In Situ Surface Modification of Emulsion Templated Bone Grafts with Hydroxyapatite Nanoparticles Improves Cell Adhesion

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Statement of Purpose: Millions of patients require bone grafting procedures to treat non-union defects every year. Our lab has previously developed a synthetic bone graft that is biodegradable and highly porous with compressive properties comparable to cancellous bone. These materials are fabricated via medium internal phase emulsion polymerization (polyMIPEs). Surfactant-stabilized MIPEs yield scaffolds with interconnected structures, but the surfactant can negatively affect cell attachment. As an alternative emulsifier, nanoparticles can be used to create Pickering emulsions. During emulsification, the nanoparticles self-assemble at the interface, which localizes the nanoparticles at the surface of the resulting emulsion-templated foam. We have demonstrated that hydroxyapatite nanoparticles (nHA) surface modification of polyMIPE grafts improve cell attachment; however, the resulting scaffolds have a closed pore structure that prevents cell migration into the scaffold. The focus of the current study is to develop a method to co-stabilize the polyMIPEs with surfactant and nHA to achieve an interconnected scaffold with improved cell attachment for bone grafting applications.

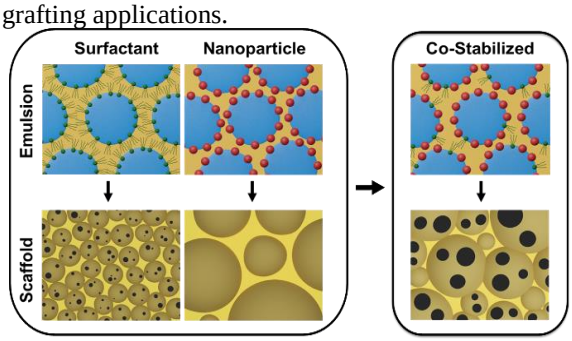

Figure 1: Schematic to produce interconnected polyMIPEs with improved cell-material interactions and interconnectivity.

Methods: PolyMIPE scaffolds were fabricated using a redox initiation method. Briefly, two emulsions containing neopentyl glycol diacrylate (90 mol % of organic phase) (NGDA-BDT), 1,4 butane dithiol (10 mol% of organic), nHA (8.5% w/w) polyglycerol polyricinoleate 4125(PGPR) (2.5% w/w) and either benzoyl peroxide (1% w/w) as an initiator or trimethylaniline (1% w/w) as a reducing agent were mixed with 69% DI water with 1% CaCl2. For nanoparticle and surfactant stabilized controls, 8.5% nHA and 5% PGPR were used. The two emulsions were mixed through a double-barrel syringe and mixing head to initiate polymerization, and the resulting composite was placed at 37 ºC overnight. Scanning electron microscopy analysis was used to determine pore size and interconnectivity. Mechanical properties were evaluated using an Instron 3300. The Injectability of the scaffold was evaluated for the micro and macro integration in a bovine bone. The attachment and proliferation of human mesenchymal stem cells (hMSCs) (day 1, 7, 14) was investigated to evaluate cell behavior on the scaffolds.

Results: Co-stabilized polyMIPEs displayed an interconnected porous structure with an average pore size of 97 ± 66 µm, which was intermediate to the surfactant and nHA stabilized controls. The compressive modulus and strength of the scaffold, 41.6 ± 6.4 MPa and 3.6 ± 0.5 MPa, respectively, were statistically similar to the control scaffolds and similar to cancellous bone. The injectability of the co-stabilized polyMIPE was demonstrated by the seamless integration of the scaffold into a bone defect. There were marked differences in cell attachment and proliferation between the different scaffolds. The surfactant-stabilized scaffold displayed a marked decrease in cell attachment compared to the nHA and co-stabilized grafts. Between days 1 and 7, there was a sharp decrease of hMSCs attached to co-stabilized polyMIPEs; however, hMSC proliferation recovered by day 14. We hypothesize that these differences were due to surfactant affected protein adsorption with corollary effects on cell attachment.

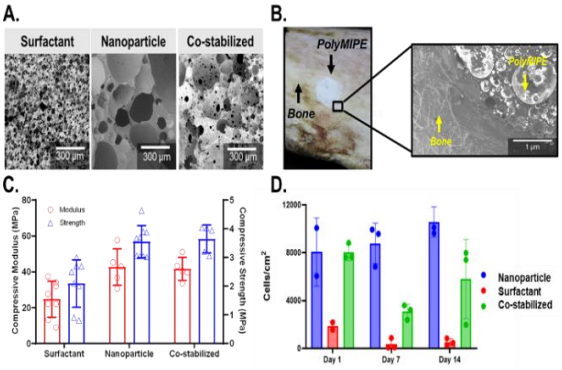

Figure 2: Co-stabilized polyMIPEs pore architecture(A), injectability(B), mechanical properties(C), and proliferation(D) compared to nanoparticle or surfactant stabilized polyMIPEs.

Conclusion: In this study, we identified a suitable formulation of co-stabilized polyMIPE that maintained an open pore structure with improved cell attachment. After characterizing the material properties (e.g., architecture, mechanical properties, and cell attachment), we found that the polyMIPE maintained the requisite properties for bone graft applications. Future studies will evaluate osteogenic differentiation and ectopic bone formation to assess the osteoinductivity of this new bone grafting material.