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Tissue biomimicry using cross-linked electrospun nonwoven fibre composites

Abstract: Mimicking the structural properties of natural tissue was aimed to be achieved by combining complementary mechanical properties of materials showing high tensile strength with those of high elasticity. By using two polymers with different properties, the advantages of both can be combined to access innovative materials for biomedical applications.

Dual co-electrospinning was established to generate biocompatible nonwoven fibre composites with fused properties from polyamide (PA6) and polyurethane based silicone elastomers (PU-co-Si), respectively. By electrospinning both compounds simultaneously, significant advantages, such as combining different fibre thicknesses and mechanical properties, are accessible. Furthermore, the effect on the fibres *via* an optional post-processing step in the shape of different thermal treatments was investigated. Initial results concerning surface morphology, biocompatibility and tensile properties of the obtained electrospun nonwovens are very auspicious. Thus, the suitability of two complementary polymer classes for tissue biomimicry through a synthetic approach was shown, offering the opportunity of novel implant materials for permanent replacement of biological tissue.

Keywords: dual co-electrospinning, nonwoven, nanofibre, microfibre, composite, posttreatment, cardiovascular, scaffold.

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1 Introduction

Biological structures, in particular tissue after decellularisation, often show fibrillary structures [1, 2]. In this context, conventional polymer processing technologies, such as extrusion, are limited to the generation of structures with fibre thicknesses larger than 10 μm [3]. To achieve the dimensions of the extracellular matrix in a range of 0.05 -10 μm , only a few polymer fabrication technologies are available, such as electrospinning as a prominent example.

Furthermore, tissue from biological sources features another special property in a manner that fibres of different thicknesses are combined, e.g. resulting in a stabilisation of the xenograft by cross-linking such fibres. Thus, the fibre thickness of the used polymers plays an outstanding role to adapt these properties to synthetic biomaterials. It was found that semi-crystalline fibres with a thickness of less than 500 nm show a higher tensile strength with decreasing fibre thickness [4-7]. Conversely, fibres with diameters of 1 μm or more show a higher tensile strength even with increasing fibre thickness [8, 9].

Co-electrospinning is frequently used in biomedical research to combine fibres with different drug loads [10, 11]. In addition, co-electrospinning has already been used to imitate biological tissue. There are few examples in the literature for the successful combination of collagen with synthetic degradable polymers such as polycaprolactone or poly-L-lactides to create a scaffold basis for tissue engineering [12, 13]. Another approach is the dual co-electrospinning of polyurethanes and polyethylene glycol (PEG) with subsequent extraction of the PEG to obtain highly porous structures for cell migration [14].

Apart from tissue engineering, the morphology of biological tissues provides further advantages, particularly regarding to their mechanical properties. The aim of this study is to combine the mechanical properties of materials with high tensile strength and those with high elasticity. Since mimicking the structural properties of natural tissue cannot be achieved simply by using solely one polymer or

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polymer blend, a dual co-electrospinning process has been developed to generate nonwoven fibre composites from PA6 and PU-co-Si with complementary properties. This offers the opportunity to develop novel implant materials that will permanently replace the function of biological tissue.

2 Materials and Methods

2.1 General Information

Chloroform (CHCl_3) and Trifluorethanol (TFE) were purchased from Fisher Scientific, Germany. Dimethylformamid (DMF) was supplied by VWR, Germany. Acetic acid (AA) and formic acid (FA) were purchased from Carl Roth, Germany. All chemicals were used as received without further purification.

Prior to spinning, PU-co-Si was dissolved in CHCl_3 :DMF:TFE (ratio 8:1:1) at 37°C under decent shaking. PA6 was dissolved in AA:FA (ratio 1:2) at 37°C .

Dual co-electrospinning was performed on a Contipro (Dolní Dobrouč, Czech Republic) 4Spin C4S LAB2 using a dual jet needleless setup at 24 cm collector distance, an applied high voltage of 43 kV and a feed rate of $50 \mu\text{L}/\text{min}$. The system was operated as received from the provider without modifications. Thermal annealing of the nonwovens was carried out at 40°C and 60°C for 24 hours in reference to room temperature (20°C).

Uniaxial tensile testing was conducted according to ISO 527 with standard dumbbell-shaped test specimens (ISO 527-1BB) and was performed on a Zwicki ZN 2.5 (Zwick, Ulm, Germany) with a 10 N load cell and a crosshead speed of $25 \text{ mm}/\text{min}$. The tensile force was measured as a function of elongation. From that the elastic modulus (E) was determined in the linear elastic region *via* linear regression. Furthermore, the elongation at break (ϵ_B) and the ultimate tensile strength (σ_M) were extracted.

