HIGH POROSITY TISSUE ENGINEERING SCAFFOLDS BY EMULSION TEMPLATING

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Statement of Purpose: It is widely recognized that the scaffold architecture (porosity, pore size, interconnectivity) can profoundly influence the behavior of cells on tissue engineered constructs. High internal phase emulsion (HIPE) polymerization affords tremendous control of scaffold morphology. In particular, pore size and interconnectivity may be readily optimized using this technology to facilitate cellular ingrowth, influx of nutrients and transport of waste throughout the scaffold. In this study, a range of highly porous, biodegradable scaffolds were prepared by polymerization of the continuous phase of HIPEs containing poly(propylene fumarate) (PPF) and the crosslinker propylene fumarate diacrylate (PFDA). The effect of composition and additives on emulsion geometry and subsequent scaffold morphology is presented.

Methods: *Synthesis:* All chemicals were purchased from Aldrich (Milwaukee, WI, USA) and used as received. PPF and PFDA were synthesized and purified as previously described.[1] The PPF synthesized for this study had a number average molecular weight (Mn) from 800 to 1000 g/mol and a polydispersity index ranging from 1.7 to 1.9.

HIPE Preparation: PPF and PFDA were mixed with toluene and the surfactant sorbitan monooleate (20 wt% Span 80™) prior to emulsification. This mixture was then stirred with a glass stirring rod fitted with a D-shaped PTFE paddle connected to an overhead stirrer motor. The aqueous solution, comprising potassium persulfate and calcium chloride in deionized water, was added dropwise with constant stirring using a peristaltic pump. Concentrations of macromer and aqueous phases were adjusted to yield 90:10 polyHIPE foams. The HIPEs were cured in a water bath at 60°C for 48 hr. The bulk of the liquid was removed in an oven at 60ºC for 12 hr and drying was completed in vacuo at room temperature.

SEM Analysis: Scanning electron microscopy was used to characterize the resulting monoliths and correlate experimental parameters with scaffold morphology. All samples were dried, mounted on aluminum stages, sputter coated with gold, and examined with an FEI-XL 30 Environmental Scanning Electron Microscope.

Results/Discussion: Toluene was used as a diluent to reduce the viscosity of the organic phase to enable HIPE formation. Fully biodegradable fumarate-based PolyHIPEs were successfully fabricated. SEM images of the rigid foam created with emulsion templating are displayed in **Figure 1**. The pore and interconnect size of tissue engineering constructs dictate cellular ingrowth and nutrient transport. There are a number of methods to modulate HIPE geometry and the resulting scaffold morphology to optimize cellular infiltration and function. To that end, we initially varied the concentrations of PFDA (25-75%) and toluene (40-60%) to examine the effect of composition on scaffold morphology, **Table 1**.

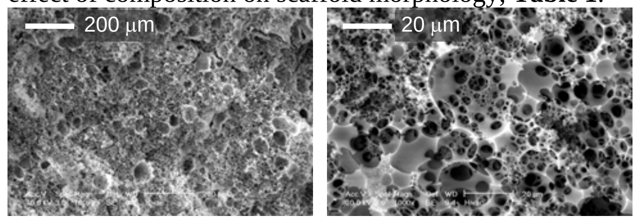

Figure 1: Porous structure of polyHIPE; 50% PFDA; 50% toluene.

Increasing the PFDA concentration resulted in greater stability of the emulsion. Consequentially, HIPEs with lower PFDA concentrations underwent more phase separation. Larger pore sizes were also correlated to this reduction in stability. Similarly, decreased emulsion stability was observed with increasing toluene concentration. This trend confirms that emulsion stability is dependent on the solution viscosity.

Table 1: Effect of crosslinker and diluent concentrations on HIPE formation and morphology.

PPF:PFDA Ratio	Toluene Concentration (%)	Morphology
3:1	40	Macroporous
3:1	50	Phase Separation
3:1	60	Phase Separation
1:1	40	Closed Cell
1:1	50	Open Cell
1:1	60	Macroporous
1:3	40	Open Cell
1:3	50	Open Cell
1:3	60	Closed Cell

The effect of PPF molecular weight on the resulting morphology of polyHIPEs was also examined (Mn = 800, 1000). Lower molecular weight PPF resulted in more stable emulsions and reduced phase separation prior to cure. Quantitative measurements of pore size, interconnect size and size distributions are currently in progress.

Conclusions: We have demonstrated that emulsion templating can be used to generate rigid, biodegradable scaffolds with interconnected pores. These scaffolds are of particular interest in craniofacial tissue engineering due to the rigidity of the resulting foams and the ease of fabrication. Further investigation of cell adhesion, proliferation, and extracellular matrix deposition on these scaffolds is currently in progress.

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References:

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