Biodegradable Composite Scaffolds for Directing Osteogenesis and Bone Formation

J. Kent Leach, Jiawei He, and Damian C. Genetos
Department of Biomedical Engineering. University of California, Davis, Davis, CA 95616

Statement of Purpose: Bone defects and nonunions caused by trauma, resection, or abnormal development pose a significant health problem worldwide with over 500,000 bone repair procedures performed annually [1]. Current approaches for graft materials face significant limitations such as limited supply, risk of immune rejection, or chronic immune response. Bioceramics, such as hydroxyapatite (HA), are highly osteoconductive and possess robust mechanical properties, but these materials can present difficulties when processing into three-dimensional structures. Hybrid biomaterials offer an exciting approach to capitalize on the beneficial aspects of various components while tailoring the mechanical and tissue-stimulative properties of the composite [2]. Previous studies have demonstrated the potential to generate composite biodegradable scaffolds by mixing synthetic polymers with HA [3], yet only one composition was investigated. We hypothesized that the material properties of composite biodegradable scaffolds could be modulated by the ratio of HA to biodegradable polymer (poly-lactide *co*-glycolide, PLG). Furthermore, we anticipated that the osteogenic potential of mesenchymal progenitor cells could be impacted by the substrate composition. To test this hypothesis, composite polymeric scaffolds were fabricated with varying compositions, and the mechanical properties and osteogenic response of human mesenchymal stem cells (hMSCs) seeded on these constructs was examined *in vitro*.

Methods: Microspheres composed of 85:15 PLG (Medisorb) were prepared using a standard double emulsion technique, and 3D porous composite scaffolds were fabricated using mixtures of microspheres, HA (average diameter = 100 nm, Berkeley Biomaterials), and sodium chloride as a porogen (250-425 μm) using a gas foaming/particulate leaching method. The amount of HA was varied while keeping the mass of PLG and salt constant, thereby allowing us to generate scaffolds of increasing HA:PLG ratios ranging from 0:1 to 5:1. Gross morphology of the scaffolds was assessed by SEM, porosity was determined using Archimedes' method, and mechanical properties of the substrates were characterized through compressive testing. The impact of these composite substrates on the osteogenic potential of hMSCs was determined through *in vitro* culture for 4 weeks and subsequent quantification of standard osteogenic markers (*e.g.*, alkaline phosphatase [ALP] and osteopontin [OPN]). Differences in mechanical properties, porosity, and production of biochemical markers were determined using the Student's *t*-Test, with $p<0.05$ as a marker for statistical significance.

Results: The addition of HA to a constant mass of PLG did not impede our capacity to generate three dimensional interconnected structures using the gas foaming/ particulate leaching technique. As expected, significant differences in scaffold morphology were observed upon increased addition of HA to PLG, with a clear reduction in porosity and pore interconnectivity present above 2.5:1 HA:PLG. We observed a decrease in porosity with increasing HA mass. Compared to the PLG control lacking HA (93 ± 3%), we did not observe appreciable reductions in scaffold porosity until 2.5:1 HA:PLG. Significant reductions in scaffold porosity were observed at 5:1 HA:PLG (83 ± 0.7%; $p<0.01$). The addition of HA to PLG composites resulted in a nearly linear increase in compressive modulus, with 5:1 HA:PLG composite scaffolds exhibiting a nearly five-fold increase in compressive modulus versus control ($p<0.001$). Finally, the addition of HA to PLG scaffolds enhanced the osteogenic differentiation of hMSCs in a dose-dependent manner up to 2.5:1 after 4 weeks of culture. Similar trends were detected in OPN secretion. The drop in ALP activity at 5:1 is likely due to decreased cell viability in a composite structure with significantly reduced porosity.

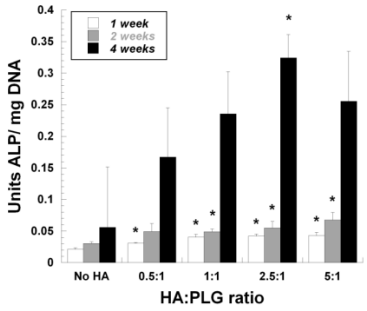

Figure 1. Alkaline phosphatase activity is increased on substrates containing more HA. Data are mean ± SEM (n=5).* = $p < 0.05$ vs. No HA at the same time point.

Conclusions: The results of this study indicate the importance of combination strategies for tissue regeneration and may provide a powerful tool to achieve desired tissue responses by modulating substrate rigidity and the rate of degradation of the synthetic extracellular matrix. Furthermore, these results have implications for regenerative strategies that require complementary signaling for simultaneous growth of multiple tissue types.

References:

1. Langer, R., and Vacanti, J.P. Tissue engineering. Science, 260, 920, 1993.
2. Leach JK. Multifunctional cell-instructive materials for tissue regeneration. Regen Med 2006;1(4):447-455.
3. Kim SS, Ahn KM, Park MS, Lee JH, Choi CY, Kim BS. A poly(lactide-co-glycolide)/hydroxyapatite composite scaffold with enhanced osteoconductivity. J Biomed Mater Res A 2007;80A(1):206-215.

Acknowledgements:
The authors acknowledge financial support from the AO Foundation (F-06-98L to JKL).