Bioactive anti-apoptotic coating: from 2D substrates to 3D commercial stent grafts for in vivo testing

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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 VSMC, compared with bare PET [3] and PTFE films. In this study, we created the bioactive CS+EGF coating on a real 3D SG. The surface-near composition of the coating, its adhesion and mechanical resistance on the SG surface were studied and optimized before implantation in a canine bilateral aneurysm model previously developed by our group [4].

Methods: To allow the grafting of biomolecules on the implant surface, an intermediate organic thin film rich in primary amines ("L-PPE:N") was deposited by plasma polymerization on the SG (ePTFE, AdvantaV12, Atrium, Hudson, NH, provided by CHS at lowered costs) [5]. During deposition, the SG was dilated and placed on a rotating mandrel in the plasma chamber, to ensure homogeneous coating (~100 nm). After creating this N-rich organic layer, amide bonds were created via carbodiimide chemistry (EDC/NHS) between carboxylic acid groups of the CS and the primary amines of L-PPE:N. EGF was then covalently immobilized on CS using the same type of chemistry [3]. Surface composition was analyzed by XPS, homogeneity of the L-PPE:N layer having first been confirmed by Acid Fuchsin staining. To ensure that our bioactive coating adhered sufficiently well on the SG to survive handling and insertion into the blood vessel via the valve of the insertion device, the SG was crimped on a balloon catheter after immersion in PBS, 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 passage through the valve, to assess possible damage to the layer. Adhesion of L-PPE:N on the SG was evaluated by a peel test followed by Acid Fuchsin staining. *In vivo* bioactivity of our CS+EGF coating underwent preliminary testing in a canine bilateral iliac aneurysm model, along with the coating's ability to reduce endoleaks (3 dogs, protocol approved by the institutional animal committee in accordance with guidelines of CCAC). In each dog, one control SG and one bioactive SG were implanted under fluoroscopy. Directly after deployment of those devices in the aneurysms, endoleaks were created at the proximal necks. The persistence and size of the leaks were assessed at 1 week, 1 month and 3 months after implantation, using Doppler ultrasound and angiography. After 3 months, the animals were sacrificed, the aneurysms were harvested and cut in 5 mm-thick slices, photographed and sent for histology.

Results: The bioactive coating, CS+EGF, was created with success on an ePTFE SG. Its composition on the SG was found to be comparable to previous results obtained on 2D substrates [5]. Moreover, this bioactive coating displayed very good adhesion on the SG, no change in composition (XPS) being observed after passage through a tight valve, and no cracking after extension or dilatation of the SG. The *in vivo* study showed that the bioactive coating helps reduce the extent and persistence of endoleaks, tissues around the bioactive SG tending to manifest improved healing, with more organized cell layers around the implant and less thrombus rich in red blood cells.

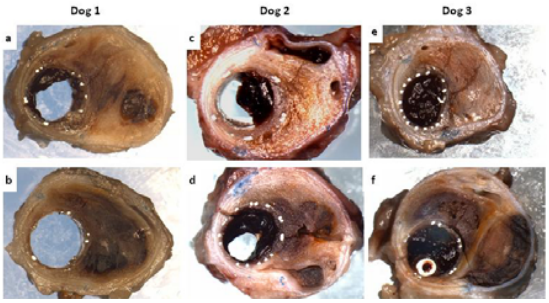

Figure 1: Macroscopic images showing healing in the middle section of aneurysm 3 months after implantation of bioactive SG (a,c,e) or control SG (b,d,f)

Conclusions: We have successfully applied a bioactive coating based on chondroitin sulfate and epidermal growth factor on the surface of a SG. This coating displayed excellent adhesion, allowing catheter-based insertion and dilatation of the SG without damage. Preliminary *in vivo* testing has shown improved healing and endoleak reduction in the presence of the CS+EGF coating, however, more animal tests will be required to fully characterize the *in vivo* bioactivity of our coating.

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