Conclusion

Newly synthesized copolymer of L-lactide and trimethylene carbonate (PLTMC 50:50) promoted faster growth and proliferation of MG 63 osteoblast-like cells in comparison with both standard culture tissue dish and commercially available poly(L-lactide). Surface roughness and topography had no significant effect on the cell adhesion and growth on poly(glycolide-epsilon-caprolactone) from day 1 to 4 after seeding. However, on day 8, the cells reached a higher population density on samples of the surface roughness between 130-180 nm, whereas on the samples of the roughness in micrometers, this density remained similar as on the polystyrene. In addition, a higher surface roughness decreased the size of the cell adhesion area. Modification of the polymeric material by hydroxyapatite deposits improved the cell proliferation.

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VASCULAR SMOOTH MUSCLE CELLS IN CULTURES ON SYNTHETIC POLYMERS WITH ADHESIVE MICRODOMAINS

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Abstract

Polyethylene terephtalate was modified by UV light irradiation produced by a Xe_2^*-excimer lamp for 10, 20 or 30 min in an acetylene atmosphere. For creation of microdomains for selective cell adhesion, a contact nickel mask (apertures of the diameter of 500 μm, centre-to-centre distance 2 mm) was used. The material was then seeded with rat aortic smooth muscle cells (passage 3, 17 000 cells/cm^2). After 1, 3 and 7 days of cultivation, the cells were homogeneously distributed on the samples without any preference of the irradiated microdomains. Moreover, on day 1, the number of initially adhered cells was similar on all tested samples. However, on day 3, the number of cells on the irradiated samples was significantly higher than that on control unmodified PET and increased proportionally to the time of exposure to UV light. On day 7 after seeding, however, the cell number on the unmodified PET exceeded the value on all irradiated samples. In the second set of experiments, polyethylene (PE) was irradiated by Ar^+ ions in order to create the adhesive microdomains (dose 10^{12}-10^{14} ions/cm^2, energy 150 and 15 keV, contact mask with holes of 100 μm diameter and distance 200 μm). The highest selectivity of the adhesion and growth of rat aortic smooth muscle cells (89% of all cells) was found on the microdomains created at the energy of 150 keV and the dose of $3 \cdot 10^{12}$ ions Ar^+. The lowest selectivity (30 %) occurred on samples irradiated with 150 keV Ar^+ ions of the dose of $3 \cdot 10^{14}$ ions/cm^2. Therefore, both methods seem to be suitable for modification of materials with highly hydrophobic surfaces in purpose to increase the cell colonization, for example when constructing bioartificial vascular replacements. The second method can be also used for the creation of domains for a regionally selective adhesion and growth of cells on biomaterials.

Key words: *UV light irradiation, ion implantation, patterned surfaces, adhesive microdomains, vascular smooth muscle cells*

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Introduction

Synthetic materials, such as polyethylene, polystyrene, polyurethane or polyethylene terephtalate, are commonly used in various industrial applications as well as in biology and medicine. They serve not only as growth supports for cell cultures *in vitro*, but also they can be used for a con-

struction of replacements of various tissues or organs, e.g. non-resorbable or semi-resorbable vascular prostheses, artificial heart valves, bone and joint replacements, implants for plastic surgery (for a review see Bacakova *et al.* 1996).

There are two approaches for application of these materials. The first one is using mostly highly hydrophobic or extremely hydrophilic surfaces not allowing the adhesion and growth of cells. This approach is used for creation of bioinert vessel replacements, where the permanent blood flow is necessary and thus the adhesion of thrombocytes or immunocompetent cells is non-desirable in order to prevent the restenosis of the graft (for a review see Bacakova *et al.* 2000). An alternative approach, widely accepted in recent tissue engineering, is creation of surfaces supporting the colonization with cells and good integration of a replacement with the surrounding tissues of the patient´s organism. This concept is used e.g. for construction of bone prostheses, persisting in the patient's organism for many years, and is developed for creation of bioartificial replacements of blood vessels, liver, pancreas and even nervous tissue (for a review see Bacakova *et al.* 2000, 2001).

