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SHORT COMMUNICATION

Cytosolic Free Ca^{2+} Concentration in Canine Aortic Endothelial Cells Lining the Polyester Arterial Prosthesis

P. ONDRUŠ[1], R. ALBERTY[2], P. POTHIER[3], V. ECHAVÉ[4], J. POISSON[4], G. BKAILY[3]

[1]Department of Cardiac Surgery, F. D. Roosevelt Hospital, [2]Department of Biology, Faculty of Science, Matthias Belius University, Banská Bystrica, Slovakia, [3]Department of Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke and [4] Department of Surgery, Sherbrooke University Medical Center, Sherbrooke, Quebec, Canada

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Summary

A rise in baseline cytosolic free Ca^{2+} in canine vascular endothelial-like cells (VEC) lining the luminal surface of the polyester arterial prosthesis is described. In one, three and six month implantation experiments we employed six adult mongrel dogs, polyester arterial prostheses *Arteknit Ra K*, fluorescent Ca^{2+} indicator *Fura-2* and digital imaging microscopy to study cytosolic free Ca^{2+} in cultured VEC. The electron microscopy scanning of the luminal surface in different regions of the graft were also performed. A rise in cytosolic free Ca^{2+} in the VEC lining the luminal surface of the prosthesis is probably the result of the immunologic reaction and mechanical stress which stimulate the proliferation activity of the endothelial cells. It seems that the baseline cytosolic free Ca^{2+} reflects the course of the endothelization process on the polyester arterial prosthesis.

Key words

Ionized calcium • Endothelial cells • Arterial prosthesis • Canine model

Implantations of polyester arterial prostheses are very common in revascular surgery. In order for the arterial prosthesis to function properly *in vivo*, it is, among other considerations, necessary to ensure the optimal endothelization of its internal surface. VEC lining the arterial prosthesis may originate from dividing endothelial cells in the perianastomotic region, from capillaries growing through the porous prosthesis, or by the adhesion of circulating cells on the surface graft (Scott *et al.* 1994). Electron microscope studies conducted on the luminal surface of arterial vascular prostheses demonstrate some structural changes of endothelial cells (Noishiki *et al.* 1994, Chafké *et al.* 1993). As cytosolic free Ca^{2+} ions are critical to most cell functions we measured baseline cytosolic free Ca^{2+} in canine aortic endothelial cells lining the luminal surface of a polyester arterial prosthesis.

Six adult female Canadian mongrel dogs (weight 16–24 kg) were implanted with polyester arterial prostheses (Arteknit Ra K, Brno, Czech Republic) as a

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thoraco-abdominal bypass for prescheduled periods up to six months as described in detail elsewhere (Goeau-Brissonniere *et al.* 1981, Bkaily *et al.* 1993). The grafts were removed after one, three and six months and specimens from proximal, medial and distal regions were cut for scanning electron microscopy observation. Isolated endothelial cells were incubated for 30 min in 0.1 % collagenase (Type V; Sigma Chemical Co., St. Louis, USA) at 37 °C under constant agitation. Thereafter, the cell suspension was centrifuged for 5 min at 800 g. After washing, the cell pellet was resuspended and plated in sterile Dulbecco′s Modified Eagle′s Medium completed with 5 % calf serum (Gibco, Grand Island, USA) and gentamicin (4 mg/150 ml; Sigma). Cells were cultured at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. They were used after 8-18 passages. *Fura-2* fluorescent Ca^{2+} indicator (Molecular Probes Inc., Eugene, USA) in Tyrode′s buffer and digital imaging microscopy (Photo Technology International Inc., Princeton, USA) were employed to determine cytosolic free Ca^{2+} in VEC (Goldman *et al.* 1990). Two dogs were used as control. After thoracotomy, isolation and opening of thoracic aorta, VEC from the aortic luminal surface were collected for Ca^{2+} analysis. All animal experiments and chemical analyses were performed in the Medical Center, University of Sherbrooke. Student-Newman-Keuls method was used for evaluation of the results. *P* values of < 0.05 were considered significant.

