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Cell adhesion on modified polyethylene

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Polyethylene (PE) was irradiated with 10 and 63 keV Ar^+ ions to fluences of 1×10^{17} to 3×10^{19} m^{-2} and then it was grafted with aminoacid (alanine). The changes of surface polarity, electrical conductivity, and oxygen concentration were examined on pristine, as-irradiated, and irradiated-grafted PE. The *in vitro* adhesion of mice fibroblasts on the modified PE was evaluated 24 hours after inoculation. It was proved that for the PE irradiated at 10 keV ion energy, the presence of chemically bound alanine increases cell adhesion and its homogenity. For PE irradiated with 63 keV ions, however, the alanine grafting leads to a reduction of the number of adhering cells. It was found that a rising surface polarity increases cell adhesion, but when its value is too high the cell adhesion starts to decrease. No correlation between electrical conductivity and cell adhesion was observed. In general, higher cell adhesion is observed on modified PE in comparison with pristine one.

1. Introduction

The use of artificial materials in medicine as substitutes of bones, joints, eye lenses, heart valves, arteries and skin, damaged by pathological or degenerative processes, is ever increasing. Artificial materials and components may substitute, stimulate or monitor some living functions (cardio-stimulators, hemodialysis membranes, biosensors, etc.). At the same time, further investigation of suitable materials is under way with the aim to develop new ones with better biofunctionality and biocompatibility.

Different polymers, including polypropylene, polyethylene, polytetrafluorethylene, and polyesthers are applied for sutures, soft tissue augmentation, and vascular prostheses. Silicones and polymethylmetacrylate are used for intraocular lenses. Membranes of regenerated cellulose are widely used as hemodialysis membranes. Several attempts have been made to replace cellulose with alternative polymers (e.g. polyacrylonitride, polycarbonate and polysulfone [1]) with better blood compatibility.

Interaction of cells with polymers is a very complicated process comprising adsorption of bioactive peptides and adhesion of cells on a polymer surface [2]. It is supposed that these processes are significantly affected by surface properties of the polymers, such as surface morphology, polarity, wettability, electrical conductivity, etc. [3]. One of several possibilities how to change these properties is irradiation of polymer with energetic ions [4–6]. The irradiation results in a cleavage of chemical bonds, creation of free radicals [7], production of excessive double bonds [8] and subsequent oxidation of irradiated material. For higher ion fluences, a polymer carbonization is observed [9].

It was shown earlier that the ion irradiation of a polymer enhances adhesion and growth of living cells on its surface [10, 11]. Increased biocompatibility of irradiated polymers was observed for smooth muscle cells [12, 13] and keratinocytes [14]. The radicals, unsaturated bonds and other species with enhanced chemical reactivity in irradiated polymer are the sites on which some other agents may be fixed. Grafting of irradiated

polymers with some suitable chemical agents may enhance the polymer biocompatibility substantially, e.g. irradiated polyethylene grafted with acrylic acid was shown to exhibit increased cell adhesion in an *in vitro* experiment [15].

In this study, the ion-irradiated polyethylene is grafted with amino acid and the adhesion of fibroblasts on the modified polymer surface is examined. Correlations between physico-chemical properties of modified polymer and cell adhesion are discussed.

2. Materials and methods

2.1. Materials and modification procedures

Oriented polyethylene (PE) foils, 10 μm thick with molecular weight of 1.8×10^5 and density of 945 $kg \cdot m^{-3}$, were irradiated with 10 and 63 keV Ar^+ ions to the fluences from 1×10^{17} to 3×10^{19} m^{-2} using a Varian 350D ion implanter. The ion beam current density was below 50 $nAcm^{-2}$ and the pressure in the implanter target chamber was 10^{-3}–10^{-4} Pa. For easier manipulation and enhancement of optical changes, the foils were irradiated from both sides. A part of the irradiated specimens was exposed to 2 wt% solution of alanine (CH_3-CH(NH_2)-COOH) in water at room temperature for 12 hours. Then the specimens were submerged into distilled water at room temperature for 2 hours to remove excessive, unbound alanine. Finally, the specimens were dried for 2 hours at 60°C in a vertical position.

