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Dedicated to Professor Claude Nicolau on the occasion of his 80th anniversary

INSIGHTS ON INTERACTION BETWEEN COLLAGEN-SILVER COLLOIDAL SOLUTIONS WITH PET FUNCTIONALIZED SURFACE

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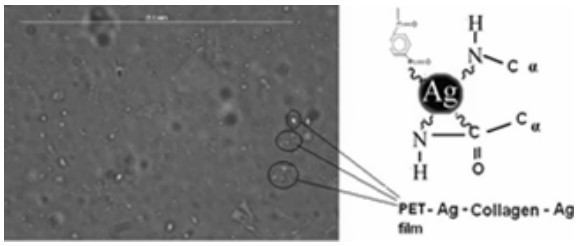
This article presents the experimental results induced by plasma action and collagen-silver solution on polyethylene terephthalate (PET) surface. Therefore, we propose a new approach for the silver nanoparticles attachment on a functionalized substrate. The FTIR spectroscopy data were used as molecular signature for physiological status, once the spectral patterns were correlated with surface properties from optical microscopy. The conjugation of collagen with AgNPs studied by FTIR spectroscopy with Fourier self-deconvolution technique revealed a compact final structure on PET substrate. These new materials can be used for patches in medical applications.



INTRODUCTION

Synthetic polymers such as polyethylene (PE), polyamides (e.g. nylon), and polyethylene terephthalate (PET) have been used clinically for making artificial devices for different applications. They were used because of their stability in the human body, flexibility with low elastic modulus, and durability.[1-4]

Due to the important influence in interaction between the modified biomaterial surface and the biological environment, several surface-modification techniques which preserve the mechanical properties of the base materials have been developed (oxidative hydrolysis, reduction, aminolysis, photochemical modification).[5-7] For example, in cardiovascular applications such as vascular prostheses, modifying the material surface results in a vascular graft with antithrombogenic properties. Reportedly, grafting on the PE liner surface of artificial joint prostheses dramatically decreases the wear. These materials are biologically inert and do not cause bone resorptive responses.[8]

Polyester surface-modified materials have recently been developed for artificial coating to enhances biological interaction of the polymers devices.[9] A unique modification of the surface topography and chemical properties can be easily achieved through plasma technology without affecting the bulk materials. The surface functionalization improves its adhesion promotion and biocompatibility, enhances

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surface energy, reduces surface friction and molecular immobilization.[10]

The polymer under plasma action changes its surface morphology with creating different functional groups, such as -OOH, -COOH.

Recently, we have shown that organic polymers including PET can be uniformly surface-modified with radiofrequency (RF) plasma followed by an adsorption step of bioactive molecules and silver-nanoparticles.[10-13]

In the current study, we investigate the modification of PET surface after He plasma and chemical treatment.[11,12] The immobilization of collagen and silver nanoparticles onto the plasma-treated PET surface was evidenced by different characterization techniques and a bonding mechanism was sketched.[13]

EXPERIMENTAL RESULTS

Materials

The experiments were realized using a plasma reactor Emitech K1050 X Plasma Asher (capacitor plasma, CPP (Emitech Ltd, UK). The plasma was generated using a low pressure, RF induced helium discharge. These devices provide up to 100 watts of continuous wave 13.56 MHz power to the reaction chamber. The chamber pressure was set at $5x10^{-1}$ mbar. PET films of 10 x10 mm^2 size were placed into the chamber and treated by helium plasma. The input power was 40W, with two different periods of time for plasma treatment: 3 and 5 minutes, respectively.

The free radicals plasma-induced are interacting, leading to the formation of polar structures and unsaturated groups onto the PET surface. Under plasma action, low-molecular weight compounds are removed or converted into high molecular products by cross-linking reactions.

