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CELL ADHESION ON HYDROPHOBIC POLYMER SURFACES

ADHEZIJA CELIC NA HIDROFOBNIH POLIMERNIH POVRŠINAH

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Adhesion of human osteosarcoma (HOS) cells on hydrophobic polymer surface was studied. Surface of polymer polystyrene (PS) was made hydrophobic by treatment in plasma created in tetrafluoromethane gas (CF_4). The PS samples were exposed for 30 s to CF_4 plasma created by RF generator powered at 200 W. This treatment time allowed for optimal polymer surface functionalization with fluorine functional groups. This caused an increase of surface hydrophobicity from initial 85° to about 110° as measured by water contact angle. The HOS cells were deposited on untreated and plasma treated samples and incubated for 1, 2 and 6 days. Both untreated and plasma treated samples were tested for biocompatibility by two different methods: optical micrographs were used to study the cell morphology and MTT test was used to study the cell viability. The results showed better adhesion of cells on plasma treated samples with more hydrophobic surface in comparison to the untreated sample. MTT test revealed about 1.6-times higher activity of cell enzymes after 6-day incubation for plasma treated sample. Optical micrographs have shown that both untreated and fluorine-plasma treated polymer surfaces are not optimal for cell proliferation, since cells need about 2 days to adapt to the surface. After this adaptation time cells start to proliferate on the polymer surface, especially, on that one treated in plasma.

Keywords: plasma surface modification, human osteosarcoma cells (HOS), biopolymers, hydrophobic surface, CF_4 plasma

Preučevali smo adhezijo rakastih (HOS) celic na hidrofobnih polimernih površinah. Hidrofobizacija površine polimera polistirena (PS) je potekala v CF_4 plazmi generirani z RF generatorjem moči 200 W. Vzorce polimera PS smo izpostavili plazmi za 30 s, kar je zadoščalo za optimalno funkcionalizacijo polimerne površine z nepolarnimi fluorovimi skupinami. To je povzročilo porast kontaktnega kota vodne kapljice iz 85° za neobdelan vzorec na 110° za plazemsko obdelan vzorec, kar je jasen dokaz, da se je hidrofobnost površine povečala. Biokompatibilnost neobdelanih in plazemsko obdelanih vzorcev smo spremljali z optičnim mikroskopom in MTT testom. Z optičnim mikroskopom smo preučevali morfologijo celic, medtem ko smo z MTT testom preučevali viabilnost celic. HOS celice smo deponirali na vzorce in jih inkubirali 1 dan, 2 dni in 6 dni. Iz rezultatov je razvidna boljša adhezija celic na plazemsko obdelanih bolj hidrofobnih površinah v primerjavi z neobdelano zmerno hidrofobno površino. Z MTT testom smo na plazemsko obdelani površini ugotovili 1,6-krat večjo aktivnost celičnih encimov po 6-dnevni inkubaciji v primerjavi z neobdelano. Posnetki z optičnim mikroskopom nakazujejo, da neobdelana in plazemsko obdelana površina nista najbolj optimalni za vezavo celic, saj celice potrebujejo precej časa (še posebej v primeru neobdelane površine), da se privadijo na okolje. Šele po tem času privajanja je opaziti proliferacijo celic po površini, ki je še posebej opazna na plazemsko obdelanem vzorcu.

Ključne besede: plazemska modifikacija površin, rakaste celice, biopolimeri, hidrofobna površina, CF_4 plazma

1 INTRODUCTION

Polymer materials are nowadays widely used in many different applications in medicine for various implants, tissue engineering, etc.[1–4] Chemical and physical properties of polymer surfaces are therefore very important since they have influence on interactions between the polymer material and a host environment which is normally composed of body fluids, proteins and various cells.[4,5] Therefore, often competitive adsorption appears. In some applications selective adhesion of cells is important. Adhesion and proliferation of cells on polymer surfaces can be controlled by preparing surfaces with particular characteristics.[7–12] This can be done by appropriate surface modification.[5,6] Polymer surfaces can be modified by different techniques. Among them plasma treatment is the most popular.[7–11] By plasma treatment we can introduce different chemical groups to the polymer surfaces and thus make surfaces either hydrophilic or hydrophobic; we can also change surface crystallinity, surface energy, roughness and morphology.[13–17] All these factors may play important (also synergistic) role in surface interactions. For making surface hydrophilic usually oxygen, nitrogen, ammonia, water or CO_2 plasma are used, while for making the surface hydrophobic the treatment is performed in a plasma created in halogens like CF_4. Hydrophilic surfaces are characterized by good wettability and good adhesion properties, while hydrophobic surfaces are known to be quite inert. Since the body liquids are normally composed of water, various proteins and cells synergistic interaction may appear so it is difficult to predict the exact interaction mechanism of hydrophilic/hydrophobic surfaces when exposed to biological system.[5,18]

In the present paper we were studying the adhesion of human osteosarcoma cells on polystyrene polymer which was treated in CF_4 plasma to make the surface

hydrophobic. Comparison of cell proliferation on plasma treated hydrophobic surface to the untreated one was performed by two different methods: optical micrographs were used to study the cell morphology and MTT test was used to study the cell viability.

