HYDROGEL BIOMATERIALS WITH INDEPENDENT AND COMBINED VARIATIONS IN MODULUS AND CELL ADHESIVE LIGAND GRADIENTS FOR GUIDED NEOVASCULARIZATION OF ENGINEERED TISSUES

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The engineering of large volume, metabolically demanding tissue requires the formation of rapid, stable, and functional neovascularization (new blood vessel formation) for oxygen and nutrient transport and removal of waste products to support viability, function, and restoration of newly formed tissue. Neovascularization is dependent on cell response to multiple spatiotemporal signals including, soluble and immobilized biochemical factors, as well as gradients of mechanical properties and physical structure provided by the 3D extracellular matrix (ECM); yet the individual and combined effects of these factors is poorly understood. Various polymerization techniques have been developed for creating gradient-based hydrogel scaffolds to promote rapid and guided neovascularization, however, most studies have focused on evaluating 3D cellular responses to scaffold embedded gradients of a single factor (i.e. growth factors). Herein we present free-radical approaches based on visible light frontal photopolymerization to synthesize synthetic poly(ethylene) glycol (PEG) hydrogel scaffolds with controllable, physiologically-relevant continuous gradients of elastic modulus and/or crosslink density, proteolytically mediated scaffold degradation (through incorporation of crosslinks susceptible to degradation by cell-secreted matrix-metalloproteinases) and immobilized concentration of cell adhesive peptide(RGD) ligands (through pendant RGD functionalized monofunctional acrylates). Scaffolds with desired gradients were created using a dual programmable syringe pump system to control the composition and flow rates of two distinct prepolymer solutions in the feed stream entering a reaction chamber simultaneously exposed to crosslinking via visible light □□ = 514 nm) using an Argon Ion laser system (Figure 1 A,B). Using this approach proteolytically degradable hydrogel scaffolds were created with (1) gradients of elastic modulus (ranging from 660-1460 Pa) and proteolytic degradation while the immobilized RGD concentration was maintained uniform (2mM), (2) with uniform modulus (600 Pa) and proteolytic degradation with gradients of immobilized RGD concentration (0.48-0.98 mM) and (3) with varying RGD gradient characteristics including magnitude and slope (steep, intermediate and shallow slopes) (Figure 2).The effect of each type of gradient on 3D vascular sprouting parameters (invasion area, sprout length and number) was evaluated using a 3D co-culture spheroid model of sprouting angiogenesis. In scaffolds containing gradients of elastic modulus, the number of vascular sprouts increased in the opposing gradient direction while RGD gradient scaffolds promoted increases in the length of vascular sprouts towards the peptide gradient. Studies are currently underway to elucidate the effects the RGD gradient as scaffold modulus and proteolytic degradation kinetics are independently modulated on vascular sprouting. Finally strategies to spatially incorporate additional gradients such as peptides that enable affinity binding of growth factors or nanoparticles that provide spatiotemporal release of proangiogenic peptides within the scaffolds will be discussed.

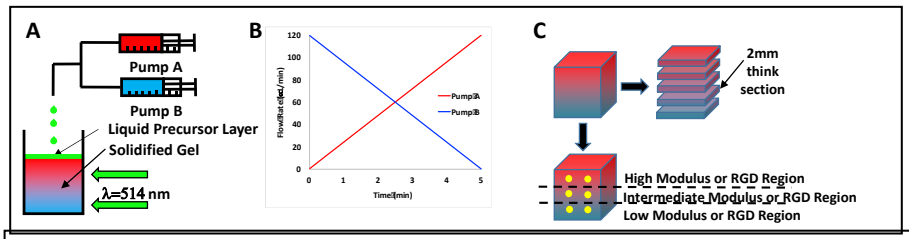

Figure 1. Gradient Hydrogel Scaffold Fabrication. (A) Schematic photo-frontal polymerization. (B) Precursor flow rate profiles during crosslinking. (C) Hydrogel sectioning along the gradient direction for quantification of spatial variation in material properties and vascular cell spheroid placement in different regions along the gradient(s).

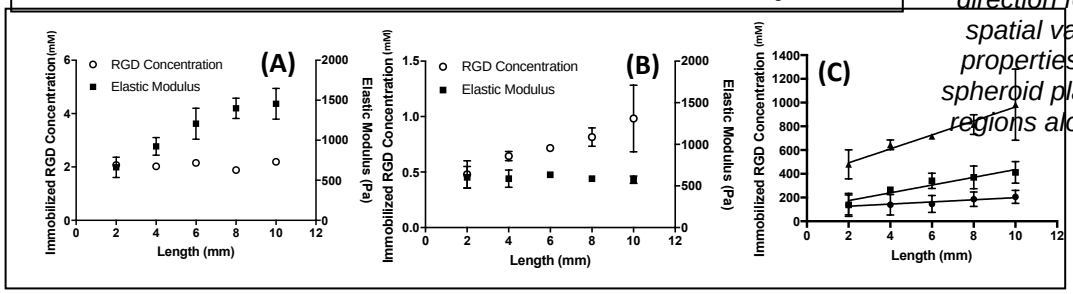

Figure 2. Hydrogels with uniform (A) RGD and modulus gradient, (B) modulus and RGD gradient, and (C) modulus and varying RGD gradient magnitude.