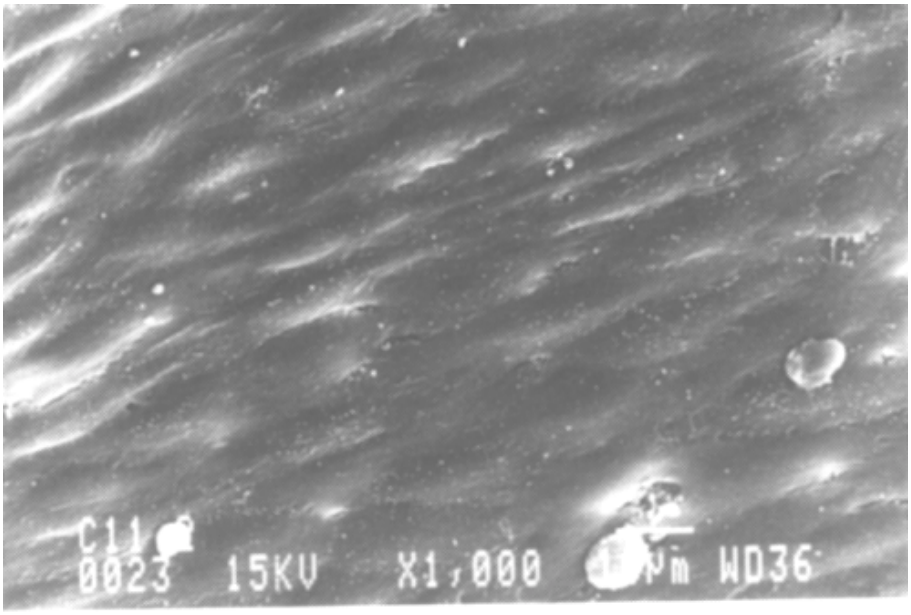

Fig. 1. Scanning electron micrograph of the explanted Arteknit Ra K prosthesis near the distal anastomosis after 1 month′s implantation showing the luminal surface covered with endothelial-like cells (x1000**).**

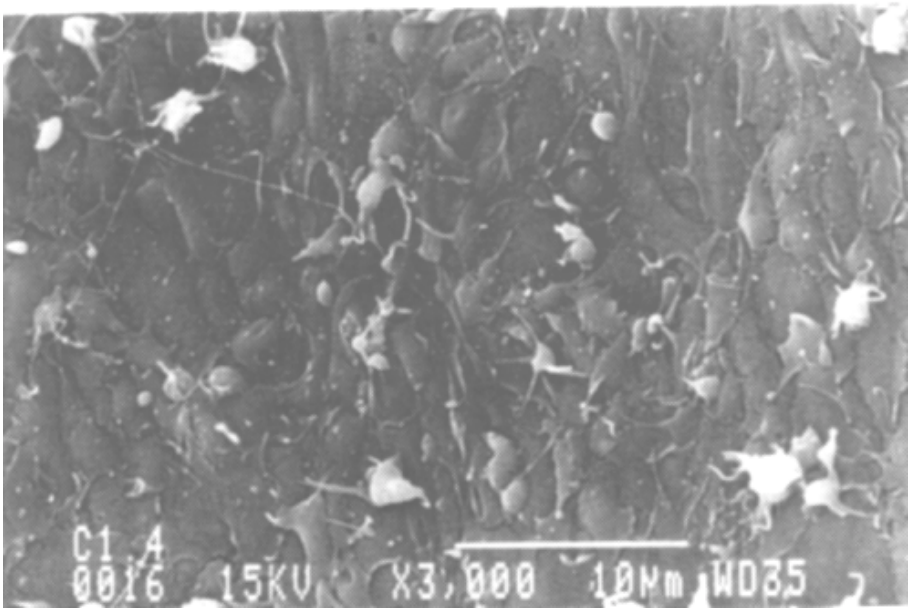

Fig. 2. Scanning electron micrograph of the Arteknit Ra K graft after implantation for 6 months showing sparsely distributed endothelial-like cells over the medial luminal surface (x3000).

After one month of implantation the endothelial-like cells completely covered the luminal surface of both anastomotic sites of the prosthesis (Fig. 1). However, a sparsely distributed endothelial-like cells were observed in mid-portion of the graft after implantation for 6 months (Fig. 2). Baseline cytosolic free Ca^{2+} concentrations in the VEC lining the luminal surface of arterial prosthesis are shown in Table 1.