There are different possibilities of modification the materials´ surfaces so they would be convenient for the cell adhesion. One of them is the creation of microdomains supporting adhesion and conducting the growth of cells (Mikulikova *et al.* 2005). For this purpose, the exposure to ultraviolet (UV) irradiation or to a beam of ions (e.g., oxygen, nitrogen, noble gases or halogens for biological applications) through a contact metallic mask with holes of a needed diameter is often used. For more pronounced changes in physicochemical properties of the microdomain surface, the process can be realised in gas atmosphere, e.g. in acetylene or ammonia.

The goal of these irradiation modifications is to create regions with functional chemical groups containing oxygen or nitrogen, like carbonyl, carboxyl or amine groups, on the material's surface, which increase the surface wettability, support the adsorption of cell adhesion-mediating extracellular matrix proteins and stimulate the cell adhesion and growth (Bacakova *et al.* 2000, 2001, Svorcik *et al.* 2004) In our case we used the UV light for surface modification of polyethylene terephtalate, a material often used for fabrication of blood vessel prosthesis, and argon ion beam for the irradiation of polyethylene, a material also perspective for biomedical use. On the modified materials we evaluated the colonization with smooth muscle cells in cultures isolated from the rat aorta.

Materials and methods

Preparation of the polymer samples

Polyethylene terephtalate (PET), currently used for fabrication of blood vessel prostheses, was exposed to UV light generated by Xe_2^*-excimer lamp (Heraeus-Noblelight, Germany) for 10, 20 and 30 min in an acetylene atmosphere. The irradiation was coming through a contact nickel mask with holes of the diameter of 500 μm and center-to-center distance 2 mm.

Polyethylene (PE) was exposed to a beam of Ar^+ ions (energy 150 keV or 15 keV, dose from $3{\cdot}10^{12}$ to $3{\cdot}10^{14}$). The implantation at room temperature was performed using Varian 350 D ion implanter with ion current density below 50 nA cm^{-2} and under the pressure of $4{\cdot}10^{-4}$ Pa. The irradiation went through a mask with holes of 100 um in diameter and the centre-to-centre distance of 200 μm.

Cells and culture conditions

The modified materials were cut into square samples of 10x10 mm in size, placed into 24-well plates (TPP, Switzerland; well diameter 1.5 cm) and fixed to the well bottom by polyethylene circles (inner diameter 0.35 cm, inner area 0.38465 cm^2). Vascular smooth muscle cells were isolated by explantation method from the aorta of young male rats of the strain Wistar SPF *(Bacakova et al. 2000, 2001)*. In the 3rd to 7th passage, they were seeded on the samples at the density of 30,000 cells/well (i.e., 17,000 cells/cm^2). The cells were cultivated in 1.5 ml Dulbecco´s Modified Eagle Minimum Essential Medium (Sigma, USA) supplemented with 10% foetal bovine serum (Sebak GmbH, Aidenbach, Germany) for 1, 2, 3, 5 or 7 days (temperature of 37°C, 5% of CO_2 in the air). The cells were then fixed by 70% cold ethanol (-20°C) and stained with hematoxylin and eosin. The number of cells inside or outside the irradiated microdomains was evaluated on pictures taken under a microscope Olympus IX 50 using the digital camera Olympus DP 70. As control materials, non-modified polymers, standard tissue culture polystyrene dishes and glass coverslips were used.

Statistics

The results were presented as mean ±SEM (Standard Error of Mean). Statistical significance was evaluated by Students´ t-test for unpaired data. Values $p \leq 0.05$ were considered as significant.

Results and discussion

On the polyethylene terephtalate irradiated with UV light in acetylene atmosphere, the smooth muscle cells did not prefer the created domains (FIG.1), but the cell numbers were significantly different on samples irradiated for different times.

