Covalent Grafting of Fibronectin on Plasma-Treated PTFE: Influence of the Conjugation Strategy on Fibronectin Biological Activity

Karine Vallières [1,2], Éric Petitclerc [1,3], Gaétan Laroche [1,2]

[1] Unité de Biotechnologie et de Bioingénierie, Centre de recherche de l'Hôpital Saint-François d'Assise, C.H.U.Q., 10 rue de l'Espinay, Québec, Québec, Canada, G1L 3L5

[2] Université Laval, Département de Génie des mines, de la métallurgie et des matériaux, Faculté des sciences et génie, Québec, Québec, Canada, G1K 7P4

[3] Université Laval, Département de Médecine, Faculté de Médecine, Québec, Québec, Canada, G1K 7P4

Statement of Purpose: Small diameter PTFE vascular prostheses implanted in humans currently have a low patency rate. Thrombosis is the major cause of failure since PTFE is not hemocompatible. In healthy arteries, the endothelium prevents the activation of platelets and the coagulation cascade in order to avoid thrombus formation. The presence of an endothelium inside synthetic prostheses could greatly improve their patency. Unfortunately, endothelial cells can't grow on PTFE. Most cells need to interact with an extracellular matrix to survive. Fibronectin (FN) is a matrix protein known to bind many other matrix components and to induce cell adhesion [1]. This protein has long been used to modify surfaces to improve biocompatibility and allow endothelialization. However, it seems that the biological activity of FN is highly dependent on its conformation [2]. The aim of this study was to compare the efficiency of two strategies to covalently bind FN to a PTFE surface. Two heterobifunctional cross linkers were used to conjugate FN to ammonia plasma-treated PTFE: glutaric anhydride (GA) and Sulfo-succinimidyl 4-[p-maleimidophenyl] butyrate (sulfo-SMPB).

Methods: Ammonia plasma treatments were performed to introduce amino groups onto PTFE surfaces. All plasma treatments were performed under 300mTorr (0,400mb) of high purity ammonia with 15W of radiofrequency (RF) power at a frequency of 13,56MHz for 60s in a cylindrical RF plasma reactor described elsewhere [3]. On separate surfaces, amino groups were made to react with the two cross linkers (GA and sulfo-SMPB). The extent of reactions was measured in a previous work in our laboratory [4]. The free end of both cross linkers was then used to bind FN either by its primary amines (GA) or sulfhydryl groups (sulfo-SMPB). A sample of each grafting step was analysed by XPS with a PHI 5600-ci spectrometer (Physical Electronics, Eden Prairie, MN, USA). To evaluate the biocompatibility of these surfaces, adhesion tests were performed on virgin PTFE and on FN bound PTFE via both cross linkers. Samples were seeded with bovine aortic endothelial cells (BAECs) for 10 minutes and washed with culture medium. Remaining cells were quantified by fluorescence with a resazurin assay.

Results/Discussion: XPS analyses confirmed the success of all PTFE modification steps. Furthermore, spectra for both FN bound PTFE surfaces suggest that the concentration of FN grafted on each cross linker is similar. Endothelial cell adhesion on virgin PTFE is very poor. For FN modified surfaces, endothelial cells adhere strongly when FN is grafted by GA. Surprisingly, cells adhere weakly when FN is grafted by SMPB, for which there is no significant difference with virgin PTFE (see Fig. 1).

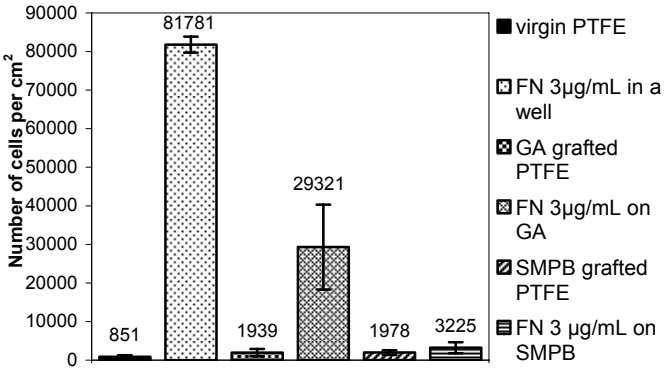

Figure 1: Adhesion of BAECs on virgin PTFE and different modified surfaces.

It seems that the FN cell binding site is available on GA-grafted FN but not on SMPB-grafted FN. Possible explanations include steric hindrance of the cell binding site either by the anchorage site or the SMPB-induced FN tridimensional conformation.

Conclusions: Covalent bonding of FN on PTFE via glutaric anhydride significantly increase cell adhesion and therefore biocompatibility of this polymer. Grafting via SMPB results in no significant improvement compared to virgin PTFE. The glutaric anhydride conjugation strategy of FN on PTFE is a promising technique to improve PTFE prosthesis patency. In future works, proliferation tests will be performed as well as AFM characterization of the FN conformation on each cross linker. Furthermore, ELISA will be performed to compare the availability of the FN cell binding site on both cross linkers.

References:1. (Sottile, J. Mol Biol Cell, 2002; **13**: 3546-59.) 2. (Garcia, A.J. Mol Biol Cell, 1999; **10**: 785-98.) 3. (Mantovani, D. Plasmas and Polymers, 1999; **4**: 207-228.) 4. (Gauvreau, V. Bioconjug Chem, 2004; **15**: 1146-1156.)