PHYSICO-CHEMICAL EVALUATION OF PHYSIOLOGICAL TEMPERATURE SETTING POLYMER-CERAMIC COMPOSITES FOR BONE REPAIR

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Introduction

Bone is a composite of an organic phase (type I collagen, proteoglycans) and inorganic phase of hydroxyapatite (calcium and phosphate salts)\(^1\). Because bone is a composite, a number of hydroxyapatite–polