ENDOTHELIALIZATION OF CARDIOVASCULAR GRAFTS

POLYDIMETHYLSILOXANE CONTENT AFFECTS ADHESION AND GROWTH OF ENDOTHELIAL CELLS ONTO POLYURETHANE GRAFTS

G. Soldani, E. Briganti, P. Losi, I. Martinelli
"Laboratory for Biomaterials & Graft Technology"
Institute of Clinical Physiology C.N.R. Massa, Italy

Introduction

It is well known that the replacement of arteries with purely synthetic vascular prostheses often leads to the failure of such reconstruction when small-diameter or low-flow configurations are concerned, due to the thombogenicity of the artificial blood contacting surface. One of the most promising approaches to improve long-term patency of these grafts is to coat the surface with endothelial cells (EC). This has been suggested because an endothelial cells monolayer provides a thromboresistant surface to circulating blood and because vascular grafts placed in humans do not or rarely spontaneously form an EC monolayer[1].

The aim of this work was to develop new polyurethane (PU)-polydimethylsiloxane (PDMS) graft materials composition to enhance cell proliferation; in particular it was assessed the influence of PDMS content on the adherence and growth of seeded EC. It has been reported that EC don't adhere to bare materials so we pre-treated polymer grafts with fibronectin (Fn) to facilitate cellular adhesion onto polymer surfaces and to develop engineered vascular grafts with improved functionality in the long-term.

Materials and Methods

Sample preparation

The new elastomeric graft material (named Silcrothane) was prepared in our laboratory by reacting a standard PU with PDMS in different content (10%, 20% and 30%). The Silcrothane material was used to realise vascular graft using a "spray, phase inversion" technique, that allows to control internal surface micro-porosity[2]. Low porosity surfaces were chosen because preliminary experiments showed that this kind of surfaces enhance cells adhesion and growth.

Grafts were sterilised by 20' of sonication in HCl 0.4N and then they were rinsed in distilled sterile water to remove HCl solution.

Endothelial cell culture

Human umbilical vein endothelial cells (HUVEC) were harvested by treatment with 0,1% collagenase as described in literature[3] and maintained in M-199 supplemented with 10% FBS. Experiments were carried out with cultures at passage earlier than fourth.

Adhesion and growth tests

The sterile grafts were cut into circular pieces of about $1cm^2$ and they were fitted into 24-wells plates. The culture plastic served as control. Pieces and controls were incubated with Fn (6μg/well) for 1h at 37°C, other pieces and controls with PBS in the same conditions. Then 0.5ml HUVEC suspensions ($1x10^5$ cells/ml) were seeded on them. After 18h (adhesion test), 3, 5 and 7days (growth test) of incubation the number of attached cells was determined.

Qualitative evaluation of adhered EC was performed by light microscopy observation and compared with controls. The cells were stained with Giemsa (0.1% in methanol) after fixation with methanol 70%.
Metabolic activity of cells was tested using the tetrazolium-based colorimetric method (MTT test) to quantify cellular viability.

Results

Light microscopy observations of the bare and Fn-coated materials showed that cell adhesion was similar on every pieces and lesser than controls. Therefore treatment with Fn was found to not induce a significant improvement of cell adhesion on the polymer surfaces.
However, no growth of HUVEC was observed on every non-coated polymers. We observed Fn-coating increased the HUVEC growth rate only on Silcrothane with 10% PDMS (Fig.1); on this surface cells appeared to be completely spread, showing their characteristic morphology. On the contrary, on the other materials cells maintained a round shape. MTT test consolidated the microscopical observations.

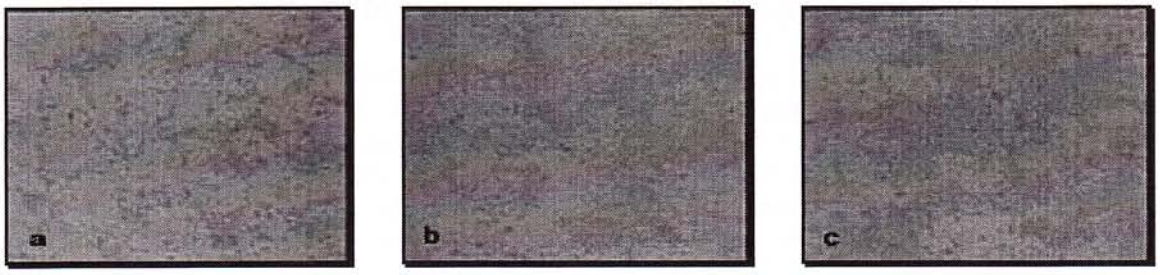

Fig.1: Distribution of EC adhering on Silcrothane with 10% PDMS Fn-coated, after 3(**a**), 5(**b**), and 7 days(**c**).

Discussion

We supposed that two mechanisms may explain the cells growth on the grafts. First, the Fn absorption is influenced by the surface free-energy or surface charge: hydrophilic surfaces accumulate less proteins then hydrophobic surfaces[4]. A second mechanism is the spatial arrangement of Fn, which is influenced by the substratum surface properties.
The EC growth rate increase may depend on Fn absorption and conformation arrangement on our polymers, so we are investigating the amount of Fn adsorption and the presence of cell binding sites by an ELISA.
This preliminary results are encouraging to develop an engineered vascular graft made by the new Silcrothane material and EC.

References

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