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Ultrathin fibre coatings on nanofibrous nonwovens by plasma enhanced chemical vapor deposition

Abstract: For the generation of tailor-made polymer coatings on nanofibrous nonwovens plasma enhanced chemical vapor (PECVD) is a promising process, even for complex geometries. The plasma coatings can greatly improve their suitability for biomedical applications by optimising biocompatibility to the local needs, especially for cardiovascular disease treatments. Therein, wound healing and endothelialisation are important steps which are connected by a complex interaction.

The monomers allylamine and hexamethyldisiloxane, as well as different process conditions were studied for the coating of nanofibrous thermoplastic silicone polycarbonate polyurethane (TSPCU) nonwovens. Aim of this study was to investigate the feasibility of plasma polymer coating under preservation of the nanofibrous morphological structure. Beside characterization of the nonwoven, biological evaluation with endothelial and fibroblast cells was performed.

The prepared nonwoven samples support the feasibility of plasma coating under preservation of the nanofibrous structure. Also, different effects of the surfaces in contact with fibroblasts and endothelial cells could be observed.

Keywords: PECVD, plasma polymer, plasma coating, hexamethyldisiloxane (HMDSO), allylamine, nonwovens, biocompatibility

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1 Introduction

Nanofibrous nonwovens with a high surface to volume ratio are being widely explored for biomedical applications, particularly for cardiovascular device implantability.

Nevertheless, often insufficient mechanical integrity, bioactivity and biocompatibility are being observed [1]. In this context, plasma enhanced chemical vapor deposition (PECVD) can apply ultrathin layers of polymers in the range of a few nanometres up to 1-2 micrometres [2] on the nanofibres to overcome these drawbacks. Layer properties and functional groups on the surface depend on the selection of the used monomers or monomer mixtures [3].

Focus of this work was studying the aforementioned potential of the plasma coatings for nanofibrous nonwovens. Therefore, PECVD processes of the common monomers allylamine [4] and hexamethyldisiloxane (HMDSO) [5] were applied on nanofibrous TSPCU nonwovens. Analyses via Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX), as well as an additional biological evaluation with endothelial cells and fibroblasts were performed.

2 Materials and Methods

2.1 Materials

Hexamethyldisiloxane (HMDSO), allylamine and all solvents (chloroform (CHCl_3), dimethylformamide (DMF) and trifluoroethanol (TFE) were purchased from Sigma Aldrich (Taufkirchen, Germany). Thermoplastic silicone polycarbonate polyurethane (TSPCU) was purchased from AdvanSource Biomaterials (Wilmington, United States). Human fibroblasts (HT-1080) and vascular endothelial cells (EA.hy926) were purchased by the American Type Culture Collection (ATCC, Manassas, USA). Minimum Essential Medium (MEM) and Dulbecco's Modified Eagle's Medium (DMEM), fetal calf serum (FCS) and penicillin/streptomycin were obtained from PAN Biotech (Aidenbach, Germany) and the CellQuanti Blue Assay from Biotrend Chemicals GmbH (Cologne, Germany).

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2.2 Methods

2.2.1 Nonwoven production

7.5 w % of TSPCU polymer was dissolved in CHCl_3 :DMF:TFE (ratio 8:1:1).

Electrospinning was performed on a 4Spin C4S LAB2 (Contipro, Dolní Dobrouč, Czech Republic) using a one jet needle setup with a collector distance between 16 and 20 cm, a voltage of 25 till 40 kV and 130-140 $\mu\text{L}/\text{min}$ feed rate of the polymer solution.

2.2.2 Plasma polymerisation

Plasma polymers were generated using the following parameters (Table 1).

Table 1: Plasma polymerisation parameters (coating duration, pressure during coating and applied generator power) for TSPCU nanofibrous nonwovens in a Femto plasma chamber from Diener electronic GmbH & Co. KG (Ebhausen, Germany) at 13.56 MHz

Monomer	Time	Pressure	Power
HMDSO I	1 min	1.2 mbar	60 W
HMDSO II	5 min	0.9 mbar	60 W
Allylamine I	10 min	0.6 mbar	10 W
Allylamine II	5 min	0.8 mbar	10 W

2.2.3 SEM images and EDX measurements

Morphological imaging analyses were performed with a QUANTA FEG 250 (FEI Company, Frankfurt/Main, Germany) scanning electron microscope (SEM) according to Teske *et al.* [6]. Fibre thickness was measured at different positions with minimum $n = 10$. EDX measurements were performed at two different areas and average changes in atom percent of the elements carbon, oxygen, nitrogen and silicon were calculated in comparison to the untreated nonwoven as reference.

