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Structure and biocompatibility of ion beam modified polyethylene

V. ŠVORČÍK, V. RYBKA
Department of Solid State Engineering, Institute of Chemical Technology, 166 28 Prague 6, Czech Republic

V. HNATOWICZ
Institute of Nuclear Physics, Academy of Sciences of Czech Republic, 250 68 Řež, Czech Republic

K. SMETANA, JR
Department of Anatomy, First Faculty of Medicine, Charles University, 128 00 Prague 2, Czech Republic

Structural changes of polyethylene (PE), induced by irradiation with 40 keV Ar^+ ions at a fluence of 1×10^{12}–1×10^{15} cm^{-2}, are characterized by different experimental methods and physical parameters of the modified PE are related to its biocompatibility. Production of oxidized structures and conjugated double bonds in the PE surface layer modified by the ion irradiation was proved using IR, UV-VIS spectroscopies and a Rutherford backscattering technique. The fusion of macrophages onto implants made of as-irradiated and chemically doped PE was studied *in vivo*. It was found that the free surface energy is not a decisive factor affecting the non-self-recognition of the modified PE by macrophages. The fusion of macrophages, however, was found to be different on the as-irradiated specimens and the specimens additionally doped with acrylic acid.

1. Introduction

Irradiation with energetic ions leads to dramatic modification of polymer surfaces. The thickness of the modified layer and the degree of structural changes depend on the ion mass and energy. Ion irradiation gives rise to splitting of chemical bonds [1], creation of free radicals and production of light, volatile fragments (H_2, CH_4, etc) which eventually may escape [2]. Some free radicals recombine, via crosslinking [3], or annihilate due to oxidation in implanter ambient atmosphere [4]. In polymers with no oxygen in the pristine macromolecule, ion irradiation leads to an increase of the free surface energy and in turn to an enhancement in surface polarity and wettability [5]. Conjugated double bonds and carbon-enriched islands created by high fluence ion irradiation are responsible for the elevated electrical conductivity of modified polymers [6]. Several *in vitro* studies have shown that ion irradiation and the induced structural changes affect the biocompatibility of polymers [7, 8].

The interaction of cells with polymers is an extremely complex process comprising adsorption of bioactive proteins from tissue fluid onto polymer and cell adhesion to the polymer surface [9]. Polymer surface properties such as surface free energy, polarity and wettability are expected to strongly influence these processes [10]. The ability of cells to adhere depends not only on the quantity of adsorbed bioactive proteins but also on their conformation. For example, hydrophobic polystyrene of bactriological grade shows significantly higher adsorption of one of the principal adhesive proteins—fibronectin—than polystyrene of tissue culture grade with moderate wettability. However, cell adhesion is much higher to the latter substrate [9]. On the other hand, highly hydrophilic polymers of poly(2-hydroxyethylmethacrylate) type are known to be non-adhesive substrates under *in vitro* conditions [10].

One of the most important aspects of *in vivo* biocompatibility is non-self-recognition of polymers since an extensive foreign body reaction represents a risk of implant failure and the induction of autoimflammatory disease in the implant host [11]. It has been demonstrated on highly hydrophilic materials that the functional chemical groups of polymers and copolymers significantly affect the extent of foreign body reaction in the rat [12–15], with considerable inhibitory effect of $-COO^-$ anions on macrophage adhesion to hydrogel and fusion into foreign body giant multinucleate cells [12–14]. Even if the $-COO^-$ anions are concentrated in a thin surface layer of poly(2-hydroxyethylmethacrylate) implants, their inhibitory effect on macrophage adhesion and fusion is preserved [16].

The properties of polyethylene modified by irradiation with 40 keV Ar^+ ions and by the subsequent

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introduction of carboxyle groups into the polymer side chain have been studied by different techniques. The biocompatibility of modified polyethylene has also been examined *in vivo* in relation to its physical properties.

2. Material and methods

2.1. Material and modification procedures

Experiments were performed on commercially available polyethylene (PE) with a density 0.945 g cm^{-3}. The 15 μm thick PE foils were irradiated at room temperature at fluences from 1×10^{12}–1×10^{15} cm^{-2} from both sides in order to amplify optical changes. The ion beam current density was kept below 50 nA cm^{-2} to prevent specimen overheating and thermal degradation. Immediately after irradiation, a part of the irradiated PE was exposed at room temperature for 12 h to 3 vol % water solution of acryl acid (CH_2 =CH–COOH) in order to introduce carboxyle group into PE side chains. Water was chosen as solvent in order to prevent eventual dissolution of the irradiated PE. After doping the specimens were thoroughly rinsed in water.

