Bio-Artificial Matrix for Therapeutic Vascularization

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Statement of Purpose: Therapeutic vascularization remains a significant clinical problem in regenerative medicine [1]. Whether the goal is to induce vascular growth in ischemic tissue or scale up tissue-engineered constructs, the ability to induce the growth of patent, stable vasculature is a key obstacle. We developed polyethylene glycol (PEG)-based bio-artificial matrices presenting protease-degradable sites, cell-adhesion motifs, and growth factors to induce the growth of vasculature in vivo. When implanted subcutaneously in rats, degradable constructs containing VEGF and RGD induced a large number of vessels to grow into the implant at 2 weeks with increasing vessel density at 4 weeks.

Methods: We modified published protocols [2-4] to design the PEG-based matrices. Acrylate(A)-PEG_{3400}-NHS was reacted with free amines on the collagenase – degradable peptide GPQGIWGQK and cell-adhesive peptide GRGDSPC. The resulting products A-PEG-GPQGIWGQK-PEG-A and A-PEG-GRGDSPC were purified by dialysis, sterile filtered, and lyophilized. A modified version of $VEGF_{121}$ was conjugated to A-PEG_{3400}-maleimide at an added c-terminal free cysteine. The $VEGF_{121}$-Cys-PEG-A contains a plasmin degradation site upstream of the free cysteine to allow for proteolytic cleavage from the matrix in cell-demanded manner [2]. A 10% hydrogel containing degradable base material, RGD, and VEGF was generated by UV photopolymerization of acrylate groups (Fig. 1).

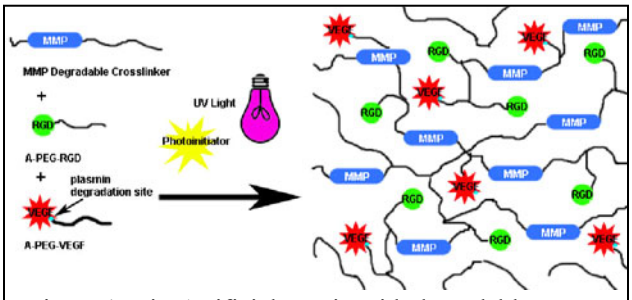

Figure 1: Bio-Artificial matrix with degradable crosslinker, RGD, and VEGF

Hydrogel constructs (8 mm x 2 mm) were implanted dorsally in Lewis rats. At the time of explantation, rats were perfused with formalin and their vasculature injected with Microfil® MV-122 (Flow Tech, Inc) radio-opaque contrast agent. Implants were scanned at 16 µm resolution with a Scanco µCT-40 to quantify vasculature.

Results: The experimental groups implanted were: non-degradable A-PEG_{3400}-A (PEGDA), non-degradable PEG plus RGD (PEGDA + RGD), degradable PEG (GPQ), degradable PEG plus RGD (GPQ + RGD), and degradable PEG plus RGD and VEGF (GPQ + RGD + VEGF). Scanning revealed significant vascular growth in the interior of implants only in the VEGF 2-week ($p<0.023$) and 4-week ($p<0.001$) groups (Fig. 2). The induced vessel growth was much denser at 4 weeks than at 2 weeks ($p<0.002$) (Fig. 3). Analysis of the microCT data revealed a higher degree of connectivity in the 4-week VEGF group ($p<0.021$) as well as increased vessel thickness for VEGF 2-week ($p<0.006$) and 4-week ($p<0.001$) groups over controls. Importantly, the bulk of the hydrogel in all groups remained intact and had only been degraded where vessels grew in.

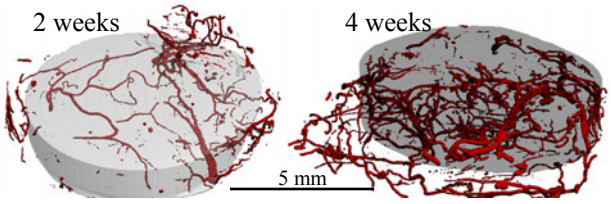

Figure 2: Representative microCT scans of vessels opacified with Microfil® in degradable implants with RGD and VEGF after explantation at 2 and 4 weeks

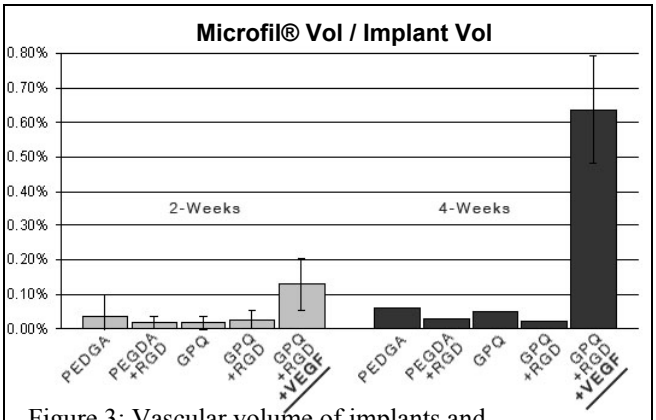

Figure 3: Vascular volume of implants and control groups at 2 and 4 weeks.

Conclusions: The implants clearly induced the growth of vasculature in a VEGF-dependent manner. This vasculature was shown to be connected with the host circulatory system by basis of Microfil® injection into the aorta. We are planning future studies to look at the ability of the material to induce vasculature in different implant sites and experimental situations. We plan to look at hind-limb ischemia, as the material is polymerizable in-situ. The material is also amendable to allogeneic cell-transplantation, as the base hydrogel outside of vessels remained intact and could provide a barrier to immune response.

References: 1. Tongers J. Circulation. 2008;118:9-16
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