Growth and Differentiation of the Vascular Smooth Muscle and Endothelial Cells Cultured on Fluorine Ion-Implanted Polystyrene

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Abstract. The rat vascular (SMCs) and bovine endothelial cells (BECs) were cultured on conventional or fluorine ion-implanted polystyrene (5×10^{12} and 5×10^{14} fluorine ions/cm^2) The cells grown on the implanted growth supports showed better adherence, higher volume and higher total protein content The immunocytochemical analysis revealed that SMCs contained more of the cytoskeletal vimentin and the vascular SMC-specific α-actin as well as several cell adhesion-mediating molecules (vinculin, talin, alpha$_V$-integrin and ICAM-1) In BECs, only the content of vimentin and talin increased, while expression of ICAM-1 was unchanged The data suggest that cells on the ion implanted polymers could be more viable and that increased expression of some adhesion molecules mediating interactions with the host immune system is cell type-dependent

Key words: Smooth muscle cells — Endothelial cells — Cell adhesion and differentiation — Ion implanted polymers

Development of new materials for tissue culture technologies and the vascular prostheses for transplantation medicine requires better understanding of interactions of cells with artificial polymers In our earlier studies we showed better adherence of vascular SMCs to the polystyrene growth supports modified by irradiation with high energy beam of fluorine ions (Švorčík *et al* 1995, Bačáková *et al* 1996) Molecular mechanisms of the better cell-polymer adhesion, as well as a possibility of uneven responsiveness of different cell types have been rarely studied Moreover, some artificial materials, as Dacron, may induce increased expression of cell surface molecules of the immunoglobulin family which may have adversed effects on immunotolerance of the transplanted cells (Margiotta *et al* 1992) Therefore, we carried out a study on size, protein content, proliferation and expression of several cytoskeletal and cell adhesion molecules in the vascular SMCs and BECs cultured on unmodified or fluorine ion-irradiated polystyrene

The vascular SMCs were derived from the primary cultures prepared from the aorta of adult rats The BECs were purchased from the American Tissue Culture Collection

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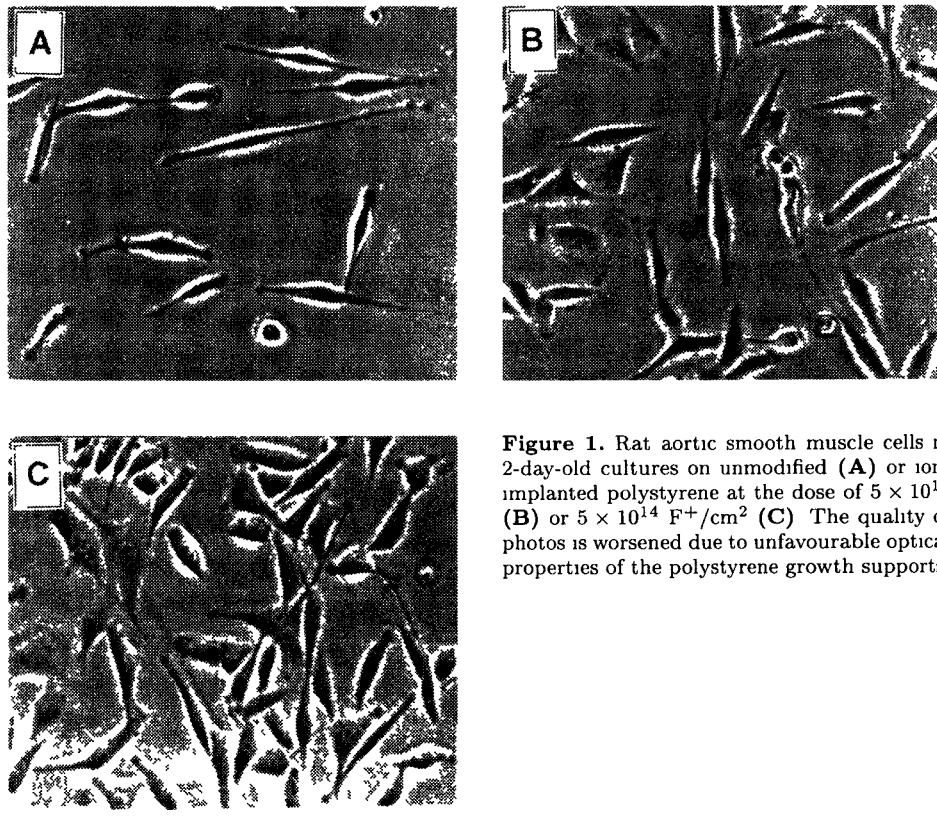

Figure 1. Rat aortic smooth muscle cells in 2-day-old cultures on unmodified **(A)** or ion-implanted polystyrene at the dose of 5×10^{12} **(B)** or 5×10^{14} F^+/cm^2 **(C)** The quality of photos is worsened due to unfavourable optical properties of the polystyrene growth supports

(CPAE line cells, CCL 209, Rockville, MD, USA) Cells were cultured in Dulbecco's MEM with 10 to 20% of fetal calf serum for 2 to 7 days Thin sheets of conventional or fluorine ion-implanted polystyrene (5×10^{12} and 5×10^{14} ions/cm^2, energy of 150 keV) were used as growth supports The cell diameters were measured in living cell suspensions prepared by mild trypsinization using an ocular microscale Proliferation of cells was measured by flow cytofluorimetry of BrDU-positive cells (Pellicciari *et al* 1996) The cytoskeletal and adhesion molecules were monitored by light microscope immunocytochemitry and ELISA The protein content was determined by the photometric Lowry's method (Lowry *et al* 1951)

Compared to cultures on conventional polystyrene both SMCs and BECs adhered to the ion-modified polymers at higher initial numbers The SMCs were also better spread and adhered by a larger area to modified growth supports (Fig 1) Both types of cells were also more resistant to the release from the irradiated polystyrene by proteolytic enzymes as well as to the spontaneous detachment when grown to confluence Compared to controls grown on the pristine polymer, the volume of SMCs amounted to 109 8 ± 1 1% ($p < 0\ 01$) and 138 4 ± 1 6% ($p < 0\ 001$) in cultures with lower and higher ion dose-irradiated polystyrene, respectively The data on the volume of BECs are not yet available though they appeared also larger The total protein content was higher by

8 1 ± 2 9% ($p < 0\ 05$) and 48 8 ± 8 6 ($p < 0\ 01$) in SMCs grown on polystyrene implanted at lower and higher ion dose, respectively In BECs, it was higher by 12 2 ± 3 2% ($p < 0\ 02$) and 18 1 ± 2 0 ($p < 0\ 001$) As shown by enzyme-linked immunosorbent assay (ELISA, Tab 1), the SMCs contained more cytoskeletal vimentin and the smooth muscle cell-specific α-actin as well as an adhesion molecule of the immunoglobulin superfamily, the intercellular adhesion molecule (ICAM-1) In BECs, the content of ICAM-1 was not significantly influenced by the ion-modified growth supports The content of vinculin, talin and α_V- integrins (i e receptors for vitronectin and fibronectin adsorbed on polymers from serum in the culture medium) in the SMCs grown on polymers treated with the lower ion dose (5×10^{12} F^+/cm^2) was higher and the increase was proportional to the changes in total content of protein On polymers treated with the higher irradiation dose (5×10^{14}) these proteins appeared even in higher concentration (Table 1) The S-phase fraction, as determined by flow cytofluorimetry of BrdU-positive cells, was in the vascular SMC populations grown on the ion-treated polystyrene (irrespectively of ion irradiation dose) by 12–13 % higher than in the control cells cultured on unmodified growth supports

We showed that the better adhesion of vascular SMCs on ion-irradiated polystyrene was accompanied by a protein content-proportional increase in the expression of α_V-integrins ICAM-1 as well as some molecules of the cortical and cytoplasmic cytoskeleton, while the proliferation of these cells increased only slightly This is a new favorable fact because of avoiding excessive proliferation of SMCs which can result in occlusion of the lumen of a vascular prosthesis (Margiotta *et al* 1995, Švorčík *et al* 1992, Bačáková *et al* 1996) These cells reached also a higher degree of chemodifferentiation indicated by a higher content of vimentin and α-actin (Skalli *et al* 1986) In BECs, the changes in cytoskeletal and adhesion molecules were less expressed and involved only talin and vimentin in populations grown on the higher ion dose-modified polymers The expression of ICAM-1, a molecule known to participate in interactions with the immune system cells (Margiotta *et al* 1992), was even unchanged in the endothelial cells This suggests a relatively good immunological tolerance of cells grown on ion-modified polymers which could be of importance for construction of polymer-supported biotransplant

Table 1. Content of cytoskeletal and adhesion molecules in cells cultured on polymers implanted with fluorine ions measured by ELISA in 7-day-old cultures[1]

The cell parameters	Smooth muscle cells		Endothelial cells	
	5×10^{12}	5×10^{14} F^+/cm^2	5×10^{12}	5×10^{14} F^+/cm^2
Vimentin	131 ± 5%$^{\heartsuit\heartsuit\heartsuit}$	130 ± 8%$^{\heartsuit\heartsuit}$	111 ± 10	165 ± 45$^{\heartsuit}$
α actin	110 ± 3%$^{\heartsuit\heartsuit\heartsuit}$	108 ± 3%$^{\heartsuit\heartsuit}$	not detected	not detected
Vinculin	111 ± 5%$^{\heartsuit}$	115 ± 6%$^{\heartsuit\heartsuit}$	100 ± 1%	126 ± 12%
Talin	101 ± 3%	107 ± 2%$^{\heartsuit\heartsuit}$	106 ± 3%	115 ± 3%$^{\heartsuit\heartsuit}$
α_V integrin	104 ± 4%	110 ± 3%$^{\heartsuit\heartsuit}$	not detected	not detected
ICAM-1	108 ± 4%	124 ± 5%$^{\heartsuit\heartsuit\heartsuit}$	106 ± 9%	105 ± 5%

[1]Absorbances (measured per cell mg protein) were expressed as percentage of values obtained in control cells cultured on unimplanted polystyrene Means ± S E M $^{\heartsuit\heartsuit\heartsuit}$ $p \leq 0\ 001$ $^{\heartsuit\heartsuit}$ $p \leq 0\ 01$ $^{\heartsuit}$ $p \leq 0\ 05$

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Different Responsiveness of Male and Female Rat Aortic Smooth Muscle Cells (SMCs) to Repeated Passaging in Culture

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Abstract. The smooth muscle cell (SMC) cultures were prepared from the aorta of male and female 8-week-old rats and used at passage 5 7 or 40–45 On day 1, low-passaged cells of both sex groups adhered to growth supports at similar numbers while after repeated passaging the adherence of female-derived cells was higher These cells had also higher total protein content and contained more of the SMC specific α-actin, vimentin and α_v integrins Compared to the male type of cultures, the high passaged cells of female origin cycled at a slower rate and were undergoing massive polyploidization Male-derived cells remained of the same morphology, ploidy and the differentiation status at all passages Their passage response consisted mainly in faster cycling and growth to higher population densities The data could be of importance for explanation of different incidence of hyperplastic vascular diseases in males and females

Key words: Smooth muscle cells — Cell adhesion — Cycling and polyploidization — Sex-related differences

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