EVENT ABSTRACT

« Back to Event

Amphiphilic coil-tags for the direct and oriented adsorption of growth factors on biomaterials

Frederic Murschel[1, 2], Aldo Zaimi[2], Samantha Noel[1, 2], Mario Jolicoeur[1] and Gregory De Crescenzo[1, 2]

[1] Ecole Polytechnique de Montréal, Department of Chemical Engineering, Canada
[2] Ecole Polytechnique de Montréal, Institute of Biomedical Engineering, Canada

Introduction: Growth factor (GF) immobilization is a crucial step towards the engineering of bioactive materials, as these molecules act as prime cues that direct cellular fate. Numerous strategies have been developed in that endeavour. They however often fail to consider technological limitations, which hinders their potential for translation to clinical use[1]. Tag-fused GF chimeras have recently emerged as an alternative that would circumvent such limitations by allowing for a simple, readily scalable and translatable grafting method[2],[3]. We have successfully used two complementary E and K peptides for the oriented attachment of E-tagged GFs on K-decorated materials via E/K coiled-coil interactions (**Fig. 1A-B**)[4]. We here report a novel strategy using the same GF chimeras, which however benefits from the strong amphiphilic nature of the coil peptides to direct a single-step tag-mediated adsorption onto materials of interest.

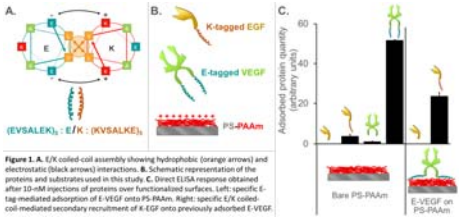

Figure 1. A. E/K coiled-coil assembly showing hydrophobic (orange arrows) and electrostatic (black arrows) interactions. **B.** Schematic representation of the proteins and substrates used in this study. **C.** Direct ELISA response obtained after 10-nM injections of proteins over functionalized surfaces. Left: specific E-tag-mediated adsorption of E-VEGF onto PS-PAAm. Right: specific E/K coiled-coil-mediated secondary recruitment of K-EGF onto previously adsorbed E-VEGF.

Materials and Methods: Coil-tagged epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) were produced by transient transfection of human embryonic kidney 293-cells as previously described[4]. Adsorbed protein after incubation was quantitated by a direct Enzyme-Linked Immunosorbent Assay. A primary human umbilical vein endothelial cell (HUVEC) line cultivated in EGM-2 medium was used for VEGF bioactivity assays.

Results and Discussion: 5 proteins – EGF, VEGF, E-EGF, E-VEGF and K-EGF – were incubated over 6 different polystyrene (PS)-based substrates with varying surface chemistries and ELISAs were performed to determine the amount of bound GF. This protein/surface-spanning experiment highlighted several protein/surface combinations of interest, among which the specific tag-mediated adsorption of VEGF fused to the anionic E peptide on positively-charged poly(allylamine) (PAAm)-functionalized PS (**Fig. 1C-left**). We further observed that E tag-adsorbed VEGF could specifically recruit a secondary layer of K-tagged EGF, demonstrating that coiled-coil interactions were not prevented following adsorption (**Fig. 1C-right**).

The stability of the adsorbed layer of E-VEGF was assayed in cell culture conditions with varying amounts of competing sera proteins (FBS), and the results indicated that a large amount of the GF remained attached to the surface for several days (**Fig. 2A**). This simple tethering strategy was successfully transferred to more relevant substrates, that is, aminated poly(ethylene terephthalate) (PET-PAAm) films (data not shown). HUVEC proliferation assays further demonstrated that E-VEGF adsorbed on PET-PAAm films retained a high mitogenic potency (**Fig. 2B**).

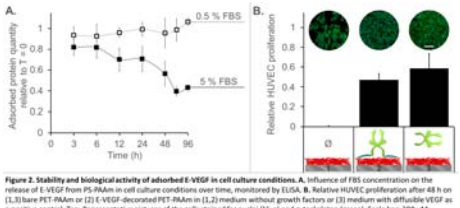

Figure 2. Stability and biological activity of adsorbed E-VEGF in cell culture conditions. A. Influence of FBS concentration on the release of E-VEGF from PS-PAAm in cell culture conditions over time, monitored by ELISA. **B.** Relative HUVEC proliferation after 48 h on (1,3) bare PET-PAAm or (2) E-VEGF-decorated PET-PAAm in (1,2) medium without growth factors or (3) medium with diffusible VEGF as a positive control. Top: Representative pictures of the cells stained for nuclei (blue) and cytoskeleton (green). Scale bar: 200 µM.

Conclusion: Engineering of GFs fused to amphiphilic peptides is of prime interest for the surface decoration of biomaterials. We here showed in particular that VEGF could be simply, though efficiently, adsorbed on aminated substrates, in an oriented and stable manner without any loss in biological activity, via a specific E coil-tag/surface interaction.

This work was supported by the Canada Research Chair on Protein-Enhanced Biomaterials (G.D.C.), the Canada Research Chair in Applied Metabolic Engineering (M.J.), by the Natural Sciences and Engineering Research Council of Canada (G.D.C., M.J. and A.Z.), by the Fonds de recherche du Québec - Nature et technologies (F.M. and S.N.) and by the MEDITIS training program (F.M.).; We thank Dr. Benoit Liberelle for technical support and fruitful discussion

References:

[1] Place, E. S.; Evans, N. D.; Stevens, M. M., Complexity in biomaterials for tissue engineering. Nat. Mater. 2009, 8, (6), 457-70.
[2] Culpepper, B. K.; Bonvallet, P. P.; Reddy, M. S.; Ponnazhagan, S.; Bellis, S. L., Polyglutamate directed coupling of bioactive peptides for the delivery of osteoinductive signals on allograft bone. Biomaterials 2013, 34, (5), 1506-13.
[3] Kumada, Y.; Shiritani, Y.; Hamasaki, K.; Ohse, T.; Kishimoto, M., High biological activity of a recombinant protein immobilized onto polystyrene. Biotechnol. J. 2009, 4, (8), 1178-89.
[4] Murschel, F.; Liberelle, B.; St-Laurent, G.; Jolicoeur, M.; Durocher, Y.; De Crescenzo, G., Coiled-coil-mediated grafting of bioactive vascular endothelial growth factor. Acta Biomater. 2013, 9, (6), 6806-13.

Keywords: self-assembly, growth factor, biofunctionalization, Polymeric material **Conference:** 10th World Biomaterials Congress, Montréal, Canada, 17 May - 22 May, 2016.

Presentation Type: General Session Oral **Topic:** Protein interactions with biomaterials

Citation: Murschel F, Zaimi A, Noel S, Jolicoeur M and De Crescenzo G (2016). Amphiphilic coil-tags for the direct and oriented adsorption of growth factors on biomaterials. *Front. Bioeng. Biotechnol. Conference Abstract: 10th World Biomaterials Congress.* doi: 10.3389/conf.FBIOE.2016.01.00062

 Received: 27 Mar 2016; **Published Online:** 30 Mar 2016.