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Adaptable Superfibers as Implant Material

Opening new paths to tailored polymer properties with optional drug incorporation

Abstract: Electrospun fiber nonwoven materials of different polymer classes provide promising perspectives in almost all fields of application, including medical science. In this paper we present the fiber generation of selected biostable polymers (PBT, TPC-ET, PA 6.12 and PVDF) by direct electrospinning, as an extremely powerful tool for manufacturing of new superfiber implant materials. This initial study includes the variation of some relevant process parameters, such as polymer concentrations or electrode spacing. The influence on fiber morphology, tensile strength and biocompatibility is shown. The results presented indicate that the choice and combination of materials is crucial for the application on load-bearing implants, independent of the processing technology and thus of the fiber bonding, delamination or fiber strength.

Keywords: electrospinning, nanofiber, polybutylene terephthalate, polyamide, polyester elastomer, polyvinylidene fluoride.

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1 Introduction

Electrospun polymeric nanofibers with tailor-made, flexible three-dimensional porous structures and a high surface-to-volume ratio offer new solutions in various fields of application such as filtration, desalination, catalysis, tissue replacement, nutrient or drug supply and textile industry already today [1,2].

New biomimetic surface structures in the sub micrometer to nanometer range, both with or without local drug release, are also debated intensively and explored in the field of medical engineering [3]. Especially in the cardiovascular field, many implant surfaces could benefit from innovative fibrous structures, but are also subject to various restrictions and regulatory barriers.

The vision of creating adaptive, implant-specific and drug-loaded surfaces that are anti-infective, flexible or expandable, chemically modifiable and cell-sensitive can be achieved relatively straightforward by using modern electrospinning or 3D-printing technologies. However, identification of chemically inert, long-term stable and yet processable materials which are clear for regulatory approval appears as an almost unresolvable challenge and has become an important topic of research worldwide.

This is the background for our endeavors to iteratively introduce extraordinary materials and systematically expand the material portfolio. In this study we present first mechanical, morphological and biological investigations of promising polymers for implant coating or covering. The processing procedures have been established and optimized for a thermoplastic copolyester elastomer (TPC-ET), polyvinylidene fluoride (PVDF), polyamide (PA 6.12) and polybutylene terephthalate (PBT). Furthermore, biocompatibility studies and mechanical tests in medium at 37°C were carried out.

Each of the selected polymer classes has unique properties, such as high mechanical strength, thermal stability and excellent chemical resistance of PA 6.12 or the rubber-like and extremely elastic properties of TPC-ET. Being extremely versatile, PBT combines stiffness and toughness, superior electrical insulation properties and exceptional surface finish [4,5].

Even though all materials have exceptional chemical and physical properties, decisive factors for their use as implant material are often missing, be it long-term stability, availability or fatigue strength. In addition, the mechanical properties of the individual polymers are often insufficient to mimic biological materials. Therefore, the combination or layered structures with tunable local and controllable drug depots are indispensable for potential applications in biomedical engineering.

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2 Materials and Methods

2.1 Electrospinning

The following solvents were selected and used as received without further purification for the electrospinning process: acetone, dichloromethane (DCM), chloroform ($CHCl_3$), trifluoroacetic acid (TFA) and trifluoroethanol (TFE) purchased from Fisher Scientific; dimethylformamide (DMF) supplied by VWR; acetic acid (AA) and formic acid (FA) purchased from Carl Roth.

All raw polymers used are manufactured under GMP conditions in medical grade. Prior to spinning, each polymer was dissolved in a specific solvent mixture at 37°C under moderate agitation. In **Table 1** all information with respect to polymer solution concentration, solvent ratio and electrode spacing are summarized.

Table 1: Composition of the polymer solution and electrode spacing.

Code	Polymer type	$c_{polymer}$ [wt%]	Solvent mixture	Electrode spacing [mm]
1a	PBT	12.5	DCM/TFA	160
1b		12.5	(ratio 1/1)	205
2a	TPC-ET	5.0	TFE/$CHCl_3$	210
2b		7.5	(ratio 4/1)	170
3a	PA 6.12	14.0	ES/ AS	80
3b		14.0	(ratio 1/1)	100
4a	PVDF 275	20.0	DMF/ acetone	100
4b		20.0	(ratio 6/4)	150

Electrospinning of PBT (DuPont) and PA6.12 (DuPont) as well as PVDF (Sigma-Aldrich) was performed on an in house-constructed spinning device using a needle setup and a rotating collector (AQ 100x100 mm), as well. The applied high voltage varied between 4 to 15 kV and a feed rate of 0.2 to 0.9 mL/h. Electrospinning of TPC-ET (DuPont) was performed on a 4SPIN C4S LAB2 (Contipro, Czech Republic) using a needle setup (E2, G19) and a rotating collector (C3), an applied high voltage of 20-30 kV and a feed rate of 200 µL/min.

