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Surface grafting of carboxylic groups onto thermoplastic polyurethanes to reduce cell adhesion

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ABSTRACT

The interaction of polymers with other materials is an important issue, being their surface properties clearly crucial. For some important polymer applications, their surfaces have to be modified. Surface modification aims to tailor the surface characteristics of a material for a specific application without affecting its bulk properties. Materials can be surface modified by using biological, chemical or physical methods. The aim of this work was to improve the reactivity of the thermoplastic polyurethane (TPU) material (Elastollan®) surface and to make its surface cell repellent by grafting carboxylic groups onto its surface. Two TPU materials were studied: a polyether-based TPU and a polyester-based TPU. The grafting efficiency was evaluated by contact angle measurements and by analytical determination of the COOH groups. Scanning electron microscopy (SEM) of the membranes surface was performed as well as cell adhesion tests. It was proved that the surfaces of the TPUs membranes were successfully modified and that cell adhesion was remarkably reduced.

1. Introduction

Biomaterials are designed to supplement, support or completely take over the functions of natural tissues or organs of human beings and are, therefore, designed to be biocompatible (non-toxic, non-immunogenic, non-inflammatory, non-carcinogenic) and to match the properties of the tissues they are replacing. The bulk of the biomaterial needs to fulfil the mechanical and physical requirements of a specific application, while its surface may be engineered to avoid future implantation problems such as immune responses or thrombogenic reactions. The surface chemistry and structure of materials and devices is mostly responsible for the biological response. Therefore, the development of surface modification procedures that allow controlling cell adhesion to that surface has been an extremely important issue.

It is known that wettability, roughness, surface charge and chemical functionalities are the main factors influencing cell adhesion to artificial materials [1]. However, the success or failure of implanted biomaterials is also dependent on cellular response, which is dependent on the concentration, composition and conformation of adsorbed proteins on its surface [2].

Cells are unable to adhere if no ligands (e.g. RGD) are present at the material surface with which integrins or other cell adhesion molecules can interact. Several serum proteins and extracellular matrix molecules (ECM) exhibit these ligand sequences. Therefore, the capacity of cells to interact and spread on surfaces is directly defined by the ability of the surface to attract and bind these molecules [3,4]. This binding capacity is mainly defined by surface chemistry and structure [3] and the ability of the cell to recognize specific ligand sequences (bio-recognition) is the mechanism behind cell–biomaterial surface interactions. Several biomedical applications including medical implant, biosensor and biochips, tissue engineering, bioelectronics and biomimetic materials are only possible assuming that bio-recognition principle is a central concern in an attempt to make a sophisticated, functional surface for specific biointeractions [5,6].

Currently, a sort of biological, physical and chemical methods have been used to perform surface modifications on materials. Surface modification generally falls into one of three categories: etching; chemical modification or coating with a different material. These approaches can be used to modify a range of properties, including wettability, permeability, biostability and/or chemical inertness, adhesion, biocompatibility, topography, electrical characteristics and optical and frictional properties [7].

Biocompatibility is also mediated by the hydrophilicity (or hydrophobicity) of the surface of a biomaterial [8]. Hydrophilicity may be the initial parameter affecting protein adsorption. It is

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well known that the adsorption of proteins from aqueous solution is improved by hydrophobic surfaces [1,8]. On the other hand, a highly hydrophilic surface may repulse any protein molecules and thus inhibit protein adsorption. Although hydrophilicity has been well known with respect to its influence on protein adsorption, its effect on cell behaviour has been quite controversial and inconsistent in experimental data. Some authors [1] state that improved surface hydrophilicity is necessary for hydrophobic materials to support cell adherence. However it has been acknowledged that both very hydrophilic and very hydrophobic surfaces are not good for cell attachment. Thus, surfaces with moderate wettability showed good cell adhesion due their ability to adsorb proteins and maintain their environmental conformation [1,9,10].

Thermoplastic polyurethanes (TPU) elastomers are versatile materials that behave as crosslinked elastomers at room temperature but can be processed by heating using several industrial polymer processing methods [11]. In this work, two different TPU were studied: Elastollan®1180A50 (a polyether-based TPU) and Elastollan®685A (a polyester-based TPU). The surface of these two TPUs was modified by grafting of carboxylic groups (either with acrylic acid or monochloroacetic acid) in order to reduce cell adhesion by reducing their surface wettability.

