

Plasma-assisted deposition of thiol-rich coatings on nanofibrous scaffolds for tissue engineering applications

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Abstract: In this study, polycaprolactone nanofibers (NFs) were coated with plasma-polymerized thiol-rich films by means of a dielectric barrier discharge using 1-propanethiol as monomer. An extensive optimization of the process parameters (monomer flow rate, treatment time, discharge power...) has led to the deposition of stable coatings exhibiting a high S content (9.6 %) while preserving the NF morphology. Finally, enhanced Schwann cell adhesion and proliferation were detected on the coated NFs, thus showing their potential in nerve tissue engineering applications.

Keywords: Tissue engineering, plasma polymerization, thiol-rich coatings, nanofibers.

1. Introduction

Tissue engineering (TE) is a rapidly emerging research area aiming at overcoming the limitations of conventional transplantation methods via the development of substitute structures so-called scaffolds for the restoration of damaged tissues and organs [1]. One of the promising approaches towards a successful tissue repair is the fabrication of a scaffold mimicking the fibrous structure of the extracellular matrix (ECM) that governs a wide range of crucial cellular performances such as adhesion and proliferation [2]. To do so, electrospinning can be used as a simple, versatile and cost-effective biofabrication technique able to produce polymeric fibrous meshes simulating the ECM morphology and nano-dimensions. Several biodegradable polymers have been employed in the generation of TE scaffolds with polycaprolactone (PCL) being by far the most considered material due to its non-toxicity and history of safe clinical use in humans [3]. Despite the afore-mentioned advantages, PCL is a hydrophobic material that does not exhibit desirable biochemical properties promoting cell-surface interactions. As a matter of fact, cells do not only recognize the fibrous topography but require as well the presence of surface biomolecules enhancing their affinity towards the scaffold. Therefore, a surface modification putting into effect appropriate biochemical properties can solve the issue. The generation of thiolated surfaces is gaining a huge popularity in TE applications since thiol groups (-SH) are known to serve as highly selective anchoring sites for the subsequent covalent immobilization of biomolecules [4]. This was previously done via self-assembly approaches that are associated with some drawbacks such as the use of organic solvents, the long reaction times and the multi-step procedure. Moreover, such methods can cause a chemically-induced damage of the delicate nanofibrous structure [5]. As an alternative, plasma-assisted polymerization of thiol-containing precursors can be employed to deposit thiol-rich coatings onto the scaffolds in a fast, solvent-free and eco-friendly way [6].

In this research, PCL nanofibers (NFs) are first fabricated by means of electrospinning. A plasma polymerization process is then performed to deposit thiol-rich coatings on the electrospun NFs by means of a dielectric barrier

discharge (DBD) using 1-propanethiol as precursor. An optimization of the deposited coatings is done via the variation of different plasma parameters (discharge power, gas flow rate, treatment time, working pressure) with a parallel extensive investigation of the physicochemical properties of the coated NFs. Finally, the optimized scaffolds were then seeded with Schwann cells (SCs), generally used in nerve TE, to assess the cell-surface interactions.

2. Materials and Methods

2.1. Scaffold biofabrication and surface treatment

To prepare the electrospinning solution, PCL pellets (molecular weight 80 000 g.mol⁻¹, Sigma Aldrich - Belgium) were dissolved in formic acid/acetic acid (9/1 v/v, Sigma Aldrich - Belgium) to reach a concentration of 20% w/v. The Nanospinner 24 (Inovenso, Turkey), that is described in detail elsewhere, was used to electrospin PCL NFs [7]. The electrospinning process was carried out at a voltage of 28 kV, a tip-to-collector distance of 15 cm and a flow rate of 0.5 ml/h. The collector rotational speed was set to 100 rpm in order to collect random NFs. Thereafter, the electrospun NFs were coated with thiol-rich films via a plasma polymerization carried out using the DBD reactor described in a previous work [8]. To do so, Ar (Alphagaz 1) was used as working gas and 1-propanethiol (99% - Sigma Aldrich - Belgium) as precursor. During the process, the precursor flow rate, chamber pressure, discharge power and treatment time were varied according to the values reported in Table 1.

Table 1: Parameters used during the plasma polymerization process.