After plasma treatments the films were introduced in a collagen/sodium citrate/silver nitrate solution. First, the solution of 1mg/mL collagen in distilled water was prepared. Second, 1mg trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) purchased from Sigma-Aldrich (99%) was added in 10 mL solution of $AgNO_3$ 0.01 M under continuous stirring. Finally, 1 µL from so-formed silver nanoparticles (AgNPs) suspension was added to the collagen solution (1 mL) and stirred for 1 hour. The final concentration of this mixture was 1mg /mL collagen (type I was purchased from Sigma-Aldrich (99, 85%)).

The biomacromolecules immobilized films were rinsed with de-ionized water for 24 h to remove the free biomacromolecules. In order to have a highest concentration in AgNPs on the substrate, the plasma-treated polymer was immersed for 7 days at room temperature in the above-mentioned mixtures, all kept in the dark. After one-week, the samples were rinsed with deionized water, subsequently analyzed by the different characterization techniques. The systems associated to two plasma treatments were denoted as follows: (P1) plasma treatment for 3 min (40W) followed by immersing in collagen/sodium citrate/silver nitrate solution and (P2) plasma treatment for 5 min (40W) followed by the same immersion procedure.

Characterization methods

The spectra were recorded on a Bruker Vertex 70 FTIR spectrophotometer, equipped with a diamond ATR device (Golden Gate, Bruker), ATR-FTIR (Attenuated Total Reflection - Fourier Transform Infrared) analysis was performed in the range 600-4000 cm^{-1}, at a resolution of 2 cm^{-1} at incidence angle of 45°. For a spectrum 128 scans were taken, with a baseline correction.

Optical microscope is the fundamental tool to study surface structures. To explore the surface morphology of treated PET, a Leica DM 2500M microscope was employed.

RESULTS AND DISCUSSION

Under the He plasma action, the chains breaking of macromolecules localized on the PET surface can occur at ester groups, generating polar groups such as hydroxyl, carboxyl and peroxidic groups.

ATR-FTIR analysis allows us to identify different species of carbonyl groups after plasma treatment. The spectra of untreated PET surface showed a substantial strong band of carbonyl bonds (C=O) stretching vibrations at 1712 cm^{-1} and a standard band at 1410 cm^{-1}, resulting from phenyl ring vibrations. The C-H band coupled with ring C-C stretch at 873 cm^{-1} and with C-C vibration at 724 cm^{-1} are also attributed to phenyl ring vibrations. At the same time, alkane moiety (C–H) corresponds to ~~a~~ stretching vibrations at 1340 cm^{-1}, and -C-O- in the ester was signalled by the presence of three strong stretching vibration bands at 1243, 1096, and 1017 cm^{-1}.

The ATR-FTIR spectra of plasma and chemical treated samples, showed a significant increase of the normalized peak intensity for amide I and amide II bands due to the collagen immobilization on PET.

Right after the plasma treatment, new inter- or intra- molecular free carbonyl groups were formed at PET surface. As a consequence of the immersion of plasma-treated polymer in the silver-containing solution and based on those reactive new groups from the surface, the PET-collagen-AgNps complex is formed. Interaction between collagen–Ag mixture and PET surface was monitored by Fourier transform infrared (FTIR) spectroscopy on the basis of the appearance of the peaks at 1540 cm^{-1} (stretching vibrations of C-N mixed with N-H bendings in the amide group) and 1526 cm^{-1} vibration signals of carboxylate anions after interactions of Ag with amide from collagen. The ability of collagen molecules to self assembled structure implied a template effect for AgNPs in molecular interaction with the –COO- groups. The interactions of biomacromolecules with silver nanoparticle on PET films show new peaks at 1612, 1665 and 1540 cm^{-1}.