2. Experimental

2.1. Materials

Hydrogen peroxide (H_2O_2) 30%, acrylic acid (AA), ferrous sulphate ($FeSO_4$), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich. Monochloroacetic acid (MCA) was obtained from Merck-Schuchart. Sodium chloride (NaCl) was purchased from Riedel-de Haen and sulphuric acid (H_2SO_4) from Fluka. The solvent, isopropyl alcohol, was ordered from José Vaz Pereira, S.A., Portugal. Elastollan®1180A50 and Elastollan®685A were obtained from BASF. All chemicals were used as received, except AA, which was purified through a basic Al_2O_3 column. Carbonate-free HEPES buffered (25 mM) and Dulbecco's modified Eagle medium (DMEM) with 10% foetal calf serum were purchased from Life Technologies, Basel, Switzerland.

2.2. Methods

2.2.1. Membranes preparation

Membranes (30 mm × 20 mm × 2 mm) were prepared using Elastollan®1180A50 and Elastollan®685A. Preparation method was by injection moulding and the procedure was the same for both materials.

Elastollans® are water absorbing materials. Thus, a high humidity and moisture in the materials results in the formation of bubbles inside the injection moulded parts. To reduce the water content of the polymers granulates, a small portion of either Elastollan® was dried in a drying chamber at 90 °C for 2 h. Immediately after the drying process, the materials were placed in the piston injection moulding machine and heated up to 190 °C. After a short heating-up time the materials were injected into a rectangular tool cavity under a pressure of 3.5 bar. For the removal of the component, the tool was cooled down and opened.

2.2.2. Surface modification by chemical grafting

Elastollan® membranes (both 1180A50 and 685A) were ultrasonically cleaned in isopropyl alcohol for 15 min prior to surface grafting. After dried, the membranes were oxidized with H_2O_2 (30%) in order to enhance the reactivity of the polyurethanes surface, as previously described [12]. Briefly, TPU membranes were immersed in a hydrogen peroxide solution (30%) and exposed for 4 h to UV-irradiation with a Mineralight®Lamp, Model UVGL-48, in the 254 nm wavelength setting. This generated a power of 6 W and the samples were placed at a distance of 4 cm from the light source. This procedure is schematically shown in Fig. 1.

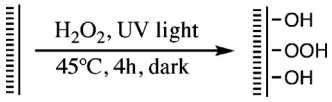

Fig. 1. Schematic representation of the photo-oxidation reaction of the TPU membranes.

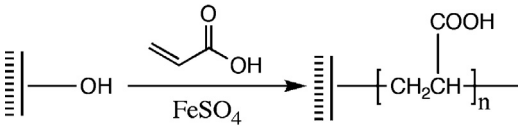

Fig. 2. AA grafting reaction on the surface of the previously oxidized TPU membranes.

After the oxidation reaction, the grafting of carboxylic groups (—COOH) onto the surface of the TPU membranes was carried by out for both Elastollans® via two approaches: either with AA or with MCA.

2.2.2.1. Grafting reaction with acrylic acid (AA). The COOH grafting reaction with AA can be illustrated by the reaction shown in Fig. 2 for both Elastollan® membranes.

TPU membranes were individually immersed in 20 mL 30% AA aqueous solution. In a glass tube purged with nitrogen was added 0.1 mL of a 0.015 M solution of $FeSO_4$/0.005 M H_2SO_4 to carry out graft polymerization reaction at 60 °C for 120 min. The carboxylic-grafted TPU membranes were rinsed with PBS (10 mM, pH 7.4) and distilled water to remove the unreacted monomer and any homopolymer that may have been formed.

2.2.2.2. Grafting reaction with monochloroacetic acid (MCA). The grafting reaction with MCA was accomplished by suspending the TPU membranes in 20 mL of 0.1 M NaCl solution and placed in an ice bath. Then, a 6 M NaOH aqueous solution was slowly added to each of the previous solutions while stirring, until the temperature reached 15 °C. The mixtures were then removed from ice bath and a 2% MCA solution was added and incubated for 70 min at 25 °C, with constant stirring. The membranes were finally washed with 0.1 M NaCl 3–4 times to remove excess reagent. Fig. 3 schematically represents the grafting of MCA.

2.3. Characterization techniques

2.3.1. Quantification of hydroxyl groups content

The procedure used for the quantification of the hydroxyl groups was based on a modification of the method of Fritz and Schenk [13]. The acetylating reagent was prepared as follows: 13 mL of perchloric acid were added to 845 mL of ethyl acetate. Then, 30 mL of acetic anhydride were allowed to react at room temperature for 30 min. The reagent was then cooled to 5 °C followed by the addition of 90 mL of acetic anhydride previous cooled to 5 °C. The reagent was kept at 5 °C for 1 h, and then was warmed up to room temperature. After the preparation of the acetylating reagent, 20 mL of the reagent was pipetted into Erlenmeyer flasks (one for each sample and one for a blank determination). Elastollan®1180A50 and 685A membranes were submerged in each flask and left to react for 1 h. Then, 5 mL of water were added followed by 20 mL of 3:1 pyridine–water mixture. The flasks were allowed to stand for 5 min. Then, 50 mL of a standard 0.5 N methanolic potassium hydroxide were pipetted into each flask (including the blank). Finally, the

