ANTIMICROBIAL EFFECT ON PET FILMS OBTAINED BY PLASMA AND SILVER NITRATE/COLLAGEN TREATMENTS

M. AFLORI¹, M. DROBOTA¹²

¹“Petru Poni” Institute of Macromolecular Chemistry, Grigore Ghica Voda Alley, Iasi, Romania,
E-mail: maflori@icmpp.ro
²“Politehnica” University of Bucharest, Romania
E-mail: miamiara@icmpp.ro

Received April 2, 2015

Abstract. The generation of an anti-colonization and biocompatible polyethylene terephthalate (PET) surface, by means of plasma followed by wet chemical modification to incorporate silver ions and collagen molecules is the focus of this publication. In order to have the highest yield of collagen and/or silver possible in a reproducible fashion, the parameters of plasma treatments (time, power) were tailored. Various surface characterization methods revealed the effects of the treatments on the polymer surface. The combined surface modifications inhibited bacterial adhesion on the treated polymer and conclusions concerning the best input parameters of plasma treatments were revealed.

Key words: Polyethylene terephthalate RF plasma, silver ions, collagen, inhibition of bacterial adhesion.

1. INTRODUCTION

An improvement of survival chances and quality of patient lives was possible by the development of the medical devices and implants. Implant-associated infections are the major impediment in the usage of biomedical devices [1–4]. These infections are difficult to treat with antibiotics and can cause necrosis of the surrounding tissue, leading to failure of the medical device/implant, and in some cases even death. The infection rate depending on the type of the implant can range from 1–2% for hip and knee prostheses to 100% for urinary catheters. In the UK alone an estimation of the costs of treating such infections are around £7–11 million per year. As an example, to date, there are only few clinical studies [4–8] evaluating the efficacy of silver-ion implanted peritoneal access devices. The potential benefit of silver-ion treated peritoneal dialysis catheters was extrapolated from favorable experience with intravascular appliances. New surface-treatment procedures for peritoneal dialysis catheters that can reduce the rate of infectious complications without adversely affecting basic design and function would constitute a major advance in the field.
It is well known that non-equilibrium plasma technologies (cold plasma), which are involved in many industrial processes from aerospace to life sciences, offer efficient routes for the modification of natural or synthetic polymeric materials, but for non-destructive operations, too. These technologies have been successfully applied to enhance or replace wet finishing processes in several domains such as biomedical and mechanical applications, optic, paper, textile and automobile industries, food packaging, etc. These extended applications are due to the fact that surface modification may be accomplished through surface activation, ablation, etching, cross-linking, functionalization, film deposition or by some combination of these effects [9–12].

The aim of this work is the obtaining anti-colonization and biocompatible PET surface, by means of plasma followed by wet chemical modification to incorporate silver ions.

2. EXPERIMENTAL

2.1. MATERIALS AND METHODS

The experiments were realized using a plasma chemistry reactor Emitech K1050 X Plasma Asher (capacitor plate plasma, CPP (Emitech Ltd, UK). The device consists of a solid state RF generator designed to provide up to 100 watts of continuous wave 13.56 MHz power to the reaction chamber. The reaction chamber system includes two pieces Pyrex chamber and two semicircular electrodes. The output impedance of the RF generator matching with the capacitive load of the reaction chamber is provided by two variable capacitors. The plasma was generated using a low pressure, RF induced helium discharge. The chamber pressure was set at $5 \times 10^{-1}$ mbar. The helium gas was introduced into the chamber at a flow rate of 50 sccm. PET films of 10 × 10 mm$^2$ size were placed into the chamber and treated by helium plasma. The input power was 30 W, while the treatment time was 5 minutes.

