

EFFECT OF COLD ATMOSPHERIC PRESSURE PLASMA GENERATED BY DIFFUSE COPLANAR SURFACE BARRIER DISCHARGE ON POLY(2-OXAZOLINE) THIN FILMS

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Abstract

Prevention of the non-specific adhesion of bacteria, cells or proteins to a solid surface is essential for sustaining the functionality and stability of medical implants. Poly(2-oxazolines) (POx) are a class of synthetic polymers, which among other features (e.g., biocompatibility, water solubility) possess anti-biofouling properties and accordingly, they are very promising for this purpose. In our contribution, we investigate the effect of cold atmospheric pressure plasma generated by diffuse coplanar surface barrier discharge (DCSBD) on thin POx-based films. Our main objective is to adjust the appropriate conditions of the plasma treatment to achieve the cross-linking of the POx layer, while the anti-biofouling properties stay maintained. For this purpose, POx-based statistical copolymer was deposited on plasma activated silicon wafers by spin-coating. Three various concentrations (2, 5, 10 wt%) of the POx solution resulted in three different thickness of the layer. Achieved POx layers were exposed to cold DCSBD plasma and chemical, and morphological changes were characterized by means of X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FTIR) and profilometry. Very similar values of roughness before and after treatment prove the minor effect of DCSBD plasma on the morphology of POx layer; however, FTIR spectra indicate the undesired etching of the POx layer during the higher exposure times. Despite that the cross-linking of POx layer was not confirmed by available methods, these results provide a good starting point for better understanding of specific processes and further adjusting the suitable conditions of plasma treatment for achieving stable POx coatings.

Keywords: Poly(2-oxazolines), coating, anti-biofouling, DCSBD, atmospheric pressure plasma

1. INTRODUCTION

Due to the biocompatibility, synthetic versatility and potential anti-biofouling properties, nowadays poly(2-oxazolines) (POx) hold a leading position as a valuable alternative to the gold standard polyethylene glycol (PEG) in the biomedical area [1]. Till now, there are a plethora of examples for POx conjugates, micelles, modified surfaces, or hydrogels in connection with their possible applications as therapeutic, imaging and protein/cell repellent agents [2]. Another important application field is surface modifications of implantable medical devices, where biocompatibility and anti-biofouling properties are important to ensure their long-term stability and functionality. POx coatings, which are very promising for this purpose, are usually prepared by challenging multi-step grafting method [3], which includes the surface pre-treatment and functionalization. In addition, these procedures often employ harmful catalysts or solvents. In contrast to this complex procedure, a recently published plasma polymerization technique for POx-based surface coatings introduced more facile, effective and solvent-free method for the modification of various materials [4]. In that case, the POx layer was deposited in one-step by low-pressure plasma generated in the capacitively coupled bell-chamber reactor. Coatings with a thickness of tens nanometers were non-cytotoxic to human dermal fibroblasts, and biofilm adhesion significantly decreased from >99 % to 9.2 % [5]. However, the current applications of plasma in industry or medicine require the more economically friendly and simpler approach; therefore, the low-pressure

work regimes are often substituted by atmospheric pressure mode. Diffuse coplanar surface barrier discharge (DCSBD) [6] is a source of cold atmospheric pressure plasma which has a potential to be involved in many industrial or medical applications. Until now, DCSBD has been successfully used for surface activation [7], hydrophilic treatment [8] and adhesion enhancement [9] of various materials, as well as for bacteria and fungi decontamination [10] and plant seeds treatment [11]. In this study, we employed DCSBD for treatment of POx thin films deposited on the model substrate (silicon wafers) with the aim to achieve the homogeneous POx layer with anti-biofouling properties. Compared to multistep grafting or low-pressure plasma polymerization, this process should be faster, easier, and economically friendly. Our main objective in this work is to monitor the chemical and morphological changes in the thin POx film after the plasma treatment by means of X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FTIR) and profilometry.