Table 1. Baseline cytosolic free Ca^{2+} in the endothelial cells harvested from polyester arterial prostheses.

Specimen	**Cytosolic free Ca^{2+}, nmol/l (mean ± SEM)**		
(n = number of cells)	**1 month**	**3 months**	**6 months**
Proximal anastomotic region	257±31 (9)[b]	217±31 (14)[a]	166±21 (11)
Middle graft	288±28 (19)[b]	missing values	252±8 (8)[b]
Distal anastomotic region	262±27 (8)[b]	208±20 (33)[a]	159±19 (7)
Control cells	156±15 (16)		

Significantly different from the concentration in control cells ([a]P <. 0.05 and [b]P <. 0.001, Student-Newman-Keuls method).

Generally, in the first three months the average values of cytosolic free Ca^{2+} in endothelial-like cells from both anastomotic regions are significant higher in comparison with control aortic cells. In the mid-section on the graft there still remained a high concentration of Ca^{2+} throughout the whole experiment. Unfortunately, the endothelial-like cells from the mid-section of the graft in the third month were not isolated for technical error during specimen preparation.

Our results indicate that the cytosolic free Ca^{2+} concentration in endothelial cells may reflect quite well the course of the endothelization process. The cytosolic free Ca^{2+} concentration in quiescent cells usually kept below 200 nmol/l (Goldman *et al.* 1990, Darnell *et al.* 1990). The implantation of a polyester, collagen impregnated vascular graft enhanced the cytosolic free Ca^{2+} concentration in surrouding endothelial cells. The increase in Ca^{2+} ions, which maintains the VEC of the thoracic aorta in an activated state, may originate from an immunologic reaction to the collagen impregnation of the polyester prosthesis (The Canadian Multicenter Hemashield Study Group 1990). Thus, this induces the proliferation of endothelial cells in order to endothelize the surface of the prosthesis in the perianastomotic region. Likewise the influence of the trombocytary products, secreted vasoactive endothelins and direct endothelium injury cannot be excluded (Hynynen *et al.* 1992).

Endothelial-like cells lining the surface of the graft can be regarded as premature cells. It is well known that other cell populations (macrophages, histiocytes, smooth muscle cells, etc) are also present on the graft surface, therefore clear identification of endothelial cells is mandatory. The isolation of VEC in our study was routinely done by an experienced researcher (P. Pothier) and VEC were identified according to their shape and size through electron microscopy observation and attachment to glass cover slips.

Endothelial-like cells maintaining their proliferation activity until the luminal surface is completely covered by a monolayer and some type of attachment stimulus is most likely necessary to stop this process. At the beginning of this endothelization process, when these attachment stimuli are absent, cells keep a more rounded shape with higher baseline intracellular Ca^{2+} concentrations. This can be evident in the middle of the graft, where even in six month implants, intracellular Ca^{2+} values were increased compared to the control cells. This finding corresponds to electron microscopy observation of the same section of the graft. In contrast, the endothelial cells lining the luminal surface of arterial vascular prostheses in both anastomotic regions were flattened and in the six month implants resembled more the parent aortic VEC. In this period, their baseline Ca^{2+} concentrations were also near to the control cells. However, the origin of the endothelial cells in the perianastomotic region differs from the middle of the graft, which may also be connected with the difference in the cytosolic free Ca^{2+} concentration as observed in our study.

The proliferating endothelial cells on the graft surface have to grow under complicated conditions always influenced by turbulence, shear stress and vasoactive endothelins originating from aortic tissue and endothelial cells. These factors could also stimulate their baseline intracellular Ca^{2+} concentration. Moreover, endothelial cells are very rich in cytokinins which may be released during the surgical procedure of implanting or

explanting the graft, the fact that may influence their Ca^{2+} concentration. Fibrin and platelet deposits over the graft surface and secretion of prostacyclin and tromboxaneA_2 will be described in our next study.

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Reprint requests

Dr. Roman Alberty, Department of Biology, Faculty of Science, Matthias Belius University, Tajovského 40, 974 01 Banská Bystrica, Slovakia. phone: + 00421 48 413 98 19. e-mail: alberty@fpv.umb.sk.