Subsequently, two kind of chemical modifications were studied: one by using a sodium citrate/silver nitrate solution and the second one by using a collagen/sodium citrate/silver nitrate solution (Fig. 1). The solution was obtained by adding 1 mg trisodium citrate in 10 ml AgNO$_3$ 0.01 M under continuous stirring. The homogenization was continued for 2 hours. 1 µl of the so-formed silver nanoparticles (AgNPs) solution was added to the collagen solution and stirred for 1 hour. A solution of 1 mg/ml collagen in pure water was prepared. The water used in the experiments was purified in a Milli-Q system (pure water). Collagen type I was purchased from Sigma-Aldrich (99.85%). Trisodium citrate (Na$_3$C$_6$H$_5$O$_7$•2H$_2$O) was purchased from Sigma-Aldrich (99%).
In order to have the highest yield of silver possible in a reproducible fashion for the wet chemical modification, the plasma-treated polymer was immersed for 7 days at room temperature in the above-mentioned solutions, protected from light. When one-week period expired the samples were rinsed with deionized water, subsequently analyzed by the different characterization techniques.

For a better monitoring of the treatments we make the notations: (T1) for 30 W 5 min plasma followed by sodium citrate/silver nitrate solution treatment and (T2) for 30 W 5 min plasma followed by collagen/sodium citrate/silver nitrate solution treatment.

### 2.2. MEASUREMENTS

FTIR analysis was performed in the range 600–4000 cm\(^{-1}\). The spectra were recorded on a BRUKER VERTEX 70 spectrometer at a resolution of 2 cm\(^{-1}\) at incidence angle of 45°. The signal-to-noise ratio was improved by coadding 128 scans per spectrum.

The polymer surface morphology was investigated by atomic force microscopy (AFM) methods which were performed at room temperature (22–24°C) on a Solver PRO-M (NT-MDT Co., Zelenograd, Moscow, Russia) setup. The last
A rectangular silicon cantilever NSG10 with the typical force constant of 11.8 N/m, resonance frequency of 213 kHz and tip curvature radius and height of 10 nm and 14–16 mm, respectively, was used. The surface textures were characterized in terms of roughness parameters, such as root mean square roughness and average height.

SAXS experiments were performed on a Bruker Nanostar instrument. The X-ray radiation employed was generated from a Cu sealed tube micro-focus X-ray source ($K\alpha = 1.54184 \text{ Å}$). The X-rays were filtered through MONTEL optics system and collimated by a system of three pin-holes. The system was equipped by an VANTEC-2000 detector (set at 107 mm from the sample) and was controlled with the SAXS software suite. The sample chamber and X-ray beam paths were evacuated and a 3600 seconds scan was preformed. The glassy carbon standard was then inserted between the sample and the detector and a 900 seconds standard scan was collected.

XPS was performed on a KRATOS Axis Nova (Kratos Analytical, Manchester, United Kingdom), using AlKα radiation, with 20 mA current and 15 kV voltages (300 W), and base pressure of $10^{-8}$ to $10^{-9}$ Torr in the sample chamber. The incident monochromated X-ray beam was focused on a 0.7 mm × 0.3 mm area of the surface. The XPS survey spectra for the samples were collected in the range of $-10$÷$1200$ eV with a resolution of 1 eV and a pass energy of 160 eV. The high resolution spectra for all the elements identified from the survey spectra were collected using pass energy of 20 eV and a step size of 0.1 eV. Data were analyzed using the Vision Processing software (Vision2 software, Version 2.2.8). The binding energy of the C1s peak was normalized to 285 eV.

The determination of the susceptibility of bacteria to different antimicrobials is performed through the Kirby-Bauer diffusimetric method which is performed on solid media (Müeller Hinton Agar) and is based on the property of antimicrobial solutions to diffuse into the culture medium at different distances from the place where they are stored. In order to determine the antibacterial effect of the substances analyzed, Gram-negative bacterial strains will be used (strains of Escherichia coli ATCC 25922). The method consists in preparing a bacterial suspension whose turbidity is equivalent to that of the 0.5 McFarland tube and its seeding in “the pitch” on the surface of the Müeller Hinton Agar medium. Finally, the disks impregnated with an anti-microbial substance are placed on the surface of the cultured medium and incubated at 37 °C for a period of 24 hours. In order to control the sensitivity of bacterial strains, chloramphenicol, streptomycin and cefotaxime are used as positive standards.
3. RESULTS AND DISCUSSIONS

