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PLASMA TREATMENT OF BIOMEDICAL MATERIALS

PLAZEMSKA OBDELAVA BIOMEDICINSKIH MATERIALOV

Ita Junkar[1], Uroš Cvelbar[2], Marian Lehocky[3]

[1]Odsek za tehnologijo površin in optoelektroniko, Institut "Jožef Stefan", Jamova cesta 39, 1000 Ljubljana, Slovenija
[2]Center of Excellence for Polymer Materials and Technologies, Tehnološki park 24, 1000 Ljubljana, Slovenia
[3]Medical Materials Research Centre, Technology Park, Tomas Bata University, Nad Ovcirnou 3685, Zlin, Czech Republic
ita.junkar@ijs.si

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Surface plasma treatment techniques for modification of biomedical polymeric materials are presented. The emphasis is on the use of non-equilibrium radiofrequency (RF) oxygen and nitrogen plasma. By variation of discharge parameters (power, discharge frequency, type of gas) and plasma parameters (density of neutrals and ions, kinetic energy of electrons, gas temperature) it is possible to produce polymer surfaces with different surface properties. Already after short plasma treatment time the surface of polymeric material becomes hydrophilic. Formation of nitrogen and oxygen functional groups is observed immediately after plasma treatment. By optimisation of plasma treatment time the number of newly formed functional groups can be increased. Plasma treatment also produces morphological changes of the surface; nanohills of different shapes and height can be formed on PET surface depending on the treatment time and type of gas. Evidently the change in surface morphology affects the change in surface roughness, which increases with longer plasma treatment time. Plasma treatment influences also on the biological response, as all plasma treated surfaces exhibit improved proliferation of fibroblast and endothelia cells. The number of adherent platelets practically does not change after nitrogen plasma treatment, however much lower number of adherent platelets is observed on oxygen plasma treated surfaces.

Key words: plasma treatment, biocompatibility, polymer, vascular grafts, endothelia cells, platelets

Predstavljene so tehnike plazemske obdelave površin, s katerimi lahko modificiramo površine biomedicinskih polimernih materialov. Poudarek je na uporabi neravnovesne radiofrekvenčne (RF) plazme dušika in kisika. S spreminjanjem razelektritvenih (moč, razelektritvena frekvenca, vrsta plina) in plazemskih parametrov (gostota nevtralnih delcev, ionov, kinetične energije elektronov, temperature plina) je mogoče pripraviti površine polimerov z različnimi lastnostmi. Že po kratkih časih izpostavitve polimernih površin dušikovi ali kisikov plazmi le-ta postane hidrofilna. Takoj po obdelavi je na površini mogoče opaziti novonastale dušikove oziroma kisikove funkcionalne skupine. Z optimizacijo časa izpostavitve plazmi je mogoče koncentracijo le-teh še nekoliko povečati. Plazemska obdelava vpliva tudi na spremembe v morfoloških lastnostih površine, tako je mogoče na plazemsko obdelanih površinah opaziti nanostrukture, katerih oblika in velikost je odvisna od časa izpostavitve plazmi, kot tudi od vrste plina uporabljenega za plazmo. Morfološke spremembe vplivajo tudi na spremembe v hrapavosti površine, ki se poveča s časom plazemske obdelave. Modifikacija površine vpliva na biološki odziv, saj se po plazemski obdelavi proliferacija endotelijskih in fibroblastnih celic na površini poveča. Število adheriranih trombocitov na površinah, obdelanih z dušikovo plazmo, se bistveno ne spremeni, medtem ko se njihovo število bistveno zmanjša na površinah, obdelanih s kisikovo plazmo.

Ključne besede: plazemska obdelava, biokompatibilnost, polimer, umetne žile, endotelijske celice, trombociti

1 INTRODUCTION

Surface properties of biomaterials play a major role in determining biocompatibility; they have a significant influence on biological response and also determine the long term performance *in vivo*. The main goal in designing biomaterials is therefore to ensure that they exhibit appropriate surface properties as well as desired physical and mechanical characteristics, which would enable them to function properly in the biological environment. It is hard to satisfy all of these characteristics; this is why surface treatment techniques are commonly employed in order to improve surface properties. It is still a highly challenging task to modify surface properties in order to produce hemocompatible surfaces, and many controversial results are reported in the literature.

