Bioactive coatings for vascular implants: a promising strategy using oriented tethering of EGF on chondroitin sulfate

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Introduction. Immobilization of bioactive molecules such as growth factors on the surface of implants is of major interest in the field of biomaterials. However, the immobilization methods that have been used so far have major limitations: non-optimal bioactivity because of growth factor denaturation occurring upon immobilization and high cost due to the large quantities of growth factor required to cover the surface. Our objective is to tackle both limitations by using a lower quantity of growth factor but in a more efficient way, where all growth factor molecules remain bioactive. In previous work, we developed a strategy to tether proteins in a non-covalent but oriented fashion; this strategy being based on two high affinity peptides, Ecoil and Kcoil. [1] A bioactive Ecoil-tagged epidermal growth factor (Ecoil-EGF) was produced and successfully captured on Kcoil-functionalized surfaces, leading to higher EGFR phosphorylation and cell adhesion compared to randomly grafted EGF. [2,3] We also showed that chondroitin sulfate (CS) and EGF, when combined in a bioactive coating, demonstrated anti-apoptotic and pro-proliferative properties on VSMC. [4] In this work, we demonstrate the advantages of CS as a sublayer for growth factor tethering thanks to its combined low-fouling and cell adhesive properties; we prove that oriented tethering can be used to immobilize EGF on CS and we establish the superior pro survival properties of the combination CS+oriented EGF.

Methods. Covalent grafting of chondroitin sulfate (CS) and carboxymethylated dextran (CMD) on aminated surfaces was obtained via carbodiimide chemistry. EGF immobilization on CS and CMD was performed either by random grafting or by oriented tethering. Covalent grafting of EGF on CS or CMD was obtained via carbodiimide activation, leading to random orientation of EGF on the surface. Cysteine-terminated Kcoil layers were grafted on CS and CMD using a heterobifunctional linker. Oriented EGF tethering was generated by capture of Ecoil-tagged EGF on the Kcoil-functionnalized surface. Water contact angle and ellipsometry measurements were used to optimize each grafting step. Cell culture and ELISA were performed on aminated 96 well plates. A direct ELISA assay using anti-EGF antibody was used to quantify immobilized EGF via both strategies. Cell culture was performed with rat VSMC (a7r5). After a 24h attachment of cells in complete growth medium, cells were exposed to serum free medium for 3, 5 or 7 days. For each timepoint, Alamar blue (Invitrogen, Burlington, ON) was added to the medium to evaluate the metabolic activity of the cells. Cells were fixed and stained with crystal violet and pictures were taken to correlate metabolic activity with cell number and observe cell morphology on each surface.

Results. Dry thickness measurements by ellipsometry confirmed the selectivity of coiled/coil tethering on CS: Ecoil-EGF capture occurred on CS+Kcoil (0.51±0.14nm) but not on CS alone (0.04±0.11 nm). EGF quantification by ELISA showed that high EGF surface densities can be reached on CS via the coiled-coil system while using relatively low EGF concentration during the incubation step, which is advantageous for realistic applications. A plateau value of 44.3±9.2 fmol/cm^2 was indeed obtained with an incubation of 22 nM of Ecoil-tagged EGF on Kcoil-CS (as compared to 1.6±0.5 fmol/cm^2 and 1μM, respectively, for EGF grafted in a random fashion). ELISA assays also showed that CS presented non-fouling properties comparable to those of CMD: EGF non specific adsorption was <0.06 fmol/cm^2 both on CS and CMD. Finally, our cell culture assays demonstrated that VSMC survival after 7 days in serum free medium was higher on CS+oriented EGF than on CS+random EGF (104±16 % of the initial cell number remained on oriented EGF, against 64±22% for random EGF). Moreover, the superiority of CS as a sub layer was successfully proven: after 7 days in serum free medium, CS+oriented EGF showed 30 times more cells compared to CMD+oriented EGF despite a similar EGF concentration on both surfaces.

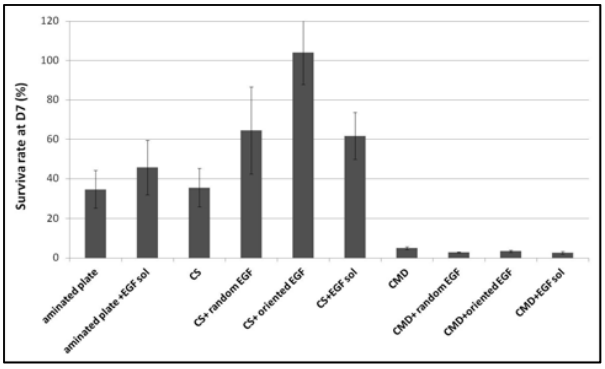

Figure 1: Cell survival rate after 7 days in serum free medium compared to initial adhesion on each surface

Conclusions. This work demonstrates the advantages of CS as a sublayer for oriented immobilization of growth factors, and the improvement of grafting efficiency and bioactivity brought by oriented tethering compared to random grafting. Thanks to the versatility of coiled/coil tethering, CS may be used as a sublayer co-capture of several Ecoil-tagged growth factors. The benefit of such a system would be tremendous since it would allow to fine-tune implant bioactivity for specific applications by changing the ratio of growth factors on the surface.

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