2.2. Experimental techniques

Some of the relevant physico-chemical properties of modified PE were determined using standard techniques. The contact angle characterizing surface polarity was determined from goniometric measurements [12]. The concentration of conjugated double bonds, created on PE molecular chain by ion irradiation, was determined by UV-VIS spectroscopy in a 200–800 nm wave interval using a Perkin-Elmer device. The sheet resistance was measured with a Keithley 487 device in a vacuum of 10^{-1}Pa using a standard two-point technique. The concentration and depth distribution of oxygen incorporated in as-irradiated and irradiated plus alanine doped PE were determined by standard Rutherford Back-Scattering method (RBS) with 2 MeV α-particles (120° laboratory scattering angle).

Adhesion of 3T3 rat fibroblasts on modified PE was studied *in vitro*. The cells were seeded in density of 543 cells mm^{-2} on a 38.5 mm^{-2} sample of PE and cultivated in H-MEMd (Inst. of Macromolecular Genetics, AS CR, Prague) with 10% of bovine serum (ZVOS Hustopece, CZ) at 37°C, 3.3% of CO_2 for 24 hours. Then the cells were harvested using 0.25% solution of trypsine (Sigma, Prague) in PBS, resuspended and mixed 1 : 2 with 0.5% solution of Trypan Blue (Sigma, Prague) in PBS. The number of cells was determined using Burker chamber. Simultaneously some of the polymer samples were fixed with methanol and the growth of 3T3 fobroblasts was visualized by May Grunwald and Giemsa Romanowski solutions. The pictures of cells adhered on unmodified and modified PE were obtained using an optical microscope at magnification of 160 times.

3. Results and discussion

Length and concentration of conjugated double bonds, which are products of PE degradation, can qualitatively be determined by UV-VIS spectroscopy [8]. UV-VIS spectra from pristine PE and PE irradiated with 10 and 63 keV Ar^+ ions and alanine-doped are shown in Fig. 1A and B, respectively. It can be seen that the irradiation results in an increased concentration and conjugation length of double bonds, both being an increasing function of the ion energy and fluence. For the PE irradiated with 10 keV Ar^+ ions, the alanine doping leads to a decrease of conjugated double bonds content. This finding indicates that the alanine preferentially adds onto double bonds in this case. The doping of PE irradiated with 63 keV Ar^+ ions to higher fluences (Fig. 1B), however, does not lead to a measurable decrease of the absorbance (compare with Fig. 1A). This effect can be explained by a supposition that the alanine is preferentially captured by free radicals [16], the concentration of which is higher in PE irradiated with more energetic ions [7]. Another reason for the observed difference may be the fact that the alanine penetration into thicker modified layer produced by 63 keV Ar^+ ions is more difficult.

The presence of conjugated double bonds and, for higher ion fluences, of carbonized regions in PE surface layer modified by the ion irradiation may affect the polymer electrical conductivity. The measured dependence of the sheet resistance on the ion energy and fluence for as-irradiated and irradiated plus alanine doped PE specimens is shown in Table I. Only the data for the specimens irradiated to the fluences above 1×10^{19} m^{-2} are given, for lower fluences the resistances were

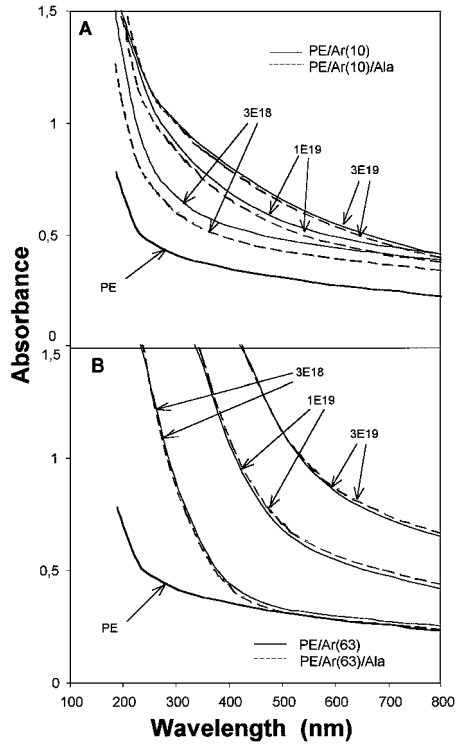

Figure 1 UV-VIS spectra of pristine PE and PE irradiated with 10 (A) and 63 keV (B) Ar^+ ions to the fluences indicated and subsequently alanine-grafted. The data were obtained as a mean from 3 independent measurements and the SD was about ±5%.

