

## RESPONSE OF ENDOTHELIAL CELLS TO POLYETHYLENE TEREPHTHALATE SURFACES ACTIVATED BY IRRADIATION

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**INTRODUCTION:** Important factor implicated in the cardiovascular graft failure is the lack of endothelial cells lining the lumen of the vessel. In the last few years several biomimetic approaches have been developed, in order to immobilize short peptide sequences, such as RGD, onto various surfaces, and produce biofunctional materials. Moreover, ion beam irradiation of polymer surfaces has been shown to induce the formation of a drastically modified altered layer, strongly affecting the cell-surface interaction [1]. The aim of this study was to investigate the *in vitro* response of endothelial cells grown onto PET surfaces modified by low energy Ar-irradiation (50 keV) and/or RGD and FBS adsorption.

**METHODS:** Polyethylene terephthalate (PET, Aldrich) was deposited as thin films by spin coating onto monopolished silicon wafers. The ion irradiation of samples was performed under vacuum of about  $7.0 \times 10^{-7}$  mbar at room temperature, with an ion fluence of  $1 \times 10^{15}$  ions/cm<sup>2</sup>. After irradiation the samples were aged in controlled laboratory atmosphere for a period of two weeks before surface characterization, peptide/protein adsorption and cell adhesion experiments. The peptide sequence Arg-Gly-Asp (RGD) was dissolved in deionized water (2.9 mM), while FBS was diluted in PBS solution (10% v/v). The immobilization of RGD or FBS onto PET surfaces was obtained by spontaneous adsorption for 1 h. The surface coverage and chemical structure of the ad-layers was investigated by XPS and AFM. Endothelial cells isolated from postcapillary venules (CVEC, coronary venular endothelial cells) were used in the study [2]. Subconfluent cells were plated onto the various PET surfaces. Cell morphology and the interaction among endothelial cells, biomaterials and extracellular matrix components were evaluated after 4 days by scanning electron microscopy (SEM) and immunofluorescence techniques [2].

**RESULTS:** The physico-chemical characterization of irradiated PET surfaces showed that ion irradiation induces a modification of the chemical structure of the outermost surface layers, while the

AFM analysis evidenced that Ar<sup>+</sup> irradiation did not affect significantly the morphology and roughness of PET surfaces. The efficiency of biofunctionalization of PET by immobilization of RGD or FBS was estimated by XPS quantitative analysis. When CVEC were cultured on unirradiated PET, they appeared retracted with altered morphology showing poor adhesion (Fig 1A). On the contrary, when cell were grown onto irradiated PET, either biofunctionalized or not with RGD or FBS, the typical endothelial cell morphology was maintained over prolonged time, showing well-being status (Fig. 1B). Moreover, the endothelial cells preserved their ability to produce cytoskeletal protein ( $\alpha$ -tubulin, Fig 1) and extracellular matrix proteins, such as fibronectin and laminin.

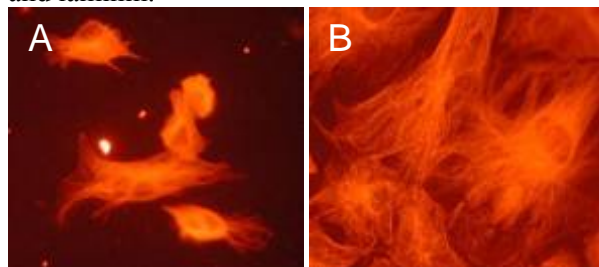


Fig. 1:  $\alpha$ -tubulin fluorescence images. Original magnification 40x.

**DISCUSSION & CONCLUSIONS:** The results show the endothelialization of the biomaterial. In fact, the altered layer produced by ion irradiation of PET is in itself able to promote enhanced CVEC adhesion, spreading and proliferation, irrespectively of the surface biofunctionalization.

**REFERENCES:** <sup>1</sup>G. Marletta and C. Satriano (2004) *NATO Science Series, II: Mathematics, Physics and Chemistry*; 152: 7. <sup>2</sup>S. Pezzatini et al. (2006) *J Biomed Mater Res A.*;76(3):656-63

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