The spinning time for all polymers varied depending on the polymer concentration in solution, always aiming for a target layer thickness of at least 100 µm.

2.2 Characterization

Scanning electron microscopy (SEM) was performed on a Quanta FEG 250 (FEI Company, Germany). Samples were mounted on aluminum carriers using pyrolytic graphite planchets and Au sputter coated prior to measurements at various magnifications.

Tensile force was measured as a function of sample elongation with a Zwick/Roell Z 2.5/TN (Zwick GmbH & Co. KG, Ulm, Germany) using a 10 N load cell and a crosshead speed of 25 mm/min. The tests were performed in a 0.9% saline solution at 37°C. According to DIN EN ISO 527, all test samples for uniaxial tensile tests have geometry according to the standard test specimen 1BB. The elongation at break (ε_B) and the ultimate tensile strength (σ_M) were extracted from the measured curves.

All cell culture reagents were purchased from PAN Biotech. Cells were maintained at 37°C and 5% CO_2 under humidified atmosphere. Human endothelial EA.hy926 cells (ATCC) were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum (FCS) and penicillin-streptomycin. For cell seeding experiments 10^4 cells per well were seeded on polymeric scaffolds placed in a 96-well plate 48 h prior to measurement. Cell viability was determined using the CellQuanti-Blue assay (BioAssay-Systems, USA) according to the manufacturer's instructions. Briefly, 10% of the total culture medium volume of CellQuanti-Blue reagent was added to each well and allowed to rest for 2 h. Samples were excited at 544 nm and the resulting fluorescence was measured at 590 nm using a microplate reader (FLUOstar OPTIMA, BMG Labtech, Germany). Data was normalized to cells grown on tissue culture-treated polystyrene.

3 Results and Discussion

3.1 Surface Characterization

To investigate the morphological structure of the nonwovens, SEM imaging was performed on the materials generated from the different polymers. After process optimization smooth and consistent fibers were observed for PBT and TPC-ET as shown in **Figure 1**. Moreover, TPC-ET is the only nonwoven fabric that has a partial fusion on fiber contact points. The manually measured fiber diameters and qualitative assessment of fiber bonding are summarized in **Table 2**.

Depending on the type of polymer, different fiber diameters, fiber arrangements and bonding are clearly visible. An increase in polymer solution concentration leads to a strong increase in fiber diameter, e.g. in the case of TPC-ET the fiber diameter doubles. The influence on the mechanical performance is shown in **Figure 2**.

The electrospinning process for PA 6.12 and PVDF still require optimization. In a further step, the polymer concentration is slightly adjusted in order to completely avoid the slight bead formation. Nevertheless, varying the electrode distance from 100 to 150 mm resulted in agglomeration and inhomogeneous deposition of the fibers. Smaller distances are preferable for both PVDF and PA 6.12 in order to obtain an optically attractive fiber pattern. Overall, it can be seen that the fiber diameters of PA 6.12 and PVDF are smaller by a factor of 4 to 10 compared to PBT or TPC-ET; the effects on the physical properties are discussed in the next section.

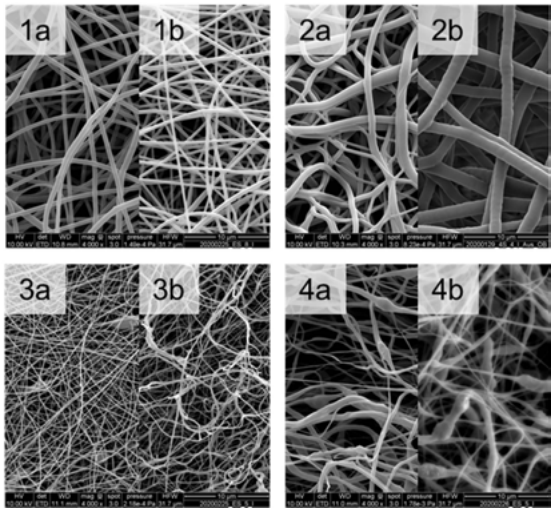

Figure 1: SEM images of electrospun PBT (1a,b), TPC-ET (2a,b), PA 6.12 (3a,b) and PVDF (4a,b) – Influence of the polymer type, concentration and electrode spacing on fiber orientation and diameter. Scale bar equals 10 µm.

On closer examination, polymer solution concentration has many different impacts on fiber structure and processing. Increasing in polymer concentration prevents the formation of beaded fibers. The process stability is also improved with higher polymer concentration, but the process window is quite narrow. A weight percentage of the polymer in solution of ± 0.5% often makes a big difference. Exceeding a concentration of 5wt% TPC-ET the nonwovens become much more uniform with increased and more constant fiber diameters for increased polymer concentration of 7.5wt% (**Figure 1:** 2a,b). Unfortunately, the spinning process became unstable and had to be interrupted every 5 minutes.

Table 2: Fiber diameter (n≥10) of the fabricated nonwovens.

