insular areas are strongly interconnected by numerous fibrous structures. The Gore Tex has very similar structures, but much larger (≥ 10 µm) and fibrous structures are mainly fiber bundles. In contrast, the Gore Dualmesh is characterized by two differently structured sides. One side is characterized by regular grooves with a rough texture similar to Gore Tex. The non-fibrous insular areas also have smaller roundish irregularities or elevations. Elongated indentations (grooves) divide the cross-linked fibrous and non-fibrous areas into larger segments that extend over the entire area under consideration (data not shown, macroscopically visible). The other side is similar to Gore Preclude.

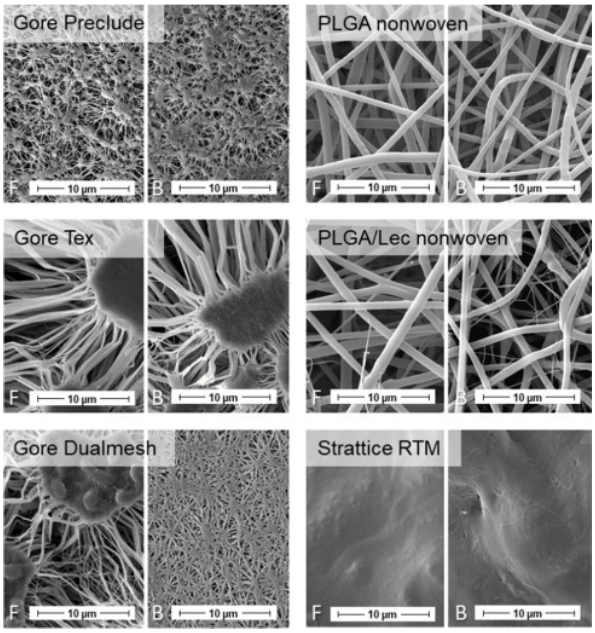

Figure 1: Representative SEM images illustrating the fiber morphology of the different membranes (F- front side, B – back side).

Both nonwovens produced are homogeneous and bead-free, whereby the PLGA fiber diameters of 710 ± 270 nm are slightly larger than the PLGA/Lec fibers at around 540 ± 150 nm. The biological membrane Strattice RTM has a continuous, closed surface on which neither fibers nor pores are visible. Merely irregular elevations are visible over the entire surface.

3.2 Mechanical behaviour

Tensile testing resulted in different maximum load and elongation at break depending on membrane types (Figure 2). Considering different membrane thickness, the thinnest Gore Preclude membrane (0.1 mm) has the highest tensile strength

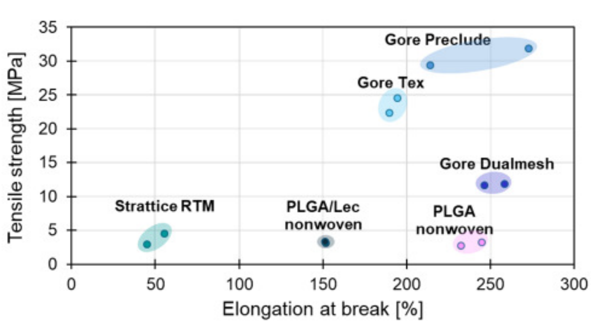

Figure 2: Scatter plot of tensile strength versus elongation at break of the six membranes (n = 2, uniaxial)

and elasticity in this comparative study. Gore Dualmesh (0.95 mm) and PLGA nonwoven (0.3 mm) have equally high elongation at break, but lower tensile strength. Gore Tex (0.4 mm) has relatively high tensile strength with lower elongation at break compared to the other two ePTFE membranes. Strattice RTM (1.4 mm), PLGA and PLGA/Lec nonwovens (0.3 mm) have a tensile strength of less than 5 MPa, whereby the porous nonwovens, in contrast to Strattice RTM achieves much higher elongation.

