VEGF-mediated Tubulogenesis in Three-Dimensional Collagenase-degradable PEGDA Hydrogels

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Statement of Purpose: A major limitation for tissue engineered products is the lack of microvascularization required for nutrient, oxygen, and waste transport. Poly(ethylene glycol) diacrylate (PEGDA) is a biocompatible, hydrophilic polymer which can be crosslinked into hydrogels with tunable mechanical properties, suitable for use as a tissue engineering matrix for soft tissues. PEG hydrophilicity prevents protein adsorption and subsequent cell adhesion. PEG can be modified with covalently-bound cell adhesive substrates[1] and growth factors to influence cell behavior[2]. Further, PEG hydrogels can be rendered biodegradable by incorporating collagenase-sensitive peptides into the framework of the hydrogel mesh, allowing cell-secreted enzymes to degrade the hydrogel in response to cell activity[3]. Endothelial tube formation in response to vascular endothelial growth factor (VEGF) is the rate-limiting step in angiogenesis[4]. Endothelial cells encapsulated within PEGDA hydrogels covalently modified with VEGF were observed over time using confocal microscopy in a timecourse experiment. Cellular behavior suggests that covalently-bound VEGF within the hydrogels increases tubulogenic cellular response including cell migration and cell-cell contact formation. These modified hydrogels have the potential to support vascularized engineered tissue in clinical settings.

Methods: Collagenase-sensitive peptide (CSP) GGGPQG|IWGQGK was synthesized on a peptide synthesizer (Aaptec) using Fmoc chemistry. The peptide was reacted with Acryl-PEG-NHS (Nektar) to flank both sides, and conjugation was confirmed via GPC. Cell adhesive peptide RGDS (American Peptide) and angiogenic growth factor rhVEGF-165 (Sigma) were each conjugated to 3.4 kDa Acryl-PEG-SMC. Conjugation of PEG-VEGF was confirmed via Western Blot and PEG-RGDS via GPC. PEG-CSP-PEG (0.1 g/ml), Acryl-PEG-RGDS (3.5 μmol/ml) and Acryl-PEG-VEGF (200 pmol/ml) were crosslinked by mixing with Irgacure 2959 photoinitiator (0.3% w/v) and exposing to long wavelength UV (365 nm) for 9 min, forming a collagenase-degradable hydrogel encapsulating human umbilical vein endothelial cells (3 x 10^7 cells/ml; HUVEC; Cambrex) labeled with Celltracker (Molecular Probes). Constructs were cultured for 5 h in EGM-2 media without VEGF at 37C in a 5% CO_2 environment and then transfered to a Zeiss LSM Confocal microscope with a stage chamber providing a regulated environment (37C and 5% CO_2). Z-stack images were collected every hour for 60 h using the Multi Time Series macro. Timelapse movies were analyzed for cell behavior.

Results: By 33 hr after encapsulation, HUVECs formed elongated multiple-cell structures in hydrogels with RGDS and VEGF homogenously and covalently bound to the matrix but less so in gels with RGDS only. Cells in VEGF hydrogels had noticeably more migatory behavior and made more cell-cell contacts than cells in hydrogels without VEGF. While endothelial tubes regressed after 51 hr, HUVEC migratory behavior in VEGF hydrogels continued until the study ended, suggesting that the covalently-bound VEGF retained bioactivity throughout the study.

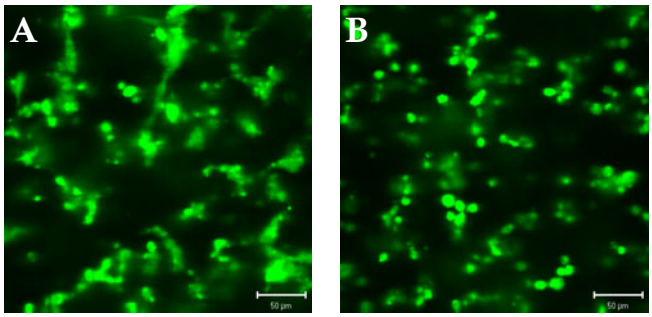

Figure 1. A) Branching networks of endothelial tubes inside collagenase-degradable PEGDA hydrogels modified with RGDS and VEGF. B) Less tubulogenesis in hydrogels modified with RGDS only. Scalebar = 50μm.

Conclusions: Collagenase-degradable PEGDA hydrogels with covalently attached RGDS and VEGF promote the first step in angiogenesis, endothelial tubulogenesis by encapsulated HUVE cells. The incorporation of a collagenase-sensitive peptide sequence within the framework of the hydrogel allows cells to remodel the matrix during migration and cell-cell contact formation. Covalently attached RGDS allows continued cell integrin-matrix connections and cell migration through the hydrogel. Covalently attached VEGF is a promising avenue for promoting tubulogenesis in engineered tissues. PEG-VEGF covalently attached to the hydrogel retained local bioactivity, as evidenced by the continued migratory behavior of cells in VEGF hydrogels, and the covalent immobilization of VEGF within the matrix ensures a predicted, local, engineered response. This research leads to future work entailing the control and stabilization of microvasculature in PEGDA hydrogels to create functional tissues and organs for clinical use.

References:

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