The FTIR spectroscopy is a powerful method to study the intermolecular interactions that exist between different components of the new groups and it is widely used in the determination of the nature of the interactions which are responsible of the formation new complexes \([13–15]\). The FTIR spectra for the conjugates have been studied, showing the entrapment or binding of protein and AgNPs. The AgNPs-containing samples show peaks at \(1612 \text{ cm}^{-1}\) (C=C groups or from aromatic rings) and \(1384 \text{ cm}^{-1}\). Furthermore, the treated samples spectra show the appearance of symmetric vibration signals of carboxylate anions situated at \(1526\) and \(1585 \text{ cm}^{-1}\), assigned to the protons transfer from the carboxylic groups/Ag, predicting the formation of ionic interactions between carboxylate and silver ions. It can be observed that there is a remarkable change in the \(\nu_{\text{asymmetric}}\) (C=O) and \(\nu_{\text{symmetric}}\) (C=O) of the carboxylate group band positions. This indicates that the carboxyl terminal group acid chain is involved in bonding with silver. This also implies that the major driving force of adsorption the silver and the formation of certain \(\text{Ag}^+\ldots\text{COO}^–\).

FTIR measurements are indicating the break of intermolecular hydrogen bonding after functionalization in contact with Ag-collagen in solution. Therefore, the appearance in FTIR spectrum of a new band centred at \(1635 \text{ cm}^{-1}\) (attributed to the complex formation between carboxylic groups from PET surface and silver nanoparticles-collagen complex) is pointed.
The symmetric stretching band of C=O in the amide I group at 1635 cm⁻¹ indicate a coordination between AgNPs-collagen and carboxyl groups. This is attributed to the electron donation of the N-H bond in the amide groups for chelating with Ag⁺ before reduction to Ag⁰, which was stabilized immediately by the complex. Therefore, the break of ester bonding produce this band centred at 1635 cm⁻¹.

The collagen–Ag interaction with PET surface is monitored by Fourier transform infrared (FTIR) spectroscopy on the basis of the appearance of the peaks at 1542 cm⁻¹ (stretching mode of C-N and blend of N-H in the amide group). The band centred at 1526 cm⁻¹ is corresponding to the vibration signals of carboxylate anions after mixing Ag with amide from collagen. The ability for molecular self alignment implied a template effect for AgNPs through the –COO-molecular interaction.

The AFM images for the pristine and treated samples are presented in Fig. 3a, 3d, 3g. In addition, the cross-section profile taken along the solid line from the height histogram and 2D height image (Figs. 3b, 3e, 3h and Figs. 3c, 3f, 3i) allowed the calculation of the average height of the samples.
The bidimensional height images of the native PET film (Fig. 3a) reveal a flat and homogeneous surface, with Sq of 1.6 nm, the value of 5.2 nm being obtained for the average height. The aspect of the statistical distribution of z-values within the image confirmed that the film surface was indeed uniform.

The surface topography of treated films as observed by AFM undergoes significant changes as a result of PET treatments, as explained in Fig. 1. The AFM images show silver nanoparticles of 70–90 nm diameters for T1 and 50–60 nm for T2. The values of Sq and average height are 5.4 nm, 25 nm respectively for T1 treatment (Fig. 3e and 3f); 22 nm, 50 nm respectively for T2 (Fig. 3h and 3i).

Analysis of SAXS data (Fig. 4) are in concordance with AFM measurements concerning the shape and the sizes of silver nanoparticles. Spherical model with a Gaussian distribution for Guinier plot was used (from DIFFRAC.NANOFIT) to simulate the scattering curves of all samples and silver nanoparticles values of 85 nm for T1, 55 nm for T2 were found.