Code	Polymer type	Fiber diameter [nm]	Fiber bonding
1a	PBT	548.9 ± 79.0	not noticeable
1b		416.0 ± 73.0	not noticeable
2a	TPC-ET	558.3 ± 206.5	pronounced
2b		1315.3 ± 318.5	weakly pronounced
3a	PA 6.12	160.2 ± 22.0	not noticeable
3b		195.0 ± 68.5	not noticeable
4a	PVDF 275	157.6 ± 30.8	not noticeable
4b		329.4 ± 312.0	not noticeable

3.2 Mechanical behavior

Tensile testing was performed in 0.9% saline solution at 37°C. Mechanical properties of electrospun TPC-ET, PA 6.12, PBT and PVDF nonwovens are shown in **Figure 2**. Here, clear differences in tensile strength were observed in the nonwovens produced from the different polymers. Whereas electrospun PBT shows low tensile strength of 6 MPa and elongation at break of 170%, PA6.12 nonwoven have similar tensile strength with lower elongation at break of only 50%.

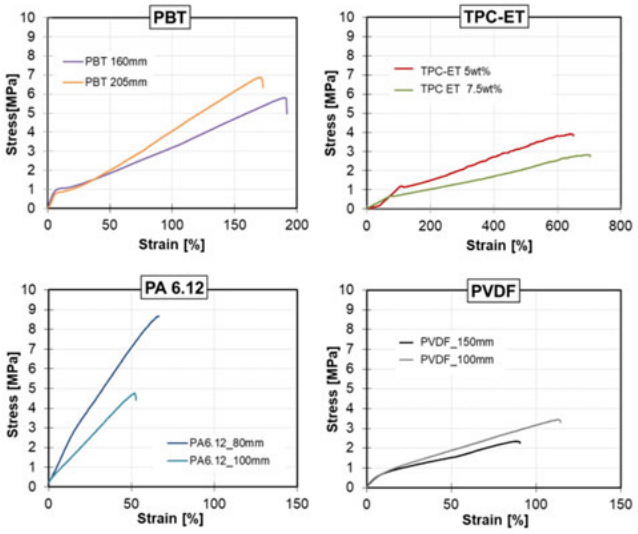

Figure 2: Stress-strain curves of electrospun PBT, TPC-ET, PA 6.12 and PVDF, measured in 0.9% saline solution at 37°C (screening results with n=1).

TPC-ET shows analogous tensile strength compared to other polymers, but a substantially higher elongation at break of about 700%. PVDF has the lowest tensile strength of all polymers analyzed at approx. 2.5 MPa.

3.3 Biocompatibility studies

To investigate the suitability of the newly established nonwovens for biomedical applications, *in vitro* biocompatibility testing was performed by accessing cell viability upon cultivation on the material (**Figure 3**).

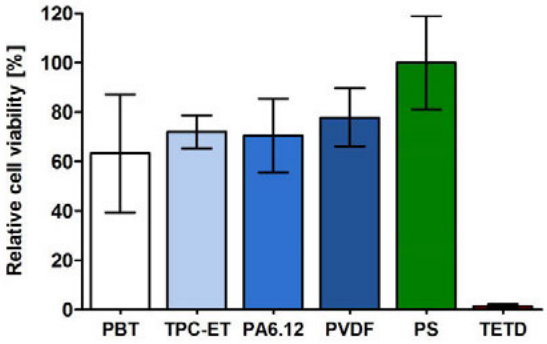

Figure 3: Relative cell viability of human endothelial EA.hy926 cells after 48 h growth on polymeric scaffolds (PS: reference material tissue culture-treated polystyrene, TETD: treatment with a reference solution of 100 µM tetraethylthiuram disulfide in cell culture medium with a strong cytotoxic effect; n = 5, MD ± SD)

All materials showed good biocompatibility with cell viabilities around 70 to 80%. Noteworthy, viability of the cells growing on PBT was slightly lower as compared to other polymers. But ultimately, it is not only the biocompatibility but also the fatigue strength and polymer stability that determine the long-term implant performance.

4 Summary and Conclusion

This parameter study show very obviously that the polymer type is much more decisive for e.g. tensile strength than the polymer concentration or other process parameters such as the electrode distance. The polymer concentration is crucial for variation of fiber thickness and processability. The collector-emitter distance should be considered for fine tuning as this factor determines the fiber bonding and the production of nonwoven layer homogeneity from a macroscopic point of view.

In summary, the different surface morphologies and mechanical properties of the nonwovens clearly show that the proper choice of polymer or the combination of different polymer types is crucial, as is the layer structure with an additional drug depot, which is often essential for biomedical applications [3,6]. Thereby, a combination of different materials is an excellent means of merging complementary polymer properties in nonwoven composites, which has already been shown for one promising material combination [3]. The results presented here indicate, that in their advantageous combination nonwovens e.g. of PVDF and TPC-ET appear very favorable for adaptable superfiber materials, especially in the application on load-bearing implants.

Author Statement

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