BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI
Publicat de
Universitatea Tehnică „Gheorghe Asachi” din Iaşi
Volumul 64 (68), Numărul 3, 2018
Secţia
CHIMIE şi INGINERIE CHIMICĂ

EFFECT OF SURFACE MORPHOLOGY ON CELL GROWTH ON POLYESTER FILMS IMMOBILIZED WITH BIOMOLECULES

BY

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Received: September 5, 2018
Accepted for publication: September 28, 2018

Abstract. There are many methods to modify the surface properties of the polymer films, such as chemical, mechanical, enzymatic or physical treatments. The polyesters are frequently used to improve the adhesion of the proteins and cells due to their excellent surfaces properties. The purpose of this study was to monitoring changed surfaces in order to improved proteins anchoring on the surface. Polyethylene terephthalate (PET) was the designated polymer due its excellent properties. The surface of this polymer has been investigated regarding the interactions of the polymer surface with proteins after UV functionalization for improving the adhesion properties using methods such as FTIR spectroscopy, scanning electron microscopy (SEM), contact angle, and biocompatibility tests.

Keywords: cells; gelatine; hybrid materials; polar groups; UV functionalization.

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1. Introduction

Different surface transformation strategies, such as chemical or physical treatments (radiofrequency plasma (Nagai *et al*., 2003), ultraviolet radiation (UV) (Lazare and Srinivasan, 1986) or laser irradiation (Fertier *et al*., 2013) have been developed over time, and materials and devices are largely controlled by the chemistry structure of the surface materials. The surface modification may involve important risks as follows: chemical methods generate some secondary inconvenient products and physical methods induce the decrease of the initial surface properties.

The polymers have many applications in the modern industry, but most of them have poorly surface hydrophilies, this fact affects the biocompatibility and the adherence of the biomolecules on surfaces. Therefore, polymer films apply superficial surface treatments for a good wettability and adhesion.

These methods have been used separately or in combination to find the most effective way. The strong affinity between polyester materials (COOH and OH active function groups) and proteins can be developed in many applications, like as antiseptic and anti-microbial textiles (Rabek, 1987).

The generation of new groups on surfaces using various methods of active polymer supports will follow optimal conditions favourable to conserve the primary properties of the polymer matrix support.

The purpose of this study was to change the surface properties of PET film with a thickness of 30 μm using UV radiation. After the films surfaces were activated, they were immersed in a gelatine solution.

Biocompatibility has been accomplished by performing a selection of the protein that acts depending on the properties desired to be imprinted. Activating the polyester surface leads to the induction of new surface groups. These polar groups facilitate the gelatine immobilization and therefore improve the biocompatibility on this polymer.

Characterization of the functionalized surfaces will be monitored by highlighting newly formed groups and the immobilization with gelatine, by means of contact angle, FTIR spectroscopy, scanning electronic microscopy (SEM), atomic force microscopy (AFM), etc. The structure-property relationships will be established, and biocompatibility tests with fibroblast cells will be performed.

2. Experimental

Materials

Irradiation of the PET films 30 μm with UV radiation was performed with a machine UV III lamp, equipped with two wavelengths of 254 and 365 nm and 200/50 mm filter (Carl-Roth, Germany).

The surface of the PET film was irradiated using different times in air atmosphere. The irradiation was performed with a wavelength of 254 nm and

the parameters were: average luminous intensity of 30 mW/cm^2 and the irradiance of 1.17 J/m^2 for 1h. The distance between the light source and the film was 10 cm. Fig. 1 shows the functionalization mechanism under UV radiation on PET film.

The gelatine solution was prepared in pH = 7 phosphate buffer at 0.5 g/L concentration. The UV activated films were kept in this solution for 24 h at room temperature. After this period the films were removed from the solution and then were washed with distilled water and ethanol. The samples were dried at room temperature.

Methods

The surface modification was measurement with contact angle and atomic force microscopy (AFM), the chemical structure with FTIR-ATR and the test cells were investigated by SEM images.

3. Results and Discussions

3.1. Characterization of the UV Functionalized Films Immobilized with Biomolecules

PET films were activated by UV irradiation and functional groups were generated on surface. It is well known that the contact angle values are affected by changes in surface morphology. Therefore, in order to investigate these changes, static contact angle with water as the test liquid was performed after 2, 4 and 6 h of irradiation.