2.2.4 Cell biological evaluation

Nonwoven specimens with a diameter of 6 mm were placed and weighted with teflon rings in wells of a 96-well plate

with four replicates per modification. Human fibroblasts (HT-1080) and vascular endothelial cells (EA.hy926) were then added. Therefore, each sample was cultured with 10.000 cells in 200 μL MEM and DMEM including 10 % FCS and 1 % penicillin/streptomycin, respectively. Cell cultivation was carried out in an incubator (ICO240med, Memmert GmbH, Schwabach, Germany) under simulated *in vivo* conditions at 5 % CO_2 , 21 % O_2 , and 37 °C. Tissue culture polystyrene (TCPS) surface served as growth control. The metabolic activity of cells was determined via CellQuanti Blue Assay after 48 h.

3 Results

3.1.1 Morphological analysis

Analyses of the fibre thickness via SEM images reveal an increase in fibre thickness for all plasma coated fibres except the second HMDSO plasma polymerisation process (see Figure 1).

Figure 1: Average fibre thicknesses of TSPCU nonwoven fibres before (reference) and after plasma coating with HMDSO and allylamine with standard deviation ($n = 10$).

3.1.2 Chemical analysis

The generation of allylamine and HMDSO plasma layers were confirmed by EDX analysis (see Figure 2) after each process. The allylamine coatings revealed a decrease in carbon and a typical nitrogen increase, related to the chemical structure of the monomer. Whereas, for the elements oxygen and silicon only minor changes were detected.

Figure 2: Chemical changes of atom percentages of carbon, nitrogen, oxygen and silicon after plasma polymerisation with allylamine and HMDSO in comparison to the reference.

Figure 3: Relative metabolic activity of fibroblast cells normalized to untreated TSPCU nonwoven (reference, n = 1 with 4 replicates).

Figure 4: Relative metabolic activity of endothelial cells normalized to untreated TSPCU nonwoven (reference, n = 1 with 4 replicates).

The carbon amount is more decreased by the HMDSO plasma coatings compared to allylamine, while an increase of oxygen and silicon was observed. The nitrogen amount did not change.

3.1.3 Cell biological evaluation

Evaluation of the metabolic activity of fibroblast cells (see Figure 3) revealed only a minor decrease in comparison to untreated nonwoven after plasma coatings. For the vascular endothelial cells (see Figure 4), a clear decrease in a similar range for all plasma coatings was observed.

4 Discussion

Morphological analysis revealed only minor changes after plasma coating without film formation (data not shown) and the fibre thickness confirmed the success of the plasma coating. Furthermore, the chemical analysis by EDX confirmed also the plasma coatings. For both allylamine and HMDSO coatings, the characteristic increase of nitrogen and silicone were observed, respectively.

Cell biological analyses showed a good biocompatibility for plasma coated TSPCU nonwovens in contact with fibroblasts. However, in contact with vascular endothelial cells a clear decrease of metabolic activity was observed for all plasma coatings, due to the new surface properties. This could be due to the surface morphology and the generated functional groups on the surface. For the application in cardiovascular diseases, further investigations are still necessary due to the complex interaction between fibroblasts and endothelial cells during wound healing and endothelialisation [7].

5 Conclusion

The study revealed the feasibility of plasma coatings on nonwovens obtaining the nanofibrous structure. Only minor influence of PECVD coatings on the relative cell metabolic activity of fibroblasts was observable. This may offer suitability of the generated materials in wound healing applications, whereas reduced activity of vascular endothelial cells may indicate unsuitability for endothelialisation. Nevertheless, further studies considering the morphology of cells and their adhesion on the surface should be performed. In prospect, by alteration of process parameters these plasma

coated materials may still be beneficial for applications in contact with endothelial cells.

Author Statement

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