Surface chemical modifications induced by treatments were determined by XPS measurements. Figure 5 shows the wide scan spectra of the samples and they mainly contain C1s, O1s, Ag3d and N1s peaks.

The elemental composition and the ratio of elements are summarized in Table 1. The untreated PET film and all the treated samples presents an O/C ratio increasing from 0.33 to 0.42, which suggests that new oxygen-containing polar groups are formed on the PET films surface after treatment [16–18].
The XPS measurements also reveal that the surface treatments lead to an increase in the N/C atomic ratio from 0.17 to 0.26. Therefore, nitrogen-containing functional groups are formed on the PET surfaces after plasma treatment and collagen immobilization. Adsorption of collagen led to the appearance of N1s peak which is increasing with plasma exposure time. The higher value of the nitrogen concentration is obtained for T2, the AFM images showing a large number of collagen grains with different sizes in this case. From the XPS measurements, the higher amount of Ag present on the treated polymer surface is in case of T1 treatment.

### Table 1

The elemental composition and ratios of all samples from XPS measurements

<table>
<thead>
<tr>
<th>Sample/Element</th>
<th>O</th>
<th>C</th>
<th>Ag</th>
<th>N</th>
<th>O/C</th>
<th>N/C</th>
<th>Ag/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>25.24</td>
<td>74.76</td>
<td>–</td>
<td>–</td>
<td>0.33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>T1</td>
<td>26.19</td>
<td>62.39</td>
<td>0.96</td>
<td>10.46</td>
<td>0.41</td>
<td>0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>T2</td>
<td>24.64</td>
<td>59.17</td>
<td>0.58</td>
<td>15.61</td>
<td>0.42</td>
<td>0.26</td>
<td>0.01</td>
</tr>
</tbody>
</table>
The pristine sample shows no inhibition activity for *E. coli* (Fig. 6a). All treatments inhibited bacterial adhesion for negative gram-bacteria (Fig. 6b and 6c), T1 having a larger diameter of the inhibition zone (Fig. 6b). As regarding the action of silver nanoparticles, is somehow similar to that of silver ions, yet higher. The accepted mechanism involves the initial attachment of silver nanoparticles to the bacterial cell membrane, interaction with the sulphur-containing proteins and DNA after membrane penetration, followed by cell death. In addition, the silver nanoparticles may release silver ions, which further enhance their antibacterial efficiency [19–20].

4. CONCLUSIONS

The polymer surfaces proprieties can be tailored in order to obtain biocompatible and antimicrobial proprieties in the same time, for various biomedical applications. In this study, PET films are modified by new and original combined treatments. In this study, PET films are activated by an RF helium plasma treatment to produce amino groups on the surface, and then plasma-functionalized polyethylene terephthalate (PET) films were placed in a silver and/or collagen solution, afterwards. The FTIR are indicating the break of intermolecular hydrogen bonding after functionalization in contact with Ag-collagen solution and the formation of the complex between carboxylic groups from PET surface and silver nanoparticles collagen. The changes in surface topography induced by the treatments on PET films surfaces were evidenced by using tapping-mode AFM experiments, as well. The roughness parameters values and the AFM images interpretation of different regions of the treated polymer surface, demonstrate the presence of the silver nanoparticles and of the complexes formed by collagen and silver at the polymer surfaces. The SAXS data are in concordance with AFM measurements concerning the shape and the sizes of silver nanoparticles.
The XPS measurements revealed the appearance of an N1s peak which demonstrates the adsorption of the collagen through the formation of nitrogen-containing functional groups on the PET surfaces after plasma treatment and collagen immobilization. A higher value of the silver was obtained for T1 treatment.

The results of this work suggest that the proposed combination of helium RF plasma and silver nitrate and/or collagen solutions for the surface modifications is effective at reducing bacterial colonization and obtaining in the same time a biocompatible polymer surface suitable for many biomedical applications.

**Acknowledgments.** The work has been funded by the Sectoral Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132395.

**REFERENCES**