Osteoinductive Modification of Injectable, PolyHIPE Bone Grafts

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Statement of Purpose: An injectable bone graft provides a space-filling substrate with the potential to integrate with native bone and stimulate regeneration. Our lab utilizes an emulsion templating technique to generate a highly porous, injectable bone grafts based on polymerized high internal phase emulsion (polyHIPE). Although these grafts provide a 3D matrix for bone regeneration, the synthetic material lacks the necessary osteoinductive cues for efficient osteogenesis. The addition of growth factors such as BMP-2 is commonly used to induce differentiation of mesenchymal stem cells into osteoblasts. Due to an initial burst release of growth factors, a high concentration of these expensive and potent proteins is necessary for continued effectiveness. Alternatively, material-induced osteogenesis has received growing interest in the community to circumvent the cost and safety concerns of growth factor use. To this end, we utilized hydroxyapatite (HA) and demineralized bone matrix (DBM) to confer an osteoinductive character to these injectable polyHIPEs. Current studies are focused on characterizing the impact of these additives on the mechanical properties and pore architecture of the grafts prior to assessing the induction of osteogenic differentiation of hMSCs.

Methods: ***PolyHIPE Fabrication:*** An organic phase consisting of ethylene glycol dimethacrylate (EGDMA) macromer, 5 wt% benzoyl peroxide (BPO) initiator, and 10 wt% polyglycerol polyricinoleate (PGPR) surfactant was mixed. An aqueous phase of water and 1 wt% calcium chloride was added and mixed using a FlackTek SpeedMixer DAC 150 FVZ-K in increments until 75% volume of water achieved. The HIPE was then placed in a 37°C bead bath to cure, resulting in a porous polyHIPE. ***Bone Demineralization:*** Rat femurs obtained from Laboratory Animal Resources and Research (LARR) were stirred with 0.6M hydrochloric acid for 24 hours and then rinsed with RO water 3 times. An Alizarin red stain which preferentially binds calcium was performed on a sample of DBM to prove removal of the mineral component. ***Incorporation of Osteoinductive Agent:*** HA nanoparticles were purchased from Sigma Aldrich. 1 and 2 wt% HA and 5, 10, and 15 wt% DBM were mixed with the macromer and initiator and polyHIPEs fabricated as discussed above. *Pore Architecture:* Gold coated specimens were imaged using scanning electron microscopy (SEM) (JEOL NeoScope 5000) to determine pore architecture. *Mechanical Properties:* Samples for mechanical testing were made by segmenting cylindrical disks with a 3:1 diameter to height ratio using a Buehler Isomet® saw. They were then compressed at 50 µm/s using an Instron 3000 equipped with a 1000-N load cell. The modulus and strength were identified as the slope of the linear region of the stress/strain curve and the stress at yield or 10% strain (whichever occurred first), respectively, according to ASTM D1621-04a.[1] *Osteoinductive Agent Location Study:* HA is mainly composed of calcium and phosphate ions, and demineralization leaves collagen as the main component of DBM. To this end, polyHIPEs with HA were stained with Alizarin red to test for the presence of calcium. Similarly, polyHIPEs with DBM were stained with Sirius red to test for the presence of collagen.

Results: *Pore Architecture and Mechanical Properties:* The addition of 1 and 2 wt% HA nanoparticles, which exceed concentrations shown to impact osteoblastic proliferation, had minimal effects on pore and interconnect size **(Figure1)**.[2]

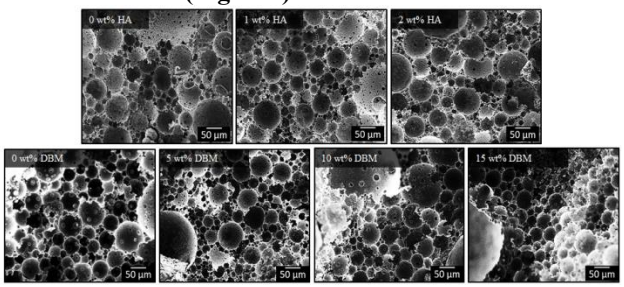

Figure 1 Effective concentrations of HA had minimal effects on pore architecture while increased DBM concentrations decrease homogeneity.

Preliminary studies indicated polyHIPEs with 2 wt% HA showed an increase in both modulus and strength compared to the control. Interconnected, porous architecture was achieved in polyHIPEs with DBM concentrations from 5 to 15 wt%; however, SEM images indicate decreasing homogeneity with increasing DBM concentration **(Figure 1)**. Current work is focused on decreasing DBM particle size and aggregation to increase pore size homogeneity. *Osteoinductive Agent Location:* Positive Alizarin red staining of polyHIPEs with HA nanoparticles compared to control foams suggests the successful incorporation into the polyHIPE. Film controls that did not promote self assembly at the surface tested negative for calcium. This suggests that utilization of the HA nanoparticles promoted preferential location of the HA at the pore surface due to self assembly at the aqueous-organic interface of the emulsion. Similarly, positive Sirius red staining of polyHIPEs indicated the incorporation of DBM into the scaffold.

Conclusion: HA and DBM have been successfully incorporated into polyHIPE scaffolds with minimal effects on pore architecture. Current work is focused on hMSC viability and osteogenic differentiation on these grafts. Specific focus will be placed on comparing the osteoinductive properties of HA and DBM. Subsequent in vivo studies will determine the effect of these modifications on bone regeneration rate in critical sized defects using a rat model.

References:

1. ASTM, Committee D20 on Plastics. Standard Test Method for Compressive Properties of Rigid Cellular Plastics (D 1621-04a). 2004.
2. (Akay, G. Biomaterials. 2004:25, 3991-4000).