Chondroitin-collagen coatings to promote healing around stent-graft in abdominal aortic aneurysms

Charbonneau C[1,2], Gautrot JE[2], Hébert MJ[1], Zhu XX[2], Lerouge S[1,2]
[1]CRCHUM, Notre-Dame Hospital, Université de Montréal, Montreal, Quebec, Canada
[2]Department of Chemistry, Université de Montréal, Montreal, Quebec, Canada

Statement of Purpose: Rupture of abdominal aortic aneurysms (AAA) is the 15th leading cause of death in North America. AAA are characterized by permanent dilatation of the aortic wall and can be treated via endovascular aneurysm repair (EVAR) using a stent-graft (SG). However, this minimally invasive technique is limited by postoperative complications such as SG migration and endoleaks. Some of our previous work **[1]** showed that these complications could be related to incomplete healing of the tissues surrounding the SG. This inadequate healing can be related to the inertness of materials currently used in SG and the pro-apoptotic environment in AAA. It is assumed that a bioactive SG including anti-apoptotic mediators could improve the healing process and improve SG incorporation into the vessel wall. A promising mediator for vascular repair is chondroitin-4-sulphate (CS). This polysaccharide has been recently demonstrated to increase cell migration and resistance to apoptosis of vascular smooth muscle cells (VSMC) and fibroblasts **[2]**. Consequently, this work consists in developing a coating incorporating CS for SG.

Methods: The preparation of the coating was done on polyethylene terephthalate (PET) surfaces, since PET is currently used in SG. To favor cell adhesion collagen was added to the coating. CS and insoluble type I collagen were crosslinked with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *n*-hydroxysuccinimide (NHS). EDC covalently attaches CS to collagen via an amide bond, whilst NHS increases EDC-mediated reaction efficiency by stabilizing the o-acelisourea amine-reactive intermediate.

Surface wettability was evaluated by static contact angle using a goniometer (First Ten Ångstroms, Portsmouth, VA). CS quantification was done by dimethylmethylene blue (DMMB) assay **[3]**. Briefly, the sample was mixed with papain to digest the interfering proteins, then a DMMB color reagent was added and the absorbance was read at 525 nm. It was then compared to a calibration curve. Morphology of the coating was observed by scanning electron microscopy (SEM) (Hitachi S-3500, Rexdale, Ontario) after Au-Pd sputtering.

The potential of the coating to promote cell adhesion, growth, migration and resistance to apoptosis was evaluated in vitro with vascular smooth muscle cells (VSMC). Cell adhesion and cell growth after 2 and 7 days on the various surfaces were determined using MTT colorimetric assay. To assess VSMC resistance to apoptosis, cells were exposed to serum-free medium (SS) for 24 hours and their viability were compared to the viability of VSMC exposed to normal medium (N).

Results/Discussion: The different surfaces evaluated were PET, collagen coating without CS (CC), collagen coating with CS (CSC) and polystyrene culture plate (PCP), a common surface used in cell culture. Both collagen coatings (CC and CSC) were shown to be more hydrophilic than PET or PCP. Their contact angles with water were approximately 60° compared to 80° for PET and PCP. SEM images of CC and CSC showed that both coatings had relatively smooth surfaces, with no apparent pores. The absence of pores could limit the cells to adhere only at the surface and not within the coating. No trace of CS was found in CC using the DMMB assay, while 121 mg of CS/g of coating was found in CSC. These results were comparable to another quantification method using a hexosamine assay **[4]**.

In cell culture experiments, adhesion of VSMC was significantly higher on CC than on PET. The addition of CS to the collagen coating (CSC) seems to limit the adhesion properties of collagen. This could be explained by the fact that CS is covalently bonded to collagen, which could limit the functional sites of collagen involved in VSMC adhesion. However, cells adhered to CSC coating exhibited higher viability when exposed to SS medium compared with cells adhered to all other surfaces tested (Figure 1). Since SS is known to induce cell apoptosis, these preliminary results suggest that the CSC coating increases resistance to apoptosis of VSMC, an important pivotal event to promote healing in such a pro-apoptotic environment. They also suggest that crosslinking of CS to collagen with EDC/NHS might preserve the anti-apoptotic activity of CS.

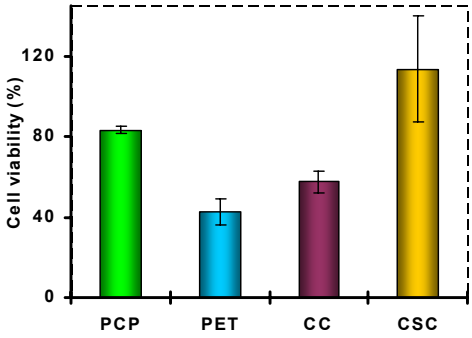

Figure 1. Viability of VSMC in SS

Conclusions: We have used recent findings in molecular biology in the field of biomaterials. The collagen-CS coating developed may be a promising way to favor vascular repair and SG incorporation after EVAR.

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References: [1] (Major A. J Endovasc Ther. 2006; 13: 457-67). **[2]** (Raymond MA. Faseb J. 2004; 18: 705-7). **[3]** (Farndale RW. Biochim Biophys Acta. 1986; 883: 173-7). **[4]** (Pieper JS. Biomaterials. 1999; 20: 847-58).