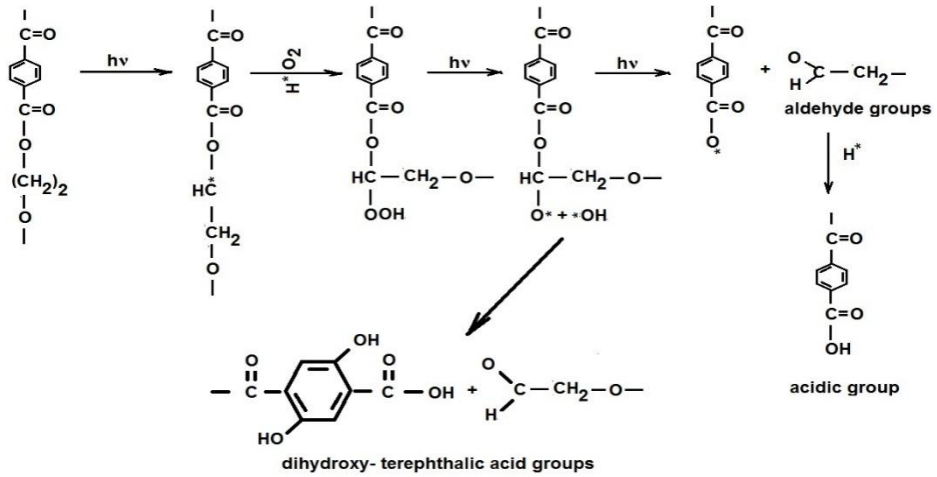


Fig. 1 − Surface functionalization PET mechanism with UV radiation action at λ = 254 nm.

Table 1
Water Contact Angle for Irradiated PET Films

Exposure UV irradiation time, [h]	Water contact angle, [°]
0	79.69
2	68.24
4	63.62
6	55.61

Table 1 shows the water contact angle values. These values are indicated for the 6 h UV irradiated sample that the angle is situated in the hydrophilic region. Topography of film surfaces undergoes important changes due to treatment action.

The topography of the functionalized film surfaces undergoes important transformations, according to Table 2. As can be seen from the table, the average roughness values are increasing. After irradiation treatment the surface it becomes rougher. It could be seen that the roughness of 4H-G and 6H-G was smaller than the functionalized films. The explanation in fact could be that the voids formed during the functionalization are then covered by gelatine immobilization, which could smooth out the surface of the film by filling the voids.

It has also been speculated that the gelatine adsorption is improved due its flexible configuration on interface allowing the surface to adsorb a large amount of protein molecule, namely by correctly changing the structural orientation at the interface. It is well-known that protein adsorption is maximized near the isoelectric pH of biomolecules.

Table 2
Roughness Values of UV-Modified Films and Immobilized Biomolecules at Different Exposure Times

Exposure UV irradiation time [h]	Rootmeansquare roughness [nm]
2 UV	1.4
4 UV	4.2
6 UV	5.0
2H-G	2.8
4H-G	3.2
6H-G	4.3

The gelatine is positively charged and behaves like a polyelectrolyte. Therefore, the adsorption of the gelatine is much higher on a slightly negatively charged surface due to electrostatic interactions.

The amount of amino-free groups in the gelatine group is much higher than the negatively groups on the activated surface, thereby there are several non-protonated amino groups than the acid groups on the polymer substrate. In general, the existence of hydrogen bonding and other unspecific interactions are important in interactions.

The sample 2H-G adsorbed gelatine layer, exhibits globular conformation and more similar into the spherical structure, while 4H-G it appears to contain elongated prominent formats (Fig. 2).

Additionally, a higher roughness is found for samples obtained after longer time anchoring. The formations were denser structuring and expand further into diameter. As we know, gelatine is a denatured form of a collagen, which was initially a triple helix with three simple-wrapped chains. During denaturation, the collagen molecule is fragmented into smaller segments.

