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Investigations on physico-chemical properties of TSPCU nonwoven for application as prosthetic venous valve

Abstract: Electrospinning is used for producing nonwovens for medical polymer-based implants, such as prosthetic valves or covered scaffolds. In this study, nonwovens for prosthetic venous valves are investigated regarding their morphology and mechanics in physiological medium. Spinning molds were developed based on previous venous valve leaflet designs, 3D printed in different sizes and covered with electrospun nonwovens. Samples were stored in a physiological 0.9% saline at 37°C to investigate the influence of fiber rearrangement and swelling in medium for several weeks. Two different nonwovens of thermoplastic silicone-based polycarbonateurethane (TSPCU) were compared. Tensile test results show that storage in medium has a relevant influence on the mechanical properties. SEM images of TSPCU show substantially increased fiber diameters after 8 days stored in medium. After detaching the valve leaflet nonwovens from the molds, shrinkage of the material of approximately 12% was detected. A suitable valve size could be identified for joining with the stent structure into an interventional prosthetic venous valve. The results demonstrate the influence of storage conditions on the morphological and mechanical properties of electrospun TSPCU nonwovens. For development and dimensioning of venous valve leaflets, this change in mechanical behavior and possible shrinkage of the material has to be considered.

Keywords: prosthetic venous valve, mechanical testing, electrospinning, nonwoven, nanofibers

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1 Introduction

Electrospinning has become an emerging method to produce micro- and nanofibers which results in fibrous polymer nonwovens for medical polymer-based implants, such as prosthetic valves or covered scaffolds.

Artificial leaflet materials present a promising option to replace tissue-based implant materials, because of their high potential for improving the surface characteristics. Polymeric nanofibers can be manufactured with high reproducibility and feature good physico-chemical properties, low thrombogenicity and appropriate biocompatibility [1].

Therefore, the use of polymeric leaflet materials, especially for heart valve prostheses has been researched over the last years [1-3].

These concepts may also be suitable for developing an interventional prosthetic venous valve based on fibrous nonwovens manufactured by means of electrospinning. Currently, there is no artificial venous valve prosthesis available for treatment of chronic venous insufficiency. Insufficient venous valves are treated with surgical procedures such as valve reconstruction or stripping. This poses a high risk for older and multi-morbid patients. Requirements on the valve and its materials include durability, proper valve opening and closing to prevent reflux and non-thrombogenicity [4].

In this study we investigated physico-chemical properties of electrospun nonwovens for prosthetic venous valves after storage in physiological environment.

2 Materials and methods

2.1 Sample preparation

A three-dimensional venous valve geometry was designed using the CAD software Creo Parametric 6.0 (PTC Inc., Boston, MA, USA). Based on this valve design, molds for electrospinning were derived. Different sizes of the molds in

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15% steps related to the target diameter were created to investigate conceivable shrinkage or swelling of the produced nanofiber nonwovens (Figure 1). The stereolithographic 3D printer Form 2 (Formlabs Inc., Somerville, MA, USA) was used for manufacturing the spinning molds.

Figure 1: 3D printed molds covered with electrospun nonwoven with different sizes based on a venous valve leaflet design

A homogenous polymer solution of 7.5 wt% thermoplastic silicone-based polycarbonateurethane (TSPCU) was obtained by dissolving the polymer in a solvent mixture of chloroform, N,N-dimethylformamide and 2,2,2-trifluoroethanol (ratio 6:2:2). Two polymer solutions with different material shore hardness (TSPCU I – 80A and TSPCU II – 55D) were compared. For the electrospinning process an in-house developed spinning device was used at room temperature and constant humidity. A high voltage of 15 kV and an emitter collector distance of 60 mm were applied. Spinning process time varied due to different mold sizes. The layer thickness of 100 μm was targeted.

All electrospun molds were stored in 0.9% saline at constant 37 °C for 4 weeks. The valve leaflet nonwovens were then detached from the molds to investigate conceivable shrinkage and identify a suitable valve size for joining with the stent structure to build a new type of interventional prosthetic venous valve. In addition, these medium-stored nonwovens were examined in mechanical and morphological studies.

2.2 Surface characterization

The morphology of the TSPCU I and II nonwoven structures was examined with a scanning electron microscope (SEM) Quattro S (Thermo Fisher Scientific Inc., Waltham, MA, USA). Samples were fixed on aluminum carriers and sputtered with a gold coating.

Fiber diameter was analyzed using the General Image Fiber Tool (GIFT), which is described in [5]. To determine the influence of storage in medium on fiber diameter, dry samples and 8 days medium-stored samples were compared, each evaluating fiber diameters of 5 different areas. Medium-stored samples were taken from the saline immediately before imaging.

2.3 Uniaxial tensile testing

Uniaxial tensile tests were performed using the universal testing machine Zwick Z2.5 (Zwick/Roell, Ulm, Germany) with a 10 N load cell. Tests were performed in 0.9% saline at 37 °C and in air at room temperature (20°C) with a crosshead speed of 25 mm/min. All laser cut test samples have specimen geometry with effective dimensions of 12 x 2 mm. Sample thickness was measured using the dial gauge Mitutoyo 543-394B with associated stand (Mitutoyo Corporation, Kawasaki, Japan) which has a measuring accuracy of 0.001 mm.

