

ATMOSPHERIC PRESSURE PLASMA CO-POLYMERIZATION OF MALEIC ANHYDRIDE AND ACETYLENE ONTO POLYCAPROLACTONE FIBERS

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Abstract

In the field of tissue engineering many different materials have been investigated as supporting scaffolds. Electrospun nanofibers prepared from polycaprolactone (PCL) exhibit promising properties, but require further processing to improve its poor wettability. Dielectric barrier discharge (DBD) is a method of choice for a low cost, upscalability and environmental friendliness. Plasma polymer thin film containing carboxyl groups significantly lowers water contact angle of PCL nanofibers. We have investigated the plasma copolymerization of maleic anhydride and acetylene in the DBD. Thin films containing anhydride and carboxyl groups were deposited on PCL electrospun nanofibers and Si substrates. The surface chemistry and the morphology of the samples were studied by analytical techniques including Fourier transform infrared spectroscopy in attenuated total reflectance mode (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). In adjusted condition the film exhibited up to 11 at.% of carboxyl groups, while being relatively stable in water. Treatment also led to improved wettability of nanofibers, as the WCA decreased from $115 \pm 2^\circ$ to 0° .

Keywords:

plasma copolymerization, maleic anhydride, polycaprolactone, nanofibers, DBD

1. INTRODUCTION

Electrospun nanofibers with a high surface area to volume ratio gain increasing interest because of their potential application for biomedical devices, tissue engineering scaffolds and drug delivery carriers[1]. They have architectures analogous to the natural environment of tissue in physical structure and biological function.[2] Therefore, the potential of electrospinning technique for tissue engineering seems to be very large. Furthermore, the electrospinning process for the nanofiber synthesis demonstrates comparatively low costs and relatively high production rate. It is worth noting that biomaterials for tissue engineering must be both bioactive and biodegradable.[2] Due to the biodegradability and good mechanical properties of polycaprolactone (PCL), this polymer is widely used for the tissue engineering.[3] In order to apply electrospun nanofibers in biomedical uses, their surface has to be modified due to the low cell adhesion to the untreated nanofibers. [2] Geux et al. have observed the cell adhesion on the PCL micro/nanofibers coated by 7 nm thick C:O:H layers using low pressure RF discharge in the CO₂/C₂H₄ - Ar mixture.[4] Recently, PCL nanofibrous meshes were coated by stable amine plasma polymers using low pressure radio frequency plasma polymerization of cyclopropylamine.[5,6] No intermediary step aimed at grafting of the bioactive molecule was required. This single-step dry process for the development of the biocompatible nanofibers suitable for the tissue engineering seems to be very promising. However, an atmospheric

pressure plasma treatment would be even more advantageous compared to the low pressure plasma process, as it avoids the utilization of vacuum technologies and can be, therefore, easily installed in the existing production lines. For example, an atmospheric pressure dielectric barrier discharge (AP-DBD) is often used for plasma processing of soft materials. DBD can be operated either in filamentary or homogeneous mode.[7] Homogeneous DBD, which was discovered and studied within last 20 years, brought new possibilities into DBD applications. It can be obtained in He or Ne and it is called atmospheric pressure glow discharge (APGD).[8,9] Another type of homogeneous DBD can be obtained in nitrogen and it is called atmospheric pressure Townsend-like discharge (APTD).[10] The homogeneous AP-DBD is more suitable for homogeneous plasma treatment of heat sensitive polymers or for homogeneous deposition.[11,12,13]

In this paper, the PCL electrospun nanofibers have been coated by a plasma copolymer layer sustaining high concentration of carboxyl groups using the atmospheric plasma process. For this purpose, maleic anhydride (MA) and acetylene C₂H₂ precursors were polymerized by means of AP-DBD. The role of acetylene as a second precursor is the enhancement of the plasma coating stability under immersion of the coated material in the water and cell medium. Indeed, the key issue is the deposition of the durable and stable coating presenting sufficient COOH concentration. Therefore, it is necessary to find the optimum between the crosslinkage of the plasma polymer (which should be obtained thanks to the ion bombardment and the free radical reactions with acetylene) and the concentration of COOH groups. Indeed, water-soluble oligomers must be avoided in spite of their high carboxyl concentration.

2. EXPERIMENT

2.1 PCL nanofibers

PCL nanofibers were prepared by electrospinning technology Nanospider™ developed by company ELMARCO s.r.o. (Czech Republic). Polycaprolactone pellets (80 g, M_w = 14 000) were dissolved in 500 ml of formic and acetic acids in ratio 17:33 and maintained at 40 °C for 6 hours and then next 12 hours at ambient temperature. The mixture was subsequently stirred with 100 ml of 1:1 solution of acetic and formic acids for next 12 hours. The PCL solution was poured into bath with wired electrode 50 cm in length. Nanofibers were collected on paper covering a grounded electrode and travelling with speed of 15 mm/min. Electrospinning was performed with electrode rotation speed of 4 RPM and voltage of 55 kV between electrode and collector.