2 EXPERIMENTAL

2.1 Preparation of HOS cells

The human osteosarcoma cell line HOS was obtained from American Type Culture Collection (ATCC). Cells were grown in DMEM (Dulbecco's modified eagle's medium, Sigma, USA) supplemented with 10 % (v/v) foetal calf serum (FCS, Sigma, USA), 2 mM L-glutamine and penicillin/streptomycin (1 000 U/mL and 1000 µg/L respectively). Cells were maintained in an incubator (Heraeus, Germany) at 37 °C, with a humid air atmosphere containing 5 % CO_2. The cells were detached from semiconfluent cultures with a 0.25 % (w/v) trypsin solution for 5 minutes. Viable cells (upon trypan blue exclusion assay) were counted on a Bürker-Türk hemocytometer and used for experiments.

2.2 Plasma treatment

Commercially available polystyrene (PS) foils supplied by Goodfellow Ltd were cut to discs with a diameter of 1 cm. The thickness of the foil was 0.25 mm. Before plasma treatment the samples were cleaned in ethanol in an ultrasound bath. No special sterilization of the samples was performed since plasma itself acts as a good method for surface sterilization.[19–21]

Samples were mounted in the glowing plasma of a radio-frequency (RF) discharge as shown in **Figure 1**. The RF generator operated at a power of 200 W and a frequency of 27.12 MHz. A discharge tube of a length 60 cm and a diameter of 4 cm is made of Pyrex glass. A rather uniform glow discharge is created within a RF coil which is 15 cm long. The impedance of the generator was optimized for such a configuration using a vacuum capacitor in parallel with the RF coil. The treatment was performed in tetrafluoromethane gas (CF_4) at a pressure of 75 Pa. The plasma treatment time was 30 s. According to our recent paper,[22] polystyrene foils become saturated with fluorine functional groups already in 10 s of treatment. Therefore, 30 s of treatment assured for optimal functionalization.

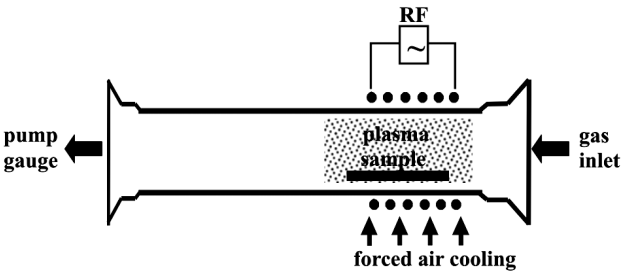

Figure 1: Optical microscopy image of polymer surface after 24 h of incubation for: (a) untreated sample and (b) plasma treated sample
Slika 1: Posnetek polimerne površine po 24-urni inkubaciji s celicami HOS: (a) neobdelan vzorec in (b) plazemsko obdelan vzorec

2.3 HOS cells viability

Cells were seeded at 2×10^4 cells in 100 µl of medium on the upper side of polymers at density of 2.55 $\times 10^4$ cells/cm^2, and were left for 3 h to attach before covering the whole polymer discs with media.[23] Cells were plated in DMEM medium supplemented with 10 % FCS and left to grown on polymer discs in an incubator at 37 °C in a humidified atmosphere of 5 % CO_2. Triplicates of cultures for each time and treatment were prepared for adhesion and cell viability assay.

Cell adhesion was monitored daily and after 1st, 2nd and 6th day of culture on the different polymer surfaces micrographs were taken. The MTT-related colorimetric assay (EZ4U; Biomedica, Austria) was used to determine cell growth and viability, according to the manufacturer's instructions and Jaganjac et al.[24] The method is based on the fact that living cells are capable of reducing less colored tetrazolium salts into intensely colored formazan derivatives. This reduction process requires functional mitochondria, which are inactivated within a few minutes after cell death.

