



Deposition of stable amine coating onto polycaprolactone nanofibers by low pressure cyclopropylamine plasma polymerization



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ARTICLE INFO

Available online 21 September 2014

Keywords:

Polycaprolactone

Nanofibers

Cyclopropylamine

Plasma polymerization

Water stability

X-ray photoelectron spectroscopy

ABSTRACT

Amine-rich films are of high interest for the bio-applications including drug delivery and tissue engineering thanks to their high reactivity allowing the formation of the covalent linkages between biomolecules and a surface. However, the bio-applications of amine-rich films require their good stability in water which is often achieved at large expenses of the amine concentration. Recently, non-toxic cyclopropylamine (CPA) has been applied for the plasma polymerization of films bearing high NH_x environment combined with the moderate thickness loss (20%) after water immersion for 48 h. In this work, the amine-rich film with the NH_x concentration over 7 at.% was deposited on Si substrates and polycaprolactone nanofiber meshes by using CPA plasma polymerization (pulsed mode) in a vertically oriented stainless steel reactor. The substrates were placed at the radio frequency electrode and the ion bombardment caused by direct-current self-bias was suppressed by using high pressure of 50 Pa. Analysis of samples by scanning electron microscopy did not reveal any cracks in the deposited layer formed during a sample immersion in water. Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) confirmed a slight oxidation of amine groups in water but the film still contained 5 at.% of NH_x (according to the N1s XPS fitting) after the immersion. The rapid oxidation of amine groups was observed during the aging experiment carried out in air at room temperature because FTIR revealed an increase of amide peaks that increased progressively with aging time. However, this oxidation was significantly reduced if the plasma polymer was stored at $-20\text{ }^\circ\text{C}$. Since the films exhibit high amine concentration and very good water stability they have great potential for applications as biocompatible functional coatings.

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1. Introduction

Electrospun nanofibers with a high surface area to volume ratio are gaining increasing interest because of their potential applications in biomedical devices, tissue engineering scaffolds and drug delivery carriers [1]. However, in order to exploit advantages of the nanofiber mesh, its surface has to be activated by reactive groups like carboxyls, hydroxyls or amines.

Among various reactive groups, primary amines are considered the most promising for immobilization of biomolecules and promotion of cell adhesion thanks to their high reactivity. Choi et al. demonstrated that polycaprolactone (PCL) or polycaprolactone/polyethyleneglycol (PCL/PEG) electrospun nanofibers can be applied for in vivo wound healing of diabetic ulcers after grafting of amine groups and immobilization of recombinant human epidermal growth factor [2]. The aminated PCL and poly-L-lactic acid nanofibers promoted most effectively the

adhesion and expansion of human umbilical cord blood hematopoietic stem/progenitor cells [3]. Amine functionalized electrospun nanofibers were also used for the adsorption of heavy-weight metals from aqueous solutions [4,5]. To date, the amine functionalization of the nanofibers has been done mostly via liquid multi-step techniques such as silanization [5] or a combination of photo-polymerization of acrylic acid and conjugation with amine compounds using two-step carbodiimide crosslinking method [3,6]. However, these methods are time-consuming and environmentally unfriendly due to a high consumption of reactants and solvents.

Potential applications of the amine functionalized biodegradable nanofibers can be significantly enhanced if the methodology of amine grafting is improved.

A deposition of stable amine-rich thin films using dry processes such as plasma polymerization could improve the range of potential applications of biodegradable nanofibers. However, the amine plasma modification of nanofibers is rarely presented in the literature. Yan et al. used N_2/H_2 plasma treatment for the grafting of NH_x onto the PCL nanofibers for increased mouse osteoblast proliferation [7]. The N_2/H_2 plasma treated PCL nanofibers demonstrated 50% higher cell proliferation compared to the unmodified PCL nanofibers. Unfortunately, the N_2/H_2

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plasma functionalization suffers from fast degradation because the concentration of grafted polar groups (including amines) rapidly decreases in both water and air [8,9]. Moreover, the amount of amine groups introduced by N_2/H_2 plasma treatment is always low, as the nitrogen concentration is generally only about 1–2 at.% [7].

On the contrary, the deposition of thin plasma coatings can suppress the aging of the deposited layer [9–12]. Nevertheless, the deposition of amine-rich plasma polymers combining a sufficiently high concentration of amine groups and essential layer stability during their application (e.g. *in vivo*) still requires certain improvements. The most widely employed methodologies of amine plasma polymer deposition rely on the allylamine, NH_3/C_2H_2 or NH_3/C_2H_4 plasmas [9,13,14]. A sufficient stability of amine layers (i.e. thickness loss below 10%) can be achieved only if the concentration of the primary amines is below 2 at.% [14]. Moreover, both ammonia and allylamine are toxic hazardous compounds. In our recent publication [15], it was shown that radio frequency capacitively coupled plasma (RFCCP) polymerization of cyclopropylamine (CPA) can be adjusted to deposit an amine-rich layer bearing 9 at.% of $NH_{x=1,2}$ functions and exhibiting only 20% thickness loss after immersion in water for 48 h. However, the biomedical applications of amine coatings require better layer stability in aqueous media and, therefore, the methodology of CPA plasma polymerization must be further optimized.

In this work, the amine-rich plasma polymer ($NH_x > 7$ at.%) were deposited on Si wafers and PCL nanofiber meshes by CPA RFCCP polymerization in a vertically oriented stainless steel plasma reactor. In this configuration, the bottom electrode bearing the substrates was direct-current (DC) negatively self-biased due to an asymmetric coupling but ion bombardment was significantly reduced by relatively high process pressure of 50 Pa. The deposited coatings exhibited the thickness loss below 2%, which is sufficiently low to employ these amine plasma polymers for biomedical applications.

