Investigation of Citric Acid-Based Calcium Phosphate Nanocomposites as an Osteogenic Biomaterial

+Chung, E and Ameer, GA
+Northwestern University, Evanston, IL
g-amere@northwestern.edu

INTRODUCTION:
The use of mesenchymal stem cells (MSC) has been increasingly popular for the treatment of bone defects. These cells can be easily expanded to high cell numbers and 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). Although PLLA is already approved by the FDA, 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 calcium phosphates. For these reasons, we have developed nanocomposites consisting of POC and up to 60% β-tricalcium phosphate or hydroxyapatite crystals (POC-nβTCP and POC-nHA). 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-nβTCP and POC-nHA composites as a suitable biomaterial for delivery and maintenance of MSC in orthopaedic applications are discussed.

METHODS:
Hydroxyapatite nanocrystals (medical grade, 100 nm) and β-tricalcium phosphate 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 to obtain 40%, 50%, 60% calcium phosphate components.

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.

In order to visualize the surface morphology of the POC-CaP composites and the adherence of MSC, scanning electron microscopy was used. For the adherence study, cells were seeded at an initial density of 10,000 cells/cm² onto 10mm diameter POC-CaP discs. Before seeding, discs were sterilized by incubation in 70% ethanol for 30 minutes, washing with sterile PBS (pH 7.4), and UV exposure for an additional 30 minutes. After sterilization, discs were incubated in cell culture media for 2 hours before cell seeding. At three days and seven days post-seeding, samples were fixed and Briefly, samples were fixed using 2.5% glutaraldehyde in PHB for at least 2 hours, dehydrated in graded series of ethanol, and free-dried. The samples were sputter-coated with a 5-nm layer of gold and observed using SEM (SEM 3500N, Electron Probe Instrumentation Center, Northwestern University). Cells were cultured in 37°C in humidified air and 5% CO₂. Culture medium was changed every 3 days.

Student t-test was used to assess statistical significance among the mechanical properties. All analyses were carried out using GraphPad Prism 4.0 and SigmaStat 3.1.

RESULTS:

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

<table>
<thead>
<tr>
<th>Property (MPa)</th>
<th>40%HA</th>
<th>50%HA</th>
<th>60%HA</th>
<th>40%TCP</th>
<th>50%TCP</th>
<th>60%TCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec</td>
<td>45 ±9</td>
<td>154±18</td>
<td>328±20</td>
<td>11±6</td>
<td>23±4</td>
<td>63±7</td>
</tr>
<tr>
<td>Sc</td>
<td>8±3</td>
<td>20±5</td>
<td>47±4</td>
<td>2±1</td>
<td>4±1</td>
<td>11±4</td>
</tr>
</tbody>
</table>

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

Both compression modulus and strength increased with increasing amounts of either HA or TCP. Between the percentage groups, all HA groups were found to be significantly different from TCP groups by p<0.0009. Only 60% HA was found to be in range of the compression modulus of human trabecular bone (325–990 MPa).

DISCUSSION:
In this study, we assessed the processability and applicability of citric acid-based CaP nanocomposites as a scaffold orthopaedic biomaterial for tissue engineering applications. Unlike CaP-polyester composites such as PLLA-HA interference screws 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 skeleton.

As CaP content increased, both POC-HA and POC-TCP increased in compression strength and modulus displaying the adjustability of composites into specific applications of interest. Between each weight percentage group, POHA-HA was significantly higher than POC-TCP, and POC-60%-HA was the only composite that displayed a similar compression modulus to that of human bone.

Scanning electron micrographs demonstrated good MSC adherence after three and seven days conforming biocompatibility of these nanocomposites. Future studies including the ability of these nanocomposites to differentiate MSC into osteoblasts and the feasibility of synthesizing porous nanocomposites will provide further support and evidence for citric acid-based, calcium phosphate nanocomposites as a biomaterial for cell-based orthopaedic applications.

REFERENCES: