WETTABILITY AND CELL SPREADING ENHANCEMENT IN POLY(SULFONE) AND POLYURETHANE SURFACES BY UV-ASSISTED TREATMENT FOR TISSUE ENGINEERING PROPOSALS

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Abstract - The surface of Poly(sulfone) (PSU) and Polyurethane (PU) films were treated with ultraviolet (UV) light in the presence of oxygen to improve their wettability, adhesion and cell spreading properties. XPS and WCA results illustrated the effective conversion of the PSU and PU surfaces from hydrophobic to hydrophilic with grafting of new oxidized functional groups during the photochemical treatments. Treated films showed a larger number of adhered cells compared to the untreated films and that number of adhered cells was comparable to the number of adhered cells in the control group. Better cell adhesion, spreading and growing on the PSU and PU substrates modified by the present UV methodology confirmed the biocompatibility of the treated surfaces.

Keywords: *Poly(sulfone), Polyurethane, UV surface functionalization, tissue engineering, cell spreading and adhesion*.

Introduction

The relationship between the surface chemistry of materials and resulting cellular response is of great importance for biomedical materials, regenerative medicine, tissue engineering and biosensors. As the surface of the biomaterial is what first comes into contact with the body when the biomaterial is used as a medical device, the initial response of the living body to the biomaterial must depend on its surface properties.[1-2] Polymers are very attractive because they have great design flexibility in composition and structure to tailor specific needs. Additionally, they have, in many cases biodegradability properties that can be imparted into polymers through molecular design. A principal drawback of many natural and synthetic polymers is their natural surface hydrophobicity, which limits their use in many applications. This important remaining problem causes inadequate interaction between polymers and cells, leading to in-vivo foreign body reactions. In order to enhance the biocompatibility and in particular the bio-functionality of materials used for tissue engineering, increasing use is being made of surface modification techniques. Many techniques have been developed and used to modify the surfaces of many different polymers. UV-assisted treatment is itself interesting because it has a simple experimental set up and low cost. Contrary to other methodologies, UV-assisted treatment is mainly based on photo-induced chemical processes, where special photo-reactive moieties have a distinct, selective and efficient reactivity. Depending on the chemistry of the photo-reactive group, controlled elimination or additional reactions are possible.[3] The mechanism of interaction between cells and substrate is very complex and surface properties have to be taken into account to make these biomaterials adequate for these kinds of cells. [4] Mesenchymal stem cells (MSCs) of dental pulp are undifferentiated cells with great plasticity potential, which give rise to several types of cells and, therefore facilitate the process of replacement or repair of diseased tissue. [5,6] In the present work, the surface properties of PU and PSU films were modified permanently to hydrophilic conditions in different degrees by UV-assisted treatment in the presence of oxygen gas. By selecting the photolysis time, the degree of hydrophilicity and surface oxidation was fixed and tests on adhesion and cell viability were carried out in untreated and treated films to evaluate the affinity of the cells to the films.

Experimental

Preparation of PSU and PU films

Thin PSU (Mw: 67,000, Sigma-Aldrich) and PU (PU 1185A10, BASF) films were prepared in glass substrates by the spin-coating technique from 10^{-4} M solutions using chloroform and tetra hydrofuran (THF) as solvents, respectively. UV irradiation was carried out using a commercial medium-pressure mercury lamp (400 W) with a modified set up that had been previously used. [7, 8] During the irradiation each sample was illuminated by UV light while a constant flux (5 $cm^3.s^{-1}$) of pure Oxygen (99.99%, White Martins PRAXAIR INC.) was flowing onto the samples.

Surface characterization

Water contact angle (WCA) of unmodified and UV-modified PSU and PU films surfaces were measured after 24 hours of treatment, using the sessile drop method. X-ray photoelectron spectroscopy (XPS) spectra were mainly obtained at the Brazilian Synchrotron Light Source (LNLS), Campinas. The SGM (Spherical Grating Monochromator) beam line, for VUV and Soft X-ray Spectroscopy (250– 1000 eV) and a Perkin Elmer 10-360 Precision Energy Analyzer was used for survey and high resolution (HR) spectra. The operation pressure was 10^{-7} Pa. To avoid charging problems due to synchrotron beam exposure, only films of about 100-200 nm thickness were used.

Cellular isolation and characterization

The protocol of Bernardi and colleagues was used for the isolation of dental pulp. [9] The extraction of the deciduous teeth was performed at the Odontology Faculty of the Federal University of Rio Grande do Sul (UFRGS) with the signed terms of consent by the patients' guardians, in accordance with the approval of the Ethics Committee of the UFRGS, registered under nº 296/08. All the biological tests were performed based on protocols already established by the group [10], as follows.

Cellular adhesion assay

For this purpose 45,000 cells were seeded on both types of films and compared to a control group. Three different cultures of MSCs were used and all the experiments were performed in triplicate. In the experiment, the cells were stained with 4'.6-diamidino-2-phenylindole (DAPI), a marker of cell core, after 6 hours of seeding on the scaffolds. Following this, photographs were taken in 9 points, randomly chosen on the scaffolds with an area of $97.2x10^{-3}$ cm^2 and the average of cells per matrix type was calculated. The result was expressed as a mean number of cells per group.

Cellular viability assay

MTT-colorimetric assay is based upon the ability of living cells to reduce 3-[4.5-dimethylthiazol-2-yl]-2.5 diphenyltetrazolium bromide (MTT) into formazan by mitochondrial succinate dehydrogenase in viable cells. The measurements were taken in triplicate on days 1, 4 and 7 after seeding the cells onto the scaffolds. Three different cultures of stem cells were used. A total of 45,000 cells were seeded onto the matrices and after the corresponding time for the analysis, the cells were incubated with 0.25 µg/mL of MTT in CMF buffer for 2 hours. Dimethylsulfoxide (DMSO) was used to dissolve the crystals and the absorbance was read on a spectrophotometer at two wavelengths: 560 nm and 630 nm. The results were calculated as the difference between them (560nm - 630nm) and expressed as a mean value of the triplicate per group. The results for the adhesion assay and cell viability were expressed as mean and standard error deviation.

Results and Discussion

Pristine PSU and PU are slightly hydrophobic polymers showing WCA of about 81° and 78° respectively. After UV irradiation in the presence of oxygen, the WCA decreased for both the PSU and PU films (see Table 1-Left). Fig. 1-A summarizes the elemental O 1s/C 1s ratio of PSU and PU surfaces calculated from the relative areas of the XPS signals as a function of the irradiation time in the presence of an oxygen atmosphere. UV-assisted treatment produced an increase in oxygen concentration on the surface, whereas the carbon concentration decreased in corresponding amounts. The rise in oxygen concentration shown in the XPS analyses correlates with the increase in the hydrophilicity measured by WCA when the photolysis time increased (see Table 1-Left). Previous studies [7, 8] have shown that the increase in oxygen concentration on the surface, measured by deconvolution of the C 1s XPS spectra, was due to the presence of C=O and COO functionalities, which reached more than 7 and 10 % relative concentrations for PSU and PU treated films, respectively in 60 minutes of irradiation. Table 1-Right presents the C 1s deconvolution results of PSU and PU samples untreated and treated at different irradiation times in the presence of oxygen.

Irradiation of PSU films in the presence of oxygen showed that shorter photolysis times lead to grafting C=O and COO groups efficiently on the surface with a corresponding decrease in C-C/C-H concentrations. When the photolysis time was equal or higher than 60 min, a conversion of the C=C into C-C functionalities was observed. The signal of C-O and C=O reduces while COO increases significantly. UV irradiation of the PSU films in the presence of oxygen produced important changes in the chemical composition of the surface, increasing and in many cases doubling, the concentration of CO_x functionalities compared to pristine PSU films (see Table 1-Right). PU films were apparently more resistant to the effect of irradiation than PSU films. Table 1-Right shows that there were no important changes in C=C and C-C relative concentrations when the irradiation time increased. Furthermore, C-N relative concentrations seemed to be almost independent of the photolysis time. Table 1-Right also shows that there was a conversion of the C-O functionality to more oxidized groups. The decrease observed in the relative C-O concentration was followed by a corresponding increase in carbonyl and in particular ester relative concentrations.

Table 1. Left - Changes in WCA as a function of UV photolysis time for films treated in the presence of oxygen; **Right -** C1s XPS deconvolution of PSU and PU samples untreated and treated at different photolysis times in the presence of oxygen.

Photolysis time (min)	WCA (degrees)	
	PSU	PU
0	81	78
5	--	76
15	74	68
30	35	43
45	--	55
60	18	49
75	--	36
90	17	28
120	10	31

Photolysis time (min)	Functional Groups (%)							
	C=C	C-C	C-N	C-S	C-O	C=O	COO	ΣCO_x
PSU								
Untreated	15	54	-	13	18	-	-	18
5	22	40	-	11	16	7	4	27
30	20	28	-	9	27	11	5	43
60	12	41	-	9	17	5	16	38
150	1	61	-	11	17	5	5	27
PU								
Untreated	19	37	20	-	18	5	1	24
5	19	41	23	-	9	2	6	17
15	20	39	20	-	12	4	5	21
60	21	34	21	-	13	6	5	24

In relation to cellular adhesion, the value observed for all the groups is expressed in Fig. 1-B. When comparing the number of adhered cells in the control group to all the other groups, the groups PSU_30 (p=0.947), PSU_120 (p=0.189) and PU_120 (p=1.) showed no statistical difference. Among the groups where the films were made with PSU, there is no statistical difference in terms of adhesion of cells (p>0.6 for all comparisons). In PU films, statistical analysis showed that the cells adhere more on films with 120 minutes of treatment (PU_120) than PU_0 and PU_30 (p<0,001).In relation to cellular viability, the cells were left in contact with the films for 1, 4 and 7 days, after which the MTT analysis was performed. The higher the absorbance was, the larger the number of viable cells. Fig. 1-C shows the result obtained for this experiment. Concerning the results, there is statistical difference in terms of viable cells in relation to time, i.e., the cells proliferate and increase their number with the passage of time for all groups (p<0.001). When comparing all the groups with the control group, without considering the time, the PSU group behaved similarly (p= 0.185). However, when comparing the groups in each day of analysis separately, on day 4 only the PSU group was similar to the control group (p=0.483). On the other days all the groups were similar (p=0.548 for day 1 and p=0.186 for day 7). In other words, only on day 4 a statistical difference was found and it occurred only between the control and PU groups. When looking at each group separately, inside the PU group there is no statistical difference. In the PSU group, the films with no treatment and with 30 minutes of treatment are similar and both are different from PSU_120. As the chemical structure of the materials could influence the response of the cells, in this work the influence of the modification of the surface by UV light treatment was evaluated as well as the consequential effect on the cells. By the results obtained it is possible to observe that the treatment influenced the adhesion of cells, as the films exposed to UV light with oxygen showed a larger number of adhered cells compared to the untreated films, although within each group this statistical difference is not present (with the exception of PU_120). The number of adhered cells in the treated films was comparable to the number of adhered cells in the control group (see Fig. 1-B).

The interaction between the tissue and the implant surface is a dynamic process. Water free biomolecules and dissolved ions surround the biomaterial surface during the initial few seconds of contact. [11] The wettability of the surface of a scaffold then plays an important role in the adhesion of extra cellular membrane biomolecules, which promote seeded or cultured cells to attach, proliferate and differentiate. [12-14] It has also been observed that cell attachment is determined by the adsorption and displacement of the extracellular proteins. [15] It is a known fact that the cell attachment ability can be linked to the type of protein at the surface of the scaffold and its ability to interact with the material surface. [4] As was demonstrated by others studies, hydrophobic materials have high affinity with a wide variety of proteins and shortly after first contact, these surfaces are covered with a layer of plasma proteins, predominantly albumin, fibrinogen, IgG, fibronectin, etc. These proteins adhere strongly to the surface and undergo changes with their conformation. It is well established that proteins tend to bind to hydrophilic surfaces in a lower amount and less lightly than to hydrophobic surfaces. [16] Following this, cells reach the surface and the adsorbed layer dictates the way the cells will respond. In general, hydrophilic functionality provides low interfacial free energy, resulting in reduced plasma protein adsorption which permits cell adhesion, with deposition of extracellular matrix proteins, such as collagen, fibronectin, laminin and others and interaction of integrins with the biomaterial. [17] In this way, the hydrophilicity increases the biocompatibility of the biomaterial. Concerning the viability assay, with the exception of day 4, there was no statistical difference among all the groups and the control. It is believed that this behavior could be attributed to the hydrophilicity of the films, since all of them had low values of contact angle with water for cells. Similar results were found by Jacobs *et al.* [18] with PLA films untreated and treated with plasma. In their work an initial difference in terms of adhesion was observed, but beyond 7 days of analysis this difference no longer exists.