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

For biocompatibility testing, all media components were purchased from PAN Biotech, Germany. Cells were maintained at 37°C and 5% CO_2 under humidified atmosphere. Human fibroblasts HT-1080 (DSMZ, Germany) were cultured in EMEM supplemented with 10% FCS and penicillin-streptomycin. For direct contact viability testing punches of scaffolds were placed in a 96-well plate. 10^4 or

2×10^4 cells per well were seeded on top of materials 48 h prior to measurement.

Cell viability was determined using the CellQuanti-Blue assay (BioAssaySystems, CA, USA) according to the manufacturer's instructions. 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 the reference material.

3 Results and Discussion

3.1 Surface characterisation

To investigate the morphological structure of the nonwoven composite, SEM imaging was performed on the materials generated solely from the individual polymers in comparison to the dual co-spun nonwoven (**Figure 1**). To our delight, the yielded material shows homogeneous and widely spread incorporation of the significantly thinner PA6 fibres into the PU-co-Si network. Moreover, the nonwoven shows partially fusion on fibre contact points.

Figure 1: SEM image of electrospun nonwovens (A) PA6 (B) PU-co-Si (C) PA6/PU-co-Si fibre composite generated *via* dual co-electrospinning process.

3.2 Mechanical behaviour

As aforementioned, biological tissue offers a highly favourable mechanical behaviour due to its fibre structure. To determine the effect when forming a composite material, tensile strength testing was performed (Table 1).

Table 1: Mechanical properties of electrospun PA6, PU-co-SI and PA6/PU-co-SI nonwoven composite compared with fixed porcine pericardium.

	modulus [MPa]	σ_M [MPa]	ϵ_B [%]
porcine pericardium	42 ± 4	5.7 ± 1.8	21 ± 5
PA6	32.2 ± 2.3	3.8 ± 0.5	36 ± 3
PU-co-Si	0.95 ± 0.03	7.9 ± 1.9	598 ± 18
PA6/PU-co-SI	19.4 ± 2.1	5.0 ± 0.6	49.5 ± 2.7

Here, clear differences in tensile strength were observed in the nonwovens produced from the individual polymers. Whereas electrospun PU-co-Si shows low tensile strength and high elasticity before rupture, PA6 nonwovens have a higher tension with lower elongation before rupture. However, when fused into a nonwoven composite, the obtained material shows mechanical properties between those of the individual materials and is also comparable with commercially available fixed porcine pericardium [15].

3.3 Effects of thermal post-processing

Thereafter, the influence of a postprocessive thermal annealing to equilibrate the nonwoven composite was investigated. For this purpose, fibre thickness was determined (Table 2). It has been shown that for a thermal treatment at 40°C in relation to room temperature (20°C), no significant changes in fibre diameter were observed (Figure 2). First results indicate shift in fibre diameter when treated at 60°C. A slight increase in fibre thickness for PU-co-Si and a decrease for PA6 was observed. These aspects will be addressed in future work.

Table 2: Averaged fibre diameter of electrospun PA6/PU-co-SI composite (n=10, MD±SD) taken from Fig. 2.

	Fibre diameter [nm]		
	20°C	40°C	60°C
PA6	199 ± 52	162 ± 32	134 ± 35
PU-co-Si	1573 ± 454	1189 ± 118	2219 ± 300

Figure 2: SEM images of PA6/PU-co-Si composite after thermal annealing (A) at 40°C and (B) 60°C, respectively. Selected fibre diameters are shown.

3.4 Biocompatibility studies

To investigate the suitability of the nonwoven composite for biomedical application, biocompatibility testing was performed (Figure 3).

Figure 3: Viability and cell adhesion of human fibroblasts growing on top of PA6/PU-co-SI nonwoven fibre composite samples after two different thermal treatments (PS: polystyrene negative control; DS: disulfiram positive control).

The material shows very high biocompatibility of around 90% in comparison to polystyrene, before and after thermal treatment as well. Furthermore, subsequent SEM analysis of seeded scaffolds revealed promising physicochemical properties of the materials with respect to cell adhesion as cells spread nicely on top of fibres building intercellular networks typical for vital and metabolically active cells.

4 Summary and Conclusion

The imitation of biological tissue is certainly a major challenge in implant design. This applies in particular to the development of synthetic implant materials. Herein, the development of polymer based nonwoven composites consisting of micro- and nanofibres offers a promising approach. In order to mimic the particular structural properties of various natural tissues in combination with a high biocompatibility, polymer fibre structures represent tailor-made materials as alternatives for xenografts in the field of application in temporary or permanent implants.

The aim of this study was to investigate the feasibility and potential of such composite materials. First of all, the dual co-electrospinning appeared to be a suitable tool to generate stable composite nonwovens with a very promising fibre arrangement. The materials indicated to be very resilient and also thermal annealing did not affect the morphology. Furthermore, mechanical and biocompatibility data indicate the possibility for emerging applications and advance improvements in the field of tissue and biomedical engineering.

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