Biological response to biomaterials is very complex and still not fully understood. As the surface of the biomaterial is responsible for initiating the primary interaction with body fluids it is of vital importance to ensure that the surface is suitably conditioned to ensure an appropriate biological response (biocompatibility). It was thought for many years that the surface of the biomaterial should be inert. However, nowadays it has been found that the contact of biomaterials with blood enables integration with the body, prevents infections, inflammatory reactions, blood coagulation and other correlated reactions. It is of primary importance that the surfaces of hemocompatible materials exhibit anti-thrombogenic properties, as this prevents thrombosis. Thrombosis is initiated with the adsorption of blood plasma proteins on the surface of the biomaterial and is strongly influenced by its physical and chemical properties.

Surface properties of implants are usually described with wettability, chemistry, surface charge and texture (roughness). These factors all influence the sequence of protein adsorption and subsequent platelet adhesion/thrombus formation. Although the mechanism of occlusion and dysfunction of artificial prostheses is multifactorial, all the studies performed suggest that fibrinogen and platelet deposition play a predominant role.[1,2] It also seems that the outermost atomic layer of the surface of an alloplastic implant is a decisive factor for determining biocompatibility.[3] One possible method to alter surface characteristics, such as wettability, chemistry, charge and morphology to improve biocompatibility of implant devices[4] is by treatment of the surface with different gaseous plasma, like glow discharge created in different gases and by variation of discharge parameters (discharge power, pressure, etc.),[5] which in turn influence plasma parameters (density of atoms, energy of plasma particles, etc.). Plasma modification has been used recently to enhance biocompatibility of implant devices made from stainless steel, titanium and various polymers.[6–14] The unique advantage of plasma modification of implant devices is that the surface can be modified without altering the bulk properties of the material.[15] It is thus possible to obtain desired mechanical and physical properties of implant material and at the same time also improve its surface properties to accomplish biocompatibility.

2 SURFACE MODIFICATION BY PLASMA TREATMENT

Combined surface treatments incorporating photons, ions and electrons and some other excited particles, are found in gas-electric discharges, often denominated plasmas. Ionised gas is usually called plasma when it is electrically neutral (i.e., electron density is balanced by that of positive ions) and contains a significant number of electrically charged particles, which is sufficient to affect its electrical properties and behaviour.[16] Therefore plasma is composed of highly excited atomic, molecular, ionic, and other native radical species. It is typically obtained when gases are excited into energetic states by radio-frequency (RF), microwave, or electrons from a hot filament discharge. To produce plasma, electron separation from atoms or molecules in gas state, or ionization is required. When an atom or a molecule gains enough energy from an outside excitation source or via interaction (collisions) with one another, ionization occurs.[17]

Plasmas are divided into thermodynamically balanced and unbalanced. Plasma characteristics are dependent upon the electrical discharge type, the type of gas or gaseous mixture, and the pressure. Thermodynamically balanced plasmas are characterized by very high temperatures of heavy particles (often about 10 000 K). These types of plasmas are not suitable for the treatment of polymeric materials, as the gas temperature is so high as to cause their thermal degradation. While in thermodynamically unbalanced plasma the gas temperature is significantly lower, as they are composed of low temperature heavy particles (charged and neutral molecular and atomic species) and very high temperature electrons (often about 50 000K). This type of plasmas are are suited for the treatment of delicate polymeric products[18–32] such as PET, PDMS or PTFE, which are used for biomedical applications.

Clark and Hutton[33] showed that with hydrogen plasma they can rapidly defluorinate fluoropolymers to a depth of 2 nm. On the other hand it was reported that plasma treatment with oxygen increased endothelia cell attachment on vascular grafts made of PTFE.[34] Comparison of plasma treatment with oxygen, nitrogen and gas mixture of nitrogen and oxygen, where conducted by M. Chen et al., where it has been shown that the mixture of gases uniquely modifies PTFE surfaces and reduces levels of inflammatory cells.[5] Surfaces having incorporated nitrogen were more effective than those of oxygen containing functional groups in promoting cell adhesion.[35] Though, appropriate surface modification is not only a function of working gas, but also of other discharge parameters, such as pressure, type of gas, power etc. Chevallier et. al. showed that nitrogen plasma treatment of PTFE at low-power (10 W) experimental conditions exhibits more alkene and less amino groups formed on the surface than a higher-power plasma treatment (20 W). Consequently, surface chemistry could be modulated through appropriate selection of discharge parameters.[36]

