Osteogenic Composites Based on Citric Acid and Calcium Phosphates

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Introduction: Although poly (L-lactide) (PLLA) is approved by the FDA, problems with using PLLA for orthopaedic applications include mechanical mismatch and slow and bulk degradation. To increase the mechanical and osteoconductive/osteogenic properties, calcium phosphates (CaP) such as hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) are incorporated. CaP make up 60-70% of our bone weight but materials based on CaP are brittle and hard to process. As a consequence, PLLA-CaP composites used in patients today consist of primarily 30% HA.

Our group has developed a biocompatible and biodegradable elastomer poly(1, 8 octanediol-co-citrate), or POC. The degradation and mechanical properties of POC can be controlled by varying the polymerization conditions (time and temperature) and the choice of diols. In addition, POC synthesis is simple 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% CaP nanocrystals. The mechanical properties of these novel composites are in range of human bone, degradation rates are faster in comparison to PLLA, and *in vivo* studies displayed biocompatibility[1].

The use of mesenchymal stem cells (MSC) is highly explored for orthopaedic use due to their ability for easy isolation and expansion. These cells enhance the synthetic replacement due to its immediate ability to express extracellular matrix, increasing the mechanical properties and integrative properties[2]. In addition, the scaffold component provides the structural support since MSC cell suspensions alone are difficult to maintain within a bone defect and do not provide any biomechanical stability. In this study, the feasibility of using POC-CaP composites as a suitable biomaterial for delivery and differentiation of MSC in orthopaedic applications is discussed.

Methods: HA nanocrystals (100 nm) were purchased from Berkeley Advanced Biomaterials, Inc., and 1, 8-octanediol (98%) and citric acid (99.5%) from Sigma-Aldrich. To prepare POC-nanocomposites, POC pre-polymer was mixed with various amounts of HA particles to obtain 40%, 50%, 60% calcium phosphate components. **Mechanical properties** were measured using a Sintech mechanical tester model 20/G (Triangle Park, N.C.) following the JIS K7208 standard. For each mechanical test, at least 6 samples were used. **Experiments using cells** were cultured in 37°C in humidified air and 5% CO_2. Culture medium (low glucose DMEM, Invitrogen supplemented with 10% FBS and 1% P/S) was changed every 3 to 4 days. **SEM** was used to visualize MSC on composites (Northwestern University). Briefly, after sterilization using ethylene oxide gas, 8,000 cells/cm^2 were seeded on composite discs. At 3 and 7 days post-seeding, samples were fixed, dehydrated, freeze-dried, and sputter-coated with a 5-nm layer of gold before observation. **ALP assays** used 30,000 MSC to seed onto 3.6 cm^2 composite discs. The amount of alkaline phosphatase was determined in 5 mL of media by measuring the release of p-nitrophenol from p-nitrophenyl (Sigma) by spectrophotometry at 410 nm.

Results: Both compression modulus and strength increased with increasing amounts of HA (Table 1). Only 60% HA was found to be in range of the compression modulus of human trabecular bone (325-990 MPa[3]).

Table 1. Compression strength and modulus of various POC-HA and POC-TCP composites.

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

Note: Ec: compression modulus; Sc: compression strength.

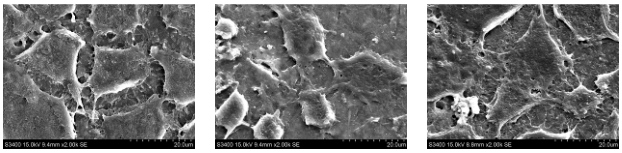

Figure 1. SEM images of MSCs after 3 days.

MSCs adhered well on all composite types after 3 days.

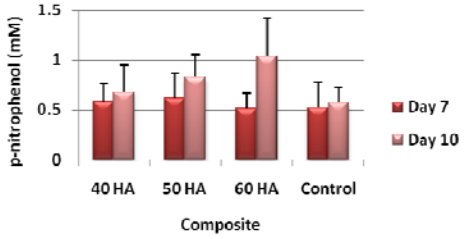

Figure 2. Akaline phosphatase assays displayed increased secretion with increasing HA.

ALP assays displayed an increasing amount of alkaline phosphatase in the media from 7 to 10 days. With increasing HA, an increase in alkaline phosphatase secretion was found (Fig. 2).

Conclusions: In this study, citric acid-based CaP-nanocomposites as an osteogenic scaffold were assessed. Our citric acid-based nanocomposites are able to incorporate up to 60% of either hydroxyapatite or β-tricalcium phosphate, matching closely to that of the CaP content in human bone. As CaP content increased, POC-HA increased in compression strength and modulus displaying the adjustability of composites into specific applications of interest. SEM micrographs demonstrated good MSC adherence conferring biocompatibility of these nanocomposites and initial ALP assays displayed an increase in alkaline phosphatase secretion with increasing HA content. In conclusion, citric acid-based CaP nanocomposites display good osteogenic potential and must be further assessed for clinical applications.

References:

[1]Chung, EJ et al. 2009. *In press*.
[2]Cooper JA et al. PNAS 2007; 104: 3049-3054.
[3]Orr TE et al. Biomaterials 2001; 22: 1953-1959.