Identification of crosstalk between inflammation and angiogenesis in bioactive hydrogels

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Statement of Purpose: Biomedical implants that facilitate communication/interaction with the surrounding tissue and/or circulatory system are rendered ineffective by the huge diffusion barrier and increased electrical resistance presented by the fibrous capsule. For successful implants, it is ideal to have the device surrounded and penetrated by highly vascularized tissue. Both angiogenesis and inflammation are inescapable *in vivo* responses to all biomaterial implants. There is emerging evidence that inflammatory cells regulate the functions of endothelial cells related to angiogenesis(*1, 2*). However, the signals initiating angiogenesis in inflammation are complex and difficult to define. The goal of this study is to addresses the hypothesis: the biomaterial-induced inflammatory response may be critical to control angiogenesis.

Methods: In order to address the hypothesis, we have fabricated porous polyethylene glycol (PEG)-cross-linked tyrosine-derived polycarbonate hydrogels with controlled crosslink and degradation properties that have been identified as variables in controlling angiogenesis(*3*). The hydrogel scaffolds has been made more bioactive via hybridization of pro/anti anigiogenic and/or anti-inflammatory synthetic peptides with extracellular matrix (ECM) materials (e.g., collagen or fibrin gel). The release of peptides from hydrogels has been measured. This system has been utilized for an *in vitro* culture of endothelial and inflammatory cells, their co-culture, and for *in vivo* implants. Using a series of *in vitro* and *in vivo* assays to investigate the effects of the degraded products on angiogenesis and inflammatory responses, we have studied endothelial cell function and behavior modulated by the interactions of inflammatory cells with the bioactive peptide-modified hydrogels.

Results: Peptides-ECM materials (e.g., collagen or fibrin gel) were stably hybridized to hydrogels. Peptides were released more slowly from collagen-hydrogels, compared to fibrin-hydrogels. Laminin-derived pro-/anti-angiogenic peptides (C16 and C16Y, respectively) regulated *in vitro* migration and tubulogenesis of human microvascular endothelial cells. Thymosin β4-derived anti-inflammatory Ac-SDKP peptides efficiently reduced in vitro phagocytosis of human blood-derived macrophages. Collagen scaffold containing lipopolysaccharide (LPS; 100 ng) or functional peptides (0 or 75 μg) were implanted subcutaneously in the backs of mice for 7 days (n=6). Fluorescent microangiography was performed to visualize vasculature and to measure vascular perfusion capacity.(*4*) Phagocytosis of the inflammatory cells that had infiltrated the implants was detected using a Vybrant® Phagocytosis assay kit. Blood vessels in the implants with anti-inflammatory Ac-SDKP peptides showed many disconnected parts with significantly less branching architecture compared to the other conditions shown in Figure (top images). In addition, a dense phagocytotic cell population was observed near blood vessels with no or pro-angiogenic C-16 peptides, whereas there were not many phagocytotic cells with anti-inflammatory Ac-SDKP peptides. Quantification of the vascular perfusion capacity and phagocytosis (Figure top and bottom graphs) showed similar patterns, in which the implants with anti-inflammatory Ac-SDKP peptides showed significantly lower levels of phagocytosis activity and perfusion capacity compared to the implants with no or pro-angiogenic C16 peptides.

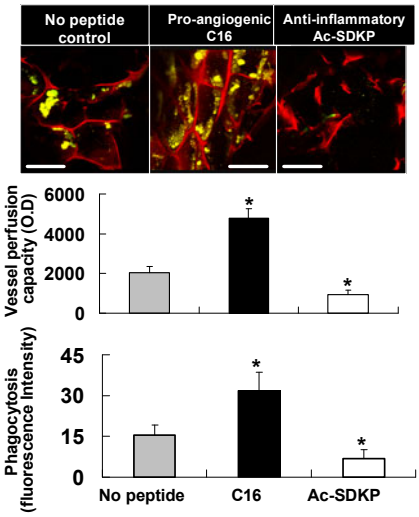

Figure. ***The Ac-SDKP peptide inhibits angiogenesis and phagocytosis in vivo.*** Images show new blood vessels (red) and activated macrophages (yellowish green) in collagen gels with no peptide (left), pro-angiogenic C16 (middle), or anti-inflammatory Ac-SDKP (right). Perfusion capacity of new blood vessels (top graph) and phagocytosis activity (yellow green fluorescence intensity) were enhanced by C16 and inhibited by Ac-SDKP. Scale bar = 100 μm. $p < 0.05$ vs. no peptide.

Conclusions: These results suggest that the inflammatory cell response contributes to the formation of functional blood vessels, and that angiogenesis and the inflammatory response influenced each other in the hydrogel implants. Elucidating a clear mechanism for angiogenesis in biomaterial-induced inflammation through further studies will provide new paradigms of design for the next generation of biomaterials.

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