3.3 Wettability

In general, high surface free energy (SFE) indicates, that the membrane can be well wetted, while low SFE indicates poor surface wettability. All ePTFE membranes are very poorly wettable independent of the different surface structures (Figure 3). Due to high water contact angles (WCA) of Gore Dualmesh (130°), Gore Tex (140°) and Gore Preclude (148°), ePTFE can be classified as hydrophobic. The PLGA nonwoven also has a similar WCA value of 138°. Compared to ePTFE, PLGA/Lec and Strattice are highly hydrophilic with a WCA of 0° within 10 seconds. Thus, electrospun polymer

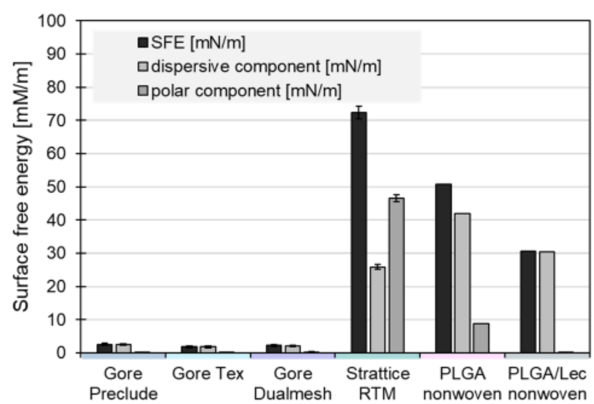

Figure 3: Wettability indicated by surface free energy (sum of dispersive and polar component) of the tested membranes.

scaffolds revealed material-dependent differences despite same structure, due to incorporated phospholipide.

3.4 Cell response

All membranes were subjected to biocompatibility testing according to DIN EN ISO 10993-5. Since blood vessels come into direct contact with implants, the human fibroblast cell line HT-1080 was used. The metabolic activity of cells in direct contact with Gore and PLGA membranes was above 70% (Figure 4). Consequently, these materials can be regarded as non-cytotoxic. In contrast, cells on Strattice RTM as well as lecithin leached PLGA/Lec nonwoven show lower cell viability, suggesting that the processing method or the testing method for these materials has to be reconsidered.

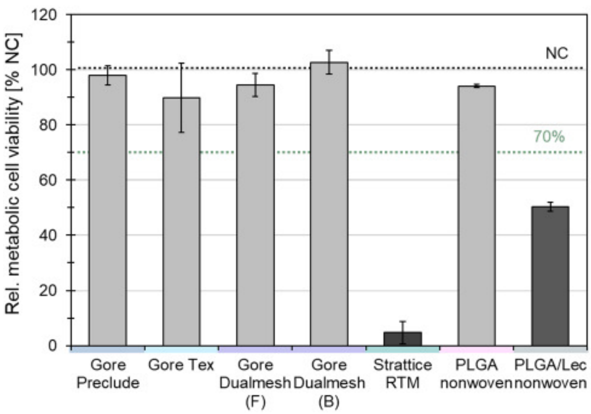

Figure 4: Relative metabolic cell viability of HT-1080 fibroblasts in direct contact on the membrane materials (n = 1 with 4 replicates, MD ± SD, NC = 100%).

The biological analysis using HT-1080 fibroblasts and THP-1 monocytes (results not shown) reveal that Strattice RTM has the most promising anti-adhesive membrane properties. This conclusion was confirmed by SEM and fluorescence images, which did not reveal any cells. However, the results of the HT-1080 cell viability test show very low values, indicating that the material has low biocompatibility. Further studies the influence of preconditioning rinsing steps (at least 2 min in saline solution) still need to follow.

4 Conclusion and Outlook

As a temporary artificial testicular anti-adhesive pouch, similar to a skin wound dressing, Strattice RTM and nanofiber membranes of midterm degradable PLGA polymers in combination with lecithin may offer advantage over bioinert, permanent ePTFE. However, this exploratory study needs to be substantiated, also including other biopolymers or composites as highly porous nonwovens compared to non-porous film membranes.

Further studies on blood compatibility or protein adsorption and the effect of wettability as well as the confirmation of the prevention of fibroblast adhesion and proliferation or the inhibition of macrophages have to follow. Beyond that, there is a need for supplementary research in this area to focus on membrane resorption, which would be advisable in the case of hernia meshes, stent coverings, tissue patches or temporary testicular pouches.

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