Parameters	Chamber pressure (kPa)	Precursor flow rate (g/h)	Treatment time (min)	Discharge power (W)
Values	15	0.125	10	3
	10	0.250	15	5

2.2. Physicochemical characterization

The surface chemical composition of the untreated and thiolated NFs was assessed by carrying out X-ray photoelectron spectroscopy (XPS) measurements using the PHI 5000 Versaprobe II spectrometer (Physical

Electronics) described thoroughly elsewhere [7]. For every condition, 4 survey scans were recorded on 4 different positions of 2 different samples. The elemental composition was then quantified using the Multipak software (V 9.6). The morphology of the untreated and coated NFs was visualized by taking scanning electron microscopy (SEM) images using the JSM-6010 PLUS/LV microscope (JEOL, Japan) [7]. After coating the NFs with a thin gold layer using the JFC-1300 autofine sputter coater (JEOL, Japan), SEM images were acquired with an accelerating voltage of 7 kV. The average diameter (AD) of the NFs before and after plasma polymerization was calculated by measuring the diameter of 100 NFs per condition using the ImageJ software. In this way, the thickness of the coatings deposited on the NFs can be calculated as follows:

$$\frac{AD \text{ of coated NFs} - AD \text{ of untreated NFs}}{2}$$

2.3. Coating stability test

To determine the stability of the thiol-rich coatings in aqueous environments, the plasma-treated NFs were soaked in distilled water at 37° C for 24 h. Afterwards, the surface chemistry of the NFs were reassessed via XPS after drying them overnight in a vacuum oven at ambient temperature.

2.4. Schwann cell culture studies

In order to evaluate the cyto-responsive properties of untreated and thiolated NFs for ultimate nerve TE purposes, SCs were cultured on the samples. To do so, the NFs were first sterilized via a UV exposure of 30 min then seeded with the RT4-D6P2T cell line (ATCC, USA) in a medium composed of Dulbecco's Modified Eagle's Medium (DMEM) and 10% of foetal bovine serum at a density of 20 000 cells/sample (Ø: 1.2 cm). At days 1, 3 and 7 post-seeding, the cell viability and adhesion to the NFs were evaluated by performing a live/dead staining assay using calcein-acetylmethoxyester (Anaspec) and propidium iodide (Sigma Aldrich). After staining the cells, the samples were imaged by means of an Olympus 1X81 fluorescent microscope.

3. Results and discussions

3.1. Effects of the chamber pressure and precursor flow rate on the physicochemical properties of the deposited coatings

In order to study the effects of the DBD chamber pressure and precursor flow rate on the properties of the plasma-polymerized coatings, they were separately varied while maintaining the discharge power and treatment time at constant values of 5 W and 10 min respectively. Table 2 contains the surface sulfur and oxygen contents as obtained from XPS data and the coating thicknesses after the different parametric plasma polymerization processes. Results reveal that when increasing the chamber pressure from 10 kPa to 15 kPa while using a precursor flow rate

of 0.250 g/h, the sulfur content of the deposited coatings significantly decreased from 9.6 % to 4.3 %. In fact, at higher pressures, more collisions between plasma species, monomer molecules and air molecules remaining in the plasma chamber occur. On the one hand, this can lead to more fragmentation and recombination of the formed monomer radicals in the gaseous phase rather than on the NFs [9]. On the other hand, this can activate air molecules which leads to the incorporation of oxygen into the deposited coating at the expenses of sulfur. This was actually observed by a higher oxygen content at a pressure of 15 kPa (16.0 %) compared to a pressure of 10 kPa (10.9 %). Next to varying the pressure, results have revealed that increasing the monomer flow rate from 0.125 g/h to 0.250 g/h could trigger a higher S content into the deposited coatings. Increasing the monomer flow rate actually results in a decreased input energy per monomer molecule which is the controlling parameter W/FM that Yasuda has proposed where W, F and M are the plasma power (W), monomer flow rate (mol/s) and monomer molecular weight (kg/mol) respectively. A lower energy triggers a less pronounced monomer fragmentation which leads to a more efficient incorporation of S-containing functionalities into the deposited coatings. When taking a look at the O content of the coatings, one can also notice significantly higher values for the low monomer flow rate (0.125 g/h). This is probably due to the higher energy input per monomer molecule which could result, given the more intense fragmentation of the monomer, in a higher amount of trapped free radicals into the deposited coatings. These free radicals, will end up interacting with the ambient air molecules post-plasma treatment, leading to the incorporation of oxygen-containing functionalities into the deposited coatings[10]–[12]. Moreover, given the fact that the coatings deposited with a monomer flow rate of 0.125 g/h are thinner than the ones deposited at a rate of 0.250 g/h, it could be that the detected oxygen is, to a low extent, due to the underlying PCL NFs. In fact, one could notice that the higher the S content is, the thicker the deposited coatings are. This is again in correlation with the Yasuda parameter which suggests that a low monomer fragmentation is associated with a high S retention degree and high deposition rate. Nonetheless, at a pressure of 15 kPa and monomer flow rate of 0.250 g/h, melted NFs were observed on the SEM images which suggests an undesirable increase in plasma temperature.