2.2 DBD set-up

The experiment was done in a metallic cube chamber with the side dimension 500 mm. The discharge was ignited between two planar metallic electrodes coated by Al₂O₃ dielectric plate (thickness of 1 mm). The upper electrode was connected to high voltage, while the bottom electrode was grounded. The gap between ceramics was set to 2.5 mm. The electrodes were rectangular, bottom with dimensions 150 mm × 60 mm, and the upper 20 mm × 60 mm. In order to improve homogeneity of the deposition upper electrode was moving above the sample and gas supply was attached to the electrode as schematically shown in Figure 1. The configuration of gas supply and exhaust was optimized in the preliminary deposition experiments.

Plasma copolymer layers were deposited in nearly homogeneous mode of DBD from mixture of maleic anhydride (MA) and acetylene diluted in argon. The MA vapours were delivered into the discharge using bubbling system. The flow rate of argon flowing through the bubbler with solid MA pellets was set to 1.8 standard litres per minute (slm). From that we can calculate flow rate of MA as 0.40 standard cubic centimetres per minute (sccm). Acetylene flow rate was set to 3.0 sccm. Before starting the experiment the discharge chamber was pumped down to a pressure of 100 Pa and then filled with gases in the same ratio as for deposition up to a pressure of 96 kPa. The plasma was ignited by sinusoidal high voltage applied to the upper electrode. High voltage was supplied by tunable generator set to 6.6 kHz with 12 W power input. The

deposition time was 10 minutes for all samples. The discharge current and voltage signal were monitored by the OWON oscilloscope.

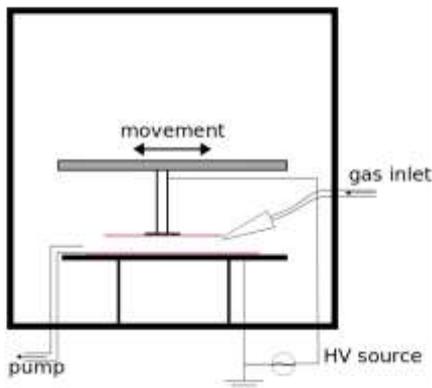


Figure 1. Schematic drawing of the DBD reactor.

2.3 Characterization

X-ray Photoelectron Spectroscopy

Surface chemical characterization was performed by XPS using the Omicron X-ray source (DAR400) and electron spectrometer (EA125) fitted on a custom built UHV system. The narrow scan measurements were performed at pass energies of 25 eV and the X-ray gun power was set to 270 W. The electrons take off angle was 50°. The maximum lateral dimension of the analyzed area was 1.5 mm. The quantification was carried out using XPS MultiQuant software [14].

The XPS C1s and N1s signals were fitted with the Casa XPS software after subtraction of the Shirley-type background. The XPS data curve fittings were performed in accordance with the available literature on binding energies (BE) of different carbon environments [15]. The fitting employed Gaussian–Lorentzian (G-L) peaks with the fixed G-L percentage 30%. The full width at half maximum (FWHM) was set to 1.85 ± 0.05 eV for all peaks.

IR spectroscopy

Fourier transformed infra red spectra in attenuated total reflectance mode (ATR-FTIR) were obtained using a Bruker Vertex 80v spectrophotometer. Accessory for ATR uses single reflection with angle of incidence 45° on interface of diamond crystal and sample. The data were collected at pressure of 251 Pa in spectral area 400 – 4000 cm^{-1} with the resolution of 4 cm^{-1} and 100 scans. The assignment of the IR absorption peaks was performed on the basis of comprehensive IR database [16].

Scanning Electron Microscopy

Samples were imaged by the field-emission scanning electron microscope (FE-SEM) Tescan LYRA 3 XM. Prior to the imaging thin gold layer was sputtered over sample by RF magnetron in order to prevent excessive charging. Electron acceleration voltage was set to 2 kV, while working distance was 5.5 mm.

Atomic Force Microscopy

Atomic force microscopy (AFM) was measured by a commercial ambient scanning probe microscope (NT-MDT Ntegra Prima) in a semicontact mode using commercial silicon cantilevers NSG-10 (NT-MDT).

WCA

The water contact angles were measured using a sessile drop technique. The system developed in our laboratory enables observation of a solid–liquid meniscus directly, using a CCD camera. The contact angles were determined from the CCD snapshots.

