# Biomimetic Materials Modulate Specific Growth Factor Signaling *In Vitro* and *In Vivo*

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**Statement of Purpose:** Vascular endothelial growth factor was originally discovered as a tumor-derived factor which is able to induce endothelial cell (EC) behaviors associated with angiogenesis. Phase II clinical trials of VEGF as a pro-angiogenic factor have shown limited efficacy due in part to detrimental side effects such as vascular leakage, edema, and hypotension, likely due in part to poor dosing. Binding and controlled release strategies have been developed to sequester and modulate the activity of VEGF. Many VEGF sequestering formulations mimic the native extracellular matrix (ECM), but ECM proteins and proteoglycans can promiscuously bind multiple growth factors (GFs). Here, we present a strategy to sequester and modulate the activity of VEGF using tethered peptide sequences derived from VEGF Receptor 2 (VEGFR2) covalently linked to poly(ethylene glycol) microspheres. We further applied this approach to sequester transforming growth factor β1 (TGF-β1), which is implicated in vascular quiescence and fibrosis during wound healing. We hypothesized that biomaterials designed to mimic the growth factor receptor can specifically bind to and regulate the activity of particular angiogenic GFs.

**Methods:** Peptides were synthesized as previously described[1] using fmoc solid phase peptide synthesis. Poly(ethylene glycol) microspheres were synthesized as previously described[1,2] using an aqueous emulsion technique employing a PEG-rich dispersed phase (containing peptide) in a Dextran-rich continuous phase. Degradable ester-containing PEG-dithiol crosslinkers were synthesized as described elsewhere[3]. Biomaterials containing VEGF-binding peptides (VBP) were assessed in a human umbilical vein endothelial cell (HUVEC) expansion assay of angiogenesis. *In vivo* angiogenesis was examined using a choroidal neovascularization (CNV) model of angiogenesis previously described[4]. Biomaterials designed to bind TGF-β1 were assayed for modulation of HT-2, NIH3T3, and HUVEC expansion.

**Results:** VEGF-binding biomaterials (Fig. 1B) efficiently sequestered VEGF and could reduce HUVEC proliferation in culture. When VEGF-binding biomaterials were pre-loaded with VEGF, the resulting VEGF release was sustained and able to increase HUVEC expansion (Fig. 1D). Biomaterials were re-engineered to contain a hydrolytically labile crosslinker, and the resulting "degradable" VBP-containing microspheres inhibited VEGF activity in culture regardless of the source of VEGF. Degradable VEGF-binding biomaterials also significantly reduced angiogenesis in an *in vivo* model of choroidal neovascularization (Fig. 1C). We also explored a biomimetic approach to modulate the activity of TGF-β1. We identified TGF-β1-binding peptides derived from TGF receptor I (TGFRI) and TGFRII, and identified that the TGFRI-derived peptide recovered HUVECs and HT2s from TGF-β1 inhibition of expansion and reduced TGF-β1-mediated expansion of NIH3T3 fibroblasts.

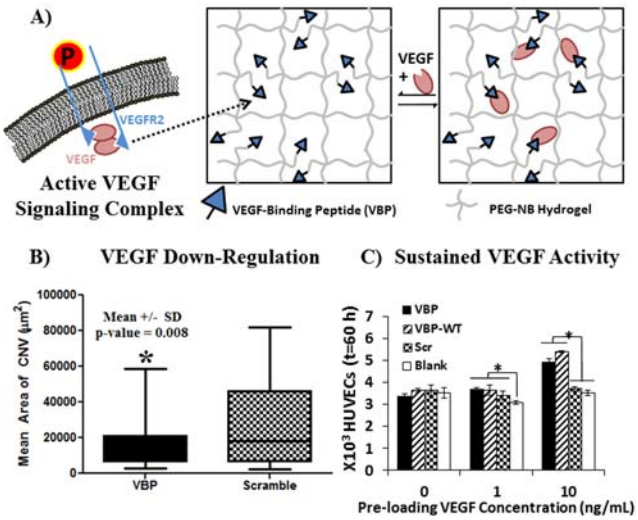


Figure 1. Biomimetic approach to sequester and modulate VEGF activity. A) Schematic of active VEGF signaling complex (phosphorylated VEGFR2 complexed with VEGF dimer). Biomaterials with covalently-attached VEGFR2-derived VEGF-binding peptides (VBPs) sequester VEGF at equilibrium with its solution. B) Down-regulation of CNV area upon injection of VBP microspheres relative to Scrambled VBP microspheres. C) VEGF sustained release in a model of HUVECs, demonstrating that VEGF released from VBP microspheres significantly increased HUVEC expansion.

**Conclusions:** Our results demonstrated that biomimetic materials can modulate the activity of specific growth factors *in vitro* and *in vivo*, and may constitute a novel method to modulate angiogenesis during tissue regeneration. Biomaterials engineered to degrade over time exhibited potent VEGF inhibition regardless of the source of VEGF, and these biomaterials inhibited angiogenesis *in vivo.* When this concept of sequestering was applied to TGF-β1, we identified a unique TGFRI-derived peptide that inhibited TGF-β1 activity in three independent *in vitro* cell models. Biomaterials containing TGFRI-derived peptides inhibited TGF-β1 activity *in vitro* and may serve as a platform to reduce TGF-β1-mediated fibrosis during tissue regeneration. Taken together, our results suggest that a biomimetic approach can efficiently modulate the activity of particular angiogenic GFs and may serve to enhance angiogenesis and reduce fibrosis for improved tissue regeneration.

**References:**

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