In our investigation surface properties of polymeric biomedical implants have been tailored by RF oxygen and nitrogen plasma treatment. The PET polymeric implants were treated in the experimental system shown in **Figure 1**. The plasma was created with an inductively coupled RF generator, operating at a frequency of 27.12 MHz and an output power of about 200 W. The plasma parameters were measured with a double Langmuir probe and a catalytic probe[36–44]. In our experiments, the pressure was fixed at 75 Pa, as at this pressure the highest degree of dissociation of molecules, as measured by the catalytic probes, was obtained. At these discharge parameters, plasma with an ion density of about $2 \cdot 10^{15}$

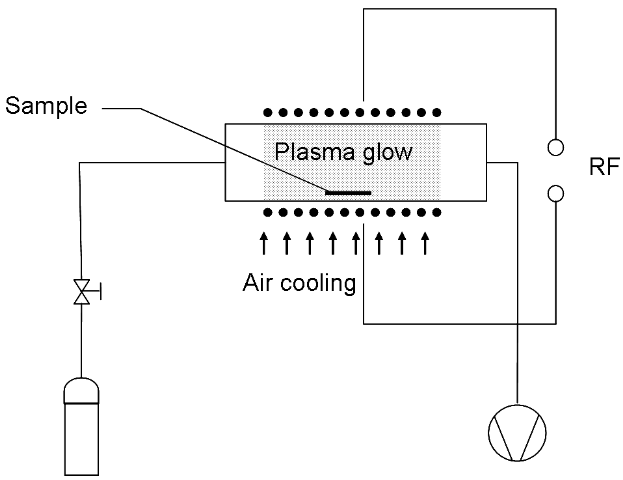

Figure 1: The RF plasma reactor chamber with the sample in position
Slika 1: Cev RF plazemskega reaktorja z vzorcem

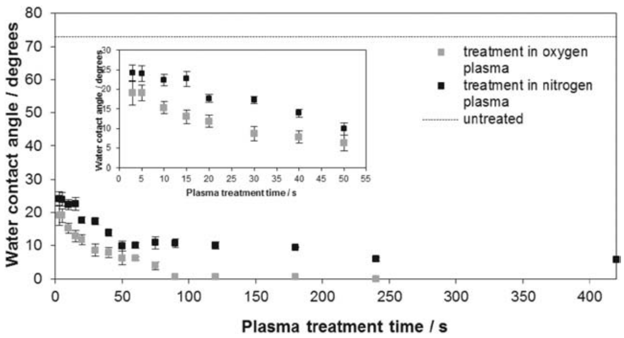

Figure 2: Water contact angle measured on the PET polymer as a function of treatment time and plasma gas, (■) oxygen plasma treatment, (■) nitrogen plasma treatment

Slika 2: Kontaktni kot vodne kapljice, izmerjen na površini PET polimera, v odvisnosti od časa obdelave in vrste plina, (■) obdelava v kisikovi plazmi, (■) obdelava v dušikovi plazmi

m^{-3}, an electron temperature of 4 eV, and neutral atoms density of about $4 \cdot 10^{21}$ m^{-3} for oxygen plasma and about $1 \cdot 10^{21}$ m^{-3} for nitrogen plasma was obtained. After plasma treatment surface chemistry, wettability and topography was altered.

Change in chemical composition was determined from (x-ray photolelectron spectroscopy) XPS. It has been shown that newly formed oxygen, or nitrogen functional groups are formed on the surface, depending on the type of gas used for modification. Already after short exposure to oxygen plasma the increase in oxygen concentration from initial mole fraction 21 % to 39 % was observed. With prolonged exposure to oxygen plasma the concentration slowly increased, and at 90 s it reached 44 %. On nitrogen plasma treated samples nitrogen concentration increased from 0 % to 12 % after 3 s of treatment time, and after 90 s of treatment it reached about 14 %. During nitrogen plasma treatment a small increase in oxygen concentration was also observed.