TABLE I The dependence of the sheet resistance (Ω) on the fluence of 10 keV and 63 keV Ar^+ ions for as-irradiated (I) and irradiated and subsequently alanine-grafted PE (G)

Ion fluence (m^{-2})	Energy (10 keV)		Energy (63 keV)	
	I	G	I	G
1×10^{19}	3.0×10^{15}	2.3×10^{16}	4.1×10^{15}	4.0×10^{15}
3×10^{19}	2.2×10^{12}	2.4×10^{13}	1.5×10^{11}	1.2×10^{11}

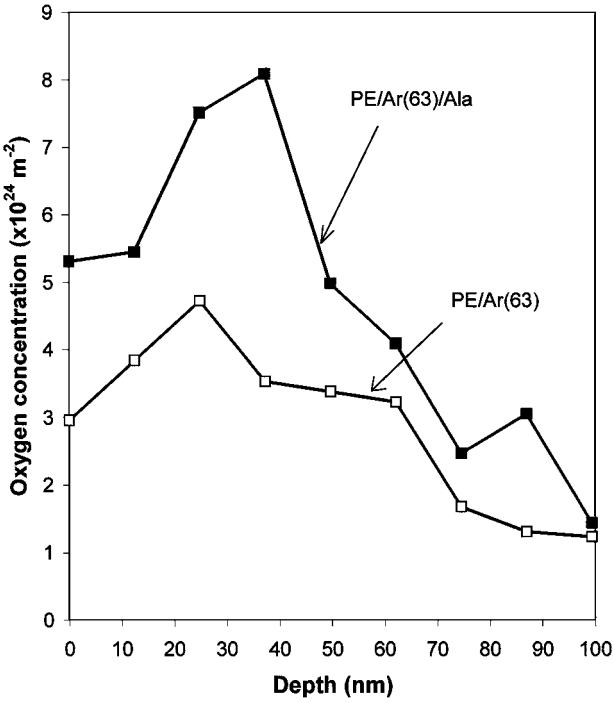

Figure 2 Concentration depth profile of oxygen from the PE irradiated with 63 keV Ar^+ ions to the fluence of $1 \times 10^{18} m^{-2}$. The profiles for as-irradiated and irradiated and subsequently alanine grafted PE are shown. Statistical errors in individual points are ±10%.

higher than 5×10^{16} Ω . The resistance is a decreasing function of the ion fluence. Alanine grafting of the PE specimens irradiated with 10 keV Ar^+ ions leads to a increase resistance which is explained by alanine addition on double bonds. No such effect, however, is observed for PE specimens irradiated with 63 keV Ar^+ ions so that the resistance measurements are in reasonable agreement with the results from UV-VIS spectroscopy.

The concentration and the depth profile of alanine incorporated in the PE surface layer are important for the characterization of the modified layer structure. It is supposed that the presence of alanine leads to local oxygen excess which can be observed in RBS spectra. The situation is somewhat complicated by the fact that even as-implanted PE is oxidized due to incorporation of oxygen from ambient atmosphere during the ion irradiation. The oxygen depth profiles measured on as-irradiated and irradiated plus alanine doped PE specimens are compared in Fig. 2 and the measured oxygen concentrations are summarized in Table II. The oxidation of as-irradiated specimen is clearly visible. Subsequent alanine grafting leads to an oxygen concentration increase in whole depth interval examined, with most pronounced increase taking place in the region of maximum polymer degradation. The grafting with