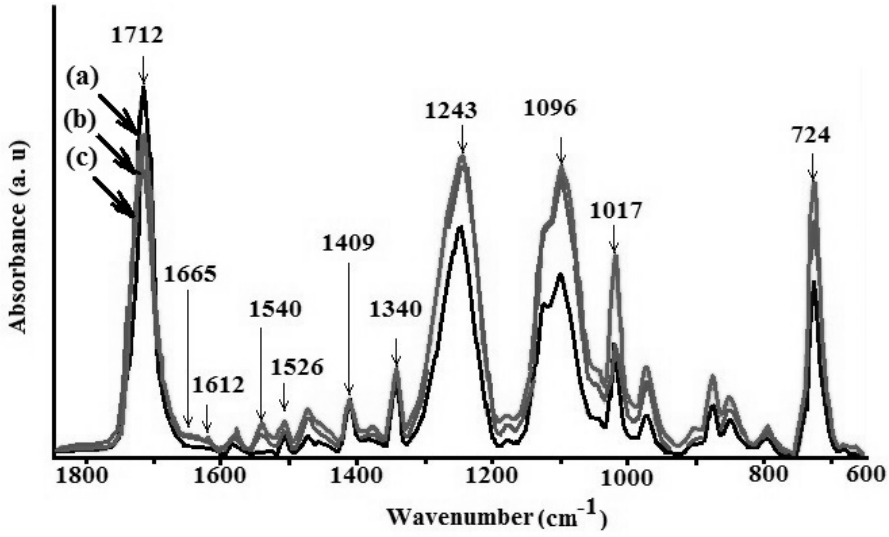


Fig. 1 – ATR-FTIR spectra for (a) untreated PET; (b) adsorbed collagen on plasma action PET film P1 and (c) P2.

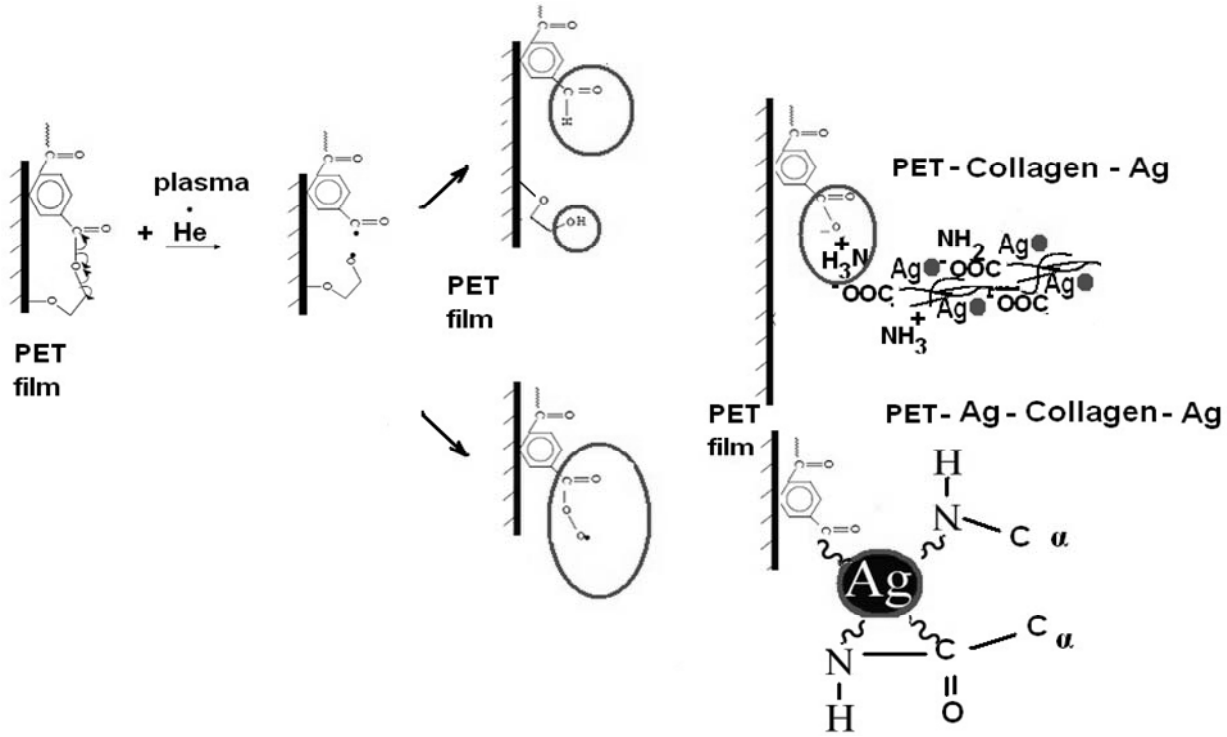
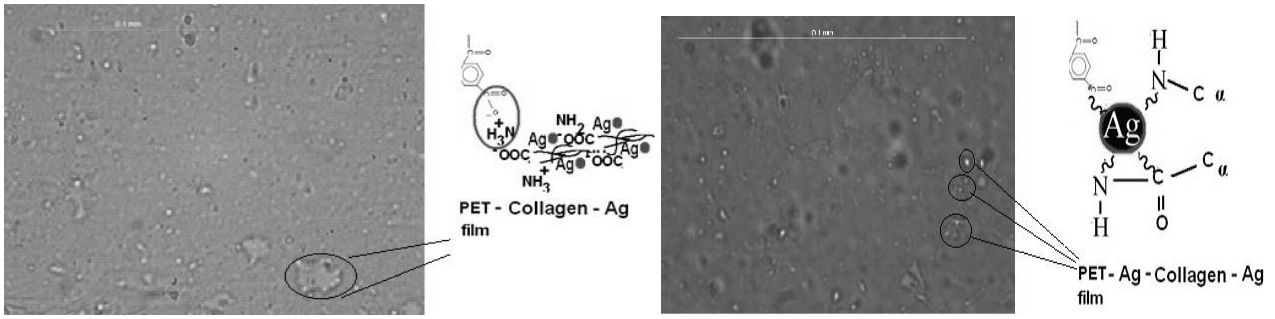


Fig. 2 – A schematic mechanism of collagen adsorption on functionalized PET film.

The complex PET-collagen-Ag was formed due the intermolecular hydrogen bonding showing a new band at 1612 cm^{-1} in ATR-FTIR spectrum. The presence of a weak band at 1612 cm^{-1} and of a strong one at 1665 cm^{-1} in the spectrum is attributed to the presence of silver in an ionized state. This indicates that the carbonyl terminal group from PET surface is involved in bonding with silver. Therefore the major driving force is one of the adsorption of collagen-silver complex on the functionalized PET surface. The result is the formation of the final complex Ag+. . . COO− bond.

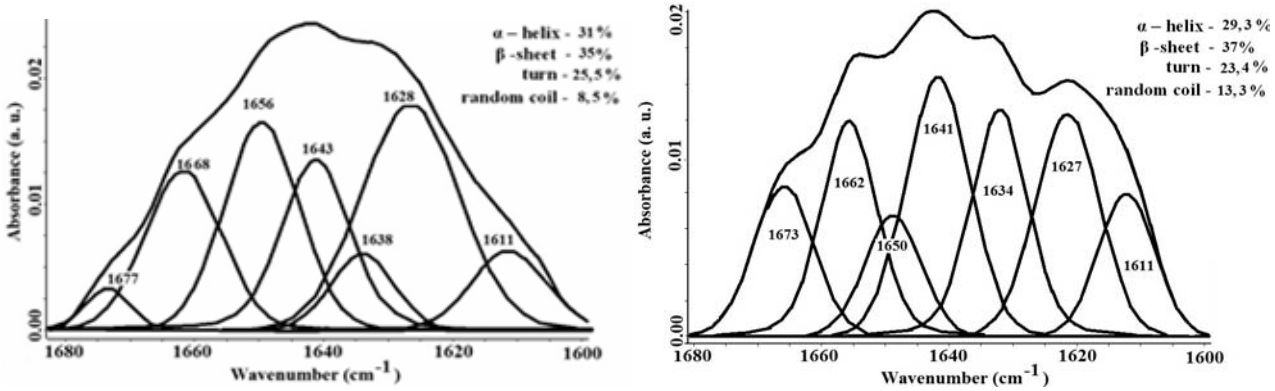
Based on these experimental findings we proposed two mechanisms of bonding between the collagen-Ag complex and plasma-treated surface of PET substrate: a mechanism by which the newly formed groups -COO- onto PET surface interact with collagen-Ag complex via ammonium-type groups belonging to collagen; the ather mechanism implying the same newly formed groups -COO- and collagen by means of the Ag nanoparticles (Fig. 2).