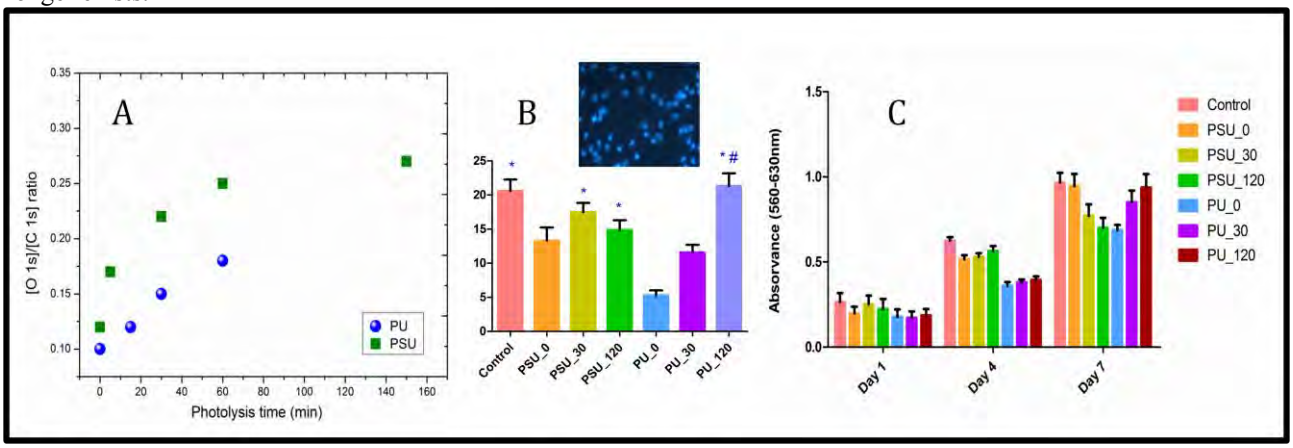

Figure 1. A - Oxygen and carbon atomic percent ratio of untreated and UV-assisted treated PSU and PU films measured by XPS; **B** - MSCs stained with DAPI in an area of 97.2×10^{-3} cm^2, and Graph of mean number of cellular adhesion after 6 hours of seeding on matrices. The sign (*) indicates no statistical difference among all the groups

compared to the control group. The sign (#) shows the statistical differences within the PU group; **C** - Cellular viability assay evaluated on days 1, 4 and 7 after seeding cells.

At the same time it was also expected that the cells would proliferate more on more hydrophilic surfaces, which did not happen to the PSU groups on day 7 of analysis. An explanation for the different behaviour between PSU and PU films could be attributed to the findings of some studies that have demonstrated that cells adhere and proliferate more on substrates which contain N-H groups in their structures. [19-21] Therefore, a highly wettable scaffold for tissue engineering does not necessarily lead to a more bio-compatible scaffold but moderately wettable surfaces result in enhanced biocompatibility. [22] The difference, therefore between PSU and PU indicates the clear influence and importance of surface chemistry. Contrary to the PU, the PSU treated with UV and oxygen per 120 minutes suffer strong alterations in their surface molecular structures, resulting in aromatic ring opening with formation of new carboxyls functionalities. Surfaces with –COOH groups display a negative charged functionality on material surfaces. But this phenomenon is dependent upon the concentration of –COOH on the surface, which was shown to inhibit cell growth. [16] Treated PSU films have a presence of –OH groups [7, 23] but for long exposure times the aromatic ring opening could lead to an increase of -C-C and -C-H groups. $-CH_3$ surfaces will most likely have unfavourable surface reaction with cells due to the magnitude of tightly bond proteins and the likelihood is that the bound proteins will expose sites responsive to inflammatory cells and also be responsible for the small number of viable cells. [16]

Conclusion

UV-assisted treatment in the presence of oxygen may be used as a rapid, simple and cost effective method to incorporate functionalities onto the surface of PSU and PU films, to increase adhesion and favor cell cultivation. The different degree of hydrophilicity and chemical grafting due to the treatments was evidenced by XPS and WCA measurements. Adhesion of cells was influenced by the treatments. It was evident when the films exposed to UV light with oxygen showed a larger number of adhered cells compared to the untreated films. Additionally, the number of adhered cells in the treated films was comparable to the number of adhered cells in the control group. The low surface free energy of the treated films may reduce protein adsorption and maintain cell adhesion and biocompatibility. The obtained data showed that the surface modification results in different behavior of cells after several days of analysis, showing the influence of the surface material. These results show that the cell response does not only depend on the hydrophilicity of the materials but also on the chemical surface alterations which occur as a result of UV-assisted treatment in the presence of oxygen. Better cell adhesion, spreading and growing on the PSU and PU substrates modified by the present UV methodology confirmed the biocompatibility of the treated surfaces.

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