Engineering Particle-based Gels for Vasculogenesis to Support Multicellular Tissue Structures

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Statement of Purpose: Blood vessels transport oxygen and nutrients to all tissues; therefore, loss of vasculature due to injury or illness can have damaging effects on tissue health and function[1]. Vascular networks are critical to the survival of cells within materials designed for regenerative medicine and tissue engineering[1]. Biomaterials that promote vascularization via the assembly of endothelial and mural cells into capillary networks are thus needed for many therapeutic approaches[1]. Current methods to establish vascular networks via vasculogenesis within engineered tissue often require degradable or porous hydrogels[2]. Recent work has shown vasculogenesis in permissive, stress-relaxing hydrogels[3]. Our work aims to develop a permissive support material (Fig. 1A, I) in which vascular networks can be formed (Fig. 1A, II), ultimately to support multicellular tissue structures for regenerative medicine (Fig. 1A, III). These hydrogels are formulated using jammed cell-scale microparticles (microgels) (Fig. 1B and C), whose unique viscoelastic properties as jammed bulks have supported bioprinting and studying cell behaviors[4]. This work aims to establish relationships between the design of the viscoelastic particles, their formulation as a bulk material, and vasculogenesis within these systems. The proposed biomaterials platform will be broadly applicable to disease modeling and tissue engineering.

Methods: We developed thiolated polyethylene glycol (PEG-SH) and acrylated PEG (PEG-acr) microgels via Michael addition during batch emulsification. We measured the viscoelastic properties of macroscale hydrogels formed from jammed microgels as a function of polymer weight percent (wt.%) in the microgels (Fig. 1D). Microgels constituted of 3wt.% was compared for viscoelastic properties against a 5wt.% with mismatch (6wt.% PEG-acr/ 4wt.% PEG-SH). RGD was added at controlled concentrations. We cultured human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) within the microgel construct at different ratios and cell concentrations to establish vascular networks that were compared to controls on 2D surfaces (Fig. 1E). Vasculogenesis was visualized via confocal microscopy of fluorescently labeled HUVECs at days 1, 2, 4. Following four days in culture, the cells within constructs were fixed with paraformaldehyde for immunohistochemistry. Cell morphologies in vascular networks were characterized via actin (phalloidin 568). The maturation of a vascular endothelium was examined via VE-cadherin (a marker of tight junctions between cells) and DAPI (nuclei). Morphologies were quantified for branching density and length.

Results: We have characterized a process for creating cell-scale microgels (Fig. 1C). The size distribution of these microgels is mostly < 40 μm ($4.0 * 10^{-5}$ m) average diameter (Fig. 1B) with 21-40 μm ($2.1\text{-}4.0 * 10^{-5}$ m) diameter constituting the majority at ~30%. Experiments have shown that macroscale hydrogel viscoelasticity is dependent on polymer concentration in individual microgels (Fig. 1D). Data above show that a 3wt.% gel exhibits more viscoelastic properties than a mismatched 5wt.% gel. Within each polymer weight percent, the bulk gel and microgels display similar viscoelastic properties. Quantification of vascular network formation and morphology show dependence on coculture ratios, cell concentration, and material properties, including viscoelastic properties and RGD concentration.

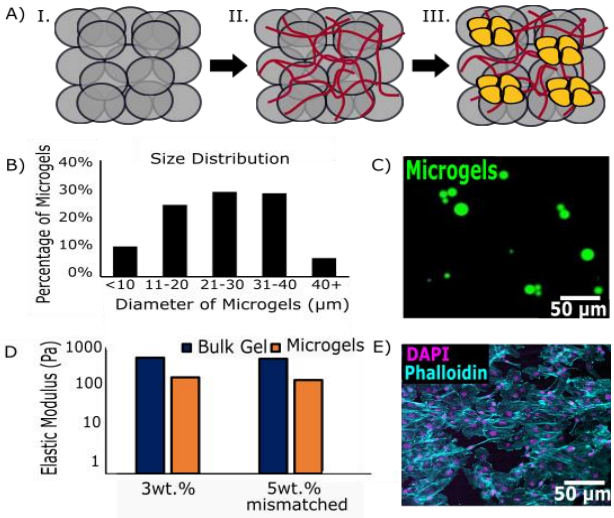

Figure 1: A. Schematic of a microgel (gray) construct (*I*) vasculaturized (red) to support (*II*) tissue (yellow) structures (*III*). **B.** Microgels and **C.** their size distribution. **D.** Elastic moduli comparison bewteen bulk gel and microgels with different weight percent concentrations (3wt.% and 5wt.% mismatched). **E.** Actin staining within vascular network cultured adjacent to microgels.

Conclusions: Vasculogenesis crucial to the success of cell-containing tissue constructs and disease models can be supported by permissive hydrogel materials. We have established a basis for engineering vascularized, particle-based structures, in which material degradation is not required to establish 3D vascular structures. We are working to optimize cell density and material design for faster (< 48 hours) vasculogenesis. Future work will continue to characterize the relationships between material properties and vasculogenesis and will work to establish vasculature within tissue constructs containing multiple cell types.

References:

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