2. EXPERIMENTAL SECTION

2.1. Materials and plasma specification

The silicon wafers with dimensions 1x1 cm² were used as the model surface for all experiments. They were ultrasonically cleaned in isopropanol, acetone, and water for 15 min and dried in the nitrogen flow prior to use. A statistical copolymer poly(2-methyl-2-oxazoline)-*stat*-(2-butenyl-2-oxazoline) (POx) with the degree of polymerization equal to 100 containing 5 % of butenyl groups in the polymer backbone (POx) was synthesized by the living cationic ring-opening copolymerization and characterized as previously described [12]. Polymer films were prepared by spin-coating the POx aqueous solutions (150, 250 and 350 μ L) with different concentrations (2, 5 and 10 wt.%) on plasma activated silicon wafers (2000 rpm, 2 min) using a spin-coater WS-650 (Laurell Technologies).

For plasma pre-treatment of silicon wafers and treatment of POx thin films, a diffuse coplanar surface barrier discharge has been used. During the experiments DCSBD was operated in ambient air at atmospheric pressure and it was supplied by sinusoidal voltage (20 kV peak-to-peak, 15 kHz) at input power 400 W. The distance between the treated silicon wafers or POx thin films and DCSBD ceramics was 0.35 mm. For pre-treatment of the wafers, time 10 s was selected to achieve sufficient wettability. POx thin films were treated for 3, 10 and 30 s.

2.2. Surface analyses

The thickness of spin-coated POx films was measured by DektakXT stylus surface profiler (Bruker). Before spin-coating, a small piece of tape was applied on the edge of silicon wafers, which was then removed before the thickness measurements. The step height between the film and wafer was evaluated on three independent wafers. Resulting thickness was determined from 5-10 spots on every wafer and expressed as an average \pm standard deviation. Atomic force microscopy (AFM) measured by Ntegra prima (NT-MDT) microscope in semi-contact mode was carried out to measure the root mean square (RMS) surface roughness of the POx films before and after plasma treatment.

Chemical analysis of POx thin films was performed by X-Ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). The XPS measurements were done on the ESCALAB 250Xi (ThermoFisher Scientific). The system is equipped with 500 mm Rowland circle monochromator with microfocused Al K α X-Ray source. An X-ray beam with 200 W power (650 microns spot size) was used. The survey spectra were acquired with pass energy of 50 eV and resolution of 1 eV. High-resolution scans were acquired with pass energy of 20 eV and resolution of 0.1 eV. In order to compensate the charges on the surface electron flood gun was used. Spectra were referenced to the hydrocarbon type C1s component set at a binding energy of 284.8 eV. The spectra calibration, processing, and fitting routines were done using Avantage software. Infra-red spectra were measured by Bruker Vertex 80V spectrometer. The samples were measured

in transmission mode. All measurements were made at a vacuum of 2.51 hPa to suppress undesirable effects caused by the presence of ambient humidity and CO₂. Each spectrum was scanned 100 times and evaluated by Bruker OPUS software (version 6.5).

3. RESULTS AND DISCUSSION

Statistical copolymer poly(2-methyl-2-oxazoline)-*stat*-(2-butenyl-2-oxazoline) (POx) was synthesized by cationic ring-opening polymerization and characterized by methods as previously described [12]. The resulting molar mass of the copolymer was 8,300 g mol⁻¹, which approximately corresponded to the required degree of polymerization equal to 100, and the fraction of 2-butenyl-2-oxazoline was 5 % what corresponded to the percentage in the feed. As the recent studies indicate, such water-soluble POx (co)polymers should possess good biocompatibility and anti-biofouling properties [13,14], therefore we have chosen this material as suitable for anti-biofouling surfaces. POx thin layer was deposited by spin-coating on silicon wafers. The thickness of the layer was controlled by the different concentration of the aqueous solution (2, 5 and 10 wt.%). The volume of applied solution (150, 250 and 350 μL) during spin-coating did not influence the thickness of the layer; hence the resulting values listed in **Table 1** were determined as an average from 6-8 samples disregarding the used volume (2-3 samples from each used volume). Samples with deposited POx layer were after that treated by cold atmospheric pressure plasma generated by DCSBD. POx samples with the lowest thickness (POx 2wt%) were excluded from the further discussion due to a nonuniform character of the film and possible removal of the thin layer by the plasma etching process. Plasma treatment often leads to morphological changes in the surface layer of many materials. Therefore, we evaluated the roughness before and after plasma treatment by AFM. Resulting values for three different plasma exposure times - 3, 10, and 30 s are summarized in **Table 1** and selected AFM images are depicted in **Figure 1**.

Table 1 The values of POx film thickness and RMS roughness before and after plasma treatment

Sample	thickness (nm)	RMS roughness (nm)			
		0	3	10	30
Si wafer		0.15			
POx 2wt%	46.6 ± 20.6	-	-	-	-
POx 5wt%	167.56 ± 55.9	0.25	0.19	0.20	1.85
POx 10wt%	361.93 ± 74.1	0.26	0.22	0.24	0.19

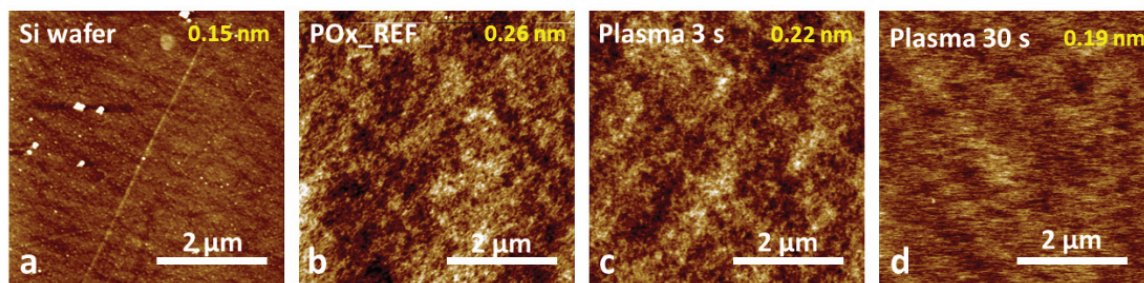


Figure 1 Selected AFM images of a) cleaned Si wafer, wafer with deposited 10wt.% POx layer b) before plasma treatment, c) after 3 s and d) after 30 s of plasma treatment. Resulting RMS roughness values are inserted in the AFM scans

Surface morphology is evidently different after the deposition of POx thin film on the silicon wafer (**Figure 1 a, b**), along with the roughness, which increased from 0.15 nm (wafer) to 0.25 or 0.26 nm for 5 and

10 wt.% solution, respectively. However, after the plasma application, only negligible changes occurred. Except the sample POx 5wt% treated for 30 s, all layers were slightly smoother compared to untreated POx film, which indicates the minor effect of gentle DCSBD plasma on the morphology of POx film.

With the aim to reveal the chemical changes in the POx layer after the plasma treatment, X-Ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR) were employed. In general, plasma modification takes place on the upper layer of material, which represents approximately several tens of nm. XPS analysis is suitable for monitoring the surface, which was in direct contact with the plasma, while FTIR spectra provide the view into the deeper layer of studied material. Accordingly, the possible chemical changes can be detected. Nevertheless, the depth of penetration of the IR beam into the sample depends on many factors (crystal, the angle of incidence, sample's refractive index, the wavelength of IR beam), and thickness of the layer limits such measurements. In our case, samples prepared from 2 wt% and 5 wt% POx solution with a thickness in the range 46.6 ± 20.6 and 167.56 ± 55.9 , respectively, were under the detection limit of FTIR measurements. Already before the plasma treatment, the intensity of characteristic signals was very low (data not shown). For this reason, we report here only the results from samples POx 10wt% before and after plasma treatment (**Figure 2**). FTIR spectra of POx demonstrate the presence of typical amide carboxyl peak (1645 cm^{-1}), which corresponds to carbonyl stretching of the amide backbone of the POx chain. Another signal in the range $1420\text{-}1478 \text{ cm}^{-1}$ is assigned to CH_x symmetrical deformations, and small signal around 1260 cm^{-1} appertains to C-N stretching [15]. The characteristics signals of the silicon wafer are in the range $500\text{-}1100 \text{ cm}^{-1}$; hence they do not overlap with POx peaks. FTIR spectra in **Figure 2** demonstrate the decreasing intensity of POx typical absorption bands with the duration of plasma treatment. Plasma irradiation probably causes the etching of the POx layer and thus the removing of the upper layer of POx film leading to decreasing of the film thickness. Accordingly, the longer treatment times (10-30 s) are not suitable for achieving stable POx coatings. Formation of the new chemical bonds, which could confirm the cross-linking, was not detected by FTIR spectroscopy.

