Development of Interconnected PolyHIPEs for Injectable Bone Grafts

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Statement of Purpose: Orthopedic surgeons have long been interested in a synthetic material that exhibits good osseointegration to reduce inflammation, decrease mechanical failure and increase integration with host tissue. Generating a scaffold that is highly porous and interconnected while retaining sufficient mechanical strength for orthopaedics is challenging. We have successfully utilized an emulsion templating technique to fabricate a polymerized high internal phase emulsion (PolyHIPE) for use as an injectable bone graft. Previously work has shown good integration between our scaffold and porcine bone, compressive properties comparable to cancellous bone, and cytocompatibility of all components except the initiator. The current study focuses on the development of interconnected polyHIPEs initiated with a cytocompatible organic-phase initiator and characterization of the resulting pore architecture, compressive properties, injectability parameters, and human mesenchymal stem cell (hMSC) behavior.

Methods: ***HIPE Fabrication:*** Propylene fumarate dimethacrylate (PFDMA) HIPEs were prepared using a FlackTek SpeedMixer. Briefly, PFDMA was mixed with 5 wt% of the organic-phase soluble initiator, benzoyl peroxide (BPO), and 5 wt% of the surfactant polyglycerol polyricinoleate, PGPR, prior to emulsification. Once thoroughly mixed, the aqueous solution of calcium chloride (1 wt%) and deionized water was then added to the organic phase in three additions and mixed at 500 rpm for 5 min each. HIPEs were transferred to a 37°C aluminum bead bath to facilitate crosslinking. ***Characterizing Interconnected PolyHIPEs:*** *Pore and Interconnect Size:* Scanning electron microscopy (SEM) was utilized to determine pore and interconnect size. Circular specimens were sectioned into quarters, fractured at the center, coated with gold, and imaged in a rastor pattern. Pore and interconnect size measurements were completed on the first ten pores that crossed the median of each 1000X magnification micrograph ensuring an n=30 for interconnects from each image. S*et time:* The tack-free time, defined as the point at which a spatula inserted into the polyHIPE was removed without any adsorbed material, was utilized to determine set time. *Compressive Modulus and Strength:* A fabricated HIPE was split into centrifuge tubes for five timepoints over two weeks and cured. HIPEs were sectioned using an Isomet saw into four disks each with 3:1 diameter to height ratio. Foam compressive properties were tested on an Instron 3300 at 50 μm/s. The compressive modulus was determined as the slope of the linear region after correcting for zero strain. Compressive strength was defined as the stress at 15% strain. ***Incorporating RGDS for Cell Binding:*** Previous results indicated PGPR had an effect on serum protein adsorption and/or conformation reducing visible binding sites for human mesenchymal stem cells (hMSC). We hypothesized an amphiphilic molecule coupled to RGDS would self-assemble at the pore walls and provide cell binding sites. RGDS functionalized oleic acid was synthesized in a two step process adapted from Shen *et al.*[1] Product purification was completed using column chromatography.

Results: ***Characterizing Interconnected PolyHIPEs:*** *Pore and Interconnect Size:* Interconnected porosity was achieved by initiation in the organic phase of the HIPE with preliminary average pore and interconnect sizes of 19 ± 13 and 4 ± 2 μm, respectively. The proposed mechanism for interconnect formation is described by the resultant macromer densification forces depending on the phase where crosslinking is initiated. During organic phase initiation, the forces generated from densification

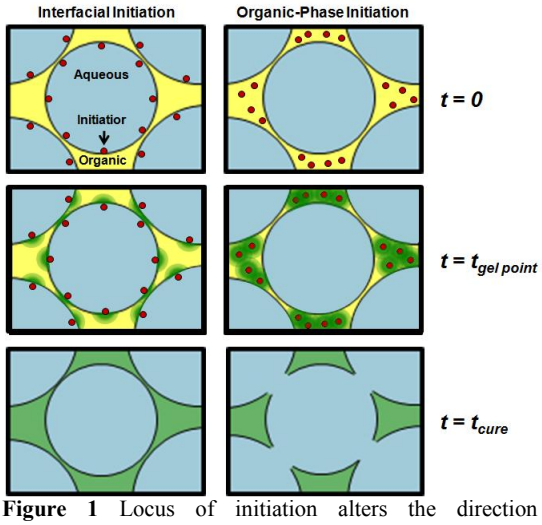

Figure 1 Locus of initiation alters the direction of densification forces resulting in interconnected porosity in organic-phase initiated PFDMA polyHIPEs.

result in tearing of the thin film between droplets and pore opening **(Figure 1)**. S*et time* The set time of polyHIPEs was approximately 3 hours. *Compressive Modulus and Strength:* An increase in compressive modulus (9 ± 3 to 43 ± 17 MPa) and strength (0.68 ± 0.04 to 2.94 ± 0.36 MPa) of HIPEs was observed over two weeks.

Incorporating RGDS for Cell Binding: Functionalization of oleic acid with RGDS was confirmed with FTIR by the introduction of an absorption peak corresponding to the amide of the conjugate (C=O, 1659 cm^{-1}) and the removal of peaks indicative of the carbonyl of bound NHS (C=O, 1786 and 1822 cm^{-1}) and NHS-ester (C=O, 1738 cm^{-1}). Purification and cell adhesion studies are ongoing.

Conclusions: Interconnected, highly porous PFDMA polyHIPEs exhibit compressive properties comparable to cancellous bone. Ongoing studies will determine the ultimate compressive properties of the polyHIPE as they increase over time. Current work focuses on determining the resultant effect of oleic acid-RGDS on hMSC adhesion and spreading and incorporating hydroxyapatite nanoparticles to develop an osteoinductive polyHIPE.

References:

1. (Shen, SI. J Drug Target. 2007:15, 51-58).