Anti-Apoptotic Bioactive Coatings Including Chondroitin Sulphate and Epidermal Growth Factor

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Statement of Purpose: The use of endovascular aneurysm repair (EVAR), for the treatment of abdominal aortic aneurysms (AAA), is currently limited by postoperative complications (such as migration and endoleaks), which was shown in previous studies to be related to an incomplete healing of the tissues surrounding the stent-graft [1]. To assure a long-term success of such treatment, tissue ingrowth around the implant is critical. However, the pro-apoptotic pathophysiology of AAA and the inertness of the materials currently used in stent-grafts, like polyethylene terephthalate (PET) or expanded polutetrafluoroethylene (ePTFE), limit the wound healing process and in turn compromise the use of EVAR. We assume that a bioactive coating including anti-apoptotic mediators could inhibit vascular cell apoptosis, promote the healing process and eventually counteract the pro-apoptotic environment of AAA.

We recently demonstrated that a bioactive coating containing both chondroitin sulfate (CS) and epidermal growth factor (EGF), immobilized on aminated glass surfaces, could increase vascular smooth muscle cells (VSMC) adhesion, growth and resistance to apoptosis [2|. The aim of this project is thus to transfer the coating on realistic biomaterial surfaces used in stent-grafts and demonstrate that the bioactivity of CS and EGF are respectively preserved. For the purpose of this study, PET was funtionalized by means of an innovative plasma polymerized thin film that yields very high primary amine content, called PPE:N for nitrogen-doped plasma-polymerized ethylene [3], to allow further grafting of CS and EGF.

Methods: PET films were utilized to prepare the coating. To create chemically reactive amino-groups on PET, PPE:N was deposited at atmospheric (HP) and low (LP) pressure [4]. Ammonia plasma surface functionalization (PETf) was also used for comparison. CS was covalently attached by means of a water soluble carbodiimide system (EDC/NHS). Stable amide linkages were created between the carboxylic acids of the CS and the primary amines of the PPE:N. EGF was subsequently immobilized on the carboxylic acid groups of CS using the same reagents. The surface properties were characterized by XPS, static water contact angle measurements and stability of coating in physiological buffer. The bioactivity of the surfaces was assessed on VSCM, by cell adhesion, cell growth and resistance to apoptosis in serum free media. Cell adhesion and cell growth was measured using crystal violet staining and resistance to apoptosis was detected using Hoechst/propidium iodide staining.

Results: CS was immobilized on HP, LP and PETf for the selection of the optimal substrate. Good stability of CS coatings on HP, LP and PETf was verified by incubating surface in physiological buffer at 37° for up to 4 weeks and measuring CS release by spectroscopy using dimethylmethylene blue. All three substrates seemed to retain CS for the duration of the experiment. The amount of sulfur (present in CS) on the 3 surfaces was compared by XPS. HP+CS (1.00 ± 0.06%) contained more sulfur than LP+CS (0.31 ± 0.04), which contained more sulfur than PETf+CS (0.05 ± 0.09).This suggests that more CS was immobilized on HP, than LP and PETf. However, VSMC apoptosis was significantly lower on LP+CS, compared to LP and HP/PETf with or without CS. LP was then chosen as the substrate for the coating. Previous work showed that CS inhibits cell adhesion but that this trend could be overturned with EGF grafting. Here again, immobilized EGF significantly increased cell growth compared to PET, PET+LP and PET+LP+CS. As illustrated in Figure 1, CS significantly increases resistance to apoptosis compared to PET, LP and our negative control. Further grafting of EGF gave similar results.

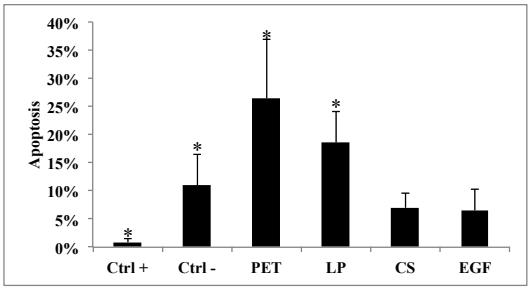

Figure 1. Percentage of apoptotic VSMC on PET, LP, LP+CS and LP+CS+EGF compared to our positive and negative controls, tissue culture plates with normal and serum free medium respectively (* $p < 0.01$ compared to LP+CS).

Conclusions: An anti-apoptotic coating was created on PET, using CS and EGF. The coating was stable and significantly increased VSMC growth and resistance to apoptosis. These results show great potiential for combined CS and EGF to create a bioactive coating on endovasular prosthesis to couter VSMC depletion and promote healing after EVAR. ***This project was supported by the FMCQ, NSERC, CIHR and FRSQ.***

References: [1] Marjor et al.. J Endovasc Ther. 2006;13:457-467. [2] Charbonneau et al. Biomaterials 2010 (accepted). [3] Girard-Lauriault et al. Plasma Process Polymers 2005;2:263-270. [4] Truica-Marasescu et al. Thin Solid Films 2008;516: 7406–7417.