Influence of the sterilization processes on the properties of RF magnetron sputtered amino-rich thin films

Anna Artemenko¹, Marta Vandrovcová², Ondřej Kylián¹, Andrei Choukourova¹, Ivan Gordeev¹, Oleksandr Polonskyi¹, Jaroslav Kousal¹, Danka Slavinska¹, Lucie Bačáková² and Hynek Biederman¹

¹Charles University in Prague, Faculty of Mathematics and Physics, Department of Macromolecular Physics, V Holešovičkách 2, 180 00 Prague 8, Czech Republic
²Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic

Abstract: Amino-rich plasma polymers have a wide range of biomedical and biotechnological applications. However, in many cases the high density of primary amines in the coatings is not the only parameter relevant for the applicability of such materials in biomedical field. In addition sufficient stability of the films both in open air and in aqueous environments as well as their resistance towards the sterilization process is often required. The later aspect is investigated in this contribution. Concretely, coatings prepared by RF magnetron sputtering of Nylon 6,6 in Ar/N₂ gas mixtures at pressure of 2 Pa and applied power of 40 W were subjected to different sterilization techniques (UV radiation, ethanol treatment, dry heat and autoclave). Sterilized samples were subsequently characterized by various diagnostics methods in order to evaluate their chemical and morphological alterations caused by the sterilization process.

Keywords: RF magnetron sputtering; amino-rich films; sterilization

1. Introduction

RF magnetron sputtering of Nylon target is a promising technique of preparation of amino-rich thin films for biomedical applications such as for instance permanent immobilization of biologically active molecules [1], improvement in the performance of living cells at different substrates, e.g. on tissue culture dishes or metal implants [2], or for production of adhesion interlayers for further functionalization [3]. However, in these applications, and in biomedical applications in general, the amino-rich character of deposited coatings (i.e. high surface density of –NH₂ functional groups) is not the only parameter determining their possible use and thus additional requirements have to be fulfilled: the coatings should be temporally stable to provide sufficient shelf-life, they should exhibit high resistance towards an aqueous environment and, last but not least, they should withstand sterilization process. Regarding the stability of amino rich coatings it has been reported by many authors that they gradually lose amino rich character when in contact with open air, which is ascribed mainly to the oxidation of primary amino groups and formation of more stable amides (e.g. [4,5]). Moreover, it has been reported recently, that the coatings prepared by plasma methods become easily soluble in water when their amino rich character increases (e.g. [4,5]). In contrast to temporal stability and solubility of amino rich coatings the resistance towards common sterilization process was not investigated in detail so far. This paper is therefore focused on this phenomenon, which is of high importance for biomedical application of amino rich films.
2. Experimental

2.1 Deposition of amino-rich coatings

The thin films were deposited by sputtering a Nylon 6,6 (Goodfellow) target (80 mm diameter, 2 mm thickness) in Ar/N\textsubscript{2} (1:1) working gas mixture, previously reported to lead to the deposition of coatings that exhibit relatively high stability in liquids [5], but maintaining still sufficient surface density of -NH\textsubscript{2} groups [1]. For the deposition was used a cylindrical plasma reactor depicted in Figure 1 and introduced in more details in previous papers (e.g. [1,6]). Plasma discharges were operated at pressure of 2 Pa, total gas flow of 5 sccm and applied RF power of 40 W (reflected RF power was kept below 2W).

The substrates (one side polished Si wafers in this study) were introduced into the deposition chamber by a load-lock system and were placed 50 mm from the sputtered target.

![Figure 1. Schematics of the deposition chamber (1-RF power supply, 2-matching box, 3-water cooling, 4-RF magnetron, 5-Nylon target, 6-plasma, 7-sample holder, 8-gas inlet, 9-pumping system)](image)

2.2 Sterilization of the samples

The deposited samples were sterilized either by UV radiation (SANKYO DENKI G20T10 (UV-C) lamp; irradiation time 2 hours), autoclave (Tutttnauer 2540 ELC; 1 hour treatment at temperature of 120°C in de-ionized water), by hot air sterilizer (HS202A; 2 hours at temperature of 160°C) and by ethanol treatment (70% ethanol for 2 hours).

2.3 Samples characterization

The thickness of the coatings before and after sterilization was measured by means of spectral ellipsometry using a variable angle spectroscopic ellipsometer (Woolam M-2000DI). The measurements were performed in the wavelength range of \( \lambda = 192-1690 \) nm at an angle of incidence AOI = 45-75° in air at room temperature. In order to obtain the thickness of the coatings, recorded spectra were fitted with multilayer model (Si/SiO\textsubscript{2}/plasma polymer) using the Complete EASE analysis software.

Morphology of the samples was evaluated by atomic force microscopy (AFM). AFM measurements were performed by Quesant Q-scope 350 atomic force microscope operated in the semi-contact mode and using NSC-16 silicon cantilevers (Schaefer Technologie, GmbH).

Characterization of chemical composition was performed on polished silicon substrates by XPS using Al K\textsubscript{\textalpha} X-ray source (1486.6 eV, Specs) equipped with a hemispherical energy analyzer (Phoibos 100, Specs). The XPS scans were acquired at constant take-off angle of 90°. All the binding energies were referenced to the C1s carbon peak at 285.0 eV, to compensate for the effect of surface charging.

3. Results

The thickness of the coatings before and after sterilization process was determined by means of spectral ellipsometry as described above. As can be seen in Figure 2, sterilization by UV radiation, ethanol or dry heat did not result in
significant changes of thickness of the coatings in contrary to autoclaved samples, for which was observed approximately 50% reduction of the film thickness.

Figure 2. Thickness of the films before and after sterilization.

Figure 3. AFM scans of the coatings after sterilization by a) UV radiation b) ethanol treatment, c) dry heat, d) autoclave.

Regarding the morphology of the coatings, only slight increase of the surface roughness was observed after the sterilization of the samples as can be seen in Figure 3. Nevertheless, from the AFM scans (7.5x7.5 µm) appeared that in all cases the root-mean-square roughness stayed below 1 nm and thus the films remained very smooth after the sterilization.

XPS measurements revealed that there are only slight variations in the elemental composition of sterilized samples as compared to not-sterilized samples (see Table 1). In all cases the oxygen content increased after the sterilization, which occurred mainly at expense of nitrogen fraction in the films.

<table>
<thead>
<tr>
<th></th>
<th>O [%]</th>
<th>C [%]</th>
<th>N [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sterilization</td>
<td>17,0</td>
<td>53,7</td>
<td>29,4</td>
</tr>
<tr>
<td>UV sterilization</td>
<td>19,8</td>
<td>51,5</td>
<td>28,8</td>
</tr>
<tr>
<td>Ethanol treatment</td>
<td>20,3</td>
<td>53,1</td>
<td>26,6</td>
</tr>
<tr>
<td>Dry heat</td>
<td>18,5</td>
<td>54,6</td>
<td>26,8</td>
</tr>
<tr>
<td>Autoclave</td>
<td>19,4</td>
<td>55,1</td>
<td>25,5</td>
</tr>
</tbody>
</table>

Table 1. Elemental composition without hydrogen of not-sterilized samples and samples sterilized by different techniques

In spite of slight changes in elemental composition of the coatings as seen by XPS, sterilization methods have diverse impact on the chemical structure of the films as can be seen in Figure 4. It was observed that only UV radiation did not make any significant changes of the chemical composition of the coatings. However, ethanol treatment, autoclave and dry heat resulted in increase of concentration of C-C, C-H chemical bonds and in decrease of concentration of nitrogen-bearing species. Apparently, these methods lead to the loss of nitrogen in the films. One of the possible pathways is hydrolysis of imines with elimination of volatile ammonia. It was found that autoclave is the most destructive technique for amino-rich plasma polymers which results in significant chemical changes and loss of mass.

4. Conclusion

In this contribution we have investigated effect of 4 commonly used sterilization methods on morphology and chemical composition of amino-rich coatings prepared by RF magnetron
sputtering of Nylon. It was found that employed sterilization methods have negligible influence on the morphology of the coatings and with an exception of autoclaving do not cause significant changes in their thickness. On the other hand it was observed that applied sterilization method strongly influences the chemical structure of the films. Regarding this phenomenon most pronounced changes were observed after dry heat sterilization, followed by autoclaving, ethanol treatment and finally by UV radiation. The relevance of such results for bioresponsive properties of sterilized samples is a subject of further investigation.

Acknowledgements

This work was supported by KAN101120701 grant from the Grant Agency of the Academy of Science of Czech Republic and partly by the Grant Agency of Charles University in Prague under the grant GAUK 110-10/251265.

References