Vascular Endothelial Growth Factor-Functionalized Biomaterials: Specificity and Stability Controlled by Coiled-Coil Interactions

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Introduction:

The development of tissue engineering is currently hampered by the lack of vascularization in thick tissues. Natural angiogenesis is indeed a complex process that involves multiple sequential and/or concomitant factors that give rise to mature "well-tempered" vessels, which conditions are difficult to reproduce *in vitro*. A few gene therapy studies however succeeded in promoting non-aberrant and uniform angiogenesis [1-2]. We report here a versatile growth factor functionalization strategy based on coiled-coil interactions, which bears the potential to promote angiogenesis by untransformed primary human cells. We focused on human vascular endothelial growth factor (VEGF), which specifically promotes the mitogenesis of vascular endothelial cells. We thus designed and produced a recombinant chimeric protein corresponding to VEGF being N-terminally fused to an Ecoil peptide tag (E5-VEGF), so as to enable its oriented and non covalent capture on surfaces bearing the complementary Kcoil (K5). 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]. Furthermore, to enable controlled release of the growth factor from the biomaterial, we designed three alternative peptides – analogs of the K5 peptide – to be grafted onto the substrate prior to E5-VEGF capture.

Materials and 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. Cell culture-compatible aminated glass surfaces and carboxylated culture well plates were used as model substrates: they were first derivatized with Kcoil peptides using LC-SPDP and EMCH linkers, respectively. Non-specific adsorption sites were blocked with bovine serum albumin [4], prior to E5-VEGF capture on the surface by one-hour incubation. Bioactivity assays were performed using a primary human umbilical vein endothelial (HUVE) cell line. Cell seeding conditions 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 ($n \geq 3$).

Results:

We evaluated the bioavailability and bioactivity of diffusible E5-VEGF on a primary HUVE cell line in pro-apoptotic conditions, that is, in an environment representative of poorly vascularized scaffolds. We observed that E5-VEGF promoted HUVE cell survival in serum-free medium with a half-maximally effective concentration (EC50) very close to the untagged growth factor EC50 (0.43±0.04nM and 0.65±0.05nM, respectively). Similarly, the chimeric growth factor tethered on glass substrates through coiled-coil interactions significantly enhanced survival of HUVE cells when

compared to Kcoil-decorated surfaces (survival rates after 48h of 37±3% and 16±3%, respectively).

We determined by direct ELISA that 31.5±16.5 and 0.13±0.07fmol/cm^2 of E5-VEGF and untagged VEGF were respectively captured onto K5-derivatized glass substrates. The specificity of E5-VEGF capture by the K5 peptides was further validated *via* an SPR-based assay (Figure 1, left). Of prime interest, numerous injections of a strong chaotropic agent (6M GdnHCl) failed to regenerate the surfaces, thus indicating a remarkable stability for E5-VEGF captured by K5 peptides.

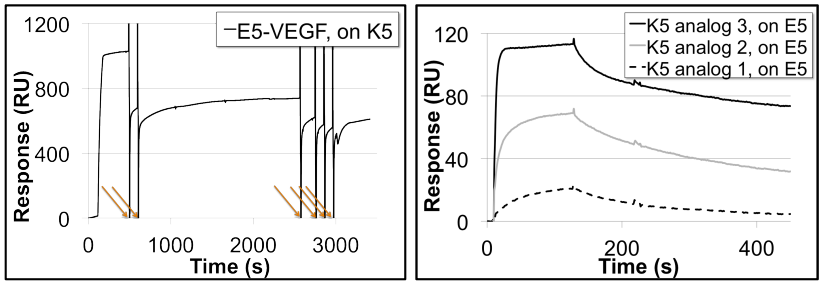

Figure 1. SPR-based assays of coiled-coil interactions: control-corrected sensorgrams of E5-VEGF injection over K5 (left, regeneration attempts are indicated with arrows) and of K5 analogs injections over E5 (right, 100nM each).

To enable controlled release of the growth factor over time, three K5 peptide analogs were designed and their ability to capture E5-VEGF in a similarly specific manner was demonstrated by a direct ELISA (Figure 2). Their interaction with E5 was evaluated by SPR-based assays: a wide range of kinetics of interactions was obtained (Figure 1, right).

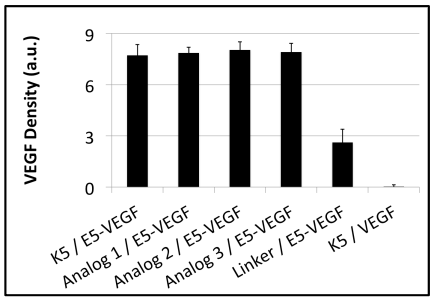

Figure 2. Growth factor density assessed by a direct ELISA, after a 1-nM incubation over Kcoil-functionalized culture wells (17±4pmol/well of Kcoil, VEGF density in arbitrary units, n=3).

Discussion:

We report here an E5-tagged chimera of human VEGF, which was specifically captured onto various Kcoil-derivatized substrates and which preserved its bioactivity when tethered. Moreover, three *de novo* designed K5 peptide analogs were synthesized to fine-control E5-VEGF stability upon grafting, and show great potential for the development of tunable biomaterials functionalization. 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.

References:

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