On the first day after seeding, the numbers of cells on the samples of irradiated PET were similar as the values

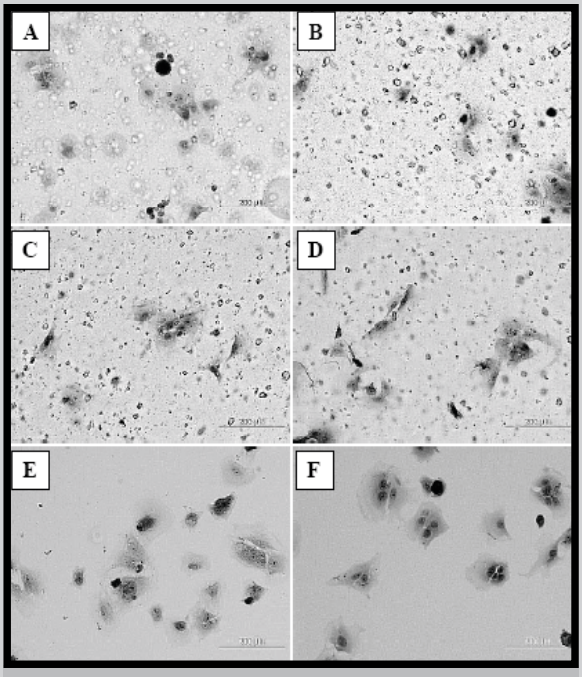

FIG.1. Morphology of rat aortic smooth muscle cells on day 1 after seeding on polyethylene terephtalate (PET). (A) non-modified PET; (B) PET exposed to UV light for 10 min, (C) 20 min, (D) 30 min, (E) polystyrene culture dish, (F) microscopic coverslip. Stained with hematoxylin and eosin, microscope Olympus IX 50, bar=200μm.

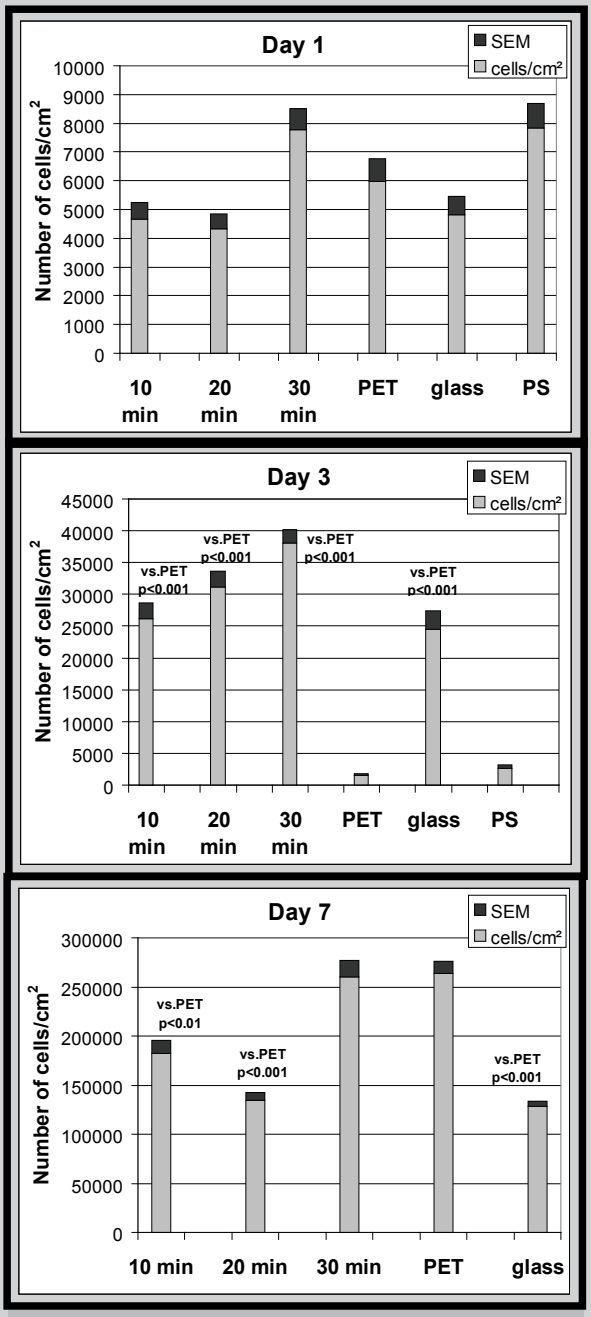

FIG.2. Number of rat aortic smooth muscle cells on polyethylene terephtalate (PET), modified by UV light in acetylene atmosphere for 10, 20 and 30 min, on day 1, 3 and 7 after the setting. Glass = microscopic glass coverslip, PS = polystyrene culture dish. Mean ±SEM from 27 microscopic fields (0.144 mm²) obtained from 3 independent samples for each experimental group. Student's t-test for unpaired data. Statistical significance ($p \leq 0.001$ etc.) was evaluated in comparison with control unmodified PET.