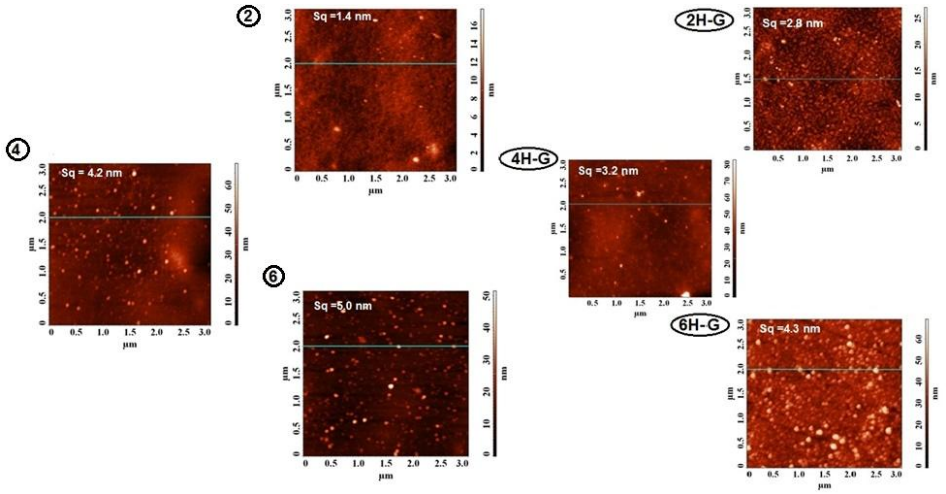


Fig. 2 − AFM 2D images (topography) obtained for UV functionalized polyethylene terephthalate films at various times and immobile with biomolecules.

After drying the samples, the immobilized gelatine molecules tend to recover partially structure from triple helix structure stabilized from collagen mainly by formation of the inter-chain hydrogen bonds between the carbonyl and amine groups. Secondly, the dielectric constant decreases significantly facilitating a stronger electrostatic bonding and interactions between charged (both intra and inter) segments of molecular gelatine.

The structure of the molecular immobilized gelatine shows that the surface is very uniform, with spherical structures immobilized on the surface at low irradiation times and with denser structures, their distribution being lower in diameter.

3.2. Structural Surface Survey of PET Film Irradiated with UV Light and Immobilized Using Infrared Spectroscopy

FTIR-ATR spectra were recorded on a Bruker Lumos FTIR microscope using Attenuated Total Reflection (ATR) module with a diamond crystal and a single reflection fixed at 45 incidence angle. These spectra were recorded at a resolution of 2 cm^{-1} with a number of 64 scans to improve the signal/noise ratio.

In the FTIR-ATR spectrum from Fig. 3, the absorption bands characteristic to the semicrystalline PET film with 30 μm thickness, activated with UV light at 2, 4, 6h, can be observed. At the surface of the film, due to the treatment action, appears changes in the FTIR-ATR spectrum. Also, in the FTIR-ATR spectrum, the structural changes occurring at the surface film are observed by increases in the vibration band of the intensities for the characteristic -COO, -CO and for peroxide groups are less noticeable. The $-C_6H_4-COO-CH_2-CH_2-$ group present the gauche and trans conformation. The bands observed at 1340 and 1370 cm^{-1} are vibrations attributed to $-CH_2-CH_2-$ ethylene glycol group in the trans and gauche conformation, while the band at 1410 cm^{-1} is attributed to the deformation plane of the aromatic ring. In order to make pertinent assessments of the superficial changes of the polyester, the spectra were normalized to the 1410 cm^{-1} band which is considered a reference band.

The stretching vibrations of the $\nu_{(C=O)}$ group of the polyethylene terephthalate ester structure present a located band at 1712 cm^{-1} which is in fact a multiband envelope belonging to the carbonyl group embedding structures which there are in different steric conformations. This band sums up the totality groups of the -COO- from the ester structure present in the PET macromolecular chains, and in different planes relative to the symmetry plane of the aromatic ring.

With irradiation time, the carbonyl band, in the same time, its expansion to the carbonyl ester region, more precisely at 1670 cm^{-1} (Fig. 4), in fact confirming the presence of several species on the PET surface due to the cleavage of the surface.

In the 3400-3100 region, ν_{NH} and ν_{OH} vibrations attributed to the amide A with a peak centred at 3292 cm^{-1}, ν_{SCH2} and ν_{asCH2} vibrations at 2964 and 2926 cm^{-1}, respectively, are observed.

The strong absorption band at 1655 cm^{-1} indicate the presence of amide I, which has a major contribution to the stretching vibration band $\nu_{(C=O)}$, whereas the amide band II represented mainly by the deformation vibrations of the δ (NH) + $\nu_{(CN)}$ is located in the spectrum at 1550 cm^{-1} from amino acid segments of the protein. Both vibrations of amide I and amide II have a markedly increasing evolution throughout the immobilization process.

Vibrations in 1234-1237 cm^{-1} region are attributed amide III from gelatine, could be associated with the loss of triple helix state of the collagen molecules.