Briefly, after 1st and 6th day of HOS cell culture on the different polymer surfaces the medium was removed and 1 ml of fresh Hanks' Balanced Salt Solution (HBSS) and 100 µl of the tetrazolium agent were added to each culture. After 2 h incubation, supernatants were transferred into 96-well plates and measured in a microplate reader (Easy-Reader 400 FW, SLT Lab Instruments GmbH, Austria) at 450/620 nm.

3 RESULTS AND DISCUSSION

Numerous samples were prepared by plasma treatment in CF_4 gas. As shown in our resent paper plasma treatment caused incorporation of about 56 at.% of fluorine to the surface of polystyrene which originally contains only carbon and hydrogen atoms.[22] Incorporation of nonpolar fluorine functional groups like CHF, CF, CF_2 and CF_3 caused increased surface hydrophobicity which was checked by water contact angle measurements.[22] The surface of untreated polystyrene is moderately hydrophobic with a contact angle of about 85°. After plasma treatment the surface hydrophobicity was increased giving a contact angle of about 110°.

Adhesion of HOS cells on plasma treated samples was monitored daily. After 1st, 2nd and 6th day of cell culture incubation on the polymer surfaces optical micrographs were taken. Some representative images are shown in **Figures 2–4**. **Figure 2a** shows optical image of the untreated sample after 24 h incubation, while **Figure 2b** reveals an optical image of the plasma treated sample incubated for the same time. We can see that in both

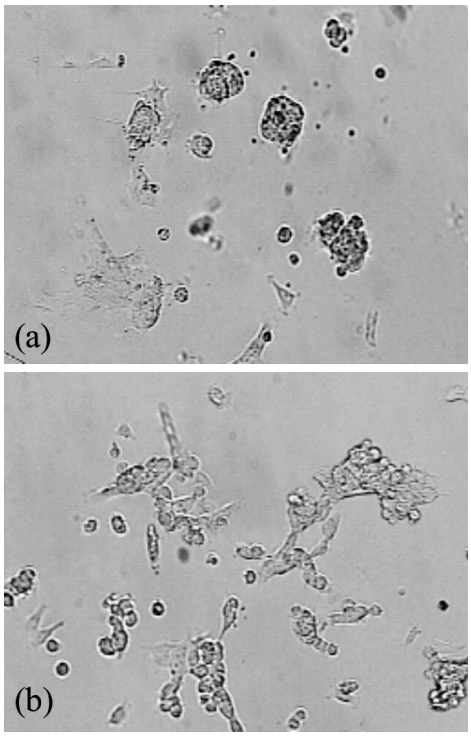

Figure 2: Optical microscopy image of polymer surface after 24 h of incubation with HOS cells for: (a) untreated sample and (b) plasma treated sample

Slika 2: Posnetek polimerne površine po 24-urni inkubaciji s celicami HOS: (a) neobdelan vzorec in (b) plazemsko obdelan vzorec

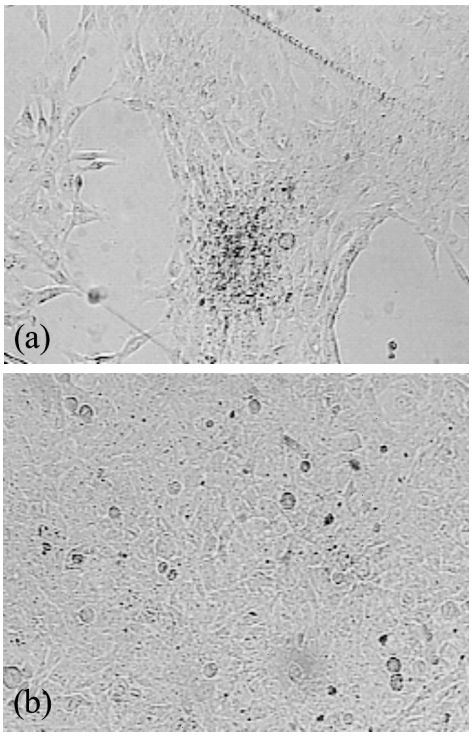

Figure 4: Optical microscopy image of polymer surface after 6 days of incubation with HOS cells for: (a) untreated sample and (b) plasma treated sample

Slika 4: Posnetek polimerne površine po 6-dnevni inkubaciji: (a) neobdelan vzorec in (b) plazemsko obdelan vzorec

