Tissue Response to Citric Acid-Based Micro-/Nanocomposites

INTRODUCTION:
There is significant demand for orthopaedic implants that are both bioactive and exhibit material properties that are compatible to those of native and healthy tissue [1]. Synthetic materials play an important role in bone repair and bone replacement. In the case of poly (L-lactide) (PLLA), one of the main problems is slow degradation, which further spark tissue loss due to revision surgeries and chronic inflammation [2, 3]. Recently, a novel elastomer poly (1, 8-octanediol-co-citrate), or POC, has been developed in our group. In particular, POC has a fast degradation rate (6 months to 1 year) and display good biocompatibility with soft tissue. The degradation and mechanical properties can be controlled by varying the polymerization conditions (time and temperature) and the choice of diols. In addition to its clinical compatibility, POC synthesis is simple, does not involve harsh solvents or catalysts, and is cost effective. Recently, we have developed POC-hydroxyapatite composites which have controllable mechanical properties and degradation rates [4]. POC-HA is malleable to meet the need of various irregular shapes of bone defects and is easily processed into a hard implant. In this study, POC composites were developed with hydroxyapatite (HA) nanocrystals and microparticles and implanted into the rabbit knee to assess biocompatibility. Because bone is composed of apatite nanocrystals, the hydroxyapatite nanocrystals have the same constituent and structure of bone may lead to engineered tissue closely resembling native tissue. Both POC nano- and micro-HA are physiologically compatible, but exhibit differences in vivo.

METHODS:
Hydroxyapatite nanocrystals (medical grade, 100 nm) and hydroxyapatite ceramic powder (medical grade, 20-50 µm) 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-HA composites, POC pre-polymer was mixed with various amounts of HA particles to obtain 40%, 50%, 60% and 65 wt.% HA components[4]. Two experimental groups, each consisting of five skelettally mature male New Zealand white rabbits (Covance, Kalamazoo), were investigated: a) the nanocomposite group and b) the microcomposite group. All animals received implants in both knees. The surgical protocol was approved by Northwestern University’s Animal Care and Use Committee. A bone defect with a 2.7 mm diameter and 4.0 mm depth was created in both medial femoral condyles. POC-HA non-porous plugs containing relatively low (40 wt.%) or high (~60 wt.%) apatite were inserted into the left and right defects, respectively via press fit. After 6 weeks, the implant and surrounding tissue were harvested and evaluated by histology and histomorphometry. Gross examination was documented with a digital camera. Hemotoxylin and eosin (HE), Masson-Goldner Trichrome (MT) and von Kossa (VK) stains were used to characterize biocompatibility. Sections were assessed via standard light or fluorescence microscopy (Nikon Eclipse TE2000-U, Japan) and quantified via histomorphometric analysis using Image-Pro plus software (version 5.0, Media Cybernetics, Inc., Silver Spring, MD, USA). The following variables were quantified: 1) ratio of active osteoid surface area to total trabecular bone surface area, 2) ratio of total osteoid surface area to total trabecular bone surface area, and 3) the trabecular bone surface area fraction. The first two variables were quantified using the linear intercept method of extrapolation. Tissue response measurements were taken at the edge of the implant (x=0) and at 200 µm from the edge of the implant (x=200 µm, where x is a distance perpendicular to the implant edge).

Student t-test and analysis of variance (ANOVA) were used to assess statistical significance among the mechanical properties and the histomorphometry data. All analyses were carried out using Graphpad Prism 4.0 and SigmaStat 3.1. A p value of <0.05 was considered to be significant.

RESULTS
POC-HA nanocomposites with HA nanocrystals of 40- 60 wt% were fabricated by mixing and molding methods. While POC-HA microparticles with 40, 50, 60% and 65wt% HA microparticles only allowed the fabrication via molding because of their high flexibility. The bonding (Eb) and compression (Ec) moduli of nanocomposites were higher than those of microcomposites (at 60 wt.% of HA, Eb=322±19 and Ec=328±20 for HA nanocomposite, and Eb=24±1 and Ec=25±2 for HA microcomposite, all values in MPa). At the tissue-implant interface, there was no evidence of a significant fibrous capsule formation or inflammation (Fig.1).

To the contrary, bone formation was evident adjacent to the implants. The bone surrounding the implant was active as osteoids with layers of osteoblasts were observed. Far from the implant, the bone histology was normal. Histomorphometry analysis of tissue within the first 200 µm from the edge of implants, revealed similar osteoblast activity between nano and microcomposites with 40 or 60 wt % HA. HA content did not have an effect on osteoid surface fraction within nanocomposites but an increase in HA microparticle content resulted in a decrease in osteoid surface area for microcomposites. The total trabecular surface area around the nanocomposites was greater than that measured around microcomposite implants (P<0.001). Compared to bone tissue response within the first 200 µm, tissue response was different between 200-400 µm from the edge of the implant. Osteoid activity was decreased in the 40 wt. % nanocomposites although it was not the case for the other composites. Although active osteoid surface area decreased significantly, trabecular bone surface area was higher than that at the bone-implant interface (0-200 µm). Far from the implant, the bone appeared normal.

DISCUSSION:
Mechanical properties of the composites with HA nanocrystals are in the range of those reported for PLLA-HA composites. The nanocomposites are more active than microcomposites in term of bone formation. At the nanostructure level, mature bone is mainly composed of collagen fibers and clusters of bone minerals, predominantly HA. From the biomimetic point of view, osteogenesis may be improved if the implant has a chemical composition and structure that is similar to bone. The finding from our in vivo experiments confirmed that all the composites demonstrated good biocompatibility and have osteostimulatory responses at the tissue-implant interface. The composites described herein are inexpensive and easy to fabricate. Future research will evaluate the long-term tissue responses during degradation of implant.

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