These two mechanisms were confirmed by morphological investigation of the modified PET surface employing optical microscopy (Fig. 3).



(a) (b)

Fig. 3 – Optical microscopy images of Ag-collagen regions immobilized on PET plasma treated (a) P1 and (b) P2.



(a) (b)

Fig. 4 – Curve-fitted amide I (1700 – 1600 cm^{-1}) band from FTIR spectra of collagen immobilized on functionalized PET films P_1 (a) and P_2 (b).

To assess how secondary structure of collagen was altered in the two situations P1 and P2, the characteristic amide I bands in the corresponding FTIR spectra were processed by OPUS 6.5 software. Spectrum decomposition of the amide bands has been performed by considering a linear baseline and a Gaussian/Lorentzian profile. The peak positions were determined using the second derivative of the spectra.

Given the fact that the amide I band offers a lot of information about the content of secondary structure of collagen, the component bands resulted after amide I band decomposition may give insights on subtle changes of protein at molecular level appeared in the final stage of the PET-collagen-silver system.

The contents of component bands for amide I, as well as the peak positions, are consistent with those reported in the literature. The percentages of different types of conformations related to all the subbands have been expressed with respect to the total area of the overall amide I band. The curve fitting procedure applied for investigation of the secondary structure of type I collagen relied on some particular wavenumbers at which these conformations are recognized in IR spectra of proteins: antiparallel β-sheet (1690 cm^{-1}), β-turns (1676 cm^{-1}), α-helix (1665 and 1653 cm^{-1}), random coil (1642 cm^{-1}), side chains (1615 cm^{-1}); the 1615 cm^{-1} band is a result of intermolecular β-strand and the 1629 cm^{-1} band corresponds to the intramolecular β-strand structure. [14,15] For our study, the abovementioned pairs were identified as follows: β-turns (1677 cm^{-1}), α-helix (1668 and 1656 cm^{-1}), random coil (1643 and 1638cm^{-1}), intermolecular β-strand (1611 cm^{-1}), intramolecular β-strand (1628 cm^{-1}) for P1 (Fig. 4a) and β-turns (1673 cm^{-1}), α-helix (1662 and 1650 cm^{-1}), random coil (1641 and 1634 cm^{-1}), intermolecular β-strand (1611 cm^{-1}), intramolecular β-strand (1627 cm^{-1}) for P2 (Figure 4b).[16-19] The results indicate a strong interaction established between the -C=O and –NH- groups of protein and AgNPs. The presence of AgNPs causes a change in conformational structure of protein, with the preponderance of β-conformations.

The FT-IR data proves the presence of the complex formed by AgNPs and collagen via

carboxylate and ammonium-type groups from collagen. Thereby, due to the role of stabilizer played by AgNPs, we believe that the conjugation of collagen with AgNPs leads to a compact final structure.

The reduction in the α-helix content in favour of the β-conformations indicates a partial unfolding of protein in the presence of AgNPs. This change of conformational is a result of hydrophobic cavities exposure to more hydrophilic environments.

CONCLUSIONS

In our study we proposed two mechanisms of interaction between aqueous collagen–Ag mixture and plasma-treated PET surface. First, the mechanism consists of binding of newly-formed carboxylate groups (onto plasma-treated PET surface) to collagen-AgNPs complex (in which there exist attractive interactions $–COO^{-}…Ag^{+}$) through the collagen ammonium-type groups; secondly, another mechanism imply the attractive interactions between the newly-formed group - COO^{-} onto PET surface and collagen via AgNPs. The observations from FTIR spectra were confirmed by optical microscopy. We suggest that the conjugation of collagen with AgNPs leads to a compact structure. The reduction in the α-helix content of collagen secondary structure in favour of the β-sheet, random coil, β-antiparallel and intermolecular β-strand indicates a partial unfolding of protein in the presence of AgNPs.

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