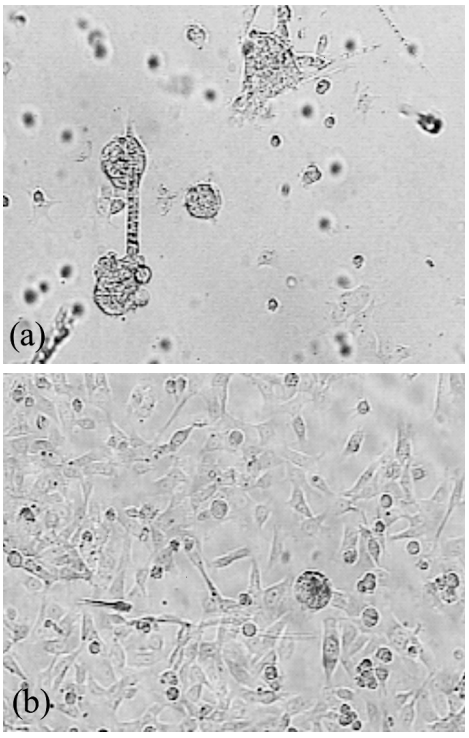

Figure 3: Optical microscopy image of polymer surface after 2 days of incubation with HOS cells for: (a) untreated sample and (b) plasma treated sample

Slika 3: Posnetek polimerne površine po dvodnevni inkubaciji s celicami HOS: (a) neobdelan vzorec in (b) plazemsko obdelan vzorec

cases the surface is not optimal for cell adhesion, since the cells tend to keep together and form agglomerates. The situation is better after 2 days of incubation as shown in **Figure 3b**, where we can observe that cells already have obtained elongated shape meaning that they have adapted to the plasma treated surface. Contrary, we can not observe this for untreated sample (**Figure 3a**) where situation is similar as after 1 day as already shown in **Figure 2a**. After 6 days of incubation (**Figure 4**) we can observe proliferation of HOS cells for both surfaces

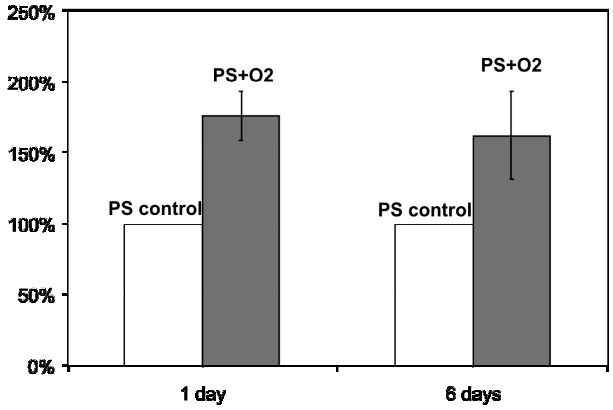

Figure 5: Results of MTT assay – comparison of untreated and plasma treated sample after 1 and 6 days of incubation with HOS cells

Slika 5: Rezultati MTT testa – primerjava neobdelanih in plazemsko obdelanih vzorcev po enodnevni in 6-dnevni inkubaciji s celicami HOS

untreated (**Figure 4a**) and treated one (**Figure 4b**). In the case of plasma treated surface (**Figure 4b**) the cells form dense structure on the surface. This is not observed for the case of untreated sample (**Figure 4a**) where we can still find empty places not covered by cells. These results clearly indicate that untreated surface is not optimal for cell adhesion, since the cells even after long incubation time did not completely adapt to the surface. In the case of plasma treated surface the cells managed to adapt to it after 2 days of incubation and then good proliferation is observed.

Qualitative results presented in **Figures 2–4** are confirmed by a quantitative technique – MTT assay. **Figure 5** summarizes the results of the cell enzyme activity which is an indicator of the cell viability. The histograms presented in **Figure 4** again indicate better proliferation of the HOS cells on plasma treated samples than on untreated ones. But within the experimental error no increase in the number of cells is observed with increasing incubation time meaning that the environment is not so optimal for cell division and multiplication although we can observe some rounded cells in Figure 4b which could be in process of division.

4 CONCLUSION

Polymer samples were treated in CF_4 plasma to make the surface hydrophobic. It is known that hydrophobic surfaces have water repealing character and worse adhesion properties. Therefore, effect of surface hydrophobicity on cell adhesion was studied. We have found that by making surface very hydrophobic we could not reduce adhesion and proliferation of HOS cells to the surface in comparison to the untreated moderately hydrophobic polymer. The cells only needed more time to adapt to the surface. The results clearly indicate that also untreated surface with moderate hydrophobicity is not optimal for cell adhesion, since the cells even after long incubation time did not completely adapt to the surface. In the case of plasma treated surface the cells managed to adapt to it after 2 days of incubation and after 6 days good proliferation is observed since the cells form dense structure on the polymer surface. Therefore, we can conclude that plasma has even little enhanced (and not prevent) proliferation of the cells on the sample treated in CF_4 plasma in comparison to the untreated one.

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