Adhesion and proliferation of keratinocytes on ion beam modified polyethylene

V. ŠVORČÍK, K. WALACHOVÁ, K. PROŠKOVÁ
Department of Solid State Engineering, Institute of Chemical Technology, 166 28 Prague, Czech Republic

B. DVOŘÁNKOVÁ, D. VOGTOVÁ
Prague Burn Center, 3rd Faculty of Medicine, Carles University, 100 34 Prague, Czech Republic

R. ÖCHSNER, H. RYSSEL
Fraunhofer-institut für Intergierte Schaltungen, 91058 Erlangen, Germany
E-mail: svorcikv@vscht.cz

Polyethylene (PE) foils were modified by irradiation with Ar^+ and Xe^+ ions to different fluences and different physico-chemical properties of the irradiated PE were studied in relation to adhesion and proliferation of keratinocytes on the modified surface. Changes in the PE surface roughness were examined using the AFM technique, the production of conjugated double bonds and oxidized structures by UV-VIS and FTIR techniques respectively. The surface polarity was determined by measuring surface contact angle and two-point technique was used for the determination of PE sheet resistance. Adhesion and proliferation of keratinocytes was characterized using the MTT-test. The ion irradiation leads to creation of conjugated double bonds which, together with progressive carbonization, contribute to the observed decrease of sheet resistance. Oxidation of the irradiated PE surface layer during the ion implantation is observed. Besides oxidation, the PE surface polarity is affected by other factors. The observed increase of the PE surface roughness due to the ion irradiation is inversely proportional to the ion size. The adhesion and proliferation of keratinocytes on the ion irradiated PE is significantly higher than on the pristine PE. Distribution of results in keratinocyte cultivation and the number of cells is related to the ion fluence applied and to ion species as well.

1. Introduction

Synthetic polymers find a variety of applications in biology, experimental medicine and clinical practice, e.g. in the production of vials and other cell culture equipment. Possibilities for production of artificial organs and tissues for humans have been studied [1,2]. Structure and surface properties of polymers may affect cell functions as proliferation and spreading, cytoskeletal building and functions, mobility and interaction with cells of the immune system [3].

Biocompatibility of polymers may besides other methods, be altered by ion irradiation [4–8]. As a result of the interaction of an ion with polymer, polymer surface layer is modified. Chemical bonds are split [9] and free radicals and low mass degradation products are produced [6]. A fraction of free radicals recombine either by cross-linking of molecular chains [10] or by polymer oxidation from ambient atmosphere in implanter target chamber. In unpolar polymers the ion irradiation leads to an increase of the free surface energy and in turn of the surface polarity and wettability [11]. Dehydrogenation leads to creation of excessive conjugated double bonds which, together with carbonization, elevate electrical conductivity [12].

Interaction of cells with polymer is a very complex process including absorption of bioactive proteins from tissue fluid onto polymer and subsequent adhesion of cells to its surface. The process is strongly affected by polymer surface properties such as surface free energy, charge and wettability [6–8, 13]. It is expected that the polymers with enhanced biocompatibility will not disturb basic cell functions and exhibit low toxicity, mutagenity, teratogenity and cancerogenity.

Thanks to the interdisciplinary approach in the treatment of extensive burns, patients burned on more than 80% of total body surface area (TBSA) may survive at present. A shortage of suitable sampling sites for permanent coverage of burned wounds is overcome by cultivation of epidermal cells-keratinocytes (HK) comprising a transfer of confluent epithelial grafts (enzymatically released and attached to a textile carrier into the injured area [14]). Recently the methods of direct HK cultivation on carriers have also been developed. The carriers may be prepared either from synthetic polymers

(e.g. hydrogels) or from biological, collagen-based materials [15].

In this work the surface properties of PE were modified by irradiation with Ar^+ and Xe^+ ions. The changes in polymer surface morphology, chemical structure, electrical resistance and surface polarity were studied by different techniques and put into relation with adhesion and proliferation of human keratinocytes.

2. Materials and methods

2.1. Materials and modification procedures

Experiments were performed on PE with a density of $0.945\ g\,cm^{-3}$. The 15 μm thick PE foils were irradiated with 63 keV Ar^+ ions (fluences from $1 \times 10^{12} - 3 \times 10^{15}\ cm^{-2}$) or with 150 keV Xe^+ ions (fluences from $1 \times 10^{12} - 1 \times 10^{15}\ cm^{-2}$) on the Varian 350D ion implanter. The irradiation was accomplished at room temperature under the pressure of 10^{-4} Pa and with ion current density below $100\ nA \cdot cm^{-2}$. The ion energies were chosen to ensure approximately the same projected range of about 100 nm for both ion species in PE (the projected range was calculated by TRIM-version 91 code [16]). The PE foils were irradiated from both sides to the same fluence to enhance changes in optical properties and to make sample manipulation in biological experiments easier.

2.2. Experimental techniques

The irradiated foils were examined using UV-VIS spectroscopy on a Perkin-Elmer device and IR spectroscopy on a Nicolet 740 device. Difference IR spectra, obtained as a difference between the spectrum from irradited foil and the pristine one, are presented throughout the paper. Surface polarity was determined from the measurement of contact angle for water on reflection goniometer. Sheet resistance at room temperature and under the pressure of 10^{-1} Pa was determined using two point technique with a Keithley 487 instrument. Surface morphology was investigated using the Nanoscope III (Digital Instruments) atomic force microscope.

Modified Green technique [14, 17] was used for the HK cultivation on polymeric carriers. A skin sample was obtained with the patient's consent and the first subculture was performed in culture flasks (Nunc, Roshilde, Denmark). Then the semiconfluent HK growth was harvested by tripsinization (0.25% solution in PBS, Sigma Prague) and obtained suspension was used for polymer testing. The PE samples were inserted into cultiwell plates and fixed by polyamide rings, so that the cultivation area was $1.5\ cm^2$. Mice fibroblasts (3T3 line), the proliferation of which was stopped by Mitomycine C (Sigma Prague), were used as feeder cells with the density of 3×10^4 cells per $1\ cm^2$ of the cultivation area. After 24 h, the HK cells ($2 \times 10^4\ cm^{-2}$) were plated onto the PE samples prepared in this way 24 h later [15]. The samples were incubated at the temperature of 37 °C in the medium for keratinocyte cultivation [18, 19]. The medium was exchanged every 48 h.

Adhesion and proliferation of keratinocytes were characterized by the MTT test [20]. The number of cells (3T3 and HK) adhered to the carriers was determined 2 d after the keratinocyte deposition. Using the same technique the cell proliferation was determined after 7 d cultivation. The samples (after rinsing of cultivation medium) were exposed at 37 °C to 1 ml of MTT dye (with 0.83 g/l H-MEMd concentration) for 2 h. Blue precipitate created in mitochondria was dissolved in 0.6 ml of izopropylalcohol (cca 15 min) and the absorbance of 250 μl of solution at the wave length of 492 nm was measured with respect to izopropylalcohol on a Titertek Uniskan photometer. Since the 3T3 cells treated by mitomycine exhibit very low absorbance in comparison with HK, their effect was neglected.

3. Results and discussion

The UV-VIS spectra of pristine, unirradiated PE and of the PE samples irradiated with with Ar^+ and Xe^+ ions ions to different fluences are shown in Fig. 1. It is well known that the absorbance in UV region is an increasing

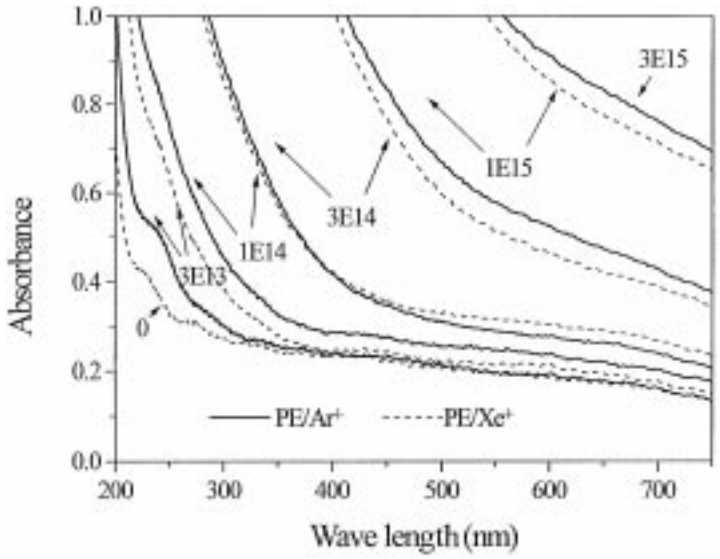

Figure 1 UV-VIS spectra of pristine and ion-irradiated PE. The numbers give the fluences of Ar^+ and Xe^+ ions (in cm^{-2}).

function of the concentration of conjugated double bonds, which in the present case are produced by radiation induced degradation of C-H bonds [21]. It is seen from Fig. 1 that the heavier Xe^+ ions produce more conjugated double bonds than Ar^+ ions.

Excess of conjugated double bonds together with progressive carbonization at higher ion fluences affect electrical conductance of the irradiated PE. The dependence of the sheet resistance R_s on the ion fluence of Ar^+ and Xe^+ ions is shown in Fig. 2. For both ion species the sheet resistance remain unchanged up to the ion fluence of $3 \times 10^{14}\,cm^{-2}$. For higher fluences the sheet resistance decreases, larger decreases being observed for Xe^+ ions. This finding correlates well with UV-VIS measurements (Fig. 1) indicating larger radiation degradation on the case of Xe^+ ions.

The ion irradiation leads to significant changes in the PE surface morphology [22] which may affect cell adhesion and proliferation. The surface morphology was studied using atomic force microscopy (AFM) on the pristine and irradiated PE. By a section technique, surface areas 20 μm in diameter (comparable to cell dimension) were examined. The results (Fig. 3) show that the irradiation with Ar^+ ions results in a significant increase of the PE surface roughness, in contrast to Xe^+ ions, for which no changes of the PE surface morphology are observed. This finding is explained by a larger effect of the heavier Xe^+ ions on the PE chemical structure which in turn may lead to a decrease of free volume fraction in the PE surface layer and subsequent surface densification and compaction. Rougher surface in the case of Ar^+ ions is expected to be more familiar to keratinocytes.

Chemical changes induced by ion irradiation were examined using Fourier transform infrared spectroscopy (FTIR). Different FTIR spectra in the interval of 1500-2050 cm^{-1} from the PE samples irradiated with Ar^+ ions to different fluences are shown in Fig. 4. For the fluences above $1 \times 10^{13}\,cm^{-2}$ an absorbance increase is observed in the region of 1650-1750 cm^{-1} which is typical for oxidized structures such as carboxyle, carbonyle (1725 cm^{-1} [23]) and esteric groups. The concentration of the oxidized structures increases up to the fluence of $1 \times 10^{15}\,cm^{-2}$. Then for still higher fluences the absorbance declines and this effect is obviously connected with degradation of the already produced oxidized structures. At highest ion fluences the deoxidation process ovecomes the oxidation one. Analogous dependence was observed also for Xe^+ ions.

The ion irradiation of unpolar polymers leads to an increase of their surface polarity [11]. The increase is, at least partly, due to polymer oxidation [24]. The surface polarity, which is closely related to the surface wettability, was determined by measuring water contact angle. The results for the PE irradiated with Ar^+ and Xe^+ ions are shown in Fig. 5. In the case of Xe^+ ions the contact angle remains unchanged up to the fluence of $1 \times 10^{14}\,cm^{-2}$ and it declines for higher fluences. For Ar^+ ions, however, a decrease of the contact angle is observed starting from the lowest fluences. Very rapid decrease occurs above the fluence of $1 \times 10^{14}\,cm^{-2}$. From comparison of Figs 4 and 5 one can see that while the concentration of the oxidized structures declines at higher ion fluences, the surface polarity (or contact angle) increases. Similar dependences were also observed on irradiated polystyrene [7]. It follows that the oxidized structures are not the only source of the enhanced surface polarity.

Adhesion and proliferation of keratinocytes is illustrated in Figs 6 and 7 for the PE samples irradiated with Ar^+ and Xe^+ ions respectively. The results were obtained as an average from measurements on four series of identical samples. The errors are standard deviations of the average. The irradiation with Ar^+ ions leads to an increase of the keratinocyte adhesion with two maxima being observed at the fluences of 1×10^{13} and of $3 \times 10^{14}\,cm^{-2}$. Similar dependence is also observed for the PE samples irradiated with Xe^+ ions but with only one maximum at the fluence of $1 \times 10^{13}\,cm^{-2}$. More pronounced differences between the PE samples irradiated by two ion species are observed on the cell proliferation. After the irradiation with Xe^+ ions to the

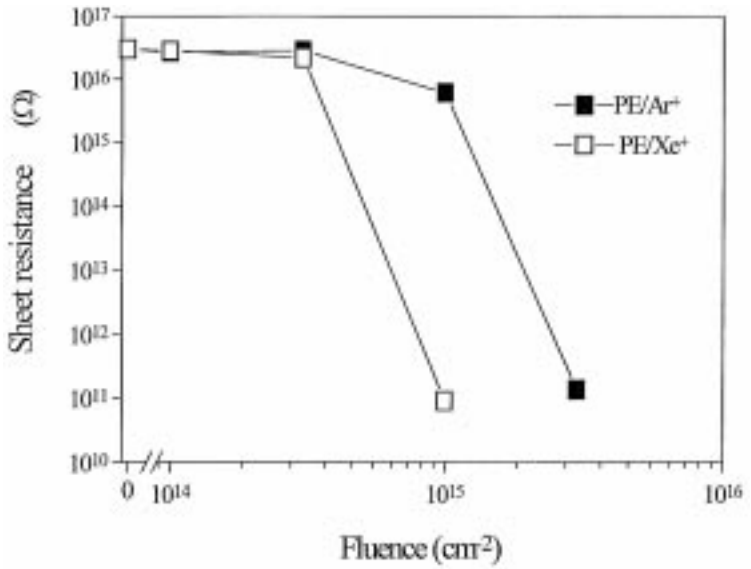

Figure 2 The dependence of the sheet resistance R_s on the fluence of Ar^+ and Xe^+ ions.

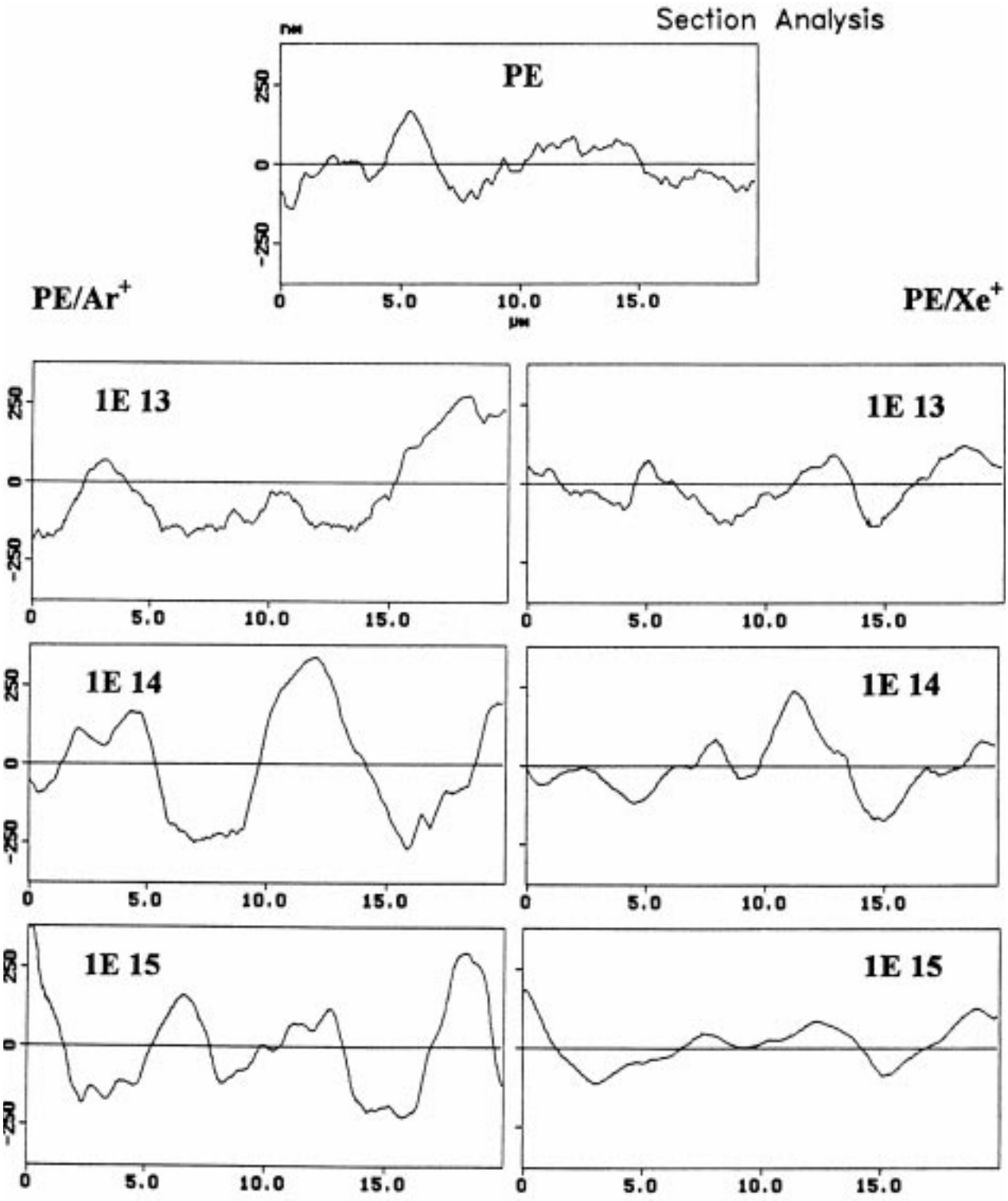

Figure 3 AFM section analysis of the pristine and ion-irradiated PE samples. The fluences of Ar^+ ions and Xe^+ ions (in cm^{-2}) are given.

fluence of $3 \times 10^{12}\,cm^{-2}$, the proliferation is elevated by about 20% in comparison with pristine PE, but for higher fluences no additional increase is observed (Fig. 7). For the PE samples irradiated with Ar^+ ions the proliferation exhibits two maxima at the fluences of 1×10^{13} and $3 \times 10^{15}\,cm^{-2}$ where the proliferation is about 40% higher than for pristine PE. Similar dependences of cell adhesion and proliferation were also observed for smooth muscle cells [7] where the enhanced cell growth was caused by altered surface polarity and an excess of carbonized clusters in the polymer surface layer.

Since the dependence of the adhesion and proliferation of HK on the ion fluence is rather complicated and the experimental errors are high, it is impossible to make any definite conclusions concerning the effect of increased surface polarity or carbonization on the HK growth. The interaction of HK with the modified PE is also affected by several other factors, such as surface roughness and by

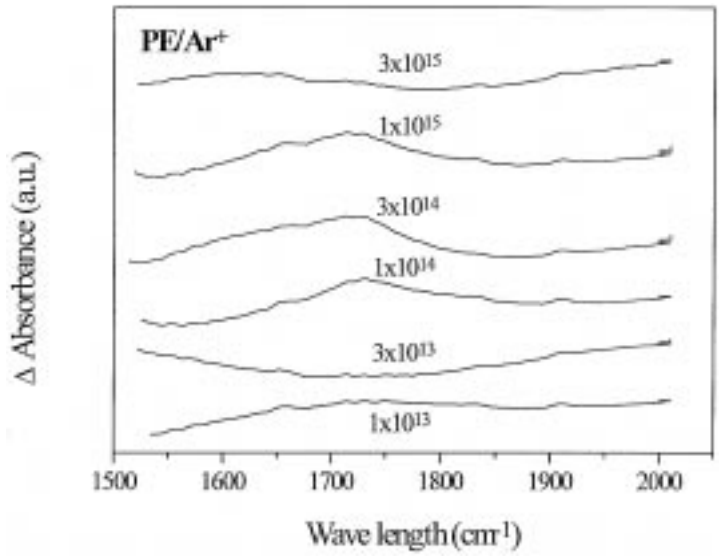

Figure 4 Difference FTIR spectra in the interval from 1500–2050 cm^{-1} for the PE samples irradiated with Ar^+ ions to different fluences.

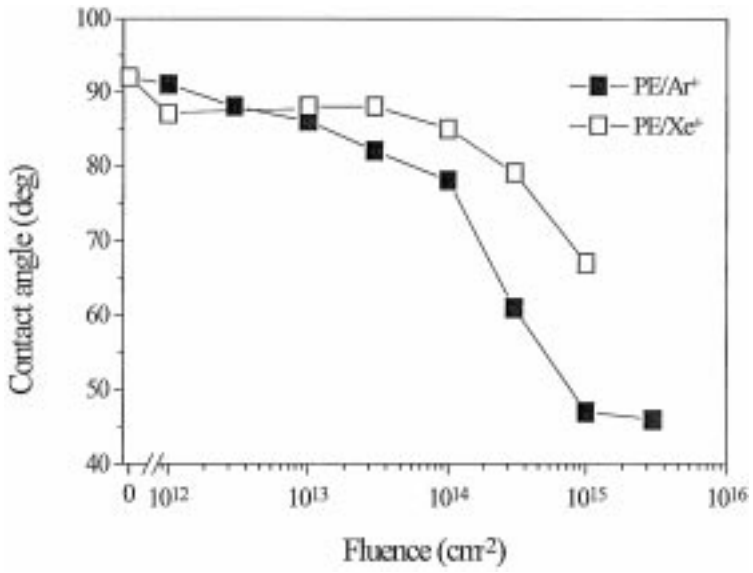

Figure 5 The dependence of the contact angle on the fluence of Ar^+ and Xe^+ ions.