3. RESULTS

3.1 Morphology of the coating

The SEM and AFM micrographs (Figure 2) revealed the morphology and microstructure of samples. According to AFM measurements, the root mean square roughness R_{rms} of MA-C₂H₂ plasma copolymers deposited on silicon wafer is around 50 nm (Figure 2a). The uncoated electrospun PCL nanofiberous mesh with the typical diameter of $\sim 1 \mu\text{m}$ and random orientation (Figure 2b). The nanofibers did not suffer significant damage during the treatment and were homogeneously coated by plasma polymer (Figure 2c). The rough powdery deposit can be observed on the top of coated fibers and it remains even after immersion in water (Figure 2d). This latter highlights the stability of plasma copolymer layer.

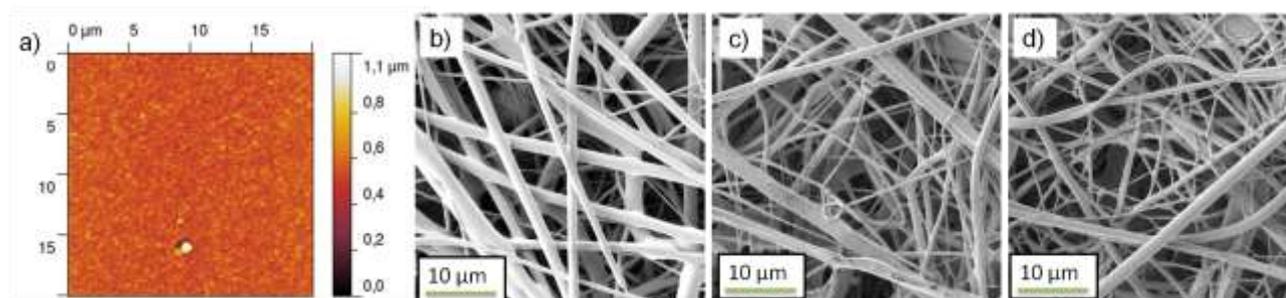


Figure 2. AFM picture of the layer on silicon (a) and SEM pictures of uncoated nanofibers (b), plasma polymer deposited on the nanofibers (c), coated nanofibers after immersion in water (d).

3.1 Chemistry of deposited layers

At first, the chemistry of deposited layers was studied by ATR-FTIR (Figure 3a). Due to the high penetration depth of $\sim 1 \mu\text{m}$, PCL signal was also visible in the spectra. The region from 1900 to 2650 cm^{-1} is excluded from the graph due to a diamond tip absorption. The absorption at 1782 cm^{-1} has confirmed the presence of a cyclic anhydride group in the polymer structure. However, after the immersion in water only the traces of the peak remained in the spectrum. It suggests that the anhydride groups were hydrolysed under the water immersion.

XPS analysis allows insight into surface chemistry of plasma polymer deposited on both silicon wafer and PCL nanofibers. The MA-C₂H₂ copolymer deposited on Si wafer exhibited 71.8 at.% of carbon and 27.2 at.% of oxygen. In case of coated nanofibers, the content of oxygen was lower (23.9 at.%) but increased to 28.3 at.% after the immersion in water. It should be noted that bare nanofibers have 23 at.% of oxygen and therefore such increase probably highlights the hydrolysis of anhydride groups to COOH functions, rather than dissolution of plasma copolymer layer.

