Novel Poly(propylene fumarate) Reinforced Brushite Composites for Bone Repair

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Statement of Purpose: Due to the limitations of bone grafts there is a critical need for the development of new bone repair strategies. While bone tissue engineering has demonstrated the potential to meet this need, the search for better scaffold materials continues. Calcium phosphate cements are particularly promising due to their osteoconductivity, and brushite (a.k.a. dicalcium phosphate dihydrate) cements have demonstrated excellent resorbability and bone formation *in vivo*. Moreover, because of their injectability, calcium phosphate cements are amenable to 3D fabrication technologies. It is possible to fabricate calcium phosphate cement scaffolds with precisely controlled architectures, which makes scaffold optimization and customization feasible. While the mechanical properties of calcium phosphate cements have previously precluded their use as scaffold materials in bone tissue engineering, we have recently shown that it is possible to reinforce these biomaterials by infiltrating their micropores with a polymer and cross-linking the polymer *in situ*. The goal of the present study was to extend our work to reinforcement with a biodegradable polymer. We chose to use poly(propylene fumarate) (PPF) to reinforce brushite scaffolds, as it is an unsaturated polyester that has previously been utilized in bone tissue engineering.

Methods: Brushite cement was prepared using a 1:1 monocalcium phosphate monohydrate:β-tricalcium phosphate molar ratio and deionized water. Powder to liquid mass ratios (P/L) of 1.0 and 1.5 were used. For reinforcement, the cement was submerged in a 4:3 mass ratio mixture of PPF (M_n = 1,700 g/mol) and the crosslinking monomer N-vinyl pyrrolidinone with 5 wt % benzoyl peroxide and 0.1 wt % butylated hydroxytoluene. Vacuum was applied to facilitate polymer infiltration. Specimens were blotted dry and then cured at 80°C for 24 hours under vacuum. Mechanical properties were characterized by three point bending and the trends were correlated to the mass of polymer incorporated. 3D scaffolds (8 mm diameter, 8.5 mm height) comprised of orthogonally intersecting beams (1mm diameter, 750 µm spacing; ~ 50% macroporosity) were prepared with P/L of 1.0 using rapid prototyping, reinforced with PPF and tested in compression. Finally, biocompatibility was evaluated *in vitro* by culturing mesenchymal stem cells in medium exposed to PPF reinforced brushite for 24 hours. The cells were stained with propidium iodide and anti-Annexin V. The percents of viable, necrotic and apoptosing cells were determined by flow cytometry.

Results: For cement prepared with P/L of 1.0, PPF reinforcement increased flexural strength from 0.75 ± 0.26 MPa to 12.40 ± 3.72 MPa, flexural modulus from 302.00 ± 139.28 MPa to 854.00 ± 312.49 MPa, maximum displacement from 0.074 ± 0.01 mm to 0.50 ± 0.09 mm, and work-of-fracture from 2.77 ± 0.99 J/m^2 to 219.34 ± 83.4 J/m^2 ($p < 0.05$; $n = 5$). In contrast, only slight improvements were seen at P/L of 1.5. This trend was due to the decreased amount of polymer incorporation. 0.38 ± 0.03 mg/mm^3 of polymer were incorporated at P/L of 1.0, whereas only 0.19 ± 0.01 mg/mm^3 were incorporated at P/L of 1.5 ($p < 0.05$; $n = 5$). Based on these results, 3D macroporous PPF reinforced brushite scaffolds were prepared with P/L of 1.0 (Fig. 1A). PPF reinforcement increased the compressive strength of these scaffolds from 0.31 ± 0.06 MPa to 7.48 ± 0.77 MPa, which is comparable to trabecular bone (Fig. 1B and C). Finally, PPF reinforced brushite with P/L of 1.0 showed good *in vitro* biocompatibility, as 96.00 ± 0.91 percent of the cells were viable after 24 hours, 3.59 ± 0.99 percent were necrotic, 0.09 ± 0.05 percent were dead by apoptosis and 0.40 ± 0.13 percent were in the early stages of apoptosis (Fig. 1D; $n = 3$). These values were not significantly different from the negative control.

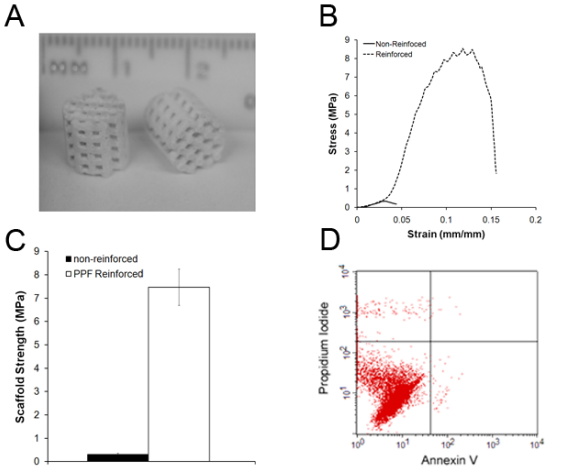

Fig. 1. (A) Macroscopic view of 3D PPF reinforced brushite scaffolds. (B) Characteristic stress-strain curves from compressive testing. (C) Average compressive strengths for reinforced and non-reinforced scaffolds. (D) Flow cytometry plot for biocompatibility assay (lower left quadrant = viable cells; upper left quadrant = cells dead by necrosis; lower right quadrant = early apoptotic cells; upper right quadrant = cells dead by apoptosis).

Conclusions: Reinforcement of brushite with PPF drastically improved the mechanical properties, and 3D macroporous scaffolds had compressive strengths comparable to trabecular bone. Thus, given that both components of the composite are resorbable, we believe that PPF reinforced brushite may be an ideal scaffold material for bone tissue engineering. Future studies will investigate mesenchymal stem cell proliferation and differentiation on the composite, as well as *in vivo* bone regeneration.