on the control non-modified polymer (FIG.2). However, on day 3 after seeding, the lowest cell population densities were found on a non-modified PET (1516±245 cells/cm²), while on the samples exposed to UV light for 10, 20 or 30 min, the number of cells was 26108±2543, 31196±2395 and 38057±2035 cells/cm², respectively, i.e. the number of the cells increased with the exposure time.

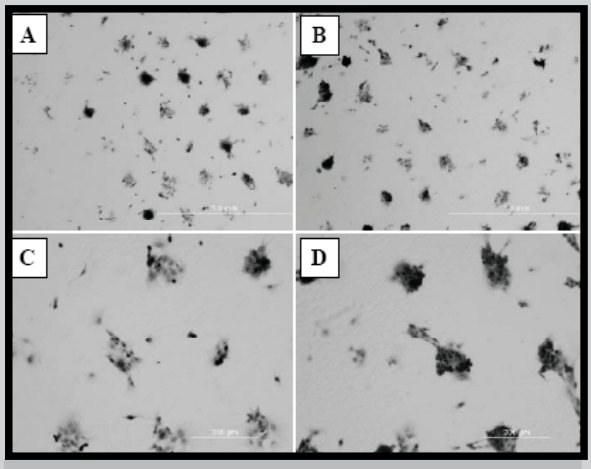

FIG.3. Morphology of rat aortic smooth muscle cells on day 1 after seeding on polyethylene modified by Ar^+ ions (energy of 150 keV, dose $3x10^{12}$, microscope Olympus IX 50. A,B: bar=1mm; C,D: bar=200 µm.

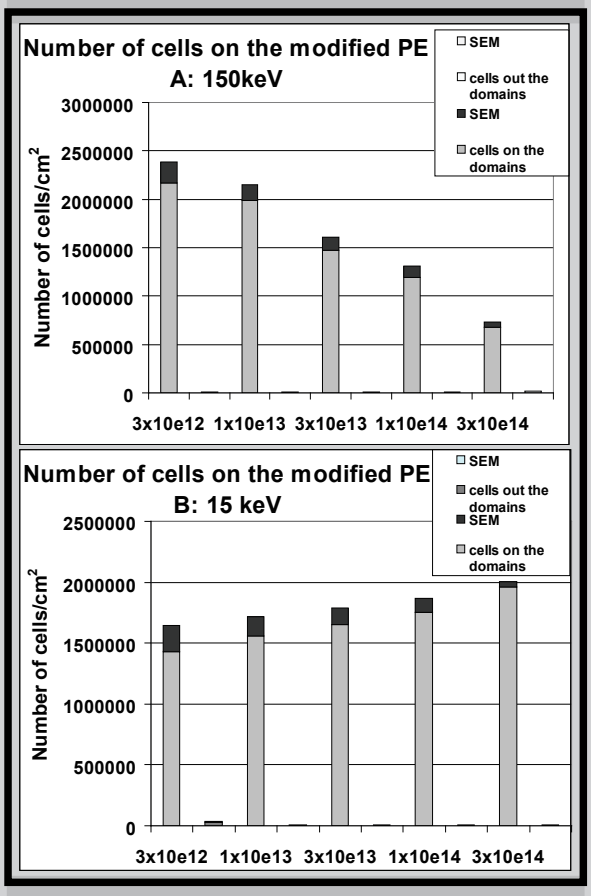

FIG.4. Number of rat aortic smooth muscle cells on polyethylene with adhesive domains created by irradiation with Ar^+ ions of the energy 150 keV (A) or 15 keV (B) and among these domains. Doses from $3x10^{12}$ to $3x10^{14}$ ions/cm². Mean ±SEM from 7 to 15 measurements obtained on 2 independent samples for each experimental group.