Table 2: Surface elemental composition and coating thickness as a function of the chamber pressure and precursor flow rate

Chamber pressure (kPa)	Precursor flow rate (g/h)	Sulfur content (%)	Oxygen content (%)	Coating thickness (nm)
untreated NFs	untreated NFs	-	22.7 ± 0.8	-
10	0.125	4.3 ± 1.4	18.1 ± 1.9	44 ± 68.1
10	0.250	9.6 ± 1.2	10.9 ± 1.4	147.6 ± 84.8
15	0.125	3.4 ± 1.0	19.2 ± 2.1	26.9 ± 76.9
15	0.250	4.3 ± 1.1	16.0 ± 2.4	Melted NFs

3.2. Effects of the discharge power and treatment time on the physicochemical properties of the deposited coatings

Since a precursor flow rate of 0.250 g/h and a chamber pressure of 10 kPa were associated with the highest S retention, they were kept constant to study the effects of the treatment time and the discharge power on the deposited coatings. **Error! Reference source not found.** contains the surface sulfur and oxygen contents and the coating thicknesses after plasma polymerization processes performed at varied treatment times and discharge powers. Results reveal that for a treatment time of 10 min, a lower sulfur content was detected for a discharge power of 3 W (6.0 %) than for a power of 5 W (9.6 %). As such, it seems that a power of 3 W, the input energy per monomer molecule was low to induce the formation of a high number of monomer radicals which led to low S retention. This is accompanied with a low deposition rate as can be seen by the considerably thinner coatings. As such, the low S content could be due to the fact that the chemistry of the underlying PCL NFs was also detected during the XPS measurements as can be confirmed by the high O content. When increasing the treatment time to 15 min, no differences in the sulfur content ($\approx 10\%$) could be detected between the different discharge powers. In fact, at extended times, more monomer molecules could be activated and thicker coatings could be deposited. When a discharge power of 5 W was used, no significant differences in the coatings chemistry could be noticed at different treatment times, as in this case the higher input energy per monomer molecule could lead to a higher deposition rate and thick coatings already for 10 min of treatment. As such, when extending the treatment time, the coatings only became thicker while retaining the same chemistry [13].

Table 3: Surface elemental composition and coating thickness as a function of the discharge power and treatment time

Discharge power (W)	Treatment time (min)	Sulfur content (%)	Oxygen content (%)	Coating thickness (nm)
0	0	-	22.7 ± 0.8	-
3	10	6.0 ± 2.2	18.2 ± 1.9	49.9 ± 70.65
5	10	9.6 ± 1.2	10.9 ± 1.4	147.6 ± 84.8
3	15	10.0 ± 0.7	10.0 ± 1.0	94.8 ± 74.9
5	15	10.9 ± 0.5	9.8 ± 0.4	122.1 ± 66.5

When taking a look at the SEM images of the untreated and coated NFs, one could notice a preserved nanomorphology for different treatment times and discharge powers, which is crucial in TE applications (Fig. 1). As such, the plasma polymerization conditions did not damage the NFs but increased their diameters as a result of the deposited coatings. These coatings nicely covered individual NFs without blocking the interfibrillar pores.

3.3. Aqueous stability of the coatings

To study the aqueous stability of the thiol coatings deposited on the PCL NFs, their surface chemistry was assessed before and after immersing the NFs in water. Fig.

1 depicts the S content on the surface of the NFs exposed to different plasma polymerization conditions pre- and post-water immersion.

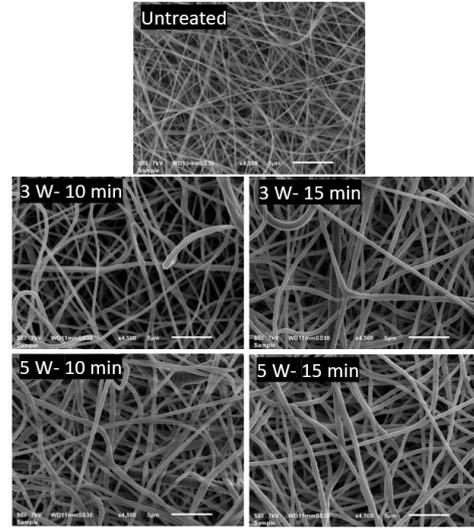


Fig. 1: SEM images of the PCL NFs before and after exposure to plasma polymerization processes conducted at different discharge powers and treatment times.

