Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 1, January – March 2012, p. 97 - 106

WATER SOLUBLE ELASTIN ADSORPTION ONTO UV-ACTIVATED GREEN POLYURETHANE SURFACES

C. CIOBANU[a], L. M. GRADINARU[a], R. V. GRADINARU[b], S. VLAD[a*]
[a]”P. Poni” Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, Iasi, 700487, Romania
[b]“Al. I. Cuza” University, Chemistry Department, 11 Carol I Street, Iasi, 700506, Romania

The UV-irradiation is an alternative approach of the polymer surface functionalization that can be easily used in laboratory. This technique doesn’t have an effect on the bulk properties of the green microporous polyurethane films. Thus, the UV-irradiation activates the surface of the green polyurethane by breaking some chemical bonds and generation of new functional groups on the surface which can be controlled by the irradiation time. The amount of water soluble elastin adsorbed on the UV-irradiated polyurethane surface, increase as a function of the exposure time. The new structures of polyurethane-elastin conjugated with specific properties for each time of irradiation were formed. The polyurethane surface has been studied by ATR-FTIR, contact angle and fluorescence spectroscopy.

(Received December 15, 2011; Accepted January 16, 2012)

Keywords: Polyurethane, UV irradiation, Surface functionalization, Elastin adsorption.

1. Introduction

Biomimetic materials based on the extracellular matrix (ECM) composition of native tissues are important in biocompatibility and tissue engineering applications because they provide appropriate biological signals for cell adhesion, migration, proliferation, and ultimately for tissue growth [1]. Elastin is an important ECM protein found abundantly in blood vessels and although its biological signaling capabilities have not been thoroughly elucidated, it is generally agreed that elastin plays both a biomechanical and biological signaling role in cellular activity [2–4]. The use of elastin-like polypeptides as a surface modification moiety represents a feasible strategy for the development of biomimetic materials in vascular applications since materials adsorbed with these polypeptides have previously demonstrated a low incidence of platelet deposition [5, 6]. Surface modification of polyurethanes with elastin-like polypeptides represents a valuable approach for the development of biomaterials in a wide range of medical applications.

This study develops the adsorption and the conjugation of the water soluble elastin-like polypeptides on the surface of UV - activated green polyurethane.

2. Experimental

2.1. Synthesis of polyurethane

Polyurethane was synthesized from poly(ethylene-adipate)diol (PEA) having a molar mass of 2000 g/mol, hydroxyl number of 56 mg KOH/g and acidity number 0.2 mg KOH/g, isopropyl

---

[*] Corresponding author: vladus@icmpp.ro

ricin-oleate diol (IRD - 98%, synthesized in our laboratory), methylene diphenyl 4,4'-diisocyanate (MDI) freshly distilled, with isocyanate concentration of 99.96% and 1.4-buthane diol (BD-99%; Sigma-Aldrich, anhydrous) in dimethylformamide (DMF-99.8% Sigma-Aldrich, anhydrous) as solvent. The synthesis was run at 60 ^{o}C according to a previously reported method [7]. The molar ratio between the components PEA: IRD: MDI: BD was 1: 0.1: 6: 4.9, the concentration of the solution PU-DMF was 33% dry weight and the viscosity 98,000 cP at 20 ^{o}C (Brookfield viscosimeter, spindle no 4). The molar mass of the resulting PU was 110,000 g/mole as determined by gel permeation chromatography. Elemental analyses calculated: C 61.28%, H 6.72%, N 4.22% and found: C 62.03%, H 6.86%, N 4.25%.

2.2. Sample preparation

Polyurethane solution was degassed for 8–12 min under vacuum (10–15 mmHg), and then the PU solution was cast onto a glass slide (220 - 240 mm) using a doctor blade with a gap of 0.6 mm. The films were precipitated in deionized and distilled water at 45 ^{o}C, for 1 h [8]. During this time, the resulted microporous films were detached from the glass plate and subsequently washed five times with 1 L of deionized and distilled water. The films were dried at room temperature and low pressure (1–2 mmHg) for 24 h. The thickness of the microporous film was 0.33 mm. The prepared PU microporous films have the water vapor permeability in the range of 280- 285 mg.dm^{-2} 24 h at 25^{o}C.

2.3. UV Treatment

Films of the PU samples were irradiated in air at 25^{o}C with a UV-Philips lamp having a polychrome emission spectrum between 200-400 nm and light intensity of 30 mW/cm^{2}. The distance between the light source and samples was kept constant at 20 cm.

2.4. Analysis methods

The ATR-FTIR analysis was performed using a Bruker Vertex 70 type spectrometer (US), with a diamond crystal having a 6 mm^{2} surface, provided with software for spectral processing. The sample surface was scanned in the 200–4000 cm^{-1} range, at 45^{o} angle. The ATR-FTIR spectra were recorded at a constant temperature of 25^{o}C. Fluorescence spectra were measured using a Perkin-Elmer LS 55 luminescence spectrometer at room temperature. The excitation wavelength was 350 nm and emission within the region from 360 to 500 nm.

Fluorescence spectra were measured using a Perkin-Elmer LS 55 luminescence spectrometer at room temperature. The excitation wavelength was 350 nm and emission within the region from 360 to 500 nm

The static contact angles were measured by the sessile-drop method, with a CAM-101 (KSV Instruments, Helsinki, Finland) contact angle measurement system equipped with a liquid dispenser, video camera, and drop-shape analysis software, at room temperature. Double distilled water, ethylene glycol and diiodomethane were used as liquids for the studies. For each kind of liquid, three different regions of the surface were selected to obtain a statistical result.

2.5. Water soluble elastin-like polypeptides (WSELP)s adsorption

In this study the water soluble elastin from bovine neck ligament (Sigma 6527) has been used. All samples of polyurethane (10 mm x 10 mm) were made in duplicate and irradiated with UV light for 2, 4, 6, 8, 10 h respectively. Immediately after irradiation, a series of polyurethane films was immersed in a solution of phosphate buffered saline, pH 7.4 and the other series in a solution of WSELPs to concentration 30 mg/ml in PBS. All samples were incubated at 37^{o}C for 42 h. After certain periods, the films were removed and the amount of elastin in the solution was determined by fluorescence using excitation at 350 nm and emission within the region from 360 to 500 nm.

3. Results and discussion

3.1. ATR-FTIR analysis

The structure of the green polyurethane is illustrated in Fig. 1.

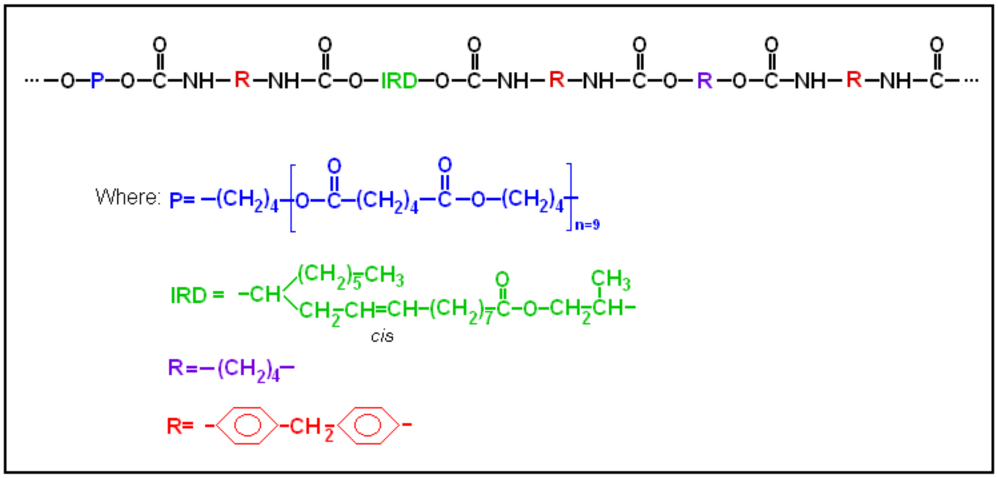

Fig. 1. Green polyurethane structure

The proteins adsorption onto UV-irradiated polyurethane surface was investigated by ATR-FTIR spectroscopy. It was previously shown [12] that after irradiation of polyurethane surfaces, on the surface appear some groups such as -NH2, -OH, -COOH and radicals, which can then form hydrogen bonds with protein groups (Fig. 6).

In Fig. 2 are illustrated the ATR-FTIR spectra of the adsorbed protein (WSELP) onto polyurethane surfaces. The surfaces were irradiated with UV-light for 2, 4, 6, 8, respectively 10 hours.

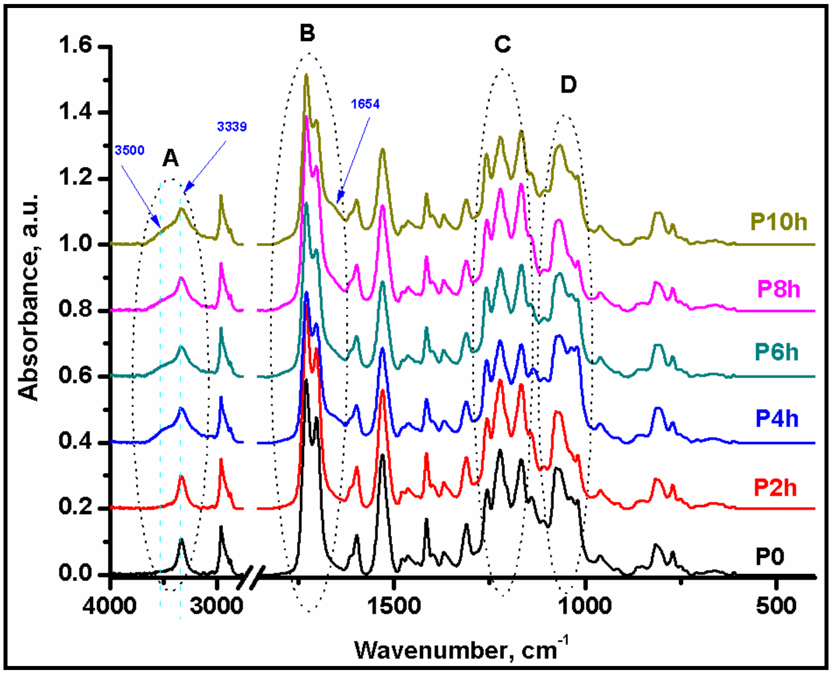

Fig. 2. The ATR-FTIR spectra of the adsorbed elastin on the UV-irradiated polyurethane surfaces

If we consider these spectra, can observe that appear a new peak at 3500cm^{-1} which increases as a function of the irradiation time, Fig. 3. This peak is characteristic to the hydrogen-free υ(N-H) stretching vibrations from the peptide (OC-**N-H**) groups which are not organized into clusters and was adsorbed on the surfaces of polyurethane.

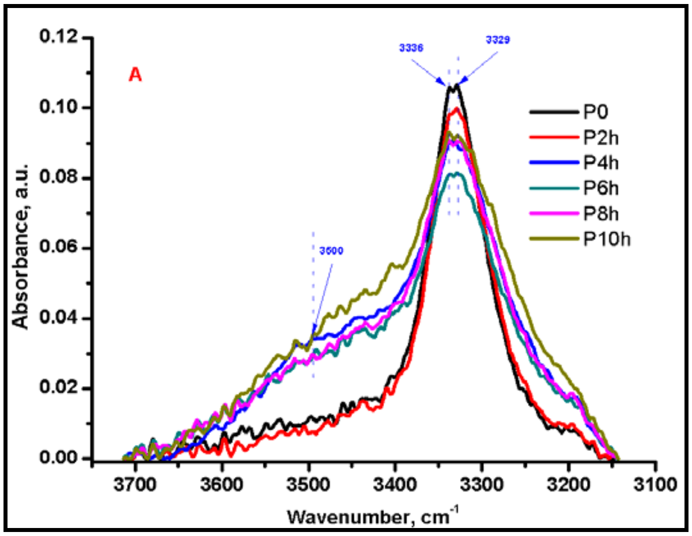

Fig. 3. The ATR-FTIR spectra (3700-3100 cm^{-1}) of the adsorbed elastin on the UV-irradiated polyurethane surfaces

The peaks at 3338 and 3328 cm^{-1} are characteristic to the H-bond between the C=O of urethane group. It is also observed that between 1800 – 1620 cm^{-1} appear a new peak at 1654 cm^{-1} (Figs. 2 and 4) which is characteristic to the stretching vibrations ν(C=O) of elastin (Amide I) [9-11] and increases with the irradiation time.

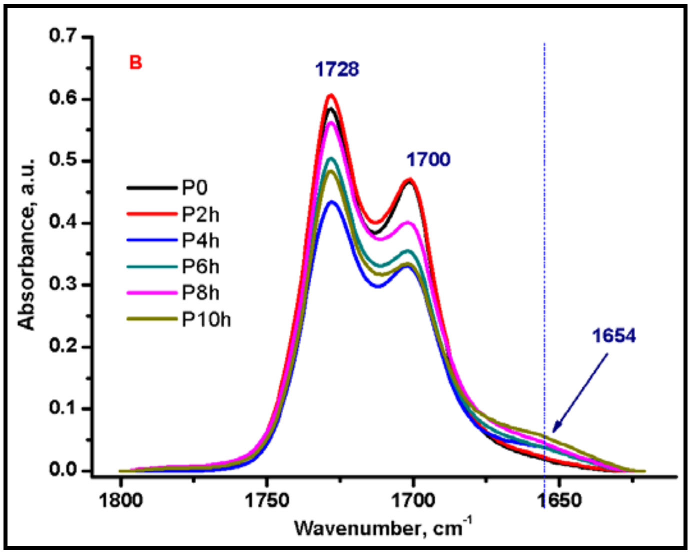

Fig. 4. The ATR-FTIR spectra (1800 – 1620 cm^{-1}) of the adsorbed elastin on the UV-irradiated polyurethane surfaces between

Also, the intensity of the peaks from 1728 and 1700 cm^{-1} which are specific for υ(C=O) stretching vibrations of carboxyl group, decrease. A major change occurs at the irradiation times of 4, 6 respectively 10 h. The absorbance intensity of the adsorbed elastin on the PU surfaces is characteristic for each sample and not decrease as a function of UV- treatment time. These phenomena suggests, that on the irradiated polyurethane surface appear reactive groups which react with reactive groups of elastin, as was observed in BSA adsorption onto of UV- irradiated polyurethane surfaces [12]. The reactive groups are characteristics for each time of irradiation. This can be explained by the fact that at any irradiation time, the reactive groups CO, $-NH_2$, -COOH, C=C formed on the surface continue to participate in photochemical processes.

Moreover, the bands with maxima at 1728, 1700 cm^{-1} are larger than those of the only irradiated samples, Fig. 7. The ratio of the peak areas between amide bands (1654 cm^{-1}) and urethane and ester carbonyls increases with the irradiation time, Fig 5.

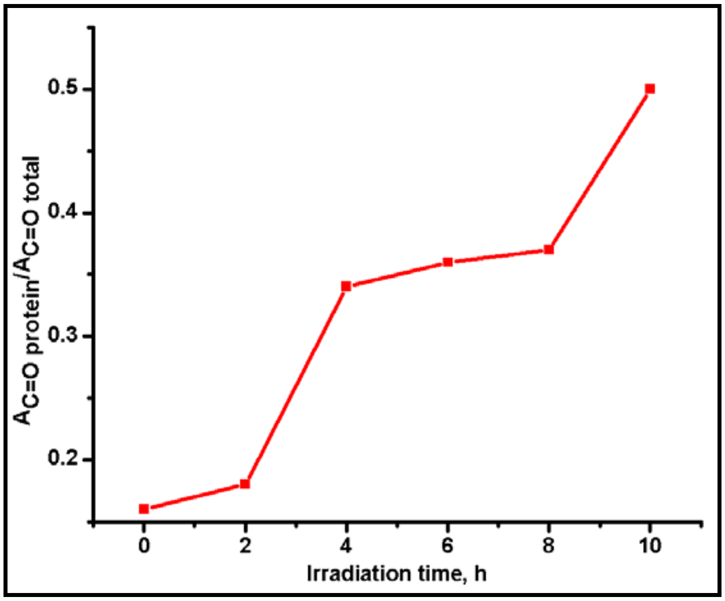

Fig. 5: The carbonyl peaks area ratio as function of the irradiation time

The amount of adsorbed elastin on surface is higher at 4 h irradiated sample. Between 4 and 8 treatment hours the amount of elastin adsorbed remains almost constant, and increases again at 10h. Probably, at 2 and 4 h of irradiation some reactive groups are formed, react with $-NH_2$ structures of elastin and leads to the formation of Schiff bases (Fig. 13). Concomitantly, some free radicals can also arise and interact with ester groups to generate amides. After this time, the new structures interact with themselves or with existing urethane groups to form another surfaces. These surfaces have a low reactivity towards elastin. For the purpose of such research, the recommended irradiation period is between 2 and 4 hours, according to the application area of polymers.

The changes of the polyurethane surfaces can be better highlight by deconvolution of the carbonyl band using the *Gauss* method. For example, the deconvolution spectra of the non-irradiated control sample (M0) and of P0 and P4h samples were performed. In Fig. 6 is observed that are three different samples, because the deconvolution peaks varies in wavenumber and wavelength.

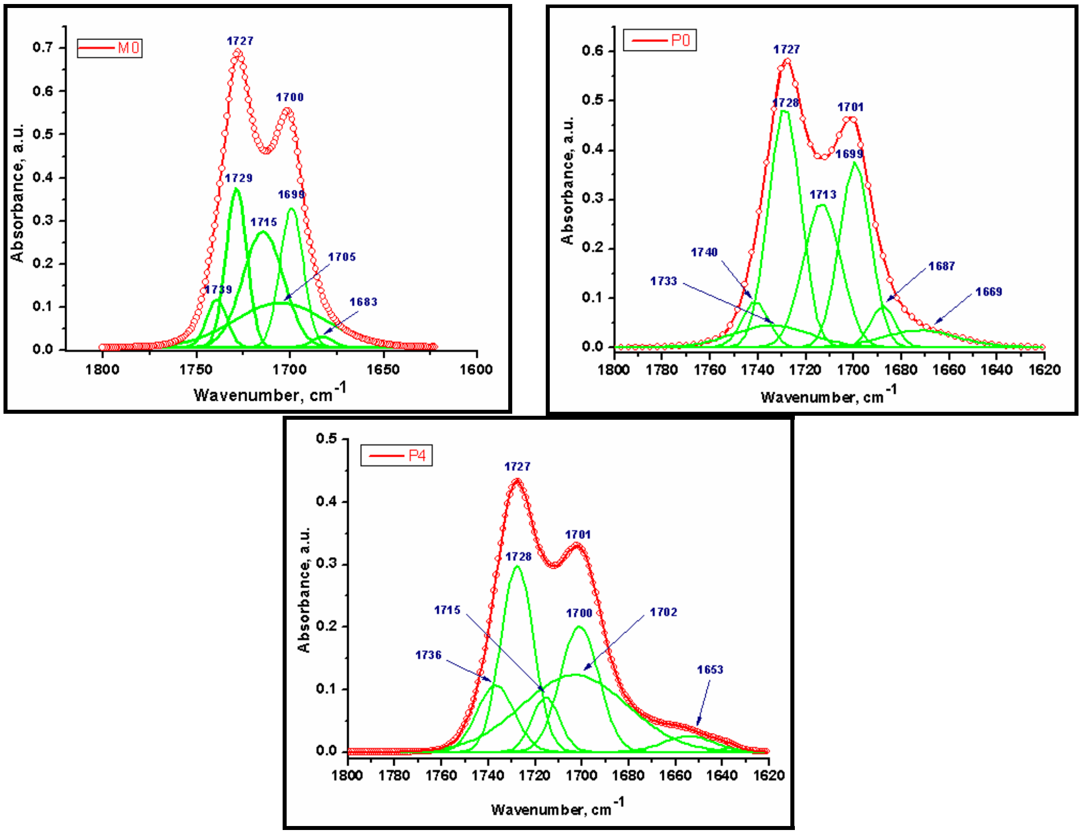

Fig. 6. The deconvolution of the carbonyl bands using the Gauss method of control sample (M0), elastin adsorbed onto non-irradiated PU surface (P0) and elastin adsorbed onto 4 hrs UV-irradiated PU surfaces (P4)

The peak at 1256 cm^{-1} (Amida III) characteristic to the stretching vibration ν(N-C) and **δ**(N-H) bending from the crystalline structures increase with irradiation time, excepting sample P4h. The intensity of the peak at 1222 cm^{-1} decreases in the same manner. The peak at 1167 cm^{-1} characteristic to the C-O-C groups increases at P2h and P8h and decreases for another samples, Fig. 7.

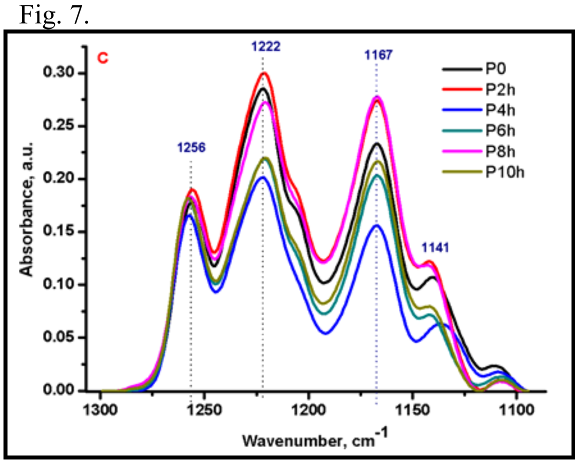

Fig. 7: The ATR-FTIR spectra (1300-1100 cm^{-1}) of the P0-P10h samples

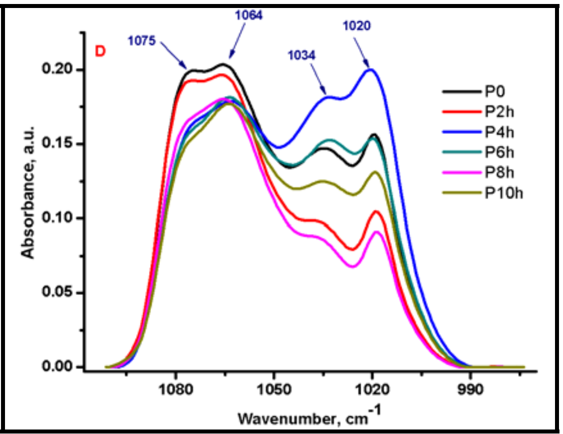

Fig. 8. The ATR-FTIR spectra (1105 – 990 cm^{-1}) of the adsorbed elastin on the UV-irradiated polyurethane surfaces

The peak at 1075 and 1020 cm^{-1} due to the C-O-C and C-O-C bond vibration (Fig. 2) are changed according to the time of irradiation as a consequence of some links generation between the ester and urethane groups and peptides groups from elastin. It can be seen that at 4 hours of irradiation, the peak intensity from 1020 cm^{-1} is higher than the other samples. This can be explained by the fact that at four hours of irradiation, the amount of the polyurethane surface reactive groups which can interact with the groups from elastin is greatest. Moreover, the absorbance intensity changes of these bands are due to the hydrogen bonds formation between the urethane structures and elastin amidic groups (1034, 1020 cm^{-1}) and between urethane or esteric groups and peptides (1075, 1064 cm^{-1}), Fig. 8.

Thus, we can formulate a linear or cyclic interaction mechanism between these structures, like in Fig. 9. The *cis* and *trans* urethane and ester structures can interact with *cis* and *trans* peptide groups through the hydrogen bonds.

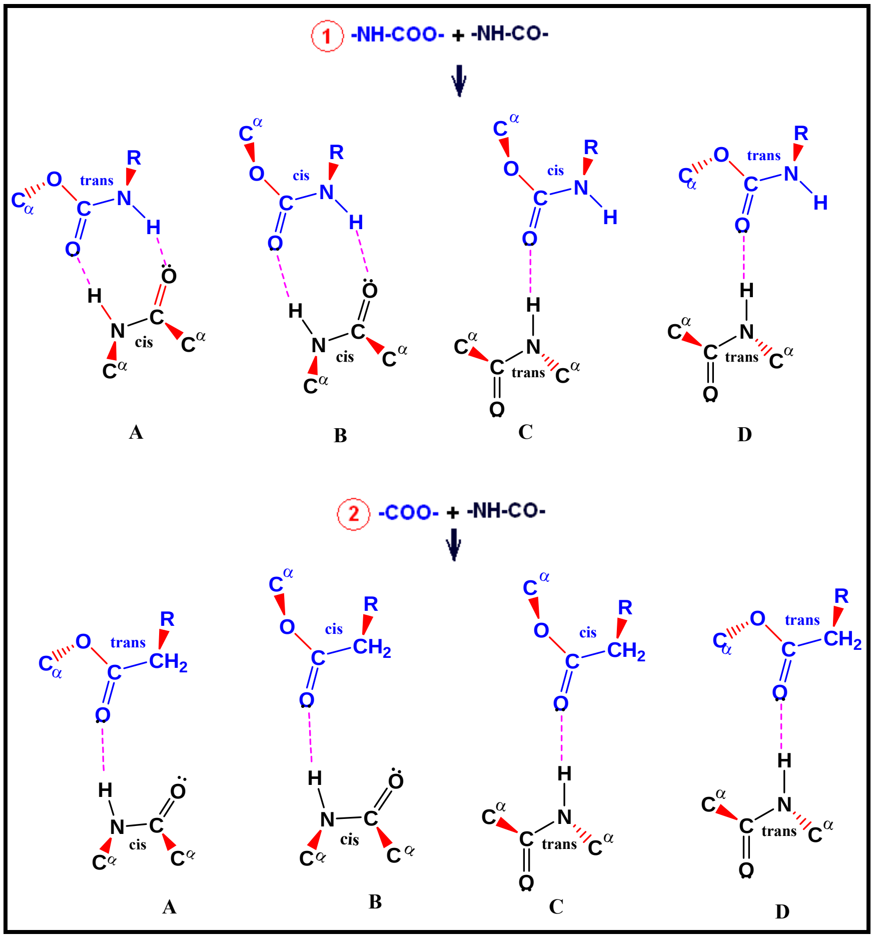

Fig. 9: The interaction mechanism between: 1-urethane and peptide groups and 2-ester and peptide groups

These surface changes are highlighted more clearly in Fig. 10, where each irradiated spectrum was subtracted from the elastin adsorbed spectrum. The subtracting spectra give us information about the arising and disappearing of the main groups. In this spectra it can be seen that appear two peaks at 1109, respectively 1032 cm^{-1}, confirming the previous remark.

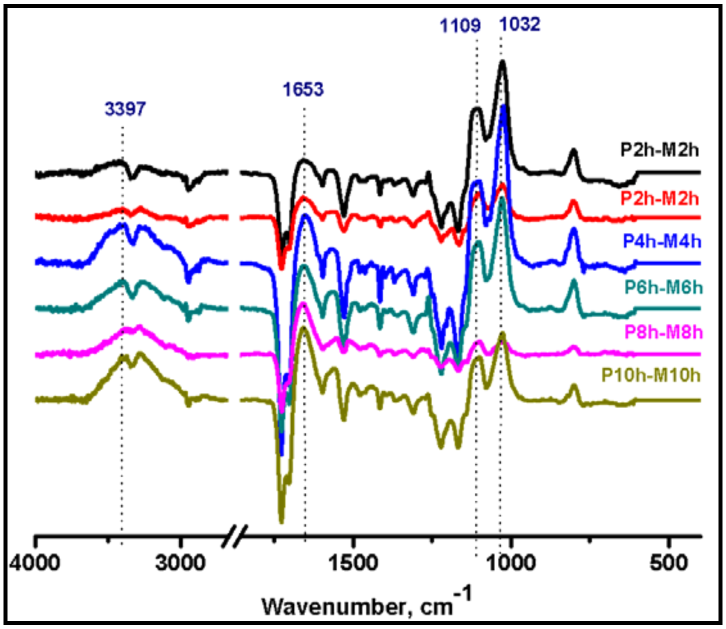

Fig. 10. The ATR-FTIR subtracting spectra

In conclusion, the UV-irradiated surface has specific properties for each time of irradiation. The reactive groups from the polyurethane surfaces interact with the peptides of elastin and form a new polyurethane-elastin matrix.

3.2. Contact angle and surface free energy

The static contact angles were measured by the sessile-drop method, using double distilled water, ethylene glycol and diiodomethane as testing liquids. The contact angle measurements data are illustrated in Fig. 11.

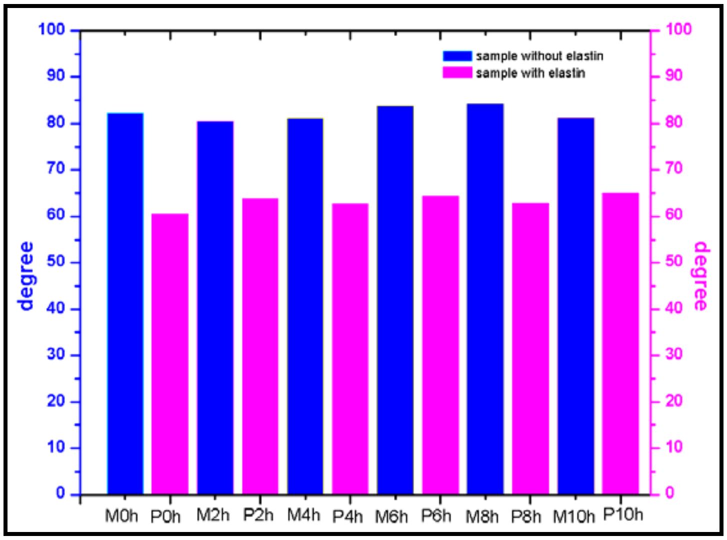

Fig. 11: The contact angle values of the samples without (M) and with elastin (P) irradiated up to 10 hrs

From the experimental data can be observed that the values of the UV-irradiated samples are around 80 degree. After elastin adsorption on the irradiated surfaces the contact angle values decrease with ~20 degrees. This behavior is because the UV-irradiated polyurethane surface that contain hydrophilic groups (–NH_2, -OH, -COOH) can interact with the adsorbing elastin molecules via hydrogen bonding and van der Waals interactions [13,14] leads to the formation of a new polyurethane matrix.

Based on these measurements, the parameters who characterized the solid surface and its absorption capacity can be calculated. The most used parameters are the surface-free energy with polar and disperse component, solid-liquid interfacial tension, work of adhesion. The resulted parameters are given in Table1.

