Bio-Artificial Matrices for Therapeutic Vasularization

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Statement of Purpose: Clinically viable vascularization therapies will lead to better treatment for patients with peripheral artery disease and coronary heart disease as well as enhanced survival of cell and tissue transplants. Many clinical trials to induce new vascular growth have focused on delivery of a single angiogenic gene or growth factor, or activation and delivery of progenitor cells. For this research, we functionalized polyethylene glycol diacrylate (PEGDA)-based hydrogel matrices with bioactive motifs to serve as engineered platforms for cell-demanded delivery of angiogenic growth factors and supportive environments for tissue ingrowth. We examined *the in vivo* release of growth factor from the bio-artificial material and the effects on reperfusion in a mouse hind limb ischemia model.

Methods: We modified published protocols [1-3] to design the PEGDA-based matrices. Degradable base material and adhesive ligand was synthesized by reacting acrylate-PEG_{3400}-NHS with free amines on the collagenase –degradable peptide GPQGIWGQK or cell-adhesive peptide GRGDSPC to make A-PEG-GPQGIWGQK-PEG-A and A-PEG-GRGDSPC. $VEGF_{121}$ was conjugated to Acryl-PEG_{3400}-maleimide. Hydrogel containing A-PEG-GPQGIWGQK-PEG-A, A-PEG-RGD, and $VEGF_{121}$-PEG-A was generated by crosslinking acrylate groups with UV light and photoinitiator. Constructs were tested for vascularization potential by subcutaneous implantation in rats, followed by microfil perfusion and microCT scanning (Fig. 1).

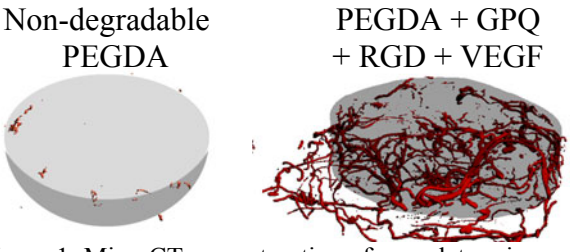

Figure 1: MicroCT reconstruction of vasculature ingrowth into subcutaneous implant of non-degradable PEGDA or bio-artificial matrix with degradable crosslinker, RGD, and VEGF at 4 weeks.

In a functional model of hind-limb perfusion, the left femoral artery was excised from male strain-129 mice and matrix precursor solution was injected into surrounding muscle tissue and polymerized in situ with UV light. Perfusion to the ischemic and non-ischemic legs was evaluated with Laser Doppler Perfusion Imaging (LDPI) (Moor Instruments).

Results: We previously confirmed that the matrices containing PEGylated RGD, GPQGIWGQK, and VEGF support cell adhesion and spreading in 3D, exhibit proteolytic-dependent degradation, and promote vascular ingrowth in subcutaneos implants (Fig 1). An *in vivo* release study indicated that proteolytically degradable matrices delivered an initial spike of $VEGF_{121}$ 24 hours after implantation, followed by sustained slow release over 2 weeks.

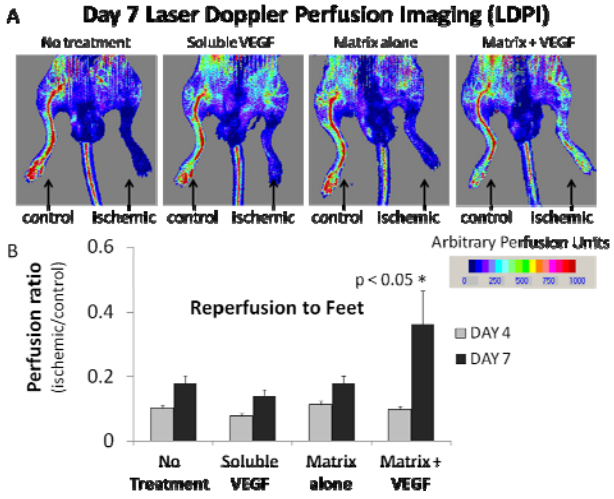

Figure 2. Laser Doppler Perfusion Imaging
A: Representative images from LDPI scans 7 days after treatment with matrix/VEGF. B: Quantification of reperfusion to the feet at 4 and 7 days post treatment.

In the hind limb perfusion model, while differences were not seen early on, by day 7 post-treatment mice receiving matrix + VEGF exhibited a significant 100% increase in perfusion to the feet compared to untreated subjects or those receiving only VEGF or only matrix (Fig. 2).

Conclusions: Blood perfusion to the ischemic limb was found to be greatest in animals that received matrices with bound VEGF at day 7 post-surgery. The result that the engineered matrix containing VEGF performs better than injection of soluble VEGF is noteworthy because it indicates that the delivery vehicle is acting synergistically to amplify the effect of the growth factor. We attribute the increased perfusion to growth factor sequestration in the matrix, resulting in prolonged exposure that persists as the matrix is degraded and remodeled, as shown with sustained release in the in vivo degradation experiment. Current experiments are examining the regenerative capacity of the matrix/VEGF combination in a model of chronic ischemia as well as detailed analysis of the matrix release kinetics.

References:

1. Zisch AH. FASEB J. 2003;17:2260-2262
2. West JL. Biotechnol Prog. 2005;21:1736-1741
3. Hubbell JA. PNAS. 2003;100:5413-5418

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