2.2. Experimental techniques

UV-VIS spectra were measured using a Perkin-Elmer spectrometer and IR spectrometry was carried out by means of FT-IR spectrometer Nicolet 740. Differential IR spectra obtained by substracting the spectra of as-irradiated samples (PE/Ar) from those of irradiated and chemically treated ones (PE/Ar/COOH) are also presented. The oxygen content and its depth profiles were determined by means of a Rutherford backscattering technique (RBS) with 2 MeV He ions and using procedures described earlier [5]. Polar component of the surface Helmholz energy, γ_s^p, characterizing polymer surface polarity, was determined by measuring a contact angle with a reflection goniometer [5]. The surface resistivity, R_s, was measured at room temperature, under the pressure of 10^{-1} Pa using a two-point method and Keithley 487 device.

For the biocompatibility study, the 5×5 mm strips of modified PE were subcutancously implanted into laboratory rats 250–350 g in weight of both sexes of Lewis strain from our breeding colony under sterile conditions as described in detail in [17, 18]. Three rats were used for each PE sample, modified under different conditions (various ion fluences and chemical treatments). The polymer samples were removed 9 days after surgery. This interval represents the time of maximal extent of foreign body reaction in the subcutaneous region in the rat [9]. The samples were fixed with 4% paraformaldehyde, stained as a total specimen with hematoxyline. The extent of the foreign body reaction was estimated histologically according to the cytological appearance of cells colonizing the implant surface. The fusion of macrophages was evaluated by the calculation of fusion index FI, which in fact is the number of nuclei in multinucleate cells versus total number of nuclei in multinucleate cells and mononuclear macrophages. The differences in FI for different implants were evaluated using an unpaired Students *t*-test.

3. Results and discussion

As mentioned in the introduction, the biocompatibility of polymers is affected by surface polarity, wettability and carbonization, which can be changed by ion irradiation and which are closely related to the structure of the modified polymer. For this reason the structural changes on the PE macromolecular chain initiated by the irradiation were studied using different techniques in order to reveal possible relationships to the biocompatibility of the system examined.

It is well known [7] that the electrical conductivity of polymers is related to the concentration of conjugated double bonds, the presence of which can be determined by UV-VIS spectroscopy [19]. The UV-VIS spectra measured on the as-irradiated PE samples and the PE samples additionally doped with acrylic acid are compared in Fig. 1. It is seen that the irradiation with Ar^+ ions produces conjugated double bonds, the concentration and conjugation length of which is an increasing function of the ion fluence. The subsequent chemical doping leads to a significant decrease of the concentration and conjugation length of the double bonds (Fig. 1), in accord with the well-known fact that acrylic acid can add onto double bonds or react with radicals created by the ion irradiation, both processes leading to incorporation of hydrophilic carboxyle groups on the PE side chain [20].

The changes in IR spectra induced by irradiation at different fluences are documented in Fig. 2, where the region of 1630–1820 cm^{-1} is depicted. One can see that irradiation at fluences above 1×10^{13} cm^{-2} leads to an absorbance increase in the region 1710–1765 cm^{-1} which is related to the presence of oxidized structures such as carboxyle, carbonyle and esther groups. It is seen that the concentration of these structures increases with increasing ion fluence up to a fluence of 5×10^{14} cm^{-2} and then it declines. The decline was confirmed more directly in RBS measurement (see

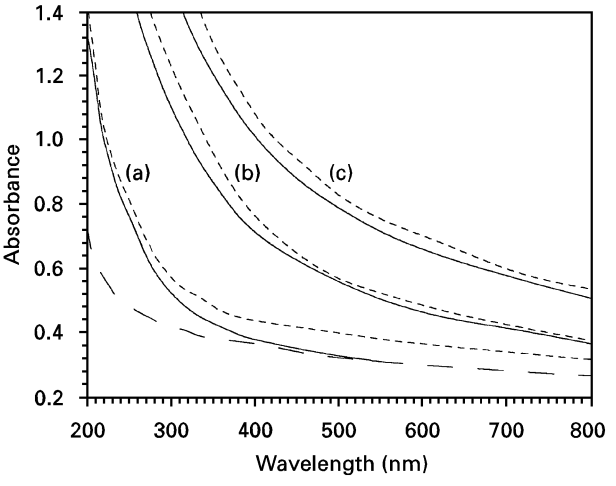

Figure 1 UV-VIS spectra of the pristine PE (– – –), PE irradiated with 40 keV Ar^+ ions at different fluences (PE/Ar,- - -) and PE irradiated and subsequently doped with acrylic acid (PE/Ar/COOH,——). Ion fluences in cm^{-2}: (a) 1×10^{14}; (b) 5×10^{14}; (c) 1×10^{15}.

