

EVENT ABSTRACT

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The addition of the collagen binding domain of fibronectin potentiates the biochemical availability of basic fibroblast growth factor for the culture of induced pluripotent stem cells

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Introduction: The culture of various cell types including induced pluripotent stem cells (iPSC), requires the use of growth factors such as bFGF (basic Fibroblast Growth Factor)^{[1],[2]}. These factors are generally added to the medium in their diffusive form and have a very short half-life^[3]: they disappear quickly from the culture medium and must be replaced at high cost. We here explore the stable and oriented tethering of bFGF on a cell culture substrate, namely gelatin (denatured collagen). Our strategy relies on the expression of two fusion proteins being labelled with two distinct peptides (the E and Kcoils) that bind to each other with great affinity and specificity^[4]. That is, a chimera corresponding to bFGF fused to the Ecoil peptide on the one hand, and the collagen-binding domain (CBD) of fibronectin fused to the Kcoil peptide on the other hand (**Fig. 1**).

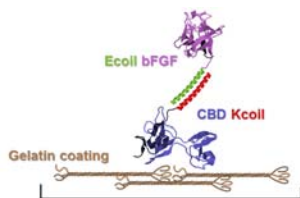


Figure 1: Schematic illustration of Ecoil-bFGF tethered in an oriented manner on a gelatin-coated surface functionalized with CBD-Kcoil.

Materials and Methods: The Ecoil-bFGF fusion protein was produced in *E. coli* and purified by Ni-NTA affinity chromatography followed by TEV protease cleavage and ion exchange chromatography. The CBD-Kcoil fusion protein was produced in *E. coli* and purified by solubilizing inclusions bodies in an 8 M urea buffer, dialyzing against PBS, and then followed by a Ni-NTA affinity chromatography. The ability of Ecoil-bFGF protein to bind to Kcoil was assessed by surface plasmon resonance (SPR), using a Kcoil-coated chip while its ability to bind to its cognate receptor was tested by subsequent injections of a recombinant chimera of the FGF Receptor (FGFR1-Fc). Finally, the suitability of the proposed approach for cell culture will be soon assessed by cultivating iPSCs on a gelatin-coated substrate to be functionalized with CBD-Kcoil/Ecoil-bFGF.

Results and Discussion: Both fusion proteins were produced and purified with high purity. The yield of the purification of Ecoil-bFGF was assessed by ELISA and reached 1.1 mg per liter of bacteria. On the one hand, SPR analyses (**Fig. 2**) demonstrated that Ecoil-bFGF specifically bound to a Kcoil-coated chip, and subsequently interacted with FGFR1-Fc with high affinity ($K_D = 1.8$ nM), in good agreement with the literature^[5]. On the other hand, the bioactivity of CBD-Kcoil was assessed via an Enzyme-Linked Immunosorbent Assay (ELISA) against bFGF: gelatin-coated substrates were incubated with CBD-Kcoil then Ecoil-bFGF, and the formation of the gelatin/CBD-Kcoil/Ecoil-bFGF complex was demonstrated by the specific recruitment of anti-bFGF antibodies. Altogether, the data indicated that our novel strategy enabled the specific capture of bioactive bFGF via collagen/CBD and coiled-coil interactions.

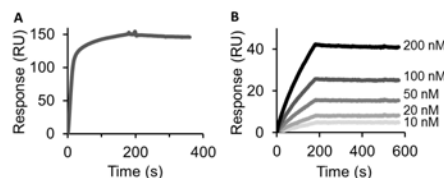


Figure 2: SPR biosensor-based assays demonstrating the bioavailability of Ecoil and bFGF moieties. Control-corrected sensorgrams are presented. (A) Injection of 10 nM Ecoil-bFGF over Kcoil. (B) Injection of increasing concentrations of FGFR1-Fc over Ecoil-bFGF immobilized by coiled-coil interactions.

Conclusion: Providing iPSCs with bioactive bFGF in a non-diffusible form may significantly decrease expenses related to cell culture. Furthermore, the modular approach we undertook may make this strategy amenable to the development of more complex scaffolds combining several growth factors (bFGF, EGF, VEGF ...), and thus pave the way to the development of novel approaches in the field of tissue engineering.

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