Wettability was examined immediately after the plasma treatment by measuring the water contact angle with a demineralised water droplet of a volume of 3 µL. The relative humidity (45 %) and temperature (25 °C) were monitored continuously and were found not to vary significantly during the contact angle measurements. Contact angle measurements show a decrease in contact angles after oxygen and nitrogen plasma treatment **Figure 2**, corresponding to a higher hydrophilicity of the polymer surface. During the water contact angle measurements room temperature was 21 °C and The oxygen plasma treated samples exhibit lower values of contact angles, and thus demonstrating that this treatment provides a higher hydrophilic character. Even after short exposure times the surfaces show an increased hydrophilicity, regardless of the type of gas used. The treatment with nitrogen plasma, however, seems to be less efficient in reaching high hydrophilicity. However the high hydrophilycity of oxygen plasma treated surfaces could be attributed to degradation products, which are formed on the surface after longer treatment times and could cause a lower contact angle, due to surface roughening.

The morphology of untreated and plasma treated surfaces was analyzed by atomic force microscopy (AFM) and scanning electron microscopy (SEM). In both cases change in surface morphology was observed. In **Figure 3** phase AFM images are shown. In **Figure 3 a** surface of untreated sample is shown, while in **Figure 3 b** and **c** change in surface morphology after treatment in nitrogen and oxygen plasma can be observed, respectively. Untreated sample has smooth surface, without any particular features on the surface, while treated samples exhibit small nanostructures on its surface. The diffe-

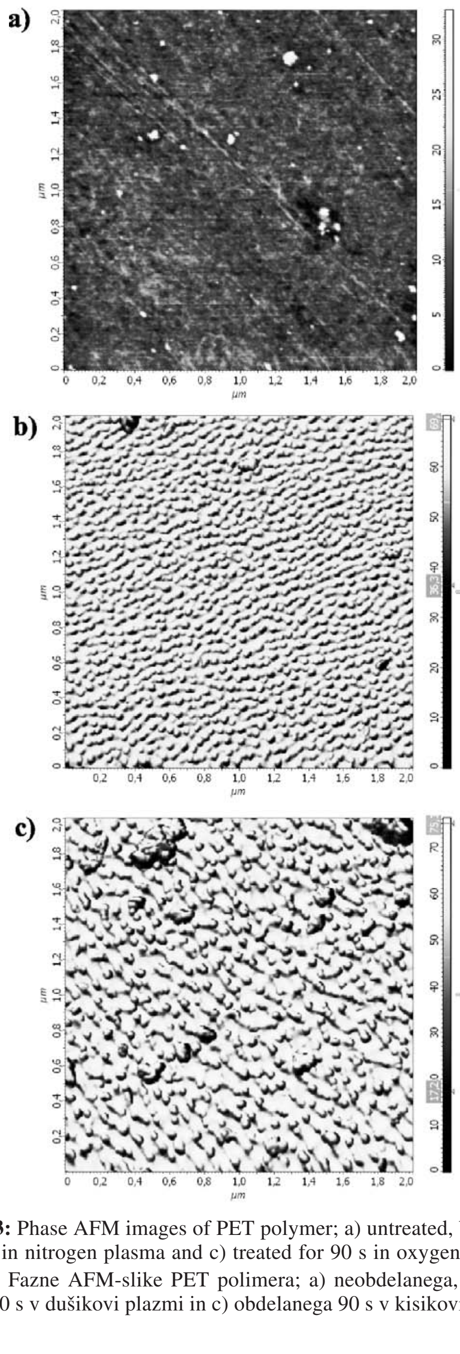

Figure 3: Phase AFM images of PET polymer; a) untreated, b) treated for 90 s in nitrogen plasma and c) treated for 90 s in oxygen plasma

Slika 3: Fazne AFM-slike PET polimera; a) neobdelanega, b) obdelanega 90 s v dušikovi plazmi in c) obdelanega 90 s v kisikovi plazmi rence between the samples treated with oxygen (**Figure 3 a**) and nitrogen (**Figure 3 b**) plasma is noticeable: the samples treated by oxygen plasma have structures which are higher and further apart, than those treated with nitrogen plasma. This could be attributed to highly oxidative nature of oxygen plasma. However the height of these nanostructures is not only dependent on the type of gas employed for treatment, but also on the treatment time. By longer treatment time the height of these nanostructures can be increased, especially for the case of oxygen plasma treatment. Due to growth of nanostructures, surface roughness is increased on these surfaces as well.