The surface energy of the films was calculated using Young equation:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos\theta \tag{1}$$

where γ_{SV} is the energy of the surface, γ_{SL} is the interfacial tension between the solid and the drop, γ_{LV} is the liquid-vapor surface tension, and cos θ is the contact angle of the drop with the surface [15,16].

The surface-free energy values (γ_{SV}) as well as the polar (γ^p_{SV}) and dispersive (γ^d_{SV}) components were obtained according to the Owens-Wendt-Rabel, and Kaelbe method [17, 18]:

$$\gamma_{SV} = \gamma^p_{SV} + \gamma^d_{SV} \tag{2}$$

$$W_a = 2\left(\sqrt{\gamma^d_{SV}\gamma^d_{LV}} + \sqrt{\gamma^p_{SV}\gamma^p_{LV}}\right) \tag{3}$$

were W_a is the work of adhesion.

Table 1: The parameters that characterise the polyurethane surface

Sample	θ (°)	W_a (mN/m)	γ_{SV} (mN/m)	γ^p_{SV} (mN/m)	γ^d_{SV} (mN/m)	γ_{SL} (mN/m)
M 0h	82.23	82.63	26.98	9.14	17.83	17.14
P 0h	60.38	108.77	45.33	42.53	2.79	9.35
M 2h	80.46	84.86	29.08	9.11	19.96	17.02
P 2h	63.75	104.99	40.45	36.37	4.07	8.25
M 4h	81.28	83.82	26.26	10.96	15.30	15.24
P 4h	62.70	106.18	40.58	35.47	5.11	7.20
M 6h	83.75	80.72	25.17	9.26	15.90	17.24
P 6h	64.32	104.34	39.56	35.09	4.46	8.01
M 8h	84.32	80.00	25.63	8.28	17.34	18.43
P 8h	62.91	105.94	43.18	40.63	2.54	10.03
M 10h	81.29	83.81	26.78	10.36	16.41	15.76
P 10h	64.97	103.60	38.40	33.21	5.19	7.60

Therefore is observed that the free surface energy (γ_{SV}) of sample M2h has a value of 29.08 mN/m and for the others is around 25-26 mN/m. For the samples with elastin, the surface energy decrease with the irradiation time. The adhesion work of the samples with elastin has higher values than of the samples only irradiated. Also, the interfacial tension solid-liquid is lower at the samples with elastin.

In conclusion, the contact angle values decrease and the surface energy is significantly higher at the samples with elastin which shows that this elastin was adsorbed onto the UV-irradiated polyurethane surfaces.

3.3. The fluorescence spectrum analysis

The intrinsic fluorescence of the proteins was applied to study the interactions of elastin-like protein with UV- activated polyurethane membranes. The emission spectra of free and adsorbed elastin are illustrated in Fig. 12.

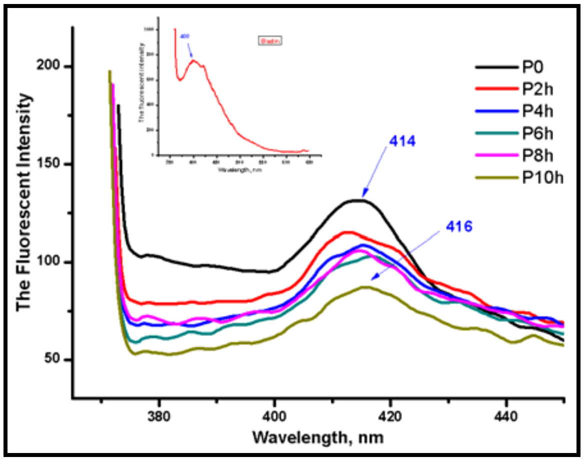

Fig. 12. The fluorescence spectra of free and adsorbed elastin onto UV-activated surface

In this spectrum, we can observe that the fluorescent intensity of the elastin is high (~800) and the wavelength is around 400 nm. For the UV-irradiated samples, the intensity is much lower, the peak is broader and its maximum moves to the higher wavelengths (416 nm). The fluorescence intensity of the adsorbed elastin on the PU surfaces is characteristic for each sample and not decrease as a function of UV- treatment time. These phenomena can be better highlight through the interaction mechanisms which is illustrated in Fig. 13.

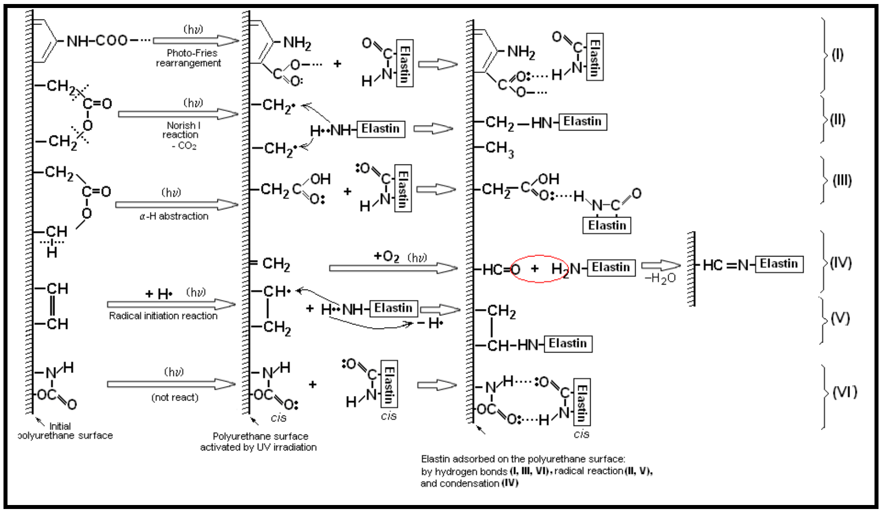

Fig. 13. Some reaction mechanisms of the macromolecules from the UV–irradiated polymer surface