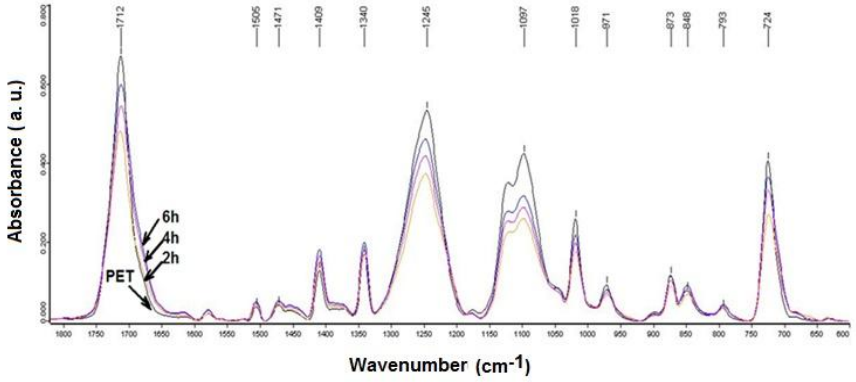
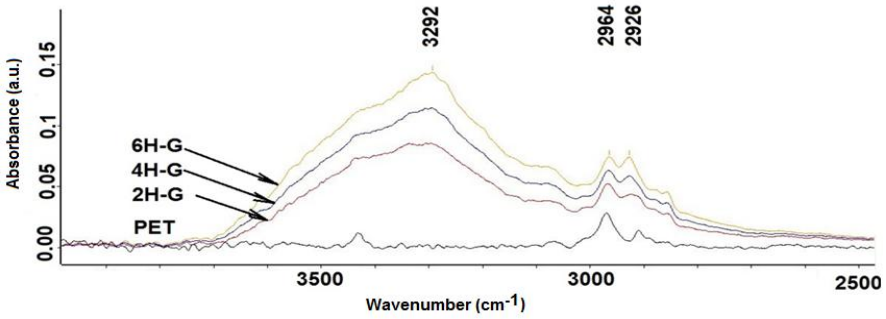
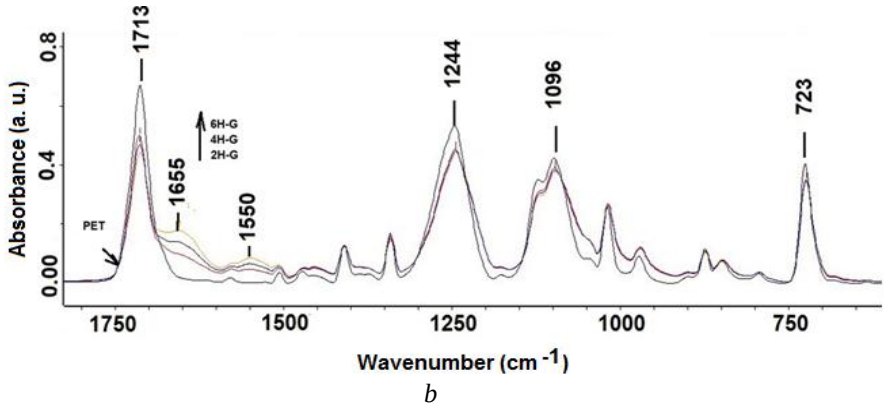


Fig. 3 − The FTIR-ATR spectra in the range of 1800-600 cm^{-1} for UV-functionalized PET film at different irradiation times (2, 4, 6h).



a



b

Fig. 4 − (*a*) and (*b*) are presented the FTIR-ATR spectra before and after immobilization with gelatine molecules.

In Fig. 5 were presented immobilized gelatine films. The immobilized films were subjected to cell growth assays. Fibroblast cells were grown at 37°C, and a humidified atmosphere until a layer of semi-confluent cells was reached. After the semi-confluence was reached, culture medium was exchanged with the extracted material in the experimental wells and the fresh medium in the control wells.

For the fibroblast cell growth assay, it was visualized by SEM imaging studies for 96 h incubation Fig. 5.