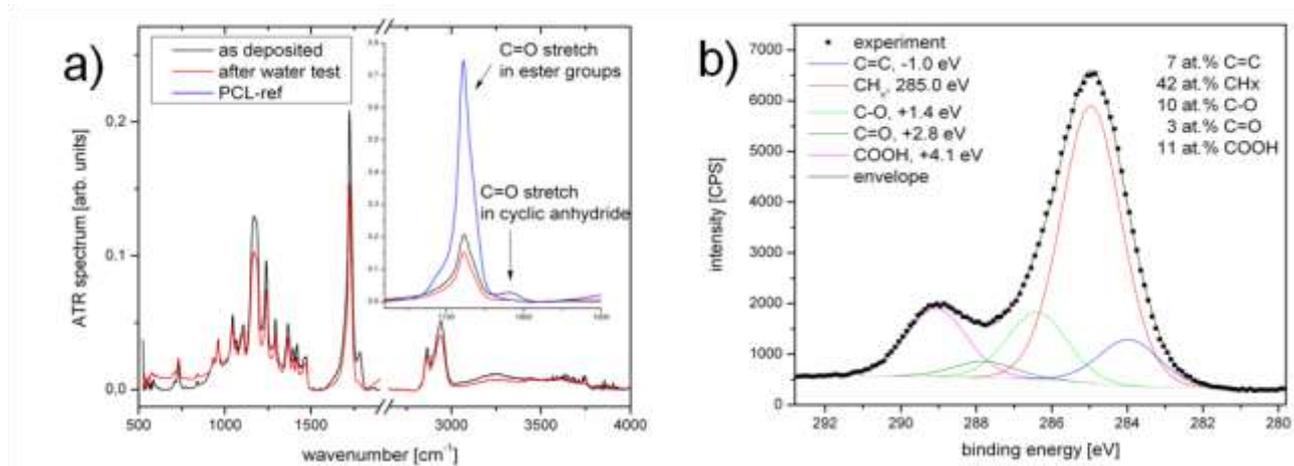


Figure 3. ATR-FTIR spectra of PCL nanofibers (a), XPS C 1s curve fitting of sample deposited on silicon wafer.

Interestingly, the concentration of oxygen in the deposited layers is relatively high compared to the atomic composition of the inserted gas mixture into the plasma. Indeed, the ratio of MA to C₂H₂ in the gas mixture is equal to 1:7.5, which corresponds to O/C ratio of 0.16, while MA-C₂H₂ copolymer exhibited 0.27 (on Si wafer) or 0.23 (on PCL nanofibers) of oxygen to carbon ratio. Hence the incorporation of the oxygen species into the plasma copolymer layer is more efficient compared to the hydrocarbon species.

Further investigation of layer chemistry was performed by means of the C1s peak deconvolution to reveal the chemical environment in the plasma copolymer (Figure 3b). The C1s signal was fitted as a sum of four components, namely aliphatic carbon CH_x at 285.0 eV, carbon with single bond to oxygen (C-O, chemical shift of 1.45 eV), carbon with a double bond to oxygen (C=O with chemical shift 2.85 eV) and carbon in carboxyl group (COOH with chemical shift of 4.1 eV). In case of Si substrate, additional component with the chemical shift of -1.0 eV was inserted to obtain the sufficient agreement of fitting with the experimental data. This peak was attributed to C=C bond. The presence of the unsaturated carbon (C=C) is related to the incomplete polymerization of acetylene, i.e. only one π-bond was opened in some cases, while one π-bond was not affected by plasma and C=C group was embedded in the coating. The C-O and C=O environments are the result of MA degradation, as these moieties are not presented in the monomer structure.

The density of COOH groups (11 at.%) is comparable with the previously reported results of MA or acrylic acid plasma polymerization using atmospheric pressure DBD [12-13,17-18]. Finally, the high concentration of COOH groups in plasma coating led to significant change of the hydrophilicity of PCL nanofibers. The uncoated nanofibers (Figure 4a) exhibited WCA of 115 ± 2°, while plasma coated PCL nanofibers were extremely hydrophilic (WCA < 10°). It should be noted that WCA of coated nanofibers was practically unmeasurable due to extreme hydrophilicity (Figure 4).

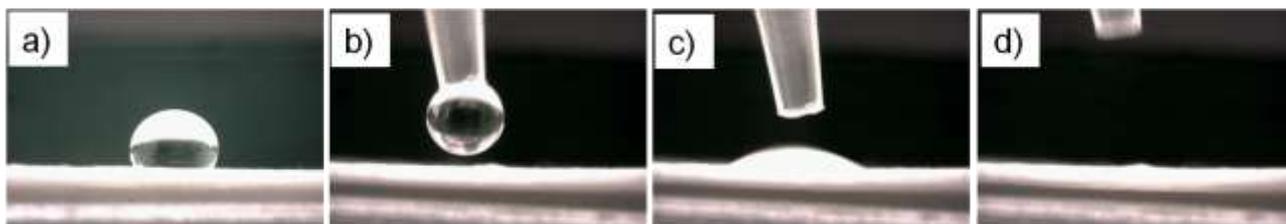


Figure 4. Snapshots from WCA measurements of PCL nanofibers: a) uncoated, b) coated before water droplet, c) 0.1s after the water droplet, d) 0.2 sec after the water droplet.

4. CONCLUSION

PCL nano/microfibers can be coated by atmospheric pressure DBD without damaging their structure. Coatings prepared by co-polymerization of maleic anhydride and acetylene contains about 11 % of COOH groups, which is comparable with result reported in the literature [12-13,17-18]. SEM and XPS revealed that plasma copolymer layers retained after water immersion. The extreme hydrophilicity of the plasma coated PCL nanofibers shown in Figure 4 should enable enhanced cell adhesion and proliferation enabling the application of this nanocomposite material for a tissue engineering.

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