BIOMIMETIC CITRIC-ACID BASED NANOCOMPOSITES FOR ORTHOPAEDIC TISSUE ENGINEERING

Eun Ji Chung and Guillermo A. Ameer*
Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA.
*g-ameer@northwestern.edu

Introduction

The use of mesenchymal stem cells (MSC) has been increasingly popular for the treatment of bone defects[1]. These cells can be easily expanded to high cell numbers and the addition of MSC facilitates the healing of bone defects. Furthermore, the isolation of MSCs from bone marrow can be easily adopted due to the routine collection of bone marrow in the clinical setting today. Because MSC cell suspensions are difficult to maintain within a bone defect and do not provide any biomechanical stability, MSC are combined with scaffolds for a tissue engineered approach.

The gold standard today for biodegradable biomaterials used for scaffolds is the polyester poly (L-lactide) (PLLA). Problems with PLLA include slow and bulk degradation, which can cause chronic inflammation, fracture, pain, tissue loss, and revision surgeries. In order to increase osteoconductive and osteogenic properties, many groups include calcium phosphates (CaP) such as hydroxyapatite (HA), an apatite that makes up 60-70% of our bone weight, into biomaterials. CaP are brittle and hard to process, and PLLA-HA composites used in patients today consist of primarily 30% HA.

Our group has developed a novel, biocompatible, and biodegradable elastomer poly(1, 8 octanediol-co-citrate), or POC. POC is an ideal material because its degradation and mechanical properties can be controlled by varying the polymerization conditions (time and temperature) and the choice of diols. In addition, POC synthesis is simple, does not involve harsh solvents, and is cost effective. And importantly, the elastomeric properties of POC can complement the brittle nature of CaP. For these reasons, we have developed nanocomposites consisting of POC and up to 60% HA crystals (POC-HA) by weight. These composites are malleable and can be shaped into a variety of bone defects. Moreover, these composites exhibit faster degradation rates in comparison to PLLA and the adjustability of its mechanical properties can be made suitable for specific orthopaedic applications of interest. In this study, the feasibility of using POC-HA composites as a suitable biomaterial for adhesion and proliferation of MSC in orthopaedic applications are discussed.

Experimental

Hydroxyapatite nanocrystals (medical grade, 100 nm) were purchased from Berkeley Advanced Biomaterials, Inc., and 1, 8-octanediol (98%) and citric acid (99.5%) from Sigma-Aldrich (St. Louis, MO, USA). To prepare POC-nanocomposites, POC pre-polymer was mixed with various amounts of HA particles (40%, 50%, 60% by weight).

Compression strength (Sc) and modulus (Ec) were measured using a Sintech mechanical tester model 20/G (Triangle Park, N.C.) following the JIS K7208 standard. Rods with a diameter of approximately 6 mm and a length of 20 mm were polished with sandpaper before testing. For each mechanical test, at least 6 samples were used and the mean values and standard deviations (SD) were calculated.

Degradation studies were performed in vitro in phosphate buffered saline (PBS), pH 7.4, at 37°C for up to 26 weeks under static conditions. Weight loss was calculated by comparing the initial weight with the weight measured at various time points ($n \geq 4$).

Human MSC were purchased from Lonza, and expansion was conducted in low glucose DMEM containing 10% FBS and 1% penicilin/streptomycin (expansion media) at 37^0C and 5% CO_2 in a humidified incubator. Culture medium was changed every 3-4 days.

In order to visualize the adherence of human MSC on POC-HA, cells were seeded at an initial density of 10,000 cells/cm^2 onto 10 mm diameter POC-HA discs. Before seeding, discs were sterilized by ethylene oxide gas (Anderson Sterilization) and discs were incubated in cell culture media over night. At day 7, samples were fixed using 2.5% glutaraldehyde, dehydrated in graded series of ethanol, and freeze-dried. The samples were sputter-coated with a 5-nm layer of gold and observed using SEM (Hitachi 3500N).