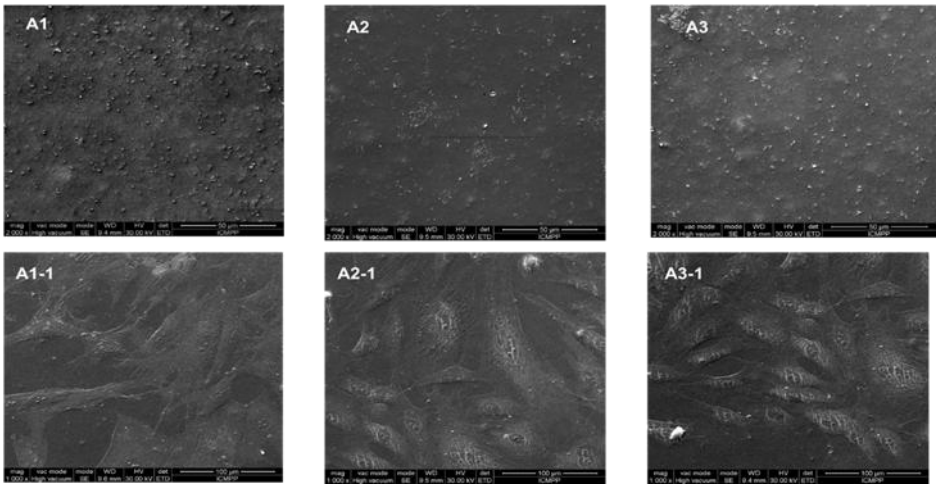


Fig. 5 − (SEM) images of immobilized modified PET films- for (2, 4, 6) gelatine A_1, A_2, A_3 followed by cell growth ($A_{1\text{-}1}$, $A_{2\text{-}1}$, $A_{3\text{-}1}$)

From the images one can see a proliferation of fibroblast cells for all treated films. In the case of tissue engineering, it is well known that cells require a surface for adherence, growth and proliferation. Therefore, cells respond and develop according to the surface, roughness and adhesion topography (Liu *et al.*, 2013). These results are caused by the rearrangement of the polar groups of the biomaterial surface and the ratio of hydrophilic to hydrophobicity in contact with the cells. It has been shown that fibroblast cells adhere and proliferate better on moderate hydrophilic biomaterials. This is also the case of the studied sample, showing the low values of the population with fibroblasts indicating a reduction of the cohesion force (Kordoghli *et al.*, 2014).

Therefore, reduced cell cohesion resulting from cell growth could be explained by the fact that the theoretical model does not contain all the interactions that occur at the biointerfaces. Non-specific forces are always present and depend on the physico-chemical characteristics of the interaction (Weidner *et al.*, 1996).

Therefore, the epithelial cells are used as substitution therapy, especially for dermatological disorders. Many of these biomaterials are easiest to accept as biological components that are found outside the body and also easier to accomplish taking into account the biological phenomena of rejecting foreign biological substitutes (Woodcock *et al*., 2005).

The important goal of applying tissue engineering is to investigate skin regeneration and all factors contributing to reconstruction without infection and other complications with artificial skin replacement or use of different cell growth therapies to implement skin reconstruction.

4. Conclusions

Although synthetic materials have been used extensively in tissue engineering fields, this work illustrates the contribution of natural materials and hybrid systems to regenerative medicine research, on the application for cell growth destined in skin regeneration research.

Acknowledgements. "The authors acknowledge the financial support of this research through the Project "Partnerships for knowledge transfer in the field of polymer materials used in biomedical engineering" ID P_40_443, Contract no. 86/8.09.2016, SMIS 105689, cofinanced by the European Regional Development Fund by the Competitiveness Operational Programme 2014-2020, Axis 1 Research, Technological Development and Innovation in support of economic competitiveness and business development, Action 1.2.3 Knowledge Transfer Partnerships.".

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EFECTUL MORFOLOGIEI SUPRAFEȚEI PRIVIND CREȘTEREA CELULELOR PE FILMELE POLIESTERE IMOBILIZATE CU BIOMOLECULE

(Rezumat)

Există multe metode de modificare a proprietăților de suprafață ale filmelor polimerice, cum ar fi tratamente chimice, mecanice, enzimatice sau fizice. Poliesterii sunt utilizați în mod obișnuit pentru a îmbunătăți aderența proteinelor și a celulelor datorită proprietăților lor excelente de suprafață. Scopul acestui studiu a fost de a monitoriza suprafețele modificate pentru a îmbunătăți imobilizarea proteinelor de pe suprafață. Polietilentereftalatul (PET) a fost polimerul desemnat datorită proprietăților sale excelente. Suprafața acestui polimer a fost investigată în ceea ce privește interacțiunile suprafeței polimerului funcționalizat cu radiație UV pentru îmbunătățirea proprietăților de aderență utilizând spectroscopia FTIR, microscopia electronică de scanare (SEM), unghiul de contact și teste de biocompatibilitate.