Results reveal that for a treatment time of 10 min, no significant differences in the S content could be detected pre- and post-immersion regardless of the discharge power. As such, one can conclude that the coatings were not delaminated from the NF surface. Nonetheless, when increasing the treatment time to 15 min, a decrease in the S-content could be observed for discharge powers of 3 W and 5 W. This is probably due to the fact that at extended treatment times, a combination of etching and deposition occur with more reactivation of the recombined monomers thus leading to a lower crosslinking degree within the coatings. As such, the coating could be partly dissolved when immersed in water [10]. Given the fact that the samples treated for 15 min at a power of 5 W present the highest sulfur content in their coating and are stable in water, they were used in next *in vitro* study.

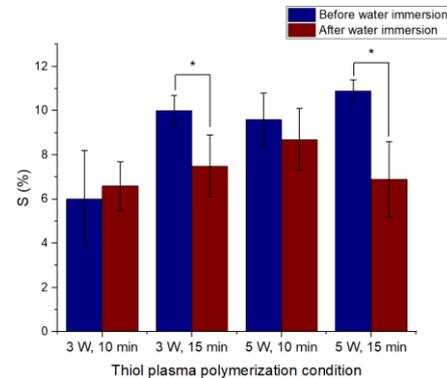


Fig. 2: Surface S content of the coated NFs at different plasma polymerization conditions pre- and post-water immersion

3.4. Cyto-responsive properties of untreated and thiolated PCL NFs

Since the ultimate goal of the study is to enhance SC-material interactions on PCL NFs for nerve TE applications, SCs were cultured on the NFs for 1, 3 and 7 days then visualised after live-dead staining. From the obtained fluorescent images, one can clearly observe a significantly higher cell density and a more homogeneous cell distribution on plasma-coated NFs compared to their untreated counterparts (Fig. 3). In fact, the coated NFs present S-containing groups, mainly thiol groups, acting as binding sites for biomolecules on which cell receptors can attach [6]. This enhances the SC attachment on the NFs, hence the high cell density. An initial good cell attachment is known to lead to a good subsequent cell proliferation, which is seen by the increased cell density on the thiolated NFs on day 3 and day 7 compared to day 1 [7]. However, the cell density on untreated NFs tended to decrease throughout the days with the occurrence of more dead cells (red colored cells). This is due to the initial bad cell attachment and subsequent mediocre proliferation or even cell death.

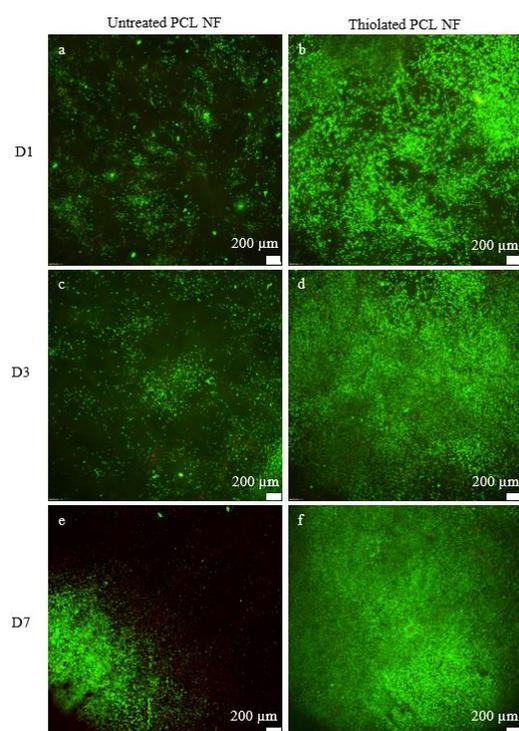


Fig. 3: Live-dead staining images of SCs cultured on untreated and plasma-coated NFs for 1, 3 and 7 days.

4. Conclusion

In this work, thiol-rich coatings were deposited on PCL NFs via a DBD plasma polymerization of 1-propanethiol for ultimate nerve TE applications. An optimization of the physicochemical properties of the deposited coatings was performed by varying several plasma process parameters including the monomer flow rate, chamber pressure, treatment time and discharge power. In parallel, the surface

chemistry and thickness of the coatings were measured via XPS and SEM measurements. The aqueous stability of the coatings was also evaluated by reassessing their surface chemistry after immersing the coated NFs in water. Results revealed that the optimal coating presenting a high S content (9.6 %) while exhibiting an aqueous stability was obtained at a chamber pressure of 10 kPa, a monomer flow rate of 0.250 g/h, a treatment time of 10 min and a discharge power of 5 W. Interestingly, the S-rich coatings were nicely deposited on individual NFs without blocking the interfibrillar pores. Finally, after culturing SCs on the NFs, a significantly higher density of viable cells was detected on the coated NFs compared to their untreated counterparts indicating better cell adhesion and proliferation. As such, one can conclude that PCL NFs coated with plasma-polymerized S-rich films are promising candidates in nerve TE applications.

5. References

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