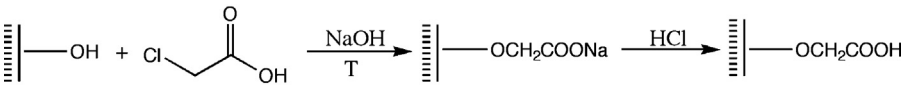

Fig. 3. MCA grafting reaction on the surface of the previously oxidized TPU membranes.

solutions were titrated with a standard 0.5 N methanolic potassium hydroxide using phenolphthalein as the indicator.

2.3.2. Quantification of carboxylic groups content

For the quantification of the carboxylic groups, 20 mL of a 0.005 M NaOH solution were pipetted into flasks. One flask was reserved for a blank determination. In the others, a sample of each modified membrane was added and incubated for 24 h. The membranes were then removed from the NaOH solution. Finally, the remaining incubation solutions as well as the blank one were titrated with a 0.005 M HCl solution.

2.3.3. Contact angle and surface free energy

The contact or wetting angle is most often assessed by placing a small liquid droplet on a flat horizontal solid surface. The contact angle is the angle which is formed by the baseline and the tangent to the drop contour at the three-phase point. This value is specific for any given system being determined by the interactions of the three interfaces. If a liquid is in contact with a plane solid surface, three interfacial free energies have to be assumed. The equilibrium of forces at the edge of a resting drop can be described by Young's equation (Eq. (1)):

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV}\cos\theta \quad (1)$$

where γ_{SV}, γ_{SL} and γ_{LV} represent the interfacial tension of the solid/vapour, solid/liquid and liquid/vapour interfaces, respectively and θ represents the equilibrium contact angle [14,15]. Owens–Wendt–Rabel and Kaelbe distinguished between disperse interaction and polar interactions [16]. Polar interactions contain Coulomb interactions between permanent dipoles and interactions between permanent dipoles and induced dipoles. Disperse interactions are caused by time fluctuations in the charge distribution within the molecules [17]. The surface energy (γ_{SV}) represents the strength of the polar forces and the dispersive forces. This quantity may be resolved into two parts, that due to polar forces and that due to dispersive forces (Eq. (2)):

$$\gamma_{SV} = \gamma_S^D + \gamma_S^P \quad (2)$$

The contact angle with water as well as surface free energy determinations of the membranes' surfaces were performed at room temperature in an OCA 20 contact angle measurement unit from Dataphysics. The tests were performed on the air-facing surfaces of the samples. The water contact angle of the samples was evaluated by static contact angle measurements using the sessile drop method. Surface free energy values as well as the dispersive γ_S^D and polar γ_S^P components were obtained according the Owens–Wendt–Rabel and Kaelbe method (OWRK) [16,17] by static contact angle measurements with four liquids: water, ethylene glycol, propylene glycol and formamide. Nine measurements on different points of each sample were performed from which the mean static contact angle and its standard deviation were determined.

2.3.4. Surface topography analysis

The topography of the membranes' surfaces was observed by scanning electron microscopy (SEM), before and after modification. Samples were sputter coated with a thin layer of gold, under argon atmosphere and examined at room temperature, in a JSM-5310 (JEOL, Japan) scanning microscope operating at 20 and 25 kV.

2.3.5. Dynamical mechanical thermal analysis (DMTA)

DMTA is a very sensitive technique, detecting different molecular events, so it is one of the main techniques for the study of the mechanical and thermal properties of polymers. DMTA analyses of thick specimens (15.0 mm × 7.5 mm × 2 mm) were performed with a Triton Tritec 2000 in the Constrain Layer Damping mode using 2 frequencies (1 Hz and 10 Hz), with a standard heating rate of 2 °C/min. The glass transition temperature (T_g) was determined as the peak of tan δ (tan $\delta = E''/E'$) where E'' and E' are the loss and storage modulus, respectively.

2.3.6. In vitro characterization

2.3.6.1. Characterization of materials cytotoxicity. The biocompatibility of all materials was tested according ISO 10993-5 [18] extract test. Briefly, 125 ml of bi-distilled water buffered at pH 7.4 were used to prepare the extract. All samples were processed separately. The samples were incubated for 24 h at 37 °C under gyratory shaking. Containers without samples but with the same quantity of bi-distilled water were used and treated identically and used as a negative control. All obtained extracts were used directly after incubation. Confluent 3T3 fibroblastic cells were trypsinized and counted. In each of the central 60 wells of 96-well plates, 200 μl contained 1500 cells were added. After 24 h the medium was replaced by 200 μl of medium containing different concentrations of the extract solutions: 0% (negative control), 10%, 20% and 50%. After an incubation period of 5 days, cellular viability was assessed through the reduction of 3-(4,5-dimethyltiazol-2,5-yl)-2,5-diphenyltetrazolium bromide (MTT) into a water-soluble formazan product.

