Proof of concept of a layer-by-layer bioactive coating for cardiovascular implants

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Bioactive coatings are increasingly used to improve biocompatibility of implants. Bioactive molecules can be either immobilized or released to trigger specific cell response in order to reach better tissue healing around implants or tissue engineering scaffolds. Our lab is thus developing bioactive coatings to improve healing around stent-grafts (SG) which are deployed in the abdominal aorta in order to prevent aneurysm rupture. This procedure called endovascular aneurysm repair, is minimally invasive and therefore minimizes the risks and recovery time for patients. Its success rate is however limited by poor healing around SG. We hypothesize that the gradual release in the aneurysm of chondroitin sulfate (CS), an ECM component shown to play a major role in vascular healing, in particular by preventing vascular smooth muscle cells (VSMC) apoptosis (Charbonneau et al. 2007), and a pro-proliferative growth factor, could stimulate fibroblasts and VSMC proliferation, resistance to apoptosis and myofibroblasts differentiation, thus allowing better healing of the vessel around the implant and preventing aneurysm progression.

Layer-by-layer (LbL) appears as an interesting strategy to achieve such bioactive coating as it enables to integrate a great amount of bioactive compounds in the film. Moreover, the layers can be degraded at physiological conditions releasing their bioactive content in surrounding tissues over an extended period of time. This simple technique consists in alternating depositions of polymers with opposite charges to create layers that are held together by electrostatic bound. LbL also allows control over various parameters such as the film rigidity, the layers size and the layers degradation rate by adjusting polymer solutions ionic concentration and pH as well as the deposition time.

We also hypothesize that a primary-amine rich plasma polymerized coating (L-PPE:N) can be promising as an initial charged substrate to create such LbL coating on any kind of biomaterial surface. Moreover, since LPPEN has shown interesting cell adhesive properties (Lerouge et al 2007), its presence may stimulate cell adhesion once the LbL coating is completely degraded.

We here present preliminary experiments conducted on a chitosan (CHI) and CS film in order to demonstrate the possibility of creating a LbL coating on L-PPE:N substrate and to validate characterization techniques. These substances were chosen for the simplicity of the LbL film they produce and for cost and availability reasons. Our long-term aim is however to create a LbL combining an anti-apoptotic agent, a growth factor, and a pro-adhesive protein.

Materials and Methods: CHI and CS LbL coatings were characterized using QCM-D (Q-sense E4, Gothenberg, Sweden), ellipsometrie, water contact angle measurement and colorimetry test using dimethylmethylene blue (DMMB) to monitor CS release in aqueous media.

To prepare the layer-by-layer samples, L-PPE:N was plasma deposited for 8 minutes on the following substrate: silicon wafers for ellipsometrie characterization, glass for contact angle characterization and on QCM-D sensors electrodes. The samples were then alternately incubated in CS solution (1mg/ml, 0,15M NaCl) and in CHI solution (1mg/ml, 0,15M NaCl with pH adjusted to ~5,5 with HCl and NaOH) for 20 minutes with MilliQ water rinsing step between each deposition. For DMMB characterization, samples were then placed in a 24 wells plate with 500µL PBS 1X for 5 days in order to release the CS embedded in the layers. DMMB colorant was then added to the PBS and absorbance data at 525 nm (Farndale et al. 1986) was taken with a spectrophotometer.

Results: The QCM-D results showed a gradually decreasing frequency shift which indicates that there is an addition of mass on the sensors when the polymers are deposited and that the film is therefore building up properly (fig 1). Films including up to 15 layers were thus created. The alternating contact angle data indicates that the film is built in a layer-by-layer fashion (fig 2). Since the angle size is directly influenced by the composition of the outermost layer, alternating angles would indicate that the layers composition changes with every depositions. Finally, when comparing DMMB absorbance data with a CS calibration curve, we calculated that for a 15 layers film, the quantity of CS embedded in the layers was around 0,5µg/mm^2.

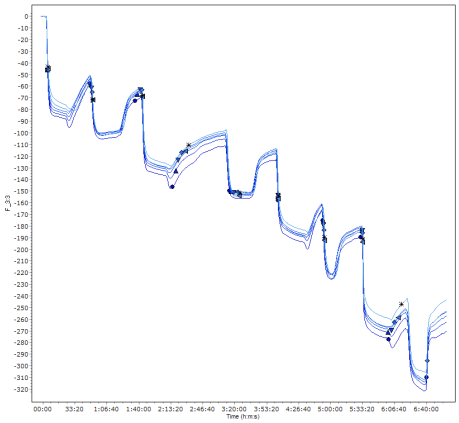

Fig 1 - The decreasing frequency shift indicates that there is a film build up

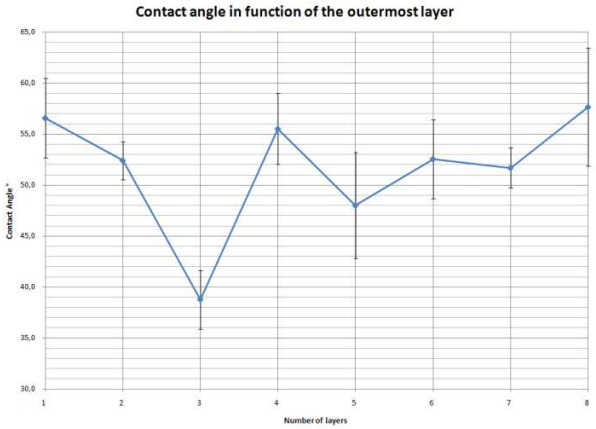

Fig 2 - Alternating contact angle indicates that the film is built in a LbL fashion

Discussion: The preliminary results showed that the chosen characterization techniques are effective and that layer-by-layer deposition can be achieved on a L-PPE:N substrate. Our next step will be to build and characterize our final coating.

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