Coil-tagging of Vascular Endothelial Growth Factor for Oriented and Tunable Biomaterials Functionalization

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Statement of purpose. Growth factor functionalization of tissue-engineered scaffolds and prostheses for regenerative medicine is of prime interest. These cytokines provide indeed instructive cues that stimulate cellular adhesion, migration and/or proliferation. In particular, human vascular endothelial growth factor (VEGF) specifically promotes the mitogenesis of vascular endothelial cells, and could thus address the pressing need for neo-vascularization within tissue-engineered scaffolds. Natural growth factors however display a limited half-life *in vivo* as well as poor retention at sites of action. The rational design of new chimeric cytokines is thus required for site-specific and lasting functionalization strategies, and, *in fine*, control of cell behavior onto/within the implant [1]. In that endeavor, we designed an Ecoil-tagged VEGF chimera (E5-VEGF) to enable its oriented and highly specific capture by its complementary Kcoil-derivatized substrate [2]. Our strategy based on E5/K5 coiled-coil interaction was previously validated with the human epidermal growth factor, which induced sustained proliferation of human corneal epithelial cells when tethered [3]. The bioavailability of tethered E5-VEGF (tE5-VEGF) was similarly assessed *in vitro*, and K5 peptide analogs were engineered to fine-control its stability upon grafting to the biomaterial.

Methods. Production of E5-$(His)_8$-tagged VEGF165 was performed by transient transfection of human embryonic kidney-293 cells. Solid-phase synthesis of cysteine-terminated peptides was performed on a CEM Liberty System microwave assisted synthesizer, using Fmoc chemistry. Surface plasmon resonance (SPR)-based assays were performed on a Biacore 3000 biosensor at 25°C, using HBS-EP as running buffer and covalent thiol coupling chemistry with a PDEA linker. Aminated cell culture-compatible glass surfaces were used as a model substrate: they were first derivatized with K5 peptides using a LC-SPDP linker, and non-specific adsorption sites were blocked with bovine serum albumin [4]. The glass surfaces were covered with E5-VEGF for one hour to enable coiled-coil-mediated protein capture. A direct ELISA assay was performed to evaluate the levels of tethered protein. Bioactivity assays were performed using a primary human umbilical vein endothelial (HUVE) cell line. Cell seeding conditions onto the glass substrates were adjusted to reach the same levels of adhesion on each surface after 4h, prior to serum starvation. After 48h, cells were fixed with formaldehyde, nucleic acids were stained with Sytox Green and fluorescence photographs of the total surfaces were taken to enable cell counting.

Results. *In vitro* bioactivity assays demonstrated that diffusible E5-VEGF (dE5-VEGF) induced HUVE cell survival in serum-free medium, i.e. in pro-apoptotic conditions [5], in a manner very similar to untagged dVEGF (EC50 values of 0.43±0.04nM and 0.65±0.05nM, respectively). We similarly observed a much higher rate of HUVE cell survival on K5/E5-VEGF-decorated glass surfaces when compared to K5-derivatized surfaces (Table 1), thus validating tE5-VEGF bioavailability.

Table 1. HUVE cell survival rates after 48h (%).

	Cell culture plate	*K5-derivatized glass*
-	12±7	16±3
31.5fmol dE5-VEGF	14±7	-
31.5fmol tE5-VEGF	-	37±3
750fmol dE5-VEGF	57±1	-
750fmol dVEGF	60±5	57±11

Specific capture by K5 peptides was validated *via* an SPR-based assay (Figure 1, left). Of salient interest, numerous injections of a strong chaotropic agent (6M GdnHCl) failed to regenerate the surfaces, thus indicating a remarkable stability for tE5-VEGF. To enable controlled release of the growth factor over time, three K5 peptide analogs were designed and their interactions with E5 evaluated by SPR-based assays: a wide range of kinetics of interactions was obtained (Figure 1, right).

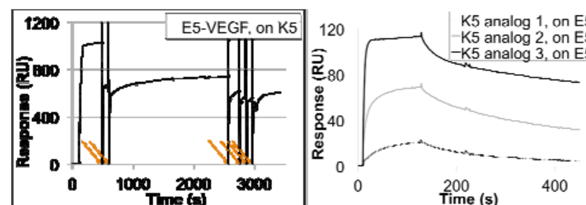

Figure 1. SPR-based assays of coiled-coil interactions: control-corrected sensorgrams of E5-VEGF injection over K5 (left, regeneration attempts are indicated with arrows) and of K5 analogs injections over E5 (right, 100nM each).

Conclusions. We report here an E5-tagged chimera of human VEGF as well as three new complementary K5 peptide analogs for biomaterials functionalization. The bioactivity of E5-VEGF was demonstrated when diffusible and tethered, and the *de novo* designed peptides show promise for a fine control of E5-VEGF release. Our tethering strategy based on coiled-coil interactions is thus an interesting avenue for many applications in the field of biomaterials. Moreover, this study paves the way for the development of a versatile platform for the study of receptor internalization and signaling pathways when the cells are exposed to soluble or tethered cytokines.

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