Challenges for the transfer of a bioactive anti-apoptotic coating for stent grafts from an in vitro model towards realistic implants

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Introduction:

Biomaterials currently used in stent-grafts (SG) for the endovascular treatment of abdominal aortic aneurysms fail to stimulate appropriate healing mechanisms in the pro-apoptotic environment of the diseased aneurismal vessel. Indeed, frequent clinical complications such as endoleaks or migration of the implant were shown to be related to insufficient healing of tissue surrounding the SG, due to apoptosis of vascular cells in aneurysms [1]. The hypothesis underlying our work is that an anti-apoptotic coating could favor healing around the SG and prevent the complications occurring with current implants. In previous work, we developed a bioactive coating by covalent immobilization of two known anti-apoptotic molecules, chondroitin sulfate (CS) and epidermal growth factor (EGF) [2], on 2D polymer films. We have shown that this coating increased *in vitro* adhesion, growth and resistance to apoptosis of rat VSMC, compared with bare PET [3] and PTFE films. However, several issues remain to be clarified to allow technological transfer on commercial implants. In this study, we first tested the bioactivity of CS+EGF coating on human vascular cells (fibroblasts, smooth muscle cells), then, we investigated the transferability of the coating from 2D substrates to real 3D SG and finally, we examined several sterilization methods that could be used for this coating in the context of an industrial manufacturing process.

Materials and Methods:

To allow the grafting of biomolecules on polymeric substrates (PET, PTFE), an intermediate organic thin film rich in primary amines ("L-PPE:N") was deposited by plasma polymerization [4]. In the case of L-PPE:N deposition on SG (ePTFE, AdvantaV12, Atrium, Hudson, NH), the SG was dilated and placed on a rotating mandrel in the plasma chamber, to ensure homogeneous coating (~100 nm). Then, CS was grafted on L-PPE:N via carbodiimide chemistry (EDC/NHS), and finally EGF was covalently immobilized on CS using the same type of chemistry [3]. Bioactivity of the CS+EGF coating deposited on PET was tested on human aortic fibroblasts (h-AoAF) and human aortic smooth muscle cells (h-AoSMC) with survival tests in serum free conditions for 4 and 7 days. This test was chosen since serum-free media mimics the depletion of nutrients in aneurysms after EVAR and since these cells are prone to death by apoptosis in aneurysmal vessels. At each time point, metabolic activity of cells was measured with Alamar blue assay and cells were fixed with crystal violet staining. To validate the transfer of our coating on 3D SG, surface composition of the L-PPE:N layer on the SG was analyzed by XPS, and homogeneity was observed by Acid Fuchsin staining, allowing us to quickly visualize the presence of L-PPE:N thanks to the interaction of the dye with positively-charged surface components such as amines. To ensure that our bioactive coating adhered sufficiently well on the SG to survive insertion into the blood vessel via the valve of the insertion device, the SG was crimped on a balloon catheter and inserted with a 8F Hemaquet introducer. Both Acid Fuchsin staining and XPS analysis were repeated after this step and the results were compared to those obtained before the valve, to assess possible damage. Lastly, several sterilization methods were tested on PET with L-PPE:N and L-PPE:N+CS, namely low-temperature hydrogen peroxide gas plasma sterilization (Sterrad®), low temperature ozone and hydrogen peroxide sterilization (3M™ Optreoz™ 125-Z), autoclave steam sterilization (121°C) and disinfection by immersion in 70% ethanol as a control. Several film properties were compared before and after

sterilization to evaluate the impact of each method (film integrity observed with Fuchsin Acid staining, surface properties studied by measurement of surface free energy and XPS analysis). Each experiment was repeated 3 times, and each time included 3 samples for each condition.

Results:

The CS+EGF bioactive coating, when created on PET, induced a clear increase in survival in human vascular cells after 4 and 7 days in serum free medium (Fig. 1). At 7 days, we obtained survival rates of 72 ± 20 % survival on CS+EGF coating compared to 33 ± 15 % on bare PET with h-AoAF; and 114 ± 23 % survival on CS+EGF coating compared to 43 ± 20 % on bare PET with h-AoSMC. Previous results with rat VSMC have shown that differences are even more drastic on PTFE films (data not shown) due to the well-known hydrophobic surface of virgin PTFE, which does not favor cell adhesion and survival. Moreover, Fuchsin Acid staining of L-PPE:N deposited on a 3D commercial SG demonstrated the homogeneity of the L-PPE:N covering on the SG surface. XPS analysis of our coating deposited on the SG showed that the composition was similar to that previously measured on 2D substrates [4], and that the compositions were consistent along the SG surface. Furthermore, the bioactive coating displayed very good adhesion on the SG, no change in composition (XPS) being observed after passage through the tight valve of an introducer, and no cracking after dilatation of the SG. Finally, among the sterilization methods tested, low-temperature hydrogen peroxide gas plasma sterilization (Sterrad®) showed best results: it preserved L-PPE:N film integrity as well as L-PPE:N and L-PPE:N+CS surface properties (water contact angle, surface free energy). To confirm the selection of Sterrad as a non-destructive sterilization method for L-PPE:N and CS surfaces, XPS measurements are under way.

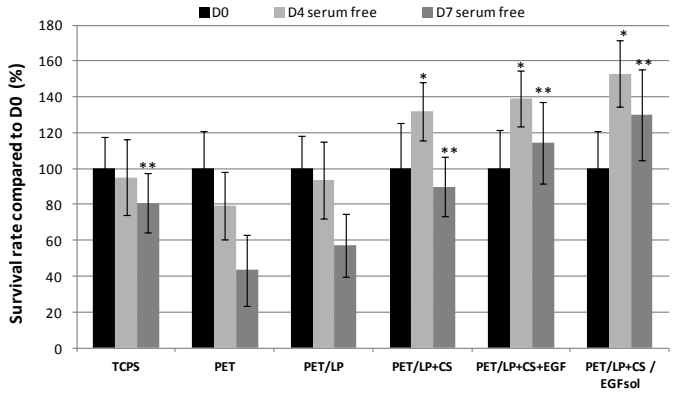

Fig 1: Survival rate of aortic smooth muscle cells on PET with bioactive coating LP+CS+EGF (*, **: statistically different from PET $p<0.0001$, resp. at D4 and D7)

Discussion:

We have demonstrated that our bioactive coating based on CS and EGF can trigger proliferative behavior over 4 days and pro-survival behavior over 7 days in several human vascular cell types when placed in serum free conditions. Moreover, we have successfully applied this bioactive coating on the surface of a commercial SG. The coating displayed excellent adhesion, allowing catheter-based insertion and dilatation of the SG without damage. Finally, we have started to investigate potential sterilization methods for our coating, and one of them, the low-temperature hydrogen peroxide gas plasma sterilization (Sterrad®), seems promising for the conservation of surface properties of both L-PPE:N and L-PPE:N+CS. However, the necessary EGF sterile grafting step after sterilization of L-PPE:N+CS still needs to be designed and optimized. *The authors are grateful for technical help from Z. Qin and J. Lefebvre, funding by NSERC/CIHR and CRC (SL), full scholarship by FQRNT (PL) and lowered costs for Atrium SG by Canadian Hospital Specialties (CHS).*

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