Biomimetic Hydrogels with Immobilized EphrinA1 for Therapeutic Angiogenesis in Hydrogels

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Statement of Purpose: The formation of a microvasculature is regulated by cell-cell interactions and migration. Here, we use immobilized ephrinA1 to induce vessel formation. Ephrins and their Eph receptors mediate cell adhesion, repulsion, and migration. Importantly in angiogenesis, ephrinA1 initiates endothelial cell migration and invasion [1]. The bioactivity of PEG-ephrinA1 has previously been verified [2] and the current work establishes the angiogenic properties of PEG-ephrinA1 in 3D by inducing human umbilical vein endothelial cell (HUVEC) tubulogenesis in matrix-metalloproteinase sensitive hydrogels in response to three different concentrations of PEG-ephrinA1. At timepoints ranging from 6 h to one week, samples were quantified and stained for extracellular matrix proteins to show the mechanism by which PEG-ephrinA1 may stabilize tubule formation. Finally, hydrogels with releasable platelet derived growth factor (PDGF) and PEG-ephrinA1 were implanted into the mouse cornea micropocket and induced a more robust vascular response with an increase in vessel density as compared to hydrogels with releasable PDGF alone.

Methods: PEG-RGDS was made by combining with PEG-SMC in a 1.1:1 PEG-SMC: RGDS molar ratio in dimethyl sulfoxide with diisopropylethylamine. Degradable hydrogels incorporating the synthesized peptide GGGPQGIWGQGK into the polymer backbone were synthesized in a similar manner by reacting at a 2.1:1 molar ratio of PEG-SMC: peptide. PEGylated ephrinA1 was made in 200 mM sodium bicarbonate pH 8.5 at a 100:1 PEG-SMC: protein molar ratio. HUVECs (10,000 cells/μl) were encapsulated into degradable hydrogels containing (1) 3.5 μmol/ml PEG-RGDS, (2) PEG-RGDS with 0.25 ng/ml PEG-ephrinA1, (3) 2.5 ng/ml PEG-ephrinA1, (4) 25 ng/ml PEG-ephrinA1. Samples were stained with DAPI and rhodamine phalloidin and processed via immunohistochemistry for collagen IV, and laminin. *In vivo* studies were performed by implanting a degradable hydrogel containing 160 ng PDGF to induce an angiogenic response with 1.6 ng PEG-ephrinA1 per gel into the cornea of *Flk1-myr::mCherry* transgenic mice, as previously described [3].

Results: PEG-ephrinA1 was shown to lead to HUVEC tubule formation when encapsulated in 3D hydrogels (Fig 1). A two-way ANOVA confirmed a significant difference in the percentage of shared borders of cells for PEG-ephrinA1 at different concentrations and times.

To investigate whether PEG-ephrinA1 may induce tubule stabilization via extracellular matrix production, collagen IV and laminin were stained and quantified. Collagen IV was shown to depend on both growth factor concentration and time (Fig 2) while laminin production increased over time.

In vivo, the presence of PEG-ephrinA1 induced a significant increase in vessel density (Fig 3; p=0.005).

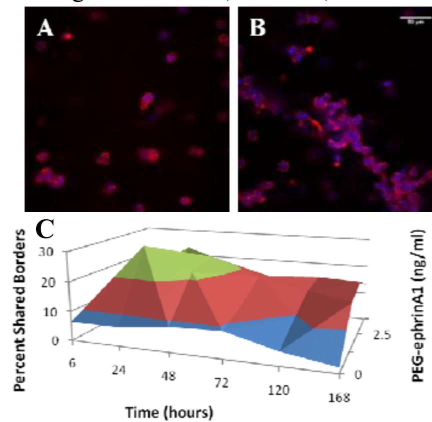

Figure 1: HUVEC tubules visualized with actin (red) and nuclei (blue) at 48 h with no 0 ng/ml (A) and 25 ng/ml PEG-ephrinA1 (B) where the presence of PEG-ephrinA1 induces tubule formation. (C) Quantification of the percentage of shared borders with a two-way ANOVA shows significant effects of both concentration and time.

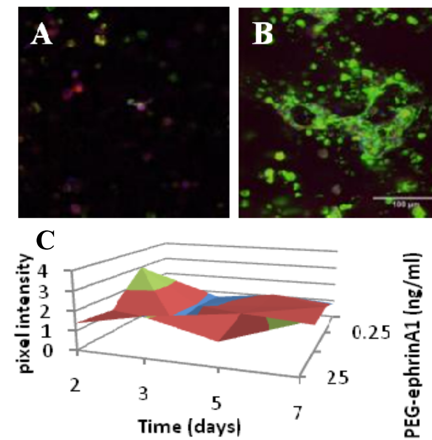

Figure 2: After 7d in culture, collagen IV staining (green) is significantly increased in tubules formed with 25 ng/ml PEG-ephrinA1 (B) as compared to no PEG-ephrinA1 controls (A). (C) Quantification of pixel intensity with a two-way ANOVA shows significant effects of both PEG-ephrinA1 concentration and time.

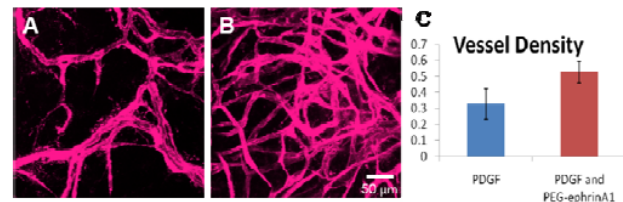

Figure 3: *In vivo* response to hydrogels incorporating releasable PDGF with PEG-ephrinA1 (B) showed a more robust vascular response than hydrogels with releasable PDGF alone (A) with a significant increase in vessel density (C; p=0.005).

Conclusions: PEG-ephrinA1 induces tubule formation in 3D degradable hydrogels and stimulates collagen IV and laminin production *in vitro*, and also promotes *in vivo* vascular formation. As such, PEG-ephrinA1 may represent a promising molecule to regulate cell adhesion and migration for formation of a microvasculature in tissue engineered constructs.

References:

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