2. Experiment

2.1. Materials

Cyclopropylamine (purity of 98%) was purchased from Sigma Aldrich and used without any further purification. Argon (purity of 99.998%) was supplied by Messer. Glacial acetic acid (99%, p.a. grade) and formic acid (98%, p.a. grade) were supplied by Penta s.r.o. (Czech Republic). Samples were cut from double-side polished silicon wafer (N-type, phosphorus doped) with (111) surface orientation substrates and the resistance of $0.5 \Omega \cdot cm$ (ON-Semiconductor, Rožnov pod Radhoštěm, Czech Republic). Polycaprolactone flakes with $M_w = 14\,000$ were purchased from Sigma Aldrich.

2.2. Preparation of nanofibers

PCL nanofibers were prepared by Nanospider™ technology using the laboratory machine NS LAB 500S from Elmarco s.r.o. (Czech Republic). The solution for electro-spinning was prepared by dissolving 80 g of PCL pellets in 500 ml of 17:33 solution of formic and acetic acids. After mixing, the solution with PCL was maintained for 6 h at 40 °C and then 12 h at 25 °C. In the last step, 100 ml of 1:1 solution of acetic and formic acids was added, and the mixture was stirred for 12 h at 25 °C. The PCL nanofibers were formed from the solution using wired electrode, 50 cm in length, rotated with the speed of 4 rpm. Nanofibers were gathered on a nonwoven polypropylene foil placed at a grounded collector, 170 mm from the wired electrode. The polypropylene foil was traveling with the speed of 15 mm/min. The voltage applied between the wired electrode and the collector was 55 kV. Nanofiber meshes were dried at 25 °C for 48 h and then peeled off from the carrying foil and stretched on glass slides.

2.3. Plasma polymerization set-up

The CPA plasma polymers were prepared in a stainless steel parallel plate reactor [16–18]. The bottom electrode, 420 mm in diameter, was capacitively coupled to a RF generator working at the frequency of 13.56 MHz. The gases were fed into the chamber through a grounded upper showerhead electrode, 380 mm in diameter. The distance between the electrodes was 55 mm. The bottom electrode with substrates was negatively DC self-biased due to an asymmetric coupling. The reactor was pumped down to 10^{-4} Pa by a turbomolecular pump with a backing rotary pump. The deposition was carried out with the rotary pump only. The leak rate including wall desorption was below 0.1 sccm for all the experiments.

The CPA was polymerized in squared pulsed CPA/Ar plasma at 100 W and the pressure of 50 Pa. The pulse duty cycle and repetition frequency were 33% and 500 Hz, respectively. The flow rate of Ar was set to 28 sccm and regulated by an electronic flow controller Hastings whereas the flow rate of CPA vapors was set to 2 sccm by a needle valve. The deposition time was 60 min. The substrates were sputter-cleaned by pulsed Ar plasma for 10 min prior to the deposition.

2.4. Characterization methods

The X-ray photoelectron spectroscopy (XPS) for a surface (2–3 nm) chemical characterization of Si and nanofibers coated by CPA plasma polymer was carried out using an Omicron X-ray source (DAR400, output power 270 W) and an electron spectrometer (EA125) attached to a custom built ultra-high vacuum system. The quantitative composition was determined from detailed spectra taken at the pass energy of 25 eV and the electron take off angle 50°. The maximum lateral dimension of the analyzed area was 1.5 mm. The quantification was carried out using XPS MultiQuant software [19]. The deconvolution of XPS C1s and N1s spectra to individual components was done in the CasaXPS software after subtraction of the Shirley background employing Gaussian–Lorentzian (G–L) peaks with the fixed G–L percentage of 30% and the full width at a half maximum set to 1.85 ± 0.05 eV.

The film on Si was characterized by ellipsometry using a phase modulated Jobin Yvon UVISSEL ellipsometer in the spectral region of 1.5–6.5 eV with an angle of incidence of 65°. The data were fitted using a parameterizing joint density of states (PJDOS) dispersion model for SiO_2 -like materials [20] and structural model of wedge-shaped non-uniform thin film [21]. The following parameters were fitted: thickness, thickness non-uniformity and dispersion model determining spectral dependencies of optical constants.

The transmittance in the spectral range from 370 to 7500 cm^{-1} was measured using Fourier Transform IR (FTIR) Bruker Vertex 80v spectrophotometer with a parallel beam transmittance accessory. The data were collected at the pressure of 250 Pa with the resolution of 4 cm^{-1} and 500 scans. The transmittance of the films on Si substrate was divided by the transmittance of bare Si substrate in order to calculate a relative transmittance. In this paper, the FTIR spectra are shown only in the range from 1490 to 3650 cm^{-1} because no significant absorption peaks belonging to the films were identified outside this range and the identification of weak absorption peaks below 1490 cm^{-1} was difficult due to strong absorption peaks in Si that could not be reliably subtracted.

Samples were imaged using the field-emission scanning electron microscope (FE-SEM) LYRA3 XM, manufactured by Tescan. The Au coating with a thickness of 10 nm was deposited by RF magnetron sputtering prior to the imaging in order to compensate for a charging. The electrons were accelerated by a 2 kV high voltage and the working distance was fixed at 4 mm in order to minimize the charging effect and to improve the resolution. Images of 1024×1024 pixels were acquired within 1 min. The measurement of the fiber and pore size was performed using the “built-in” Tescan software.

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