3 BIOLOGICAL RESPONSE

To achieve a desired biological response, the attachment of cells to the surface of biomaterials is of primary importance. When a biomaterial is exposed to a living organism many extremely complex reactions may occur at the cell-biomaterial surface. These reactions include coagulation, healing, inflammation, mutagenicity, and carcinogenity and play an important role in the successful implementation of the implemented material or device[45-48]. Surface parameters, such as surface chemistry, wettability, surface topography and surface roughness influence protein adsorption, and the adsorbed protein layer further dictates subsequent cellular reactions. Thus, by carefully tailoring surface properties by plasma treatment one could engineer the surface for a specific protein adsorption which would lead to a desired cellular response.

Gas plasma treatment is one of the strategies for enhancing surface properties by enriching the surface with new functional groups known to enhance cell proliferation – such as oxygen or nitrogen.[49,50] One of the strategies to improve biocompatibility/hemocompatibility of the surface is to introduce new functional groups, such as hydroxyl (-OH), amine (-NH_x), methyl (-CH_3) sulphate (-SO_4) or carboxylic (-COOH).[51–54] This is either employed to tailor the biological response (improve cell proliferation, reduce platelet adhesion etc.) or to enable immobilisation of biomolecules (enzymes, proteins etc.). The effects of functional groups on hemocomaptibilility have been extensively studied, but again results are not always consistent. The study by Wilson et. al. has shown that treatment of polymer (polyethyetherurethane- PEU) surface with RF ammonia and nitrogen plasma (incorporation of nitrogen groups) significantly reduces contact activation[54]. However, no changes in thrombogenicity, as compared to the untreated surface, were observed after oxygen and argon plasma (incorporation of oxygen groups). Similar results were obtained for RF plasma treatment of polydimethylsiloxane (PDMS) by Williams.[55]

On the other hand surface wettability is also believed to be one of the important parameters which affect biological response to a biomaterial. It is established that protein adsorption is the first event that takes place on the surface of a biomaterial with biological fluids,[52–54] and that the biological response is controlled by the nature and confirmation of the proteins adsorbed to the surface. Thus, wettability is believed to play an important role in the amount and conformational changes of adsorbed proteins[59] platelet adhesion/activation, blood coagulation[60] and adhesion of cells.[61,62]

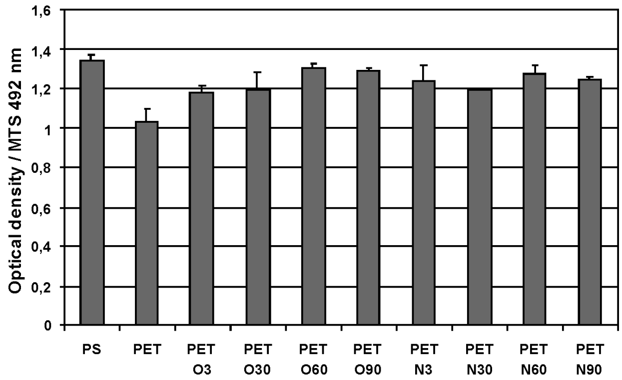

Figure 4: Viability of endothelia cells (HUVEC) cultured on surfaces treated by oxygen and nitrogen plasma for different treatment times
Slika 4: Viabilnost endotelijskih celic (HUVEC) na površinah, obdelanih s kisikovo in dušikovo plazmo pri različnih časih obdelave

Generally hydrophobic surfaces are considered to be more protein-adsorbent than hydrophilic surfaces, due to strong hydrophobic interactions occurring at these surfaces.[63–65]

Nevertheless surface morphology should also be taken in account when talking about biological response to biomaterials. Surface morphology is important in protein adsorption and subsequent cell response. Reidel and colleagues showed that adsorption of albumin dramatically increased due to presence of nanoislands.[66] While Vertegel et. al. showed that the adsorption of lysozyme to silica nanoparticles decreased with decreasing nanoparticle size[67]. Surface topography plays an important role in providing three-dimensionality of cells[68]. For instance the topography of the collagen fibres, with repeated 66 nm binding, has shown to affect cell shape.[69]

It has been shown that RF oxygen and nitrogen plasma treatment improve proliferation of fibroblast as well as endothelia cells. **Figure 4** shows the measured absorbance, which is directly proportional to viability of endothelia cells, cultured on different samples. These results show that proliferation of endothelia cells is improved on all plasma treated surfaces, which is in accordance with the results published in the literature.[5, 70] Improved proliferation of cells can be attributed to newly formed functional groups (oxygen and nitrogen) introduced after short plasma treatment time (3 s), as well as to higher hydrophilicity of the surface, surface morphology etc. It seems that longer treatment time (longer than 30 s) by oxygen plasma is more affective in promoting endothelia cell attachment than nitrogen plasma.