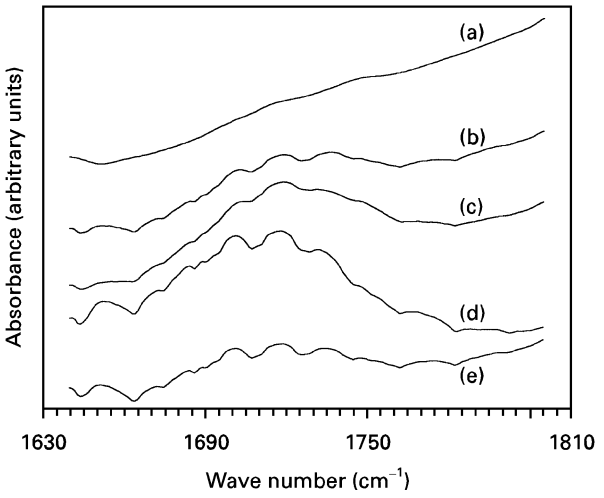

Figure 2 Part of the IR spectrum of the PE specimens irradiated with 40 keV Ar^+ ions at different fluences in cm^{-2}: (a) 1×10^{13}; (b) 5×10^{13}; (c) 1×10^{14}; (d) 5×10^{14}; (a) 1×10^{15}.

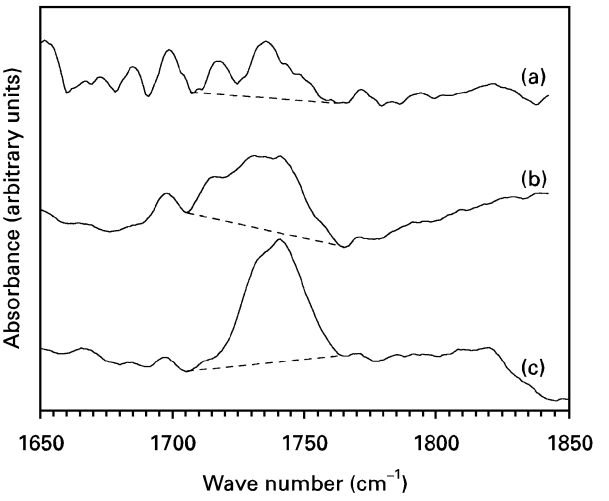

Figure 3 Differential IR spectra obtained by substracting the IR spectra of as-irradiated PE (PE/Ar) from those of the PE additionally doped with acrylic acid (PE/Ar/COOH) at ion fluence of: (a) 1×10^{14}; (b) 5×10^{14}; (c) 1×10^{15} cm^{-1}.

TABLE I Oxygen areal density (N_0) in the surface layer 200 nm thick measured as a function of the ion fluence on the as-irradiated PE (PE/Ar) and the PE iradiated and subsequently doped with acrylic acid (PE/Ar/COOH)

Fluence Ar^+ ions	N_0 (1×10^{16} cm^{-2})	
(cm^{-2})	PE/Ar	PE/Ar/COOH
1×10^{13}	1.1	1.8
5×10^{13}	1.3	2.0
1×10^{14}	3.2	4.4
5×10^{14}	4.8	6.5
1×10^{15}	3.6	5.5

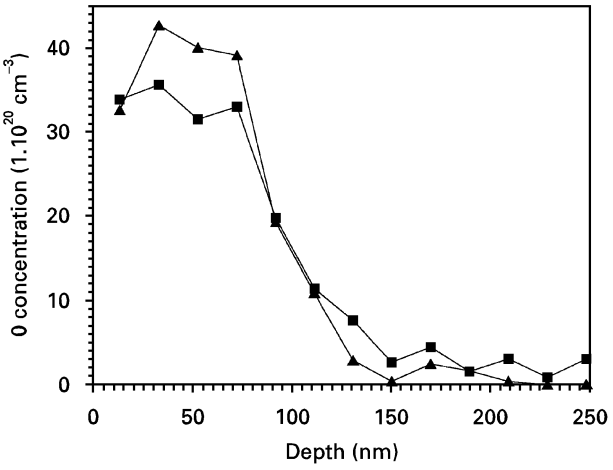

Figure 4 The concentration depth profile of oxygen incorporated in the PE irradiated at a fluence of 5×10^{14} cm^{-2} (PE/Ar, –■–) and in the PE irradiated at the same fluence and doped with acrylic acid (PE/Ar/COOH, –▲–).

Table I). This leads to the conclusion that at an ion fluence of 1×10^{15} cm^{-2}, the oxidized structures created in the early stages of ion irradiation are also degraded, i.e. deoxygenation processes prevail over oxidation processes [4].

The electrical sheet resistivity, R_s, measured on the PE samples irradiated at fluences below 5×10^{14} cm^{-2} was found to be practically unchanged and equal to 6×10^{14} Ω, typical for pristine PE. At a fluence of 1×10^{15} cm^{-2} R_s decreases to 3×10 Ω probably due to progressive carbonization of the PE surface, which is also seen on the RBS spectra.

The effects of chemical treatment in acrylic acid are seen in Fig. 3, where the difference between IR spectra from the chemically treated PE samples and as-irradiated samples are shown for different ion fluences . A significant increase of the absorbance in the region 1710–1765 cm^{-1}, attributed to the presence of oxidized structures, is clearly observed. It may be therefore concluded that the acrylic acid is chemically bound to the PE macromolecule or to its fragments produced by the preceding ion irradiation. The elevated oxygen concentration in chemically treated specimens was proved also by RBS measurements (see also Table I and Fig. 4).

From the point of view of the surface properties of the modified PE, the depth of oxygen penetration is of interest. In Fig. 4 the concentration depth profiles of incorporated oxygen, measured by RBS technique, in the PE sample irradiated at a fluence of 5×10^{14} cm^{-2} and the sample subsequently doped with acrylic acid are compared. In both samples the oxygen depth profile has a broad concentration plateau from the sample surface to a depth of about 100 nm, which is followed by an abrupt concentration decrease. A mild concentration decrease near the sample surface is an experimental artefact due to the limited depth resolution of RBS technique. It may be concluded that the substantial oxygen portion is incorporated within the surface layer about 100 nm thick and that the doping with acrylic acid results in about 25% increase of the oxygen concentration in the near surface region.

The present results show that irradiation with 40 keV Ar^+ ions leads to the production of oxidized (i.e. polar) groups in the PE surface layer. Since pristine PE is a non-polar polymer, one can expect

hydrophilic behaviour of the ion irradiated and chemically treated PE. The measured dependence of the free surface energy γ_s^p on the irradiation fluence for the as-irradiated and chemically treated specimens is shown in Fig. 5. The free surface energy is nearly constant up to a fluence of 5×10^{13} cm^{-2}. For higher fluences , free surface energy increases rapidly and for the highest fluence of 1×10^{15} cm^{-2} it is about four times higher than for the pristine, non-irradiated PE. It is seen, at the same time, that treatment with acrylic acid further increases the free surface energy regardless of the irradiation fluence. It is concluded that irradiation to higher fluences leads to significant increase of the PE free surface energy, i.e. that the surface of the modified PE is hydrophilic. These properties can further be enhanced by treatment with acrylic acid.

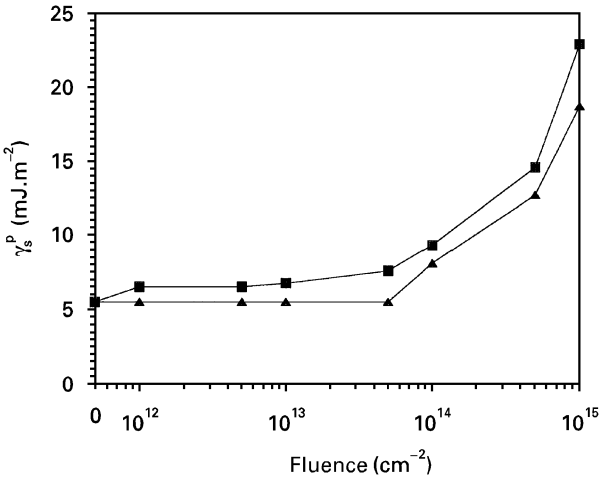

Figure 5 The dependence of polar component of the surface free energy on the ion fluence for the as-irradiated PE (PE/Ar, –▲–) and that doped with acrylic acid (PE/Ar/COOH, –■–).

The pristine PE, the PE specimens irradiated to different ion fluences and those eventually doped with acrylic acid were used in subsequent *in vivo* bicompatibility experiments, when the 5×5 mm strips of modified and un-modified PE were subcutancously implanted into laboratory rats (for more details see above). All types of implants (including pristine PE as a control sample) induce foreign body reaction with prevalence of macrophages on the implant surface. However, lower reaction is observed on the surface of the pristine PE as can be judged from the occurrence of macrophages and foreign body giant multinucleate cells (Fig. 6a). Ion irradiation enhances both the number of macrophages and foreign body giant multinucleate cells as well (Fig. 6b). Ion irradiation influences the ability of macrophages to fuse and form giant foreign body multinucleate cells. At a fluence of $1–5 \times 10^{14}$ cm^{-2} significant enhancement of macrophage fusion is observed, which is documented in Fig. 7, where the fusion index FI is shown as a function of the ion fluence. The implants additionally doped with acrylic acid exhibit a biphasic dependence, with maxima at fluences of 5×10^{12} and 1×10^{14} cm^{-2}. These extreme values of fusion index are significantly higher that that for pristine PE.

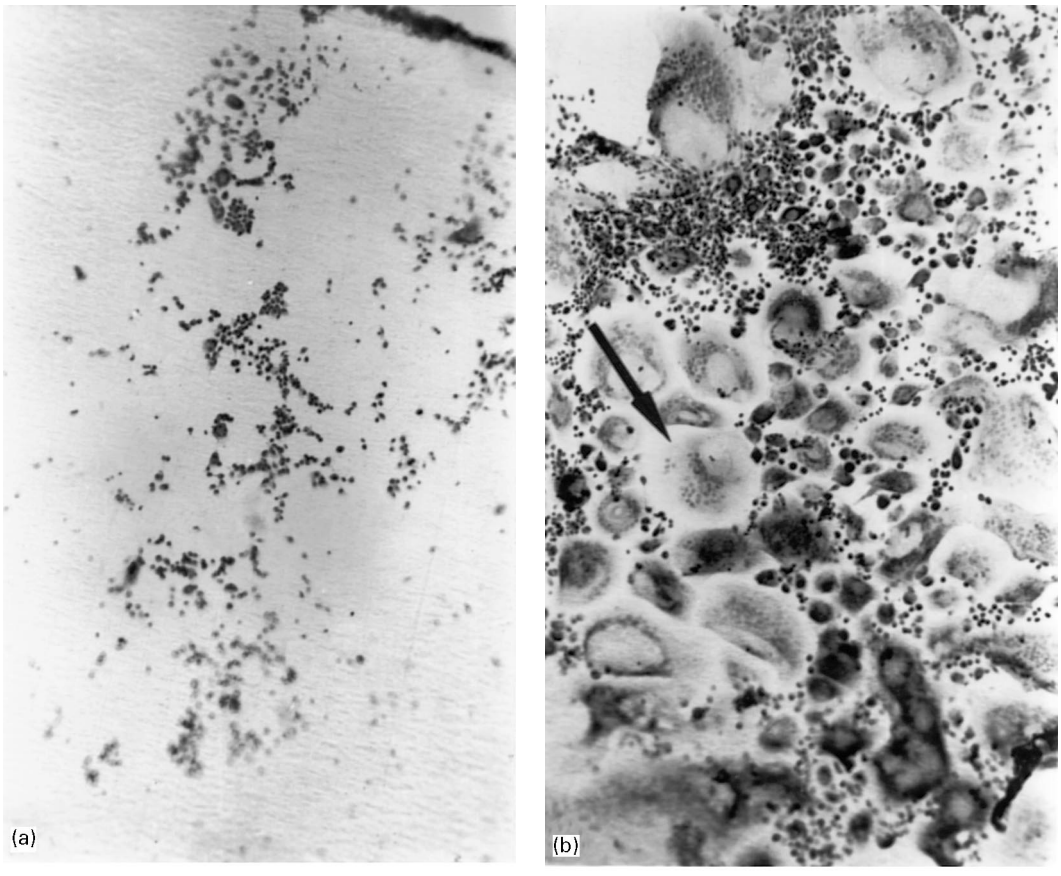

Figure 6 Foreign body reaction against the non-irradiated PE (a) and the irradiated at a fluence of 1×10^{14} cm^{-2} and doped with acrylic acid (b). The foreign body giant multinucleate cells (arrow) cover predominantly the irradiated and doped strip (b). Hematoxyline magnification $\times 90$.

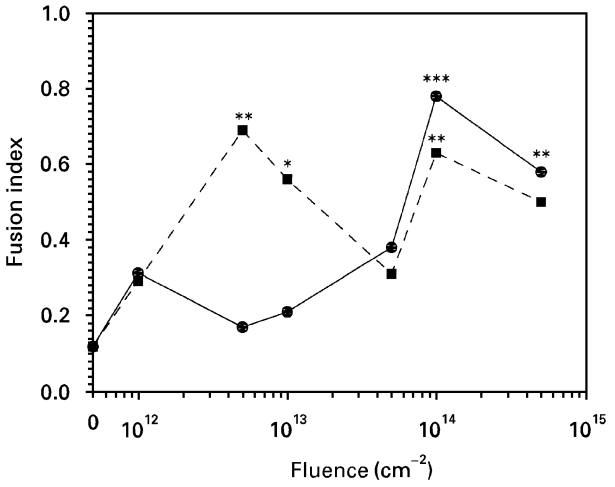

Figure 7 Fusion index as a function of the Ar^+ ion fluence measured on the irradiated PE (PE/Ar, –●–) and the PE subsequently doped with acrylic acid (PE/Ar/COOH, –■–). The statistically significant differences from the non-irradiated PE (student *t*-test) are signed ***$p < 0.01$, **$p < 0.02$, *$p < 0.05$.

Earlier [7, 8, 21] it was observed that ion irradiation enhances the surface polarity and wettability of polymers and stimulates, at the same time, adhesion of endothelial cells [21] and smooth muscle cells [7, 8] studied *in vitro*. In the present *in vivo* experiment, the PE modifications, either by ion irradiation or by subsequent doping, stimulate the fusion of macrophages on the surface of PE used as implants in the laboratory rat. This observation differs significantly from the results of earlier *in vitro* experiments [7] in which a significant effect of the ion irradiation on the smooth muscle cell adhesion and proliferation was reported for polypropylene and polystyrene, but no such effect for polyethylene modified under the same conditions. The discrepancy may simply indicate the fact that the cell interaction with the polymer surface modified by ion irradiation depends on the cell type and polymer structure as well. In our earlier studies, performed on the laboratory rat with a set of hydrogels, the $–COO^-$ anions clearly inhibited adhesion, spreading and fusion of macrophages [12–14, 16]. It should, however, be noted that the materials used in these experiments were highly hydrophilic, much more than the modified PE with added $–COO^-$ anions. It is known from another *in vitro* experiment on adhesion of endothelial cells to hydrophobic polymers that both anionic and cationic ions, present on the polymer surface, stimulate cell adhesion [22] by the modification of wettability and surface free energy. Seems to be that it is necessary for the adhesive proteins adsorption and control of their correct conformation which could be recognized by cell receptors [9].

4. Conclusion

The principal results of this study can be summarized as follows:

- irradiation with Ar^+ ions leads to dehydrogenation of PE and production of conjugated double bonds which may serve as bonding sites in subsequent doping with acrylic acid;
- electrical conductivity of irradiated PE remains unchanged for ion fluence below 5×10^{14} cm^{-2} and increases by about 1.5 orders of magnitude for a fluence of 1×10^{15} cm^{-2} due to polymer carbonization;
- irradiation leads to significant oxidation of the PE surface layer, about 120 nm thick, and production of different oxidized structures;
- maximum oxygen concentration is situated near the sample surface and the oxygen content can be increased further by doping with acrylic acid, enhancing the hydrophilic character of the PE surface;
- different dependencies of the macrophage fusion index, determined from our *in vivo* experiments, on irradiation fluence were observed in as-irradiated PE samples and those additionally doped with acrylic acid;
- the inhibitory effect of $–COO^-$ anions in implants on macrophage adhesion, spreading and fusion is not a general phenomenon and is observed only on particular materials.

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