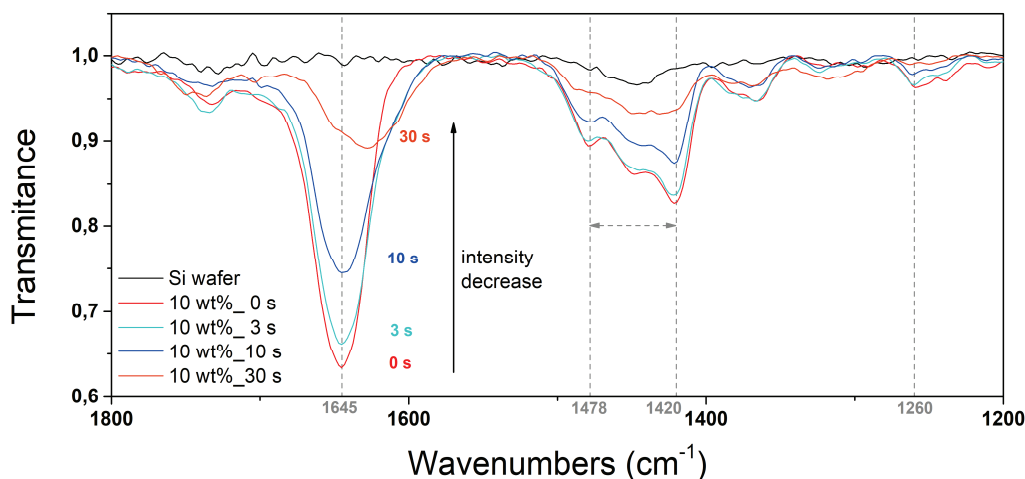


Figure 2 FTIR spectra of Si wafer and POx 10wt% thin film before and after the plasma treatment

Chemical composition acquired by XPS measurement is listed in **Table 2**. Average N/C and O/C ratio, as an indicator of surface oxidation, slightly increased due to the plasma treatment. The influence of the layer thickness was in all cases negligible, whereas with the increasing treatment time the new oxygen groups were formed and O/C ratio increased (e.g. POx 10wt% - 0.24, 0.29 and 0.46 for treatment time 3, 10 and 30 s, respectively). Additionally, a detailed analysis of the high-resolution C1s peak was performed. The C1s peak of untreated POx layer was deconvoluted into three components (**Figure 3**, POx_REF), which were attributed to C-C/C-H (284.8 eV), C-N (285.8 eV) and N-C=O (287.7 eV) bonds [14]. Air plasma is a great source of reactive oxygen and nitrogen species, therefore also in our case plasma treatment induced the formation

of additional C=O / N-C=O (287.7 eV) groups and new C-O (286.1 eV) and O-C=O (289.3 eV) functionalities (**Figure 3**, 0 days). In general, detection of the plasma-induced cross-linking is a serious challenge. Besides UV radiation, the plasma electrons interacting with the substrate can initiate a formation of radicals in the polymer chains and starting the cross-linking. However, also the chain scission can simultaneously occur. In our case, the most pronounced increase was achieved in a number of C-O and N-C=O/C=O groups, while the percentage of C-C and C-N bonds decreased. OH radicals produced in air plasma can abstract the hydrogen from polymer backbone and formed alkyl radicals, which initiate the incorporation of O-containing groups onto the polymer surface. On the other hand, there is an assumption that reduction of the percentage of C-C bond and forming of new C-O can indicate the disruption of the polymer chain and following creation of ether (C-O-C) cross-linking junctions.

