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L929 Fibroblast cells response on plasma modified TPE polyurethanes surfaces: morphology study

Sakineh Rahmani [1], Mohammad Taghi Khorasani [2*], Farzin Etemadi [3]

[1]School of Biomedical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Biomaterial Department of Iran Polymer and Petrochemical Institute, Tehran, Iran
[3]Graduated of Textile Engineering, South Tehran Branch, Islamic Azad University, Tehran, Iran

*corresponding author e-mail address: ***m.khorasani@IPPI.ac.ir***

ABSTRACT

The aim of this article is investigating of morphology, surface chemical composition and cell growth of unmodified (Shore A and Shore D), oxygen plasma treated TPE PU. Modified and unmodified samples were characterize with ATR-FTIR, SEM, AFM, DMTA analysis and cultivating L929 fibroblast cell test. Apply oxygen plasma on Polyurethanes changing surface properties such as increasing surface roughness and hydrophobicity with increasing treatment time. Oxygen plasma process make a base for attaching and growing cells on treated samples. Observing cells growth through the surface and shape of them demonstrate that on plasma treated samples cells growth uniformly on whole films surfaces but on untreated samples cell growth uneven and shape of them are more like spherical. Result noticed that oxygen plasma is a suitable process for surface modifying and increasing biocompatibilities of polyurethane that contacting straight with cells.

Keywords: *Polyurethane, Rubber, Plastic, Plasma Treatment, Surface Morphology, Fibroblast Cell.*

1. INTRODUCTION

Surface properties such as surface chemistry, surface topography and thermodynamic properties could be critical for biomaterial biocompatibility and need to considered in their selection for medical application, together with bulk properties characteristics. In fact, several studies have investigated the relationship between surface chemical composition with the complex process of cell adhesion onto biomaterials, and their subsequent influence on the attachment and spreading of cells that ultimately determine the success or failure of the implant in service [1-3].

Polyurethane elastomers are bulk copolymer that using in a wide application such as industrial and medical and because of its excellent mechanical properties as well as non-toxicity have spurred intensive studies of this potential material in the biomaterial fields [4-6]. The first polyurethane produced by chemical reaction of aliphatic diisocyanate and diamine. The polyurethane that Otto Bayer et al. introduced was very hydrophilic thus could not be used in application that plastic characteristic is needed. The first polyurethane with plastic and fiber characteristic was produced by reaction of aliphatic diisocyanate and Glycol. Afterward by reaction of Aromatic diisocyanate and high molecular Glycol, a major family of thermoplastic elastomers introduced. Polyurethane specifications change from hard thermoplastic to supple elastomeric and have been extensively used in blood-contacting materials, such as a coating for cardiac pacemaker leads, breast implants, vascular devices, artificial heart implants, and heart valves. This is due to the fact that polyurethanes show both relatively well-biocompatibility and excellent mechanical properties such as tensional strength, toughness and abrasion resistance. However, the surface of medical PU is, to some extent, hydrophobic, not completely thrombo resistant, and needs to be improved for more applications. It is a common practice in many applications to alter the surface of a material with special molecules or agents to enhance the surface biocompatibility, while still maintaining the bulk physical and mechanical properties of the raw material as a whole [7-10].

Studies have suggested that the interactions between the biological environment and artificial materials are most likely dominated by the materials' surface properties such as morphology and surface chemical bonding. Hence, surface modification of existing biomaterials with an aim towards improving a materials' biocompatibility has been a major focus of biomaterials research in recent years. In contrast, the plasma surface modification process has been shown to be able to modify the surface properties of a biomaterial without affecting its bulk physical properties [11].

Plasma or fourth state of matters consist of atoms, molecules and highly excited radicals and for first time introduced by Longmuir in 1923. These high density reactive particles change surface characterization of materials that naturally inactive like ceramics, metals and polymers. Surface modifications by plasma enhance wetting, biocompatibility and adhesion of polymers. Furthermore, plasma process can use for applying non-porous coating and fixing molecules and bio-macromolecules [12-15].

2. EXPERIMENTAL SECTION

2.1. Materials.

Table 1. Materials.

Sample	Shore	Grade	Company
PU	A	Polyether–Polyurethane(KU_2-8670)	Bayer - Germany
PU	D	Polyester–Polyurethane(LARIPUR-7025)	Milan - Italy
Dicumyl Peroxide	---	99%	Bayer
DMF	---	N,N-Dimethyl formamide	Merck KGaA
Ethyl Alcohol	---	96% (V/V)	Kimia Alcohol Zanjan (PSS)
Glutaraldehyde	---	25%	Merck

2.2. Polyurethane films preparation.

To prepare PU films, we solved 10 g polyurethane into 100 cc dimethyl formamide (DMF) with magnetic mixer in temperature of 40°C within 24 hours. Top of Beakers covered with Para film to prevent solvent evaporation (All stages done under the ventilator).

Afterward PU solutions poured into Petri dish. After 2 hours samples put into oven with temperature of 40 °C and duration of 4 days until samples dry. Afterward samples were prepared by cutting the films for removing thick areas.

2.3. Surface modification of polyurethane films by oxygen plasma.

PU films were placed inside oxygen plasma reactor (Plasma Enhanced Chemical Vapor Deposition) with exposure time of 30s and 60s on two samples for evaluating effect of process time. Reactor parameters were 20 W, DC power supply, 40 mA current, 500 V, 3×10^{-3} tor pressure and a rotary pump for achieving vacuum condition.

2.4. Surface characterization.

2.4.1. ATR-FTIR spectroscopy.

Attenuated total reflectance Fourier Transformer Infrared (ATR-FTIR) analyses were done by a Nexus 870 Model spectrophotometer. The spectra were recorded at an incident angle of 45°, using a KRS-5 prism as an internal-reflection element. Scanning was carried out in the range of 3500-700 cm^{-1}.

2.4.2. Scanning Electron Microscopy (SEM) analysis.

Scanning electron microscopy (SEM) was performed on gold coated PU samples using a positron sputter coater. A Seron Technology AIS2100 SEM operating typically at 10 kV employed for morphology study. Samples mounted onto the sample holder, sputter coated with gold and studied with SEM.

2.4.3. Surface topography and structure.

For surface structure and properties of materials at the nanometer scale, we used an Atomic Force Microscopy (AFM) VEECO Model.

2.4.4. Dynamic Mechanical Thermal Analysis (DMTA).

Mechanical properties test to check the structural interactions used and the results of storage modulus versus temperature was obtained by DMTA-Triton, Tritec 2000 Airoven.

2.4.5. Cell culture assay.

Cell culture reaction of the prepared films was evaluated by an in vitro cell culture test. Therefore samples were placed in plates and 4×10^4 cells/ml put on each of them, then poured cell growth medium (RPMI) on them that contain all cell necessary nutrition. Afterward kept them into CO_2 incubator (Incubator conditions consist of CO_2 5%, temp. 37 °C and humidity about 80~90%). After 24 hours brought out the films and images of them were taken by 100x magnification light microscopy (Nikon), then bring back them into incubator (Memmert) for another 24 hours [16, 17]. Then, brought out samples again and taken image of plates bottom and films surfaces by light microscopy. Then drawn off cell culture medium by micropipette and for fixing adhered cells on the surface, 2.5 % glutaraldehyde solution was used and left them 1 hour in a dim location. Then all films were washed with PBS (Phosphate Buffered Saline) to remove non-adhere cells. Then used graded ethanol (50-60-70-80-90 and 100%) respectively within 5 minutes for each of them to fixing all cells on the surface. Finally, SEM analysis performed and cell growth on samples surface compared with unmodified PU films.

3. RESULTS SECTION

3.1. FTIR-ATR study.

ATR-FTIR analysis results of modified and unmodified PU (Shore A) with plasma was shown in Figure 1. As shown in Figure 1 the absorbance peak of modified PU (shore A) at 1748 cm^{-1} and unmodified PU peak at 1745 cm^{-1} refer to ester bond and peaks at 2575 and 2210 cm^{-1} related to carboxylic bond (COOH) which in plasma modified PU these peaks increase significantly. Also absorbance peak at 3507 cm^{-1} depending to N-H bonding and peak at 3577 cm^{-1} as for hydroxyl groups (O-H). In the unmodified PU spectrum can be seen that the peak at 3120 cm^{-1} shift to shorter wavelength that demonstrate added hydrogen and nitrogen bonds that cause increasing surface hydrophilicity [18]. Figure 2 shows the ATRFTIR spectrum of shore D TPEPU. As can be seen in this spectrum the peaks at 2565, 2643 and 2764 cm^{-1} refer to hydroxyl groups and peak at 3456 cm^{-1} represent the N-H groups on the film surface and absorbance peaks at 847 cm^{-1} related to aromatic rings and peaks at 2565, 2640 cm^{-1} refer to COOH groups and peak at 2555, 3440 and 3662 cm^{-1} representing carboxylic groups, N-H bonding and hydroxyl groups respectively and in plasma modified

spectrum the intensity of these peaks increases which noticed to increasing of oxygen enrich functional groups.

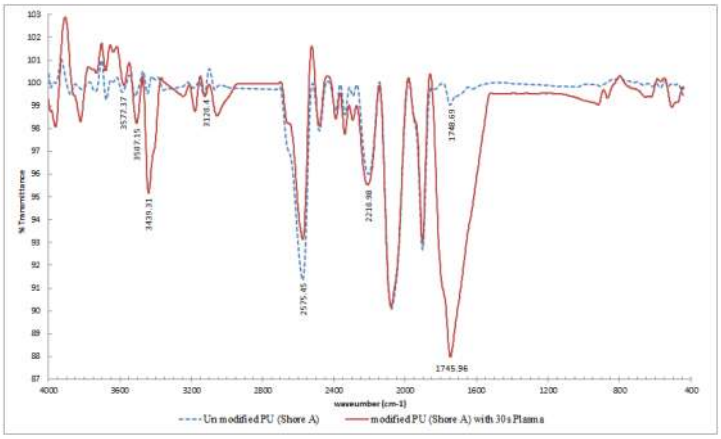


Figure 1. ATRFTIR spectrum of PU (Shore A) before and after 30s oxygen plasma treatment.

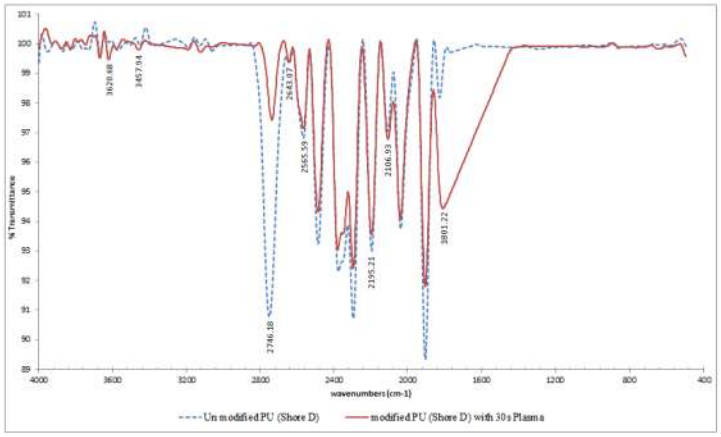


Figure 2. ATRFTIR spectrum of PU (Shore D) before and after 30s oxygen plasma treatment.

3.2. Study of surface morphology.

Scanning electron microscopy (SEM) and atomic force microscopy images (AFM) of unmodified PU (Shore A) and treated samples with plasma exposure time of 30s were shown in Figure 3. As can be seen in SEM images, after plasma treatment and because of plasma effect and chain destruction, the roughness increased uniformly through the surface. Also as seen in this Figure separated micro phase structure observed that proving existence of plasma coating on whole surface [18]. Figure 4 shows that unmodified PU (shore D) before plasma treatment has a small amount of roughness but after treatment high velocity plasma particles etching surface and therefore deep roughness created. Also modified PU showed that after plasma treatment surface roughness increased and in some areas separated micro phase structure is observing and in some regions etching phenomenon occurred that observing in brighter area on the image [19-20].

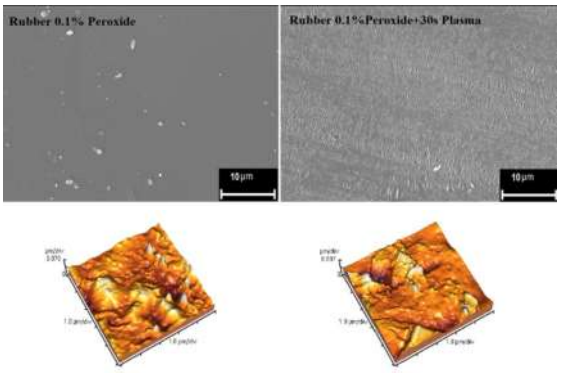


Figure 3. SEM and AFM images of unmodified PU (Shore A) and modified PU with 30s oxygen plasma treatment.

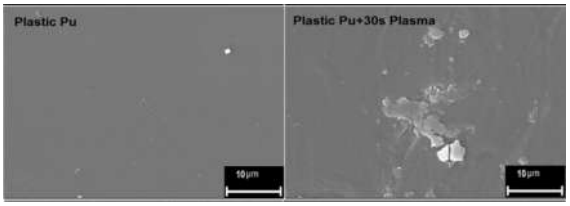


Figure 4. SEM images of unmodified PU (Shore D) and modified PU with 1% peroxide and 30s oxygen plasma treatment.

3.3. Atomic force microscopy (AFM).

As seen in Figures 3, 5 and Table 2, generally after plasma treatment on unmodified rubber PU some roughness created on surface spread in whole surfaces.

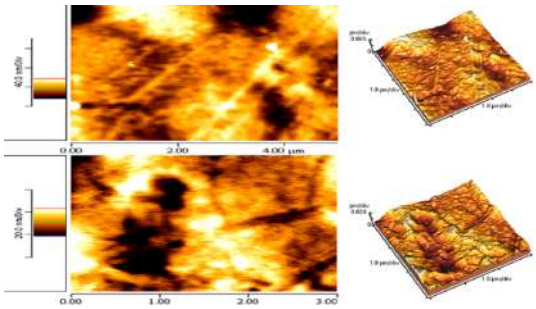


Figure 5. AFM images of plasma treated (Shore A) PU within 60s (Left) beside their 3D images (Right).

As seen in Table 2, after plasma treatment on PU (Shore A) some roughness created on the surface that avg. roughness value increasing from 4.294 to 15.591 nm confirms this matter. PU (Shore A) itself has more roughness compare to unmodified sample but after plasma treatment avg. roughness increase from 10.356 to 14.95 nm shows that roughness increasing too, and gaining value to 17.95 nm demonstrate more roughness appear with more uniform array and spread across the surface.

Table 2. AFM analysis results of PU (shore A).

Number Sample	Sample	Rms Rough (nm)	Mean Ht	Median Ht	Ave Rough (nm)	R_{p-v}	Surface area(μm^2)
1	PU	5.863	25.265	25.875	4.294	29.255	$25\mu m^2$
2	PU + 30s plasma	26.59	72.465	70.185	15.591	324.4	$25\mu m^2$

3	PU 1% Peroxide	13.725	43.97	42.565	10.3565	115.195	$25\mu m^2$
4	PU + 1% Peroxide + 30s plasma	21.54	127.9	127.7	14.95	245	$25\mu m^2$
5	PU + 1% Peroxide + 60s plasma	25.315	148.12	148.02	17.95	260.26	$25\mu m^2$

3.4. DMTA study.

In Figure 6 tan(δ)'s peak and sudden decrement of storage modulus in higher temperature than -50°C on both samples shows that glass transition temperatures (Tg) of unmodified rubber PU and modified sample are -49°C and -50.2°C respectively.

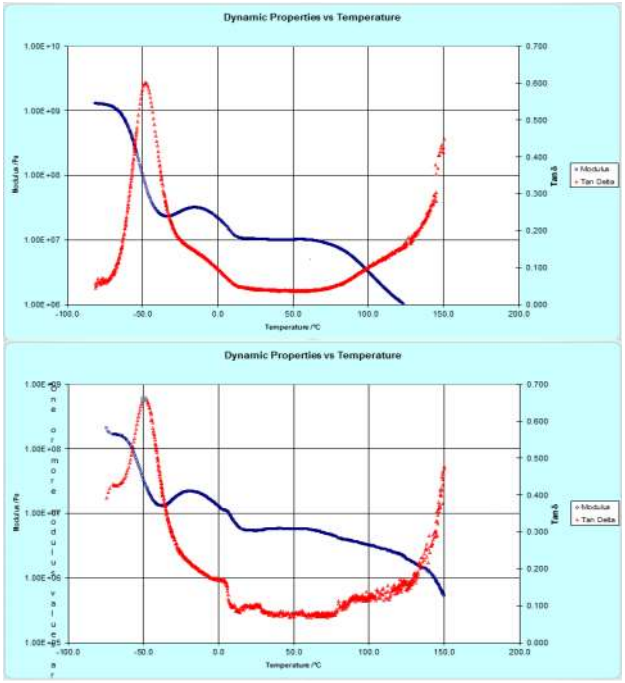


Figure 6. DMTA diagram of unmodified PU (Shore A) [Top] and PU (Shore A) 1% peroxide [Bottom].

Values of Table 3 shows that storage modulus of modified PU in all three selected temperature of -50°C, 37°C (human body temp.) and 50°C was decreased that indicate higher chains mobility and decrement of rubber polyurethane stiffness [21-24].

Table 3. Storage modulus (E′) values at selected temperatures.

Sample	Storage modulus (Pa) at -50 °C	Storage modulus (Pa) at 37 °C	Storage modulus (Pa) at 50 °C
PU (shore A)	10.830×10^7	1.003×10^7	1.008×10^7
PU (shore A) + %1 peroxide	3.547×10^7	0.5847×10^7	0.5824×10^7
PU (shore D)	1.729×10^9	0.3937×10^9	0.2733×10^9
PU (shore D)+ %1 peroxide	1.539×10^9	0.4322×10^9	0.3224×10^9

As can be seen in Figure 7 glass transition temperatures of unmodified plastic PU and plasma modified sample are 60 °C and 72 °C respectively. In addition, regard to Table 3, storage modulus of modified sample is higher when compared to unmodified plastic PU, thus we can conclude that applying plasma can causing formation of covalent cross-link bonds between the polymer chains.

As result, macromolecules mobility in un-oriented regions decreases and stiffness of plastic polyurethane slightly increases [21-24]. Also the reason why tan(δ)'s peak of modified plastic PU sample at Tg temperature range (60.9°C and 72.5°C) was narrowed, is reactions between peroxide and polymer chains that cause decreasing of macromolecules semi-viscous mobility.

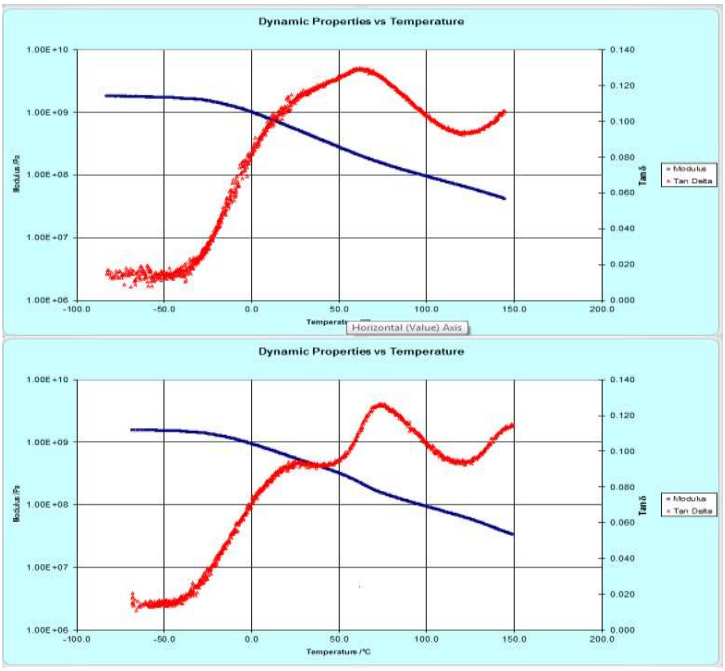
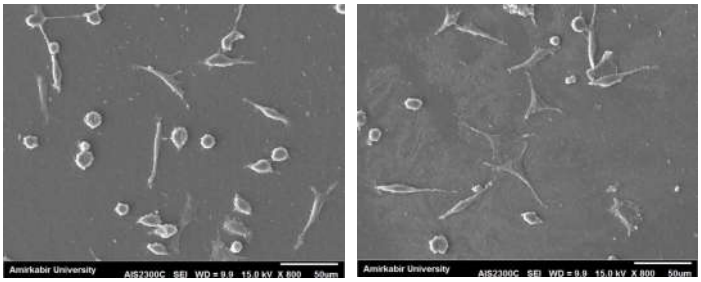


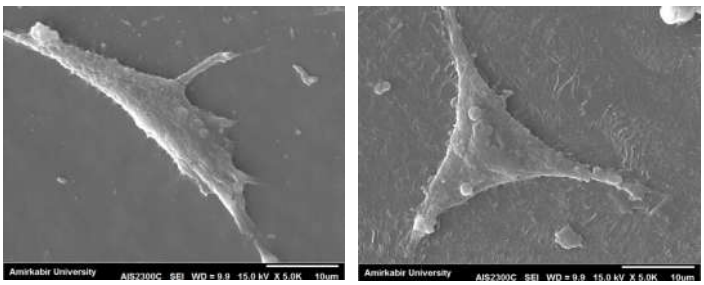
Figure 7. DMTA diagram of unmodified PU (Shore D) [Top] and PU (Shore D) PU 1% peroxide [Bottom].

3.5. Cell culture study.

SEM photomicrographs of unmodified, and plasma treated PU (shore A) films that immersed in cell culture plates within 48 hours are shown in Figure 8. As seen in Figure 8 (a) on samples that 48h immersed, cells are more like spherical and located apart each other that indicate separated local small clusters but on plasma treated sample (b) cells grown uniformly stretched and attached together very well [25].



a) b)



c) d)

Figure 8. SEM images of how cells grown and adhered on surface of a) PU (Shore A) and b) their plasma treated sample within 30s after 48h immersion in cell suspension (magnification is 1kX) and c) and d) magnification is 5Kx.

Figure 9 shows that contrast to unmodified plastic PU (shore D) cells growth on plasma modified and joint to each other well and spread on whole surface in uniform array. In other word most cells are active and are in webbing, filopodia and flattening status and surface sample make a good base for cell growth, whereas on unmodified samples most cells are in spherical shape and located apart each other.

As result, we concluded that plasma treatment could enhance cell growth and adhesion very well compared to unmodified one.

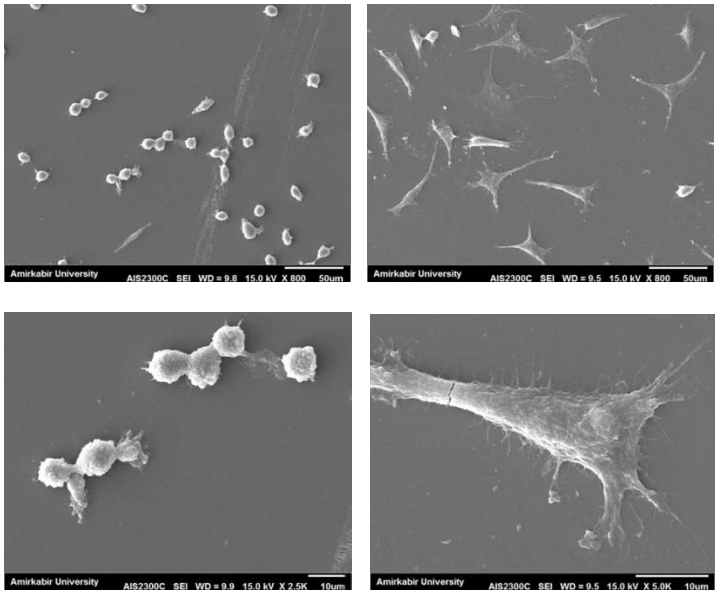


Figure 9. SEM images of how cells grown and adhered on surface of (Shore D) PU and their plasma treated sample within 30s after 48h immersion in cell suspension by 1Kx (Top) and 5Kx (Bottom) magnification.

4. CONCLUSIONS

After plasma treatment, absorbance peaks of oxygen and hydrogen increases and wavelength of polar bonds such as hydroxyl, carboxyl, carbon-carbon and hydrogen bonds increase, thus surface going to more hydrophilic manner. After plasma treatment, generally surface morphology changes and some roughness appear. DMTA results show that storage modulus of plasma modified polyurethane rubber decreases and in polyurethane plastic (shore D) increases. Surface modifying PU with oxygen plasmas is an effective procedure for enhancing cell growth, expanding on surface and their adhesion. Plasma modification is a suitable process in tissue engineering because just modify uppermost atomic layer of surface and has little change on bulk properties. For achieving uniform coating on polyurethane surfaces, cold oxygen plasma process can be useful.

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