Results and Discussion

Compression modulus and strength increased with increasing content of HA (Table 1). Only nanocomposites with 60% by weight HA were in range of human trabecular bone with a compression modulus of 328 MPa[2].

Table 1. Compression data of POC-HA composites.

Mechanical Property (MPa)	40%HA	50%HA	60%HA
Modulus	45 ±9	154±18	328±20
Strength	8±3	20±5	47±4

Degradation profiles depicted that an increase in HA content decreased the degradation rate. The mechanism of degradation of POC-HA composites is through the degradation of POC (hydrolysis) consistent with the reported degradation rate of POC[3].

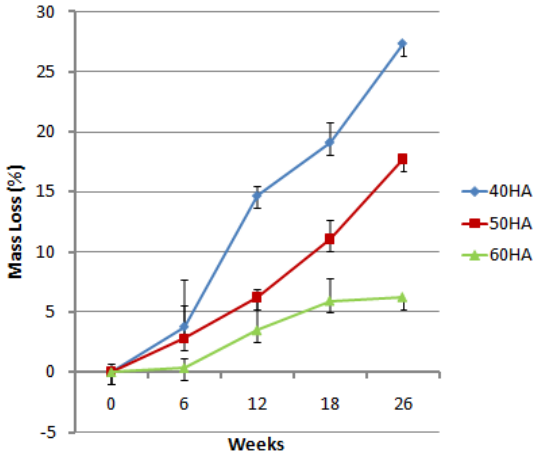

Fig. 1. Degradation profiles of POC-HA composites for up to 26 weeks.

At 7 days, MSC adhered well on all POC-HA composites (Fig. 2). However, only nanocomposites with 60% HA by weight displayed MSC with elongated morphology. MSC on both nanocomposites with 40% and 50% by weight HA displayed similar morphology by 21 days (data not shown). Not until after 7 days did MSC proliferate for all composite types (Fig. 3)

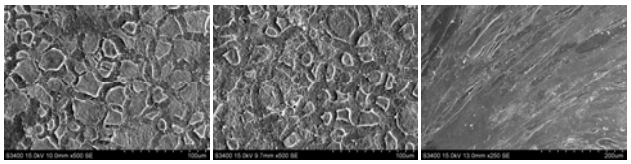

Fig. 2. MSC on nanocomposites with (L-R) 40, 50, and 60% by weight HA at 7 days.

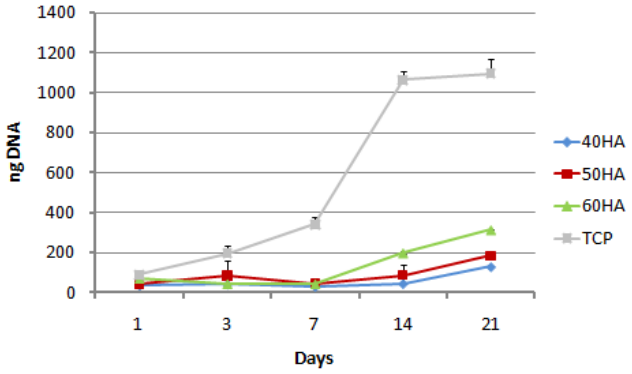

Fig. 3. Proliferation kinetics of MSC on all POC-HA composites for up to 21 days.

Conclusion

In this study, we assessed the processability and applicability of citric acid-based CaP nanocomposites as a scaffold for orthopaedic tissue engineering applications. Unlike CaP-polyester composites such as PLLA-HA interference screws that are used in clinical settings today, our citric acid-based nanocomposites are able to incorporate up to 60% of either hydroxyapatite or β-tricalcium phosphate. This percentage matches closely to that of CaP content in the human bone. These data confirm the design flexibility of our nanocomposites and their biocompatibility with MSCs. Future studies will investigate the ability of these nanocomposites to directly differentiate MSC into osteoblasts.

References:

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3. Yang et al. Advanced Materials 2004, 16: 511-516.