Nanocomposite Bone Scaffolds Based on Biodegradable Polymers and Hydroxyapatite

Johannes Becker, Mahrokh Dadsetan, M. Brett Runge, Heng Zeng, Lichun Lu, Michael J. Yaszemski
Mayo Clinic, College of Medicine, Rochester MN

Statement of Purpose: The creation of synthetic biomaterials that mimic bone matrix is a promising approach for minimally invasive surgery and is necessary to restore skeletal continuity in defects such as vertebral body fracture. Materials such as poly (methyl methacrylate) (PMMA) or calcium-phosphate (CaP) bone cement have been widely used for percutaneous procedures such as vertebra- or kyphoplasty. However, several disadvantages including short injection time, potential to remodel surrounded bone and high exothermic heat release may lead to complications. Recent work in our laboratory has focused on developing novel polymers which have mechanical properties similar to trabecular bone with optimal setting time and heat release. These polymers are synthesized with copolymerization of polypropylene fumarate (PPF) and polycaprolactone (PCL). The mechanical property of PPF-co-PCL varies with change in PPF/PCL ratio in copolymer structure and PCL molecular weight [1]. It has been shown that hydroxyapatite (HA), the major mineral bone component, promotes biomineralization and stimulates cell adhesion and proliferation. However, HA on its own, has a limited use due to its frangible nature. In this study, we hypothesized that the incorporation of HA favors cell attachment and proliferation on PPF-co-PCL scaffolds. To test our hypothesis, PPF-co-PCL was blended with different amounts of nano-HA, and crosslinked using a chemical crosslinker. The chemical structure and mechanical property of HA/ PPF-co-PCL composites were characterized using Fourier transform infrared spectroscopy and dynamic mechanical analyzer (DMA). Furthermore, attachment and differentiation of W20-17 pre-osteoblast cell line was evaluated.

Methods: PPF and copolymers of PPF and PCL were synthesized as previously described [1]. Briefly, hydroxyapatite of 0, 100, 200 or 300 mg was mixed with 1g of copolymer and crosslinked in the presence of benzoyl peroxide (BPO), methyl methacrylate and dimethyl toluidine (DMT). After crosslinking at 37°C for 24 hours, all disks were washed in 50:50 acetone and ethanol for 24 h, followed by 100% ethanol for 4 h and finally in 70% ethanol for 30 min.

Mechanical testing: For compression testing, cylindrical samples were made with a 1:5 ratio (diameter vs. length). Dog-bone shape samples were punched out of each PPF-co-PCL/HA formulation for measurements of tensile strength. Compressive and tensile modulus was determined from slope of linear region of stress/ strain curve.

Cell attachment: For cell attachment each disk was immersed in 100% ethanol for 24 h and soaked in sterile phosphate-buffered saline (PBS) for 30min before plating. All samples were placed in a 24-well plate, secured with silicon rings, and kept under 500 µL medium in an incubator at 37°C. The medium was aspirated after 2 h and an aliquot of fresh 300 µL media containing 15,000 W20-17 cells was added on top of each sample. After 2 h, 500 µL additional medium was added. Media was changed every three days, thereafter. Cell numbers and viability were determined by using the MTS Cell Proliferation Assay kit at days 1, 3 and 7. The cell morphologies were characterized using a confocal microscope after staining with Live/Dead kit.

Results: Figures 1a and 1b reveal the mechanical properties of HA-modified nanoparticles. Fig. 1a exhibits an increasing compressive modulus with increasing HA concentration in scaffold formulation. Furthermore, tensile strength (Fig. 1b) increases with increasing HA concentration.

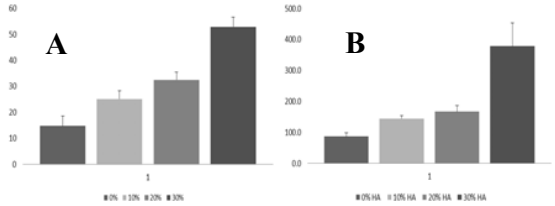

Figure1. (A) Compressive moduli of HA-modified copolymer measuring the linear region at 3-5% strain, (B) Elastic modulus measurements at 2% strain.

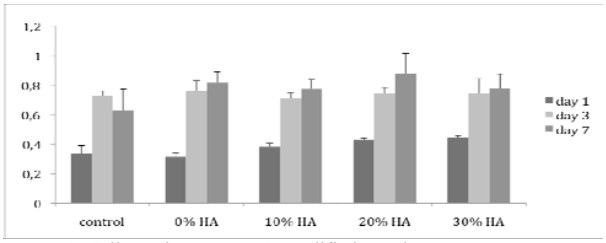

Figure 2. Cell attachment on HA-modified copolymers.

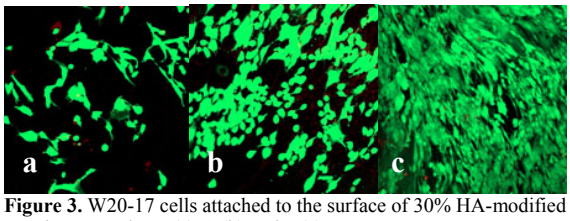

Figure 3. W20-17 cells attached to the surface of 30% HA-modified copolymer on day 1 (a), 3 (b) and 7 (c).

We demonstrated that incorporation of HA into PPF-co-PCL improved cell attachment significantly, and cells had greater proliferation rate after 3 and 7 days in culture (Figure 2). Additionally, we demonstrated that cells remained viable throughout the 7 day of culture and revealed fibroblast-like morphology by day 7 (Figure 3). An increased alkaline phosphatase activity and collage type I expression was observed on scaffolds with 30% HA.

Conclusions: Our results show that incorporation of HA into PPF-co-PCL changed mechanical property of scaffolds, and significantly enhanced attachment, proliferation and differentiation of osteoblasts. This approach could be further investigated in vivo for treatment of bone defects.

Reference: [1] Yan et. al Biomater Sci Polym 2011; 22(4-6): 489-504