2.3.6.2. Cell adhesion and spreading. Cell spreading behaviour on the different materials was analysed by inoculating the samples with 3T3 Swiss albino embryo mouse fibroblast cells at a density of 15,600 cells/cm^3 in carbonate-free HEPES buffered (25 mM) Dulbecco's modified Eagle medium (DMEM) with 10% foetal calf serum (FCS). The samples were thereafter incubated for 48 h under cell culture conditions (95% humidified air/5% CO_2, 37 °C). Afterwards, the cells were fixed and were stained for DNA, actin and microtubuli in order to visualize the cells and their cytoskeleton by the method previously described by Kaiser and co-workers [3]. The cell spreading was visualized by confocal laser scanning microscopy in the fluorescence mode.

3. Results and discussion

3.1. Determination of the hydroxyl and carboxyl groups content

Surface modifications were quantitatively analysed. The -OH groups as well as the COOH groups were determined. In Tables 1 and 2 the obtained results are presented.

The reaction with H_2O_2 results in the formation of —OH groups on the surfaces. Thus is possible to suggest that, for

Table 1
Amount of —OH groups (mol/cm^2) present on TPU surfaces after the oxidization reaction with H_2O_2.

Elastollan® membrane	n_{-OH}/cm^2 ($\times 10^4$)
1180A50 + H_2O_2	25.13 ± 0.06
685A + H_2O_2	2.36 ± 0.03

Table 2
Amount of —COOH groups (mol/cm^2) present on TPU surfaces following grafting with AA or MCA.

Elastollan® membrane	n_{-COOH}/cm^2 ($\times 10^7$)
1180A50 + AA	41.7 ± 0.90
1180A50 + MCA	4.13 ± 0.01
685A + AA	29.2 ± 0.09
685A + MCA	3.38 ± 0.10

the same condition of reaction, the number of —OH groups grafted to the surface is higher for Elastollan®1180A50 than for Elastollan®685A. This difference in the grafting efficiency might be explained by the different chemical structures of the two TPUs. It is known that polyester urethanes (Elastollan®685A) are more susceptible to hydrolytic degradation and polyether urethanes (Elastollan®1180A50) to oxidation [19,20].

According to the different amount of —OH groups present on both Elastollan® surfaces, the larger number of grafted —COOH groups obtained for Elastollan®1180A50 membranes was already expected. Therefore higher grafting efficiency was achieved for this material.

Interestingly, it was also observed a relation between the amount of —COOH and the type of grated monomer. AA grafting led to a higher —COOH content than MCA for both materials. This result might be associated with the AA ability to polymerize, which may lead to a more significant number of AA molecules attached to the surface.

3.2. Contact angle measurements

It is widely recognized that surface energy is an important parameter affecting polymers adhesion, material wettability and even cell adhesion and biocompatibility [21].

The measurement of contact angles is considered the most convenient method for determining the surface free energy of solid samples. This technique relies on the determination of the interactions between the solid sample of interest and liquids with well determined surface tensions.

During this work, water contact angles and surface energies of the membranes before and after chemical grafting (AA or MCA) were determined. The obtained results are presented in Table 3.

As shown in Table 3, both modification steps (oxidation and grafting of —COOH groups) led to a decrease of the water contact angle. Contrarily, surface energies increased after both reactions. These results are explained by the presence of hydroxyl and carboxylic groups which are hydrophilic and may therefore establish significant ionic, H-bonding and polar forces. These interactions are the reason of obtaining high energy surfaces when in contact with air. However, these variations are more significant for membranes prepared with Elastollan®1180A50 which is consistent with the results obtained and described in the previous section. Such results indicated that the amount of —OH groups as well as of -COOH grafted onto the surfaces was significantly higher for this material. The results also suggest that, for both Elastollan®, the variation in water contact angle and surface energy was higher when AA was used as the grafting monomer. Once again, this result is consistent with the quantification of —OH and —COOH groups already referred.

Furthermore, while the original membranes present a very low polar component, this value considerably increased when —OH and —COOH groups were grafted. The presence of polar functional groups such as —OH or —COOH increases the hydrogen bounding interactions and therefore increases the polar component of the surface energy [2]. In this study, oxidation with H_2O_2 (introducing —OH groups) and grafting of AA or MCA (containing carboxylic groups) onto the films surface increased the polar component of the surface energy while decreasing their dispersive one.

3.3. Surface topography analysis

In order to observe any differences in the surface of the materials before and after chemical grafting, the samples were analysed by scanning electron microscopy (SEM). In Fig. 4 are presented the images obtained during this analysis at the magnification of 500×.

From Fig. 4 can be seen that different modifications led to different surface topologies. It is known that a surface becomes smoother after grafting, when compared with its original state [22]. Therefore, the smoother the surface becomes, the higher density of monomer is grafted to the surface. In this study, after the grafting reactions, the surfaces do look smoother than the unmodified materials, which is an indication of the grafting efficiency. Also, as expected from previous results, AA grafting resulted in smoother surfaces, which is consistent with the suggestion of a higher grafting efficiency with this monomer.

3.4. Thermal analysis

The DMTA technique has been intensely used and recognized as a powerful tool to identify the materials thermal transition due to its extremely high sensibility. The main goal of this analysis is to obtain information about the mechanical and/or thermal properties of the materials by applying a sinusoidal load to a specimen and measuring the resultant deformation, while the sample is subjected to a controlled temperature programme. This information allows inferring about the extension of the modification along the materials bulk.

The results presented in Fig. 5 show the damping factor (tan δ) traces obtained for the unmodified as well as for the correspondent AA and MCA grafted membranes.

These traces indicate that the TPU bulk material was not affected by the grafting with MCA since the glass transition temperature (T_g) remains the same (only 2 °C difference between unmodified and MCA grafted surfaces), meaning that only the surface of the membranes was modified. However, modification with AA caused a more significant decrease on T_g value for both materials (approximately 10 °C). This decrease in the T_g indicates that the resultant membranes were more flexible than the unmodified one. The increase in the flexibility of the membranes may be explained by the fact that the grafting might lead to the formation of branched chains

Table 3
Water contact angle, surface free energies and respective components (dispersive and polar) obtained for the unmodified and grafted TPU membranes.

Elastollan® membrane	θ (°)	γ_{sv} (mN/m)	γ_S^D (mN/m)	γ_S^P (mN/m)
1180A50	95.4 ± 1.8	19.34 ± 2.30	11.01 ± 1.43	8.33 ± 0.97
1180A50 + H_2O_2	86.7 ± 1.1	25.99 ± 2.22	8.52 ± 1.35	17.47 ± 1.77
1180A50 + AA	82.3 ± 0.4	30.71 ± 1.63	8.70 ± 0.59	22.01 ± 1.18
1180A50 + MCA	84.7 ± 0.8	25.42 ± 1.36	11.11 ± 0.77	14.31 ± 1.12
685A	99.6 ± 1.2	19.02 ± 2.14	16.54 ± 1.98	2.48 ± 0.8
685A + H_2O_2	92.3 ± 1.0	25.37 ± 2.24	9.84 ± 0.74	15.53 ± 1.74
685A + AA	88.2 ± 1.1	31.82 ± 1.44	8.46 ± 1.05	23.35 ± 0.98
685A + MCA	91.8 ± 0.3	25.40 ± 0.90	7.27 ± 0.24	18.14 ± 0.55

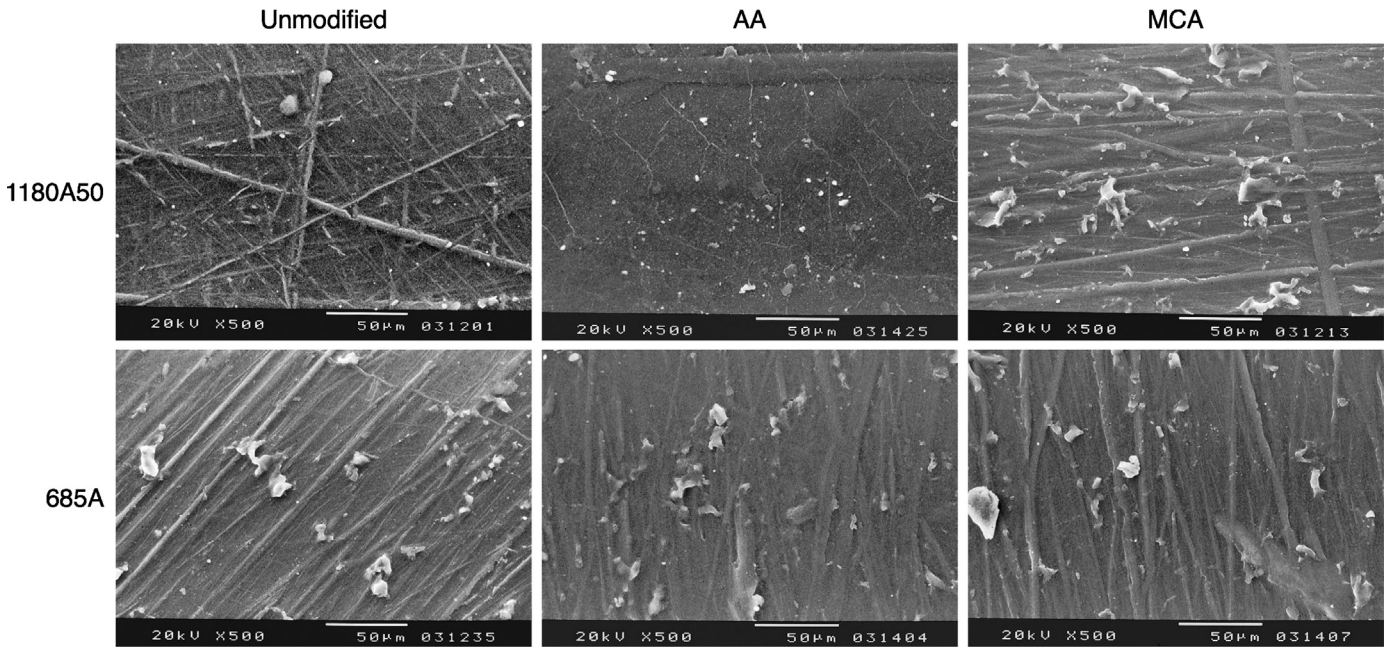

Fig. 4. SEM pictures of unmodified and grafted TPU membranes.

structures on the surfaces. These branched chains may increase in the space available for molecular motion and therefore lead to a decrease in T_g. Similar results have been reported by other authors [23].

3.5. *In vitro characterization*

The results of the MTT assay are presented in Fig. 6, which represents the cellular activity after incubation with the extracts of the membranes. To calculate the relative cytotoxicity, the absorbance of the control well (0%) was regarded as 100% of cellular viability. From Fig. 6 is possible to see that a small decrease in cellular activity was noted when cells were exposed to the membranes extracts obtained from all the samples. However, considering standard deviation such variations are not significant. These results show that the extracts did not contain any component which may cause cell death. This suggests that the molecules linked to surface of the materials are covalently linked and therefore are not released into the incubation medium. Consequently, both the unmodified and modified materials did not reveal cytotoxic profile during these tests.

Cell adhesion on the surfaces was evaluated with 3T3 Mouse fibroblasts cells. Cell shape and spreading (cytoskeleton organization) was analyzed by staining of the cell components i.e., actin (green) and nuclei (blue) using fluorescent dyes. The images obtained by confocal laser scanning microscopy in the fluorescence mode are presented in Fig. 7.

In the case of Elastollan® 1180A50, cells developed a well spread actin cytoskeleton. The fibroblast cells formed star-like as well as elongated actin bundles, similarly to the polystyrene culture

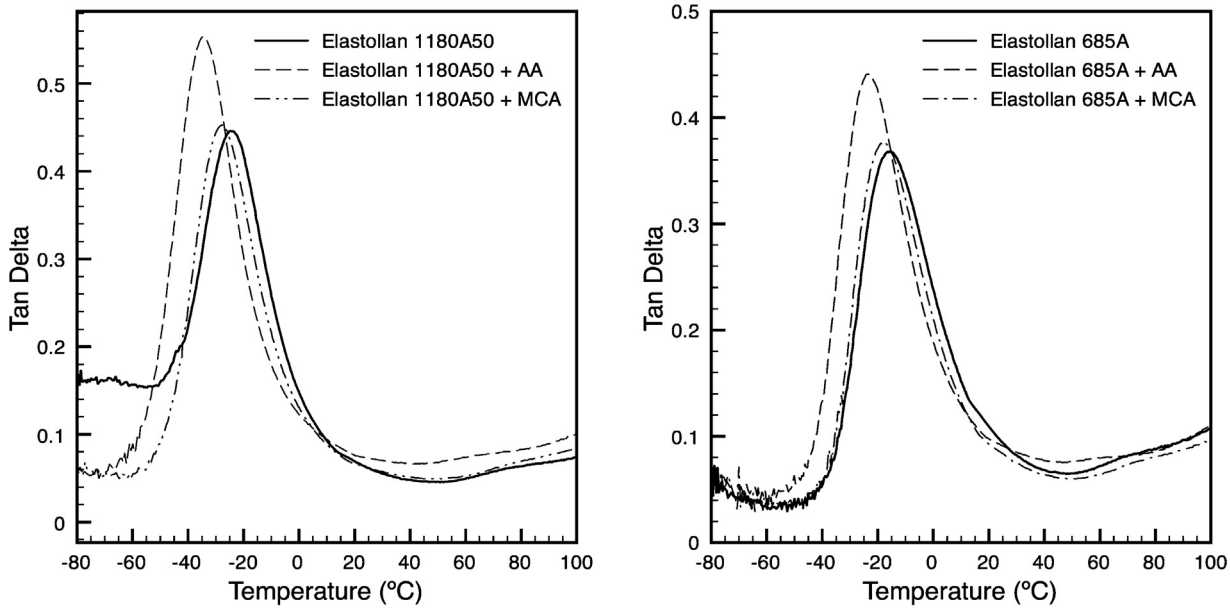

Fig. 5. Plots of tan δ versus temperature for the unmodified and grafted TPU membranes obtained at 1 Hz.

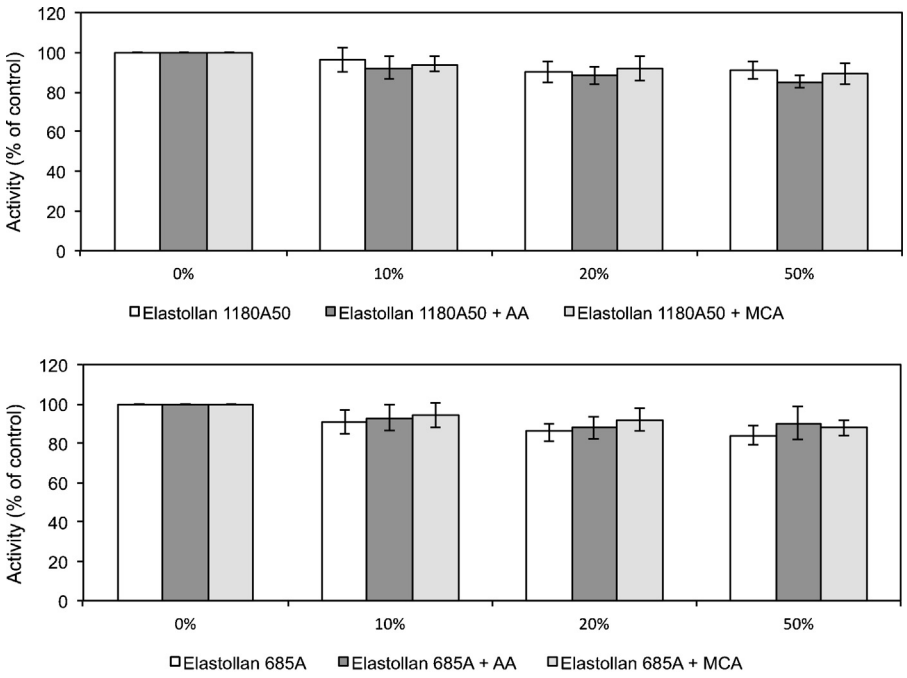

Fig. 6. Effect of unmodified and grafted TPU membranes extracts on cell performance.

dish surface (Fig. 7). Cells inoculated on Elastollan®685A material developed an actin cytoskeleton, which was poorly established. Nevertheless can be assumed that both Elastollan® materials promoted cell support, mainly Elastollan®1180A50.

Regarding the modified surfaces, in Fig. 7, cells exhibited a weaker spreading in the presence of modified surfaces when compared to the unmodified membranes. The grafting effect on cells adherence was more pronounced for Elastollan®1180A50, since no cells could be observed on this surface when grafted either with AA or MCA. This means that the grafting procedures described were effective in producing a cell repellent surface for Elastollan®1180A50 independently of the amount of COOH groups (AA – 41.7×10^{-7} n_{-COOH}/cm^2; MCA – 4.13×10^{-7} n_{-COOH}/cm^2) and water contact angle within the range 82–84°.

The effect of these grafting procedures was not so obvious for Elastollan®685 membranes since, despite the obvious reduction in cell spreading, cell could still be observed at the surface of this material. However, in these cases, cell colonization was sparse and cells developed an actin cytoskeleton, which was poorly established and the most circular cell shape was mainly reduced to a small area around the nucleus, which might indicate that cell