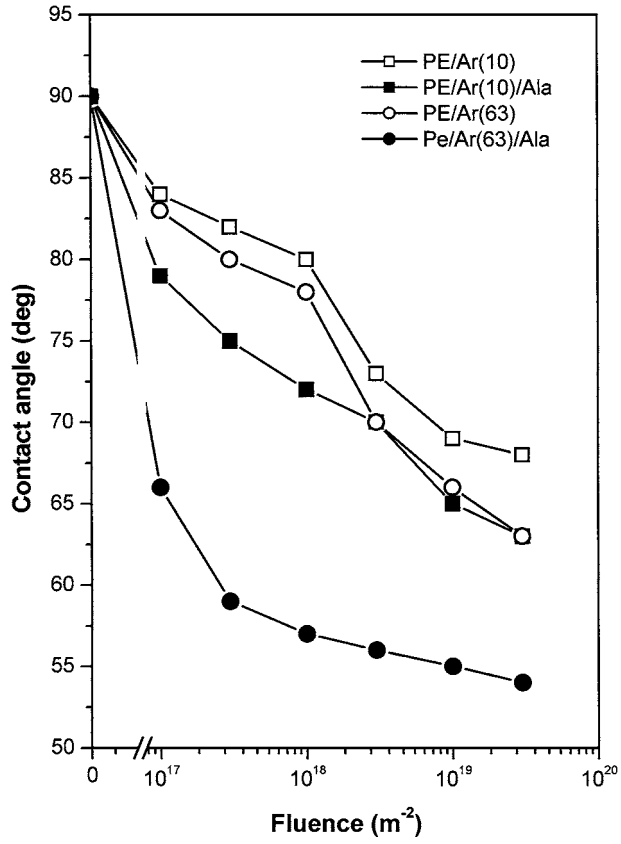

Figure 3 The dependence of the contact angle on the ion fluence measured on PE irradiated with 10 and 63 keV Ar^+ ions and subsequently alanine-grafted. The data were obtained as an mean from 5 independent measurements and the SD was about ±5%.

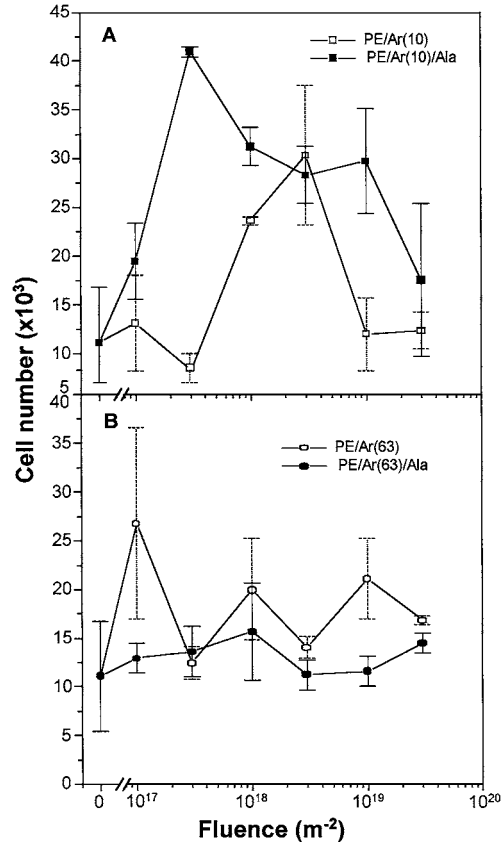

Figure 4 The dependence of the number of adhering cells on the ion fluence measured on PE irradiated with 10 (A) and 63 keV (B) Ar^+ ions and subsequently alanine-grafted.

aminoacids in whole specimen layer modified by the irradiation was observed (FTIR, UV-VIS and RBS spectroscopic technique [17]). The observed oxygen excess proves the presence of alanine with an oxygen- rich – COO- group. From Table II one can see that the oxygen concentration is an increasing function of the ion energy and fluence. For the ion fluences below 1×10^{18} m^{-2} the oxygen content was below the present RBS sensitivity.

The presence of oxidized structures on the PE surface should affect the surface polarity, which in turn may affect the polymer biocompatibility [18]. The polarity is characterized by the size of the contact angle, whose dependence on the ion fluence for irradiated and subsequently alanine grafted PE is shown in Fig. 3. It is seen that the contact angle decreases with increasing ion energy and fluence. The alanine grafting leads to an additional contact angle decrease. Most pronounced decrease is observed on specimens irradiated with higher energy ions. By comparing these results with the data on oxygen content (Fig. 2 and Table II) one can see that the surface polarity, measured as contact angle, is correlated with the concentration of oxidized structures on the PE surface.

TABLE II The dependence of the oxygen concentration ($\times 10^{19} m^{-2}$) on the fluence of 10 keV and 63 keV Ar^+ ions for as-irradiated (I) and irradiated and subsequently alanine-grafted PE (G)

Ion fluence (m^{-2})	Energy (10 keV)		Energy (63 keV)	
	I	G	I	G
1×10^{18}	0.9	1.2	4.5	6.5
1×10^{19}	70	110	130	140