Interestingly, on the 7th day, the cell population density on the unmodified polyethylene terephtalate exceeded the value on all examined samples and reached the

value 263587±12218 cells/cm^2, whereas the values on the samples irradiated for 10, 20 and 30 min were only 182705±3202, 134321±8446, 259976±16765 cells/cm^2. This result, as well as the homogeneous distribution of cells on the material surface, was surprising, because the preferential adhesion of cells on the irradiated microdomains was expected. It is possible that on PET in the acetylene atmosphere, modification of the entire material surface occurred, and the whole surface of the sample gained the physical and chemical characteristics originally supposed only for domains, such as the creation of oxygen-containing groups, changes in the surface wettability etc. On the other hand, the UV light-irradiation in acetylene atmosphere leaded to the formation of hydrogenated amorphous carbon (a-C:H) (Bacakova *et al* 2001, Svorcik *et al.* 2004, Kubova *et al*. 2005). Amorphous carbon has been found to be relatively hydrophobic and not well supporting cell adhesion in comparison with conventionally used cell culture plastics (*Bacakova et al. 2001*, *2004*), so that the cell number was not increased on the irradiated microdomains in comparison with surrounding non-modified polymer.

In all groups of ion-irradiated polyethylene, i.e., at all doses and energies of ions, the cells were growing preferably on the modified domains (FIG.3), although they were found also among them. The selectivity of the cell growth on the domains differed in samples irradiated by ions of different energies and doses.

At the energy of 150 keV, the highest selectivity was observed at a relatively low dose of 10^{13} ions/cm^2. On these samples, 80±0.04% of cells adhered to the domains. The cells on these domains reached the density of 1986325±160223 cells/cm^2, while outside them it was only 4161±1239 cells/cm^2. At the energy 15 keV, the highest selectivity was noticed at the dose of $3\cdot10^{14}$ Ar$^+$/cm^2, where 75.8±0.06% of cells adhered to the domains.

The lowest selectivity at the energy of 150 keV was registered on polymers modified by the highest doses of $3\cdot10^{14}$ ions/cm^2, where only 22.80±0.03% of cells adhered to the domains. At the energy of 15 keV, the lowest selectivity was registered at the lowest doses of $3\cdot10^{12}$ ions/cm^2 (62.4±0.07% of cells on the domains). The lowest doses were probably less sufficient to increase the polymer attractiveness for cell colonization (FIG.4)

Therefore, the cells preferred the domains, on which oxygen-containing functional groups were created by the influence of Ar$^+$ ion irradiation. These groups are known to increase the surface wettability and improve the adhesion and subsequent growth of cells (*Bacakova et al. 1996, 2000, 2001*). Differences between the tested samples in the selectivity of cell adhesion on ion-irradiated domains were caused by the doses of Ar$^+$ ions. At the energy of 150 keV, higher doses of Ar$^+$ might lead to the formation of amorphous carbon, which is known not to increase the material's attractiveness for cells, and thus these domains were less convenient cells colonization (*Bacakova et al. 2001, 2004, Rockova–Hlavackova et al. 2004*).

In case of the ion energy of 15 keV, i.e., a value one order lower, it seems that a lower ion doses were insufficient to create the adhesive microdomains. On the contrary, higher ions doses were better for this purpose and the cells preferred the domains created this way.

Conclusion

The method of irradiation with Ar$^+$ ions appears as convenient for surface modification of polyethylene and other relatively hydrophobic synthetic polymers. Vascular smooth muscle cells preferred the ion irradiation-created microdomains for their growth. However, it is very important to choose appropriate combination of the ion dose and energy, which will be the goal of our subsequent researches.

On the polyethylene terephtalate exposed to UV light in acetylene atmosphere, we obtained a homogeneous colonization of the material surface with vascular smooth muscle cells instead the expected selective adhesion and growth of these cells on the modified microdomains. Nevertheless, on day 3 after seeding, the cell number on irradiated samples increased with the time of exposure to UV light, although on day 7, these differences disappeared. Therefore, these events need further investigation.

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HUMAN ENDOTHELIUM ON VASCULAR PROSTHESES MODIFIED BY EXTRACELLULAR MATRIX PROTEINS IN A FLOW EXPERIMENT

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