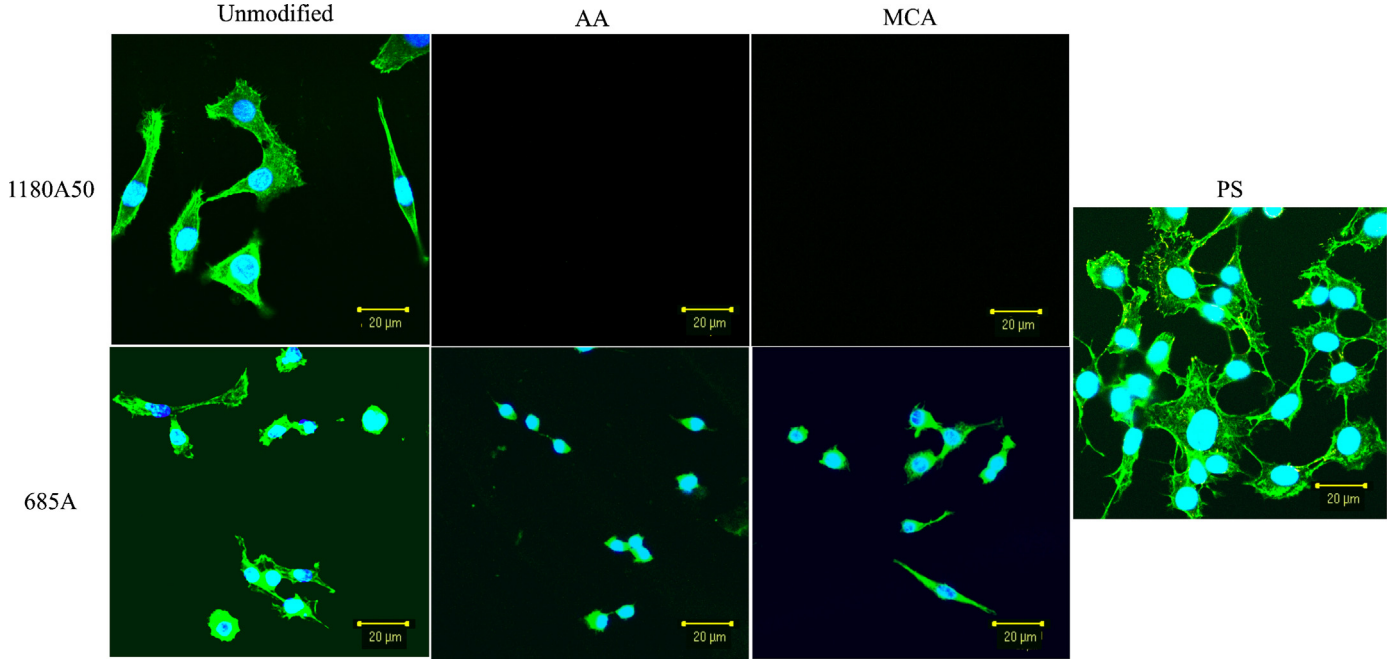

Fig. 7. 3T3 Mouse fibroblasts cell behaviour on unmodified and functionalized TPU surfaces.

show bad adherence to the surface. The cells adhesion on modified Elastollan®685, within the obtained range, is also independent of the amount of COOH groups (AA – 29.9×10^{-7} n_{-COOH}/cm^2; MCA – 3.38×10^{-7} n_{-COOH}/cm^2) and water contact angle (AA – 88.1°; MCA – 91.8°). Several studies such as the works of Haddow et al. [24] and Daw et al. [25], indicated that human keratinocyte cells and ROS17/2.8 osteoblast-like cells improved their adherence and growth on substrates with COOH group. The reason for the improvement was attributed to the enhanced of the surface wettability. However, Li et al. [26] found that, even with low COOH groups grafting densities and the improvement of wettability of the substrate, cell adhesion was reduced on PET substrates. These findings are inline with the results presented in this here. Despite the COOH groups grafted into Elastollan® surfaces and the improvement of hydrophilicity, the cell adhesion was reduced.

Therefore, in the present work, in vitro studies suggested that for both Elastollan®, the COOH grafting decreases or even eliminates cells adhesion to the surface while maintains their non-cytotoxic nature.

4. Conclusion

During this work, chemical grafting of –COOH groups was performed on the surface of membranes prepared from either Elastollan®1180A50 or Elastollan®685A. According to the obtained results, one can state that grafting of acrylic acid (AA) was more efficient than monochloroacetic acid (MCA).

Thermal analysis showed a small shift of the T_g value after grafting. This variation was more pronounced for AA and is associated with the diluting effect of the branched chains that result from grafting reaction on the surface [23]. Since AA tends do polymerize on the surface, this effect becomes more evident leading to a more significant variation on T_g.

Surface wettability analysis revealed a small decrease of water contact angle and a considerable increase in the polar component of the surface energy, due to the presence of grafted polar groups (COOH). In addition, SEM analysis illustrated that surface grafting created smoother surfaces.

Cell adhesion tests showed that both modifications decreased cell adhesion. This effect was, however, more significant for the membranes prepared with Elastollan®1180A50. It was also found that cell adhesion on modified surfaces was not directly related with the amount of COOH groups present on the surface, within the studied range (4.13×10^{-7}–41.7×10^{-7} n_{-COOH}/cm^2).

As an overall conclusion, it is possible to state that the modifications performed on the original membranes resulted in cell repellent profiles meaning that these modification methods are suitable to reduce cell adhesion on a biomaterial.

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