Table 2 Concentration of elements (at%) and relative area of C1s chemical bonds (%) before (POx REF) and immediately after plasma treatment (POx 5wt%, POx 10wt%)

Sample	plasma treatment time (s)	N1s	C1s	O1s	N/C	O/C	C-C	C-N	C=O	C-O	COO	N-C=O
POx REF	0	14.2	69.7	16.1	0.20	0.23	38.1	48.6	0	0	0	13.3
POx 5wt%	3	14.5	66.5	19.0	0.22	0.29	27.1	22.4	3.3	22.8	2.3	22.1
	10	14.7	40.6	44.7	0.36	1.10	18.6	20.2	8.6	30.5	4.5	17.5
	30	11.2	70.6	18.2	0.16	0.26	13.1	15.4	0	35.4	2.2	33.9
POx 10wt%	3	13.7	69.5	16.8	0.20	0.24	21.9	20.3	5.8	24.9	0.8	26.4
	10	16.7	64.5	18.8	0.26	0.29	29.3	24.7	4.2	22.6	1.5	17.8
	30	17.5	56.4	26.1	0.31	0.46	22.5	18.0	8.4	24.3	3.3	23.6

Ageing of POx layer in ambient air was monitored during the first 3 days and it is depicted in **Figure 3** for the selected sample POx 10wt% treated by DCSBD plasma for 30 s. Considerable increasing of C-O groups after three days could involve the post-oxidation. However, concerning the O/C ratio, no markedly changes occurred, values almost in all cases stayed maintained (data not shown). In summary, no considerable changes after three days were revealed. POx film exposed to plasma remained chemically stable while stored in air.

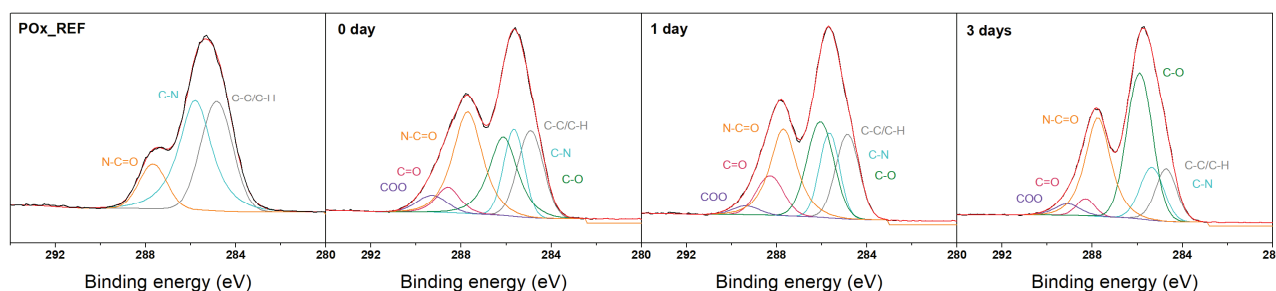


Figure 3 Deconvolution of high-resolution C1s spectra of initial POx 10wt% film and its ageing (0, 1, 3 days) after 30 s of plasma treatment

4. CONCLUSION

The influence of cold atmospheric pressure plasma generated by diffuse coplanar surface barrier discharge on thin poly(2-oxazoline) films was evaluated during the different exposure time (3, 10 and 30 s). Very similar values of roughness before and after plasma treatment proved the minor effect of DCSBD plasma on the morphology of POx layer. However, FTIR spectra indicated the possible etching of the POx layer and thus the removing of the upper layer of POx film in the case of higher exposure time (10 and 30 s). Air plasma is great

source of reactive oxygen and nitrogen species, thus induced the oxidative reactions in the POx layer, which was revealed by the XPS. To detect the plasma-induced cross-linking is not trivial by using the FTIR and XPS, and in the future experiments, the additional measurement techniques as MALDI or SIMS could be utilized. Accordingly, using the inert gases instead of ambient air could enhance the cross-linking of the film, and it will be subject of further experiments.

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