Force-displacement curves were measured to calculate stress-strain functions resulting in Young's modulus (E) between 1% and 3% strain. Tensile strength (σ_{max}) and elongation at break (ϵ_{B}) were also determined. The resulting stress-strain curves were averaged by means of the software Origin 2018b (Originlab Corporation, Northampton, MA, USA). Table 1 summarizes the number of test samples (n), the used test medium and storage time in 0.9% saline (t_{M}) for each group of TSPCU nonwoven.

Table 1: Test conditions for uniaxial tensile testing of TSPCU nonwoven

polymer	n	medium	t_{M} [d]
TSPCU I / II	3	air	0
TSPCU I / II	6	saline	0
TSPCU I / II	6	saline	31

3 Results and discussion

3.1 Morphological investigation

To investigate the morphological structure of the nonwovens, SEM imaging was performed as summarized in Figure 2. TSPCU I nonwoven of dry and 8 days medium-stored samples (Figure 2 A and B) were compared with similarly

stored samples of TSPCU II (Figure 2 C and D). Dry samples showed a structure of smooth and homogeneous fibers, with TSPCU I and TSPCU II having an averaged fiber diameter of approximately 890 nm and 422 nm, respectively. Due to fiber swelling medium-stored samples showed a substantial increase in fiber diameter (TSPCU I 23% and TSPCU II 39%). In addition, crystalline sodium chloride has been deposited on the fibers. The following mechanical investigations showed conceivable effects of fiber swelling.

Figure 2: Representative SEM images of electrospun TSPCU nonwoven dry and 8 days medium-stored with A) TSPCU I_0d, B) TSPCU I_8d, C) TSPCU II_0d and D) TSPCU II_8d

3.2 Mechanical behavior

Averaged stress-strain curves from uniaxial tensile tests up to maximum tensile strength and material failure are shown in Figure 3. Figure 4 shows strain range up to 20% strain. This range was relevant for the mounting process in which the electrospun valve leaflet was pulled over the stent structure. Because of expected material shrinkage, corresponding strains occurred.

Mechanical parameters are summarized in Table 2. It could be seen that storage in medium had a relevant influence especially on Young's modulus and tensile strength. Elongation at break was not substantially effected.

Figure 3: Stress-strain curves of TSPCU I and II uniaxial tensile testing measured in air and saline

Figure 4: Stress-strain curves of TSPCU I and II uniaxial tensile testing in relevant strain range for prosthetic valve use

Comparing dry samples measured in air and in saline, a significant decrease in Young's modulus and tensile strength could be seen for TSPCU I of 63% and 58% and for TSPCU II of 87% and 35%, respectively. On the one hand, this occurred due to improved sliding behavior of the fibers in medium. On the other hand, the hydrogen bonds split in contact with water, so the material became more compliant.

Comparing dry and medium-stored specimens, both measured in medium, it could be seen that Young's modulus and tensile strength increased with increasing storage time for TSPCU I of 47% and 28% and for TSPCU II of 188% and 59%. Both chain and fiber mobility was probably an

influencing factor. Due to increased chain mobility, the polymer chains rearranged and were thereby more densely packed, which enhanced the tensile strength. In addition, the swollen fibers were also more densely packed, as can be seen in the SEM images (Figure 2). This might inhibited interfiber mobility.

In relevant strain range, mechanical parameters of TSPCU II were substantial higher, which was partly due to higher material hardness.

Table 2: Mechanical parameters of TSPCU I and II measured in air and saline as a result of uniaxial tensile tests

Sample	E [MPa]	$\sigma_{\max}$ [MPa]	ϵ_B [%]
I_0d_air	2.2 ± 0.2	12.9 ± 0.7	210.4 ± 21.9
I_0d_saline	0.8 ± 0.2	5.4 ± 1.9	224.1 ± 43.7
I_31d_saline	1.2 ± 0.4	6.9 ± 1.7	232.9 ± 5.5
II_0d_air	11.7 ± 1.3	11.1 ± 0.8	220.3 ± 23.1
II_0d_saline	1.5 ± 0.1	7.2 ± 0.9	265.8 ± 23.2
II_31d_saline	4.4 ± 0.7	11.5 ± 1.2	263.4 ± 19.1

After detaching the nonwoven from the molds the electrospun venous valve leaflet was joined with the stent structure. Figure 5 shows the stent covered and welded with TSPCU I nonwoven using the initial mold size of 0%. Material shrinkage of approximately 12% was detected for both material harnesses after 4 weeks. Shrinkage was clearly visible in areas around the stent structure where the nonwoven was curved inside. This fact can be useful for further investigations, because the venous valve prosthesis is implanted in a vessel with smaller diameters. Thus, it may not leave any folds in the leaflet, which can affect a proper valve function.

Figure 5: Detail of joined venous valve stent structure with electrospun valve leaflet (TSPCU I) with mold size 0%

4 Conclusion

This study demonstrates that storage of electrospun TSPCU leaflet material in physiological medium has relevant influence on its morphological und mechanical properties. So material testing should always be performed with physiologically stored samples, as in further implant application.

Further investigations are necessary in order to decide which material is most suitable for use in the venous system. In addition to the static uniaxial tensile tests, cyclic tests should therefore also be conducted. These tests can even better represent the physiological material stress. To ensure reproducibility of visualizing fiber swelling, further investigations in SEM imaging are also necessary.

The results of this study form a basis which should be considered for development and design adaptations of the venous valve. Shrinkage behavior is an important factor in dimensioning a prosthetic venous valve.

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