Bioactive anti-apoptotic coating for vascular implants combining chondroitin sulfate and EGF: optimization by oriented immobilization of the growth factor

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Introduction

Despite recent progress, healing around implants and biocompatibility remain a major issue. In that endeavour, one of the most promising technologies that have been developed is the immobilization of bioactive molecules such as growth factors at the implant surface. However, most of immobilization methods that have been used so far are suboptimal: bioactivity issues due to growth factor denaturation occurring upon immobilization and high cost due to the large quantities of growth factor required to cover the surface have been reported. Our objective is to tackle both limitations by using low quantities of fully-bioacitve growth factors. In previous work, we have already reported the development of an experimental approach aiming to tether proteins in a non-covalent but oriented fashion through the use of peptides, namely the E and K coils, that heterodimerize with high specificity and affinity. [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 randomly tethered 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.

Materials and Methods

Covalent grafting of CS and carboxymethylated dextran (CMD) on aminated surfaces was achieved 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 done via carbodiimide activation, leading to random orientation of EGF on the surface. For oriented immobilization, cysteine-terminated Kcoil layers were first grafted on CS and CMD using a heterobifunctional linker. EGF tethering was then 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. Resistance to apoptosis of VSMC on each surface was tested in serum free conditions with Hoechst 33342/Propidium iodide staining.

Results

First, the selectivity of EGF coiled/coil tethering on CS was confirmed with dry thickness measurements by ellipsometry after each grafting step: Ecoil-EGF capture occurred on CS+Kcoil

(0.51±0.14nm) but not on CS alone (0.04±0.11 nm). Then, EGF quantification by ELISA showed that high EGF surface densities can be reached on CS via our E/K coiled-coil system while using relatively low EGF concentration during the incubation step, a considerable advantage in the perspective of large-scale applications. We indeed observed a plateau value of 44.3±9.2 fmol/cm^2 with a 22 nM incubation 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. Moreover, our cell culture assays demonstrated that VSMC survival over 7 days in serum free medium was higher on CS+oriented EGF than on CS+random EGF (at D7: 110±17 % survival on oriented EGF, against 66±17% for random EGF) (Fig.1). This test also successfully proved the superiority of CS as a sublayer: 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. Finally, we verified that the oriented EGF increased survival by decreasing of apoptotic death in VSMC.

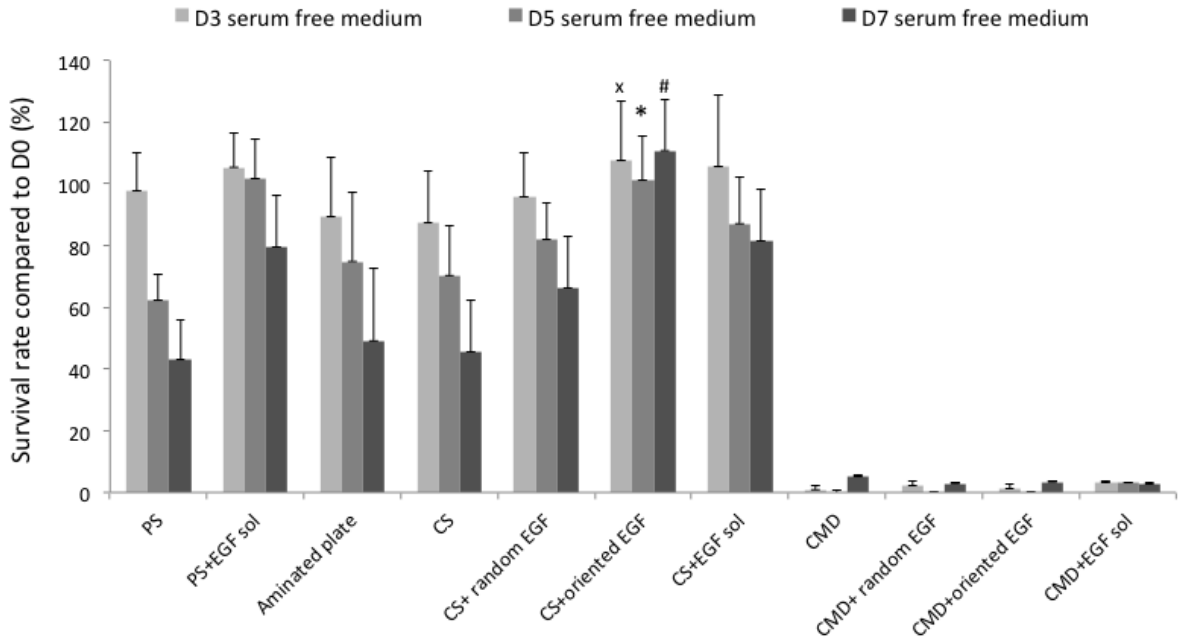

Fig.1: VSMC survival in serum free conditions over 7 days on CS and CMD in the presence of oriented or random EGF (x, *, #: different from CS+random EGF at D3 ($p<0.05$), D5 ($p<0.005$) and D7 ($p<0.005$) respectively)

Discussion

In this work, we demonstrated the advantages of CS as a sublayer for oriented immobilization of growth factors. We also showed the improvement of grafting efficiency and bioactivity brought by oriented tethering when compared to random grafting. Thanks to the versatility of coiled-coil tethering, CS may be used as a sublayer to simultaneously combine 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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References

[1] De Crescenzo G. Biochem. 2003. 42;1754-63.[2] Boucher C. Biomat.2010. 31 :27;7021-31. [3] Liberelle B. Bioconj. Chem. 2010. 21:12;2257-66. [4] Charbonneau C. Biomat. 2011.32 :1591-1600.