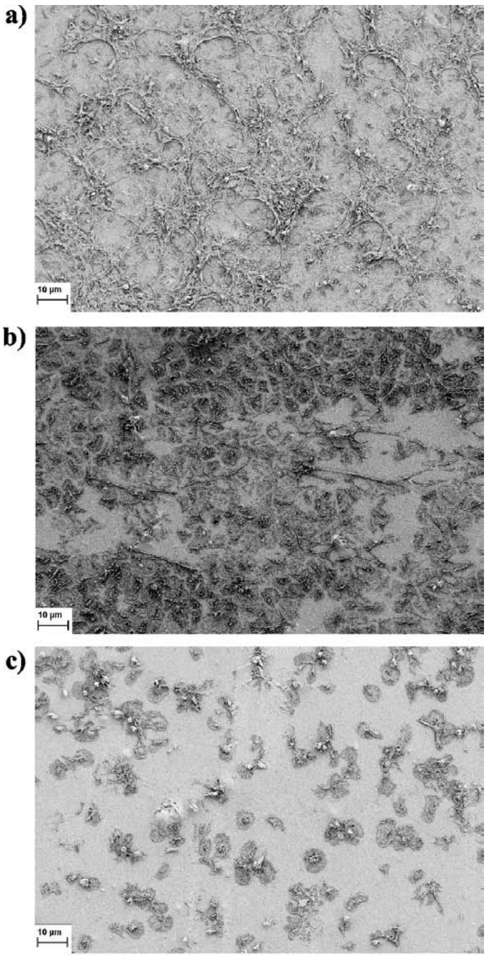

Figure 5: SEM images of platelets interacting with: a) untreated, b) 3 s nitrogen plasma treated (PET N3), c) 3 s oxygen plasma treated (PET O3) surface of PET foils
Slika 5: SEM-slike trombocitov v kontaktu s PET polimerom; a) neobdelanim, b) obdelanim 3 s v dušiku in c) obdelanim 3 s v kisiku

Endothelia cell seeding is a common approach to improving hemocompatibility of vascular grafts, as endothelia cells are thought to be an ideal hemocompatible surface. However adhesion of platelets is not desired for hemocompatible surfaces. Thus lower or practically no adhesion of platelets would be desired. Interestingly our study showed significant differences in adhesion of platelets to oxygen and nitrogen plasma treated surfaces. Observable differences in the number of adherent platelets and their shape can be seen in **Figure 5**. The number of adherent platelets decreased dramatically on oxygen plasma treated surfaces; as can be seen from **Figure 5 c** after only 3 s of oxygen plasma treatment, a lower number of platelets was observed. Those that did adhere seemed to be in a more round form, which is thought to be attributed to low platelet activity. On the contrary there were many aggregated platelets on untreated (**Figure 5 a**) and nitrogen plasma treated surfaces (**Figure 5 b**). Fibrin formation was also observed on these surfaces, especially on the untreated surface. The platelets on the untreated polymer surface are mostly in well spread form and start to aggregate.

4 CONCLUSIONS

It has been shown that plasma treatment techniques enable surface modification of biomedical materials and thus enable desired biological response of the surface. Therefore many biomedical materials have been treated by plasma in order to improve their surface properties to accomplish biocompatibility. By fine tuning the discharge and plasma parameters the surface can be appropriately modified.

Our study showed that by oxygen and nitrogen plasma treatment surface chemistry, wettability and morphology can be altered. Furthermore plasma treatment enables improved proliferation of fibroblast and endothelia cells and influences on adhesion properties of platelets. Interestingly adhesion of platelets was noticeably reduced on oxygen plasma treated surfaces, while adhesion on nitrogen plasma treated surfaces was similar to the untreated ones. It has been shown that oxygen plasma treatment is a promising way to improve hemocompatible properties of PET surface, as surfaces modified in this manner exhibit improved proliferation of endothelia cells and reduced platelet adhesion.

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