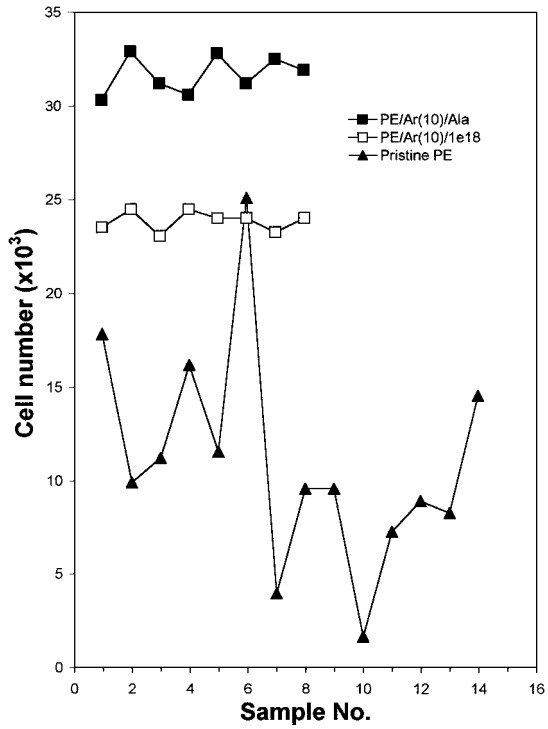

Figure 5 The number of adhering cells for pristine PE and PE irradiated with 10 keV Ar^+ ions to the fluence of $1 \times 10^{18} m^{-2}$ and alanine-grafted.

The cell adhesion on pristine PE and the PE modified by the ion irradiation and alanine grafting is shown in Fig. 4 as a function of the ion fluence. For the PE irradiated with 10 keV Ar^+ ions the dependence on the ion fluence exhibits a clear maximum (Fig. 4A). Analogous dependence was reported for adhesion of smooth muscle cells on polystyrene irradiated with F^+ ions [12]. For the PE irradiated with 63 keV Ar^+ ions the number of adhering cells does not depend on the ion fluence within the experimental errors (Fig. 4B). For the specimens irradiated with 10 keV Ar^+ ions to low fluences the alanine grafting leads to pronounced increase of the number of adhering cells. For the ion fluences above $3 \times 10^{17} m^{-2}$, however, the number of adhering cells decreases linearly (Fig. 4A). A comparison

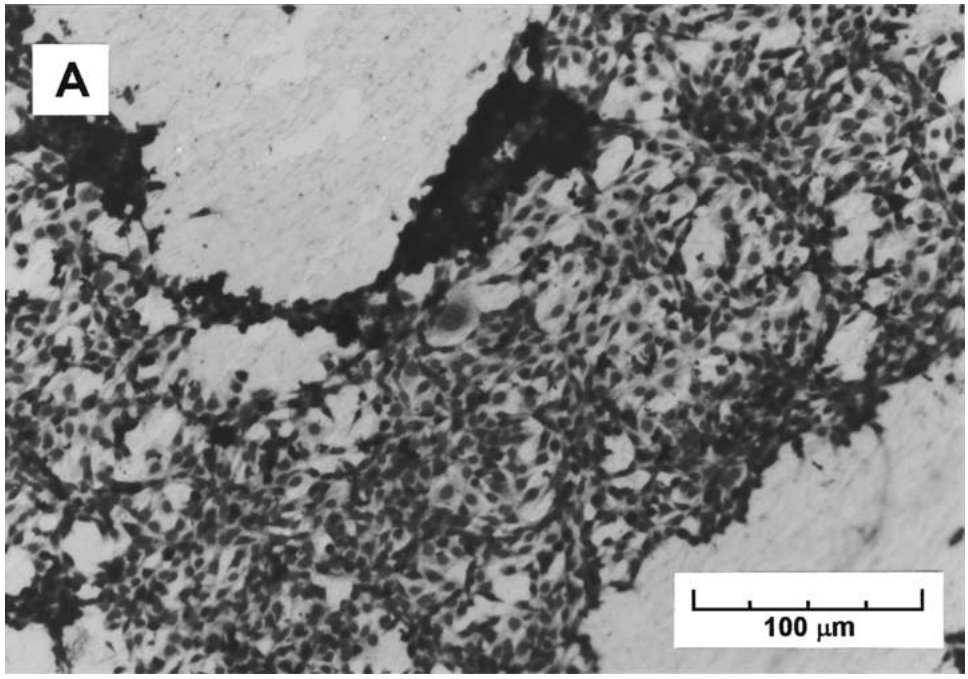

Figure 6 The photographs of adhering cells on pristine PE (A) and PE irradiated with 10 keV Ar^+ ions to the fluence of $1 \times 10^{18} cm^{-2}$ (B) (*Continued.*)

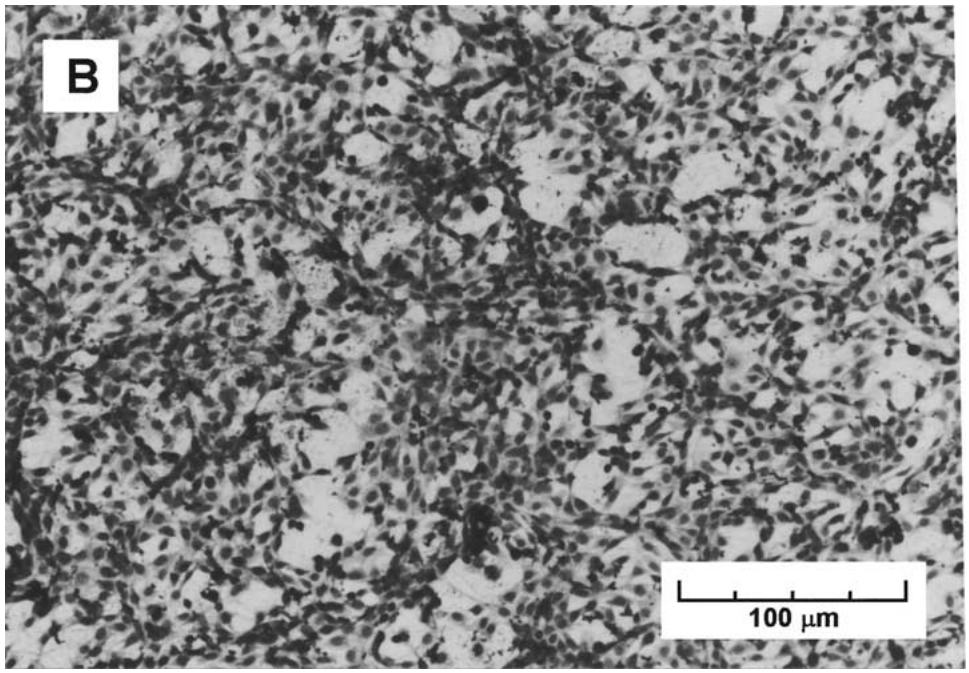

Figure 6 (*Continued*.)

of the present results on the surface polarity (Fig. 3) with those on cell adhesion (Fig. 4A) shows that the high surface polarity on the PE specimens irradiated to high fluences and alanine grafted leads to a decline in the cell adhesion. The same effects are observed for the PE specimens irradiated with 63 keV Ar^+ ions (Fig. 4B vs. Fig. 3). These results are in agreement with those published earlier, i.e. that mild increase of the polymer polarity leads to an increase of the cell adhesion [12], but the cell adhesion declines on strongly hydrophilic material [18]. No correlation between the cell adhesion and the specimen electrical conductivity was observed (compare Fig. 4 with Table I).

The above discussed results are further illustrated in Figs 5 and 6. Fig. 5 shows the number of adhering cells on pristine PE and PE irradiated with 10 keV Ar^+ ions to the fluence of $1 \times 10^{18} m^{-2}$ and subsequently alanine grafted. The above fluence was chosen since the results obtained for this fluence exhibit lowest statistical scattering. It can be seen that the ion irradiation increases the number and especially the homogenity of adhered cells and that the subsequent alanine grafting results in an additional increase of the cell number without a worsening of their homogenity. The large scatter of cell number for pristine PE could be explained by local inhomogeneities of pristine PE which are eliminated by subsequent ion irradiation. These conclusions are confirmed by optical microscope pictures shown in Fig. 6. Microscope examination clearly shows that the PE modifications by ion irradiation and alanine grafting increase the number of adhering cells as well as their homogenity.

4. Conclusion

The results of this study can be summarized as follows:

- the ion irradiation results in an increase of surface polarity and electrical conductivity of PE,
- the radiation damages on the polymer chain, such as free radicals and excessive double bonds are the sites on which the alanine is grafted. The alanine is incorporated in whole layer which was modified by the irradiation,
- the alanine grafting increases surface polarity and decreases electrical conductivity of PE,
- the number of adhering cells depend on the ion energy. At lower ion energy, the cell adhesion depends on the ion fluence and the dependence exhibits pronounced maximum,
- the effects of alanine grafting depends strongly on the ion energy and fluence. For PE irradiated with 10 keV Ar^+ ions with low fluences, the subsequent grafting leads to strong increase of cell adhesion. For PE irradiated with 63 keV Ar^+ ions, however, the grafting results in cell adhesion decline which may be due to too high surface polarity,
- cell adhesion does not depend on the electrical conductivity,
- ion irradiation increases the number and homogeneity of adhering cells. Additional increase may be achieved by alanine grafting.

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