The urethane groups under the influence of the light give a Photo-Fries rearrangement in *ortho* position and forms an amino ester structure. Thus, after UV irradiation, polyurethane becomes a poly (urethane amino ester) copolymer (I). Another photo-chemical reaction at the UV-irradiated surface is Norrish I reaction which gives radicals that are reacting with elastin obtaining elastin-conjugate (II). The carboxyl and carbonyl groups which are obtained after the cleavage of the hydrogen from *alpha*-position and under the influence of oxygen from the air were formed H-bonds with peptide groups (III). The exposure of green polyurethane surface to ultra violet light alters the physical behavior and chemical composition of the green polyurethane surfaces. Irradiated surfaces of green polyurethane become hydrophilic and unstable radicals together with stabile carboxyl, carbonyl and amino groups are formed. The carboxyl, carbonyl and urethane groups react with amide group from elastin and form hydrogen bonds (I, III, VI). The radicals interact with elastin and form elastin-green polyurethane conjugates (II, V). Also, the aldehyde groups from the polyurethane surface can interact with the elastin $-NH_2$ groups leads to the formation of some Schiff base (IV).

Thus, the fluorescent data are in agreements with the ATR-FTIR data that were previously reported.

4. Conclusion

After adsorption of water soluble elastin-like polypeptides (WSELP)s onto UV-irradiated polyurethane surfaces, these becomes more hydrophilic and have specific properties for each time of irradiation. Thus, the contact angle values decrease and the surface energy is significantly higher at the samples with elastin. The reactive groups from the polyurethane surfaces interact with peptides of (WSELP)s and form a new polyurethane-elastin matrix which contained two types of structures: i) H bonded polyurethane-elastin and ii) polyurethane-elastin conjugated. Also, these new matrixes have specific properties for each time of UV-treatment. This method is very simple and cheap and can be applied in any lab for the surfaces functionalization of the polyurethanes used as biomaterials

Acknowledgments

This work was funded under 7th FWP (Seventh Framework Programme), NMP-2007-2.3-1, BIOSCENT, nr. 214539/01.01.2009.

References

[1] M. P. Lutolf, J. A. Hubbell, Nat. Biotechnol., **23**, 47 (2005);
[2] L. Robert, Pathol. Biol. (Paris), **3**, 399 (2005);
[3] B. S. Brooke, A. Bayes-Genis, D. Y. Li, Trends Cardiovasc. Med., **13**, 176 (2003);
[4] S. Mochizuki, B. Brassart, A, Hinek, J. Biol. Chem., **277**, 44854 (2002);
[5] K. A. Woodhouse, P. Klement, V. Chen, M. B. Gorbet, F. W. Keeley, R. Stahl, J. D. Fromstein, C. M. Bellingham, Biomaterials, **25**, 4543 (2004);
[6] L. Buttafoco, N. G. Kolkman, P. Engbers-Buijtenhuijs, A. A. Poot, P. J. Dijkstra, I. Vermes, J. Feijen, Biomaterials, **27**, 724 (2006);
[7] C. Ciobanu, Polyurethane for Synthetic Leather, *PhD Thesis*, “Petru Poni” Institute of Macromolecular Chemistry, Iasi (1979);
[8] V. Melning, O. M. Apostu, V. Tura, C. Ciobanu, J. Membrane Sci., **267**, 58 (2005);
[9] B. H. Stuart, Infrared Spectroscopy: Fundamentals and Applications, John Wiley & Sons Ltd Ed., England (2004);
[10] V. Hlady, J. Buijs, H. P. Jennissen, Methods Enzymol., **309**, 402 (1999);
[11] D-A. Wang, Adv. Polym. Sci., **209**, 179 (2007);
[12] C. Ciobanu, L. M. Gradinaru, R. V. Gradinaru, M. Drobota, S. Vlad, Dig. J. Nanomater. Bios., **6** (4), 1751 (2011);
[13] L-Q. Wang, G-Z. Liang, G-C. Dang, F. Wang, X.-P. Fan, W-B. Fu, Chinese Journal of Chemistry, **23**, 1257 (2005);
[14] L. Irusta, M. J. Fernandez-Berridi, Polymer, **40**, 4821 (1999);
[15] L. H. Sperling Introduction to Physical Polymer Science, Ed 4, Wiley-Interscience, England (2005).

[16] M. Stamm, Polymer Surfaces and Interfaces: Characterization, Modification and Applications, Springer (2008);
[17] H. Y. Erbil, Surface Chemistry of Solid and Liquid Interfaces, Blackwell Publishing, England (2006);
[18] K. L. Mittal, Contact Angle, Wettability and Adhesion, Vol. 2, VSP; Utrecht, Boston (2002);