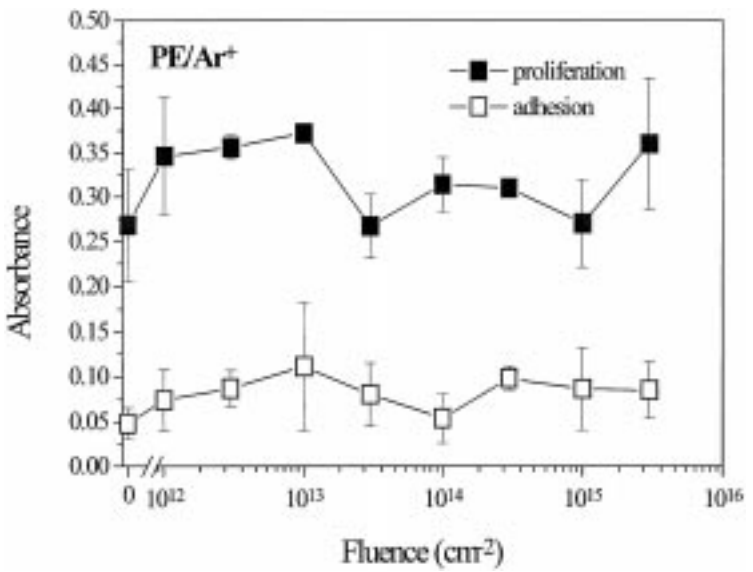

Figure 6 The dependence of the HK adhesion and proliferation on the fluence of Ar^+ ions.

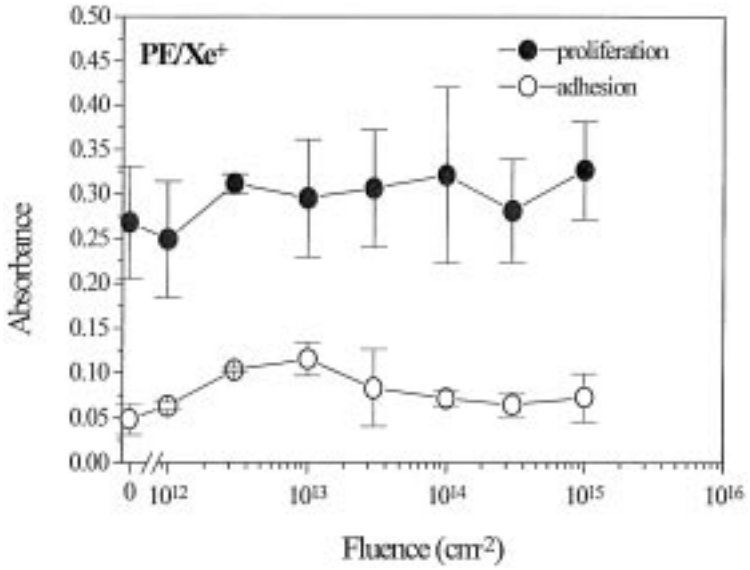

Figure 7 The dependence of the HK adhesion and proliferation on the fluence of Xe^+ ions.

the presence of 3T3 cells, which have got weak analytical signals but affect strongly the adhesion and proliferation of HK.

4. Conclusion

The present experimental results show that:

- The irradiation with heavier Xe^+ ions leads to higher degradation of PE in comparison with Ar^+ ions. The higher degradation is indicated by enlarged concentration of conjugated double bonds;
- Sheet resistance is a monotonously decreasing function of the ion fluence, with a rapid drop being observed at the fluences of 3×10^{14} and 1×10^{15} cm^{-2} for Ar^+ and Xe^+ ions, respectively;
- The PE samples irradiated with Xe^+ ions exhibit smoother surface. The effect is probably due to the densification and compaction of the PE surface layer.
- During the irradiation to low and medium fluences the PE is oxidized. The oxidized structures are destroyed at higher fluences.
- The surface polarity (measured via contact angle) is an increasing function of the ion fluence. Rapid increase of the contact angle is observed for the fluences above 1×10^{14} cm^{-2}.
- Keratinocyte adhesion and proliferation is enhanced by the ion irradiation. The cell proliferation is higher for the samples irradiated with Ar^+ ions.
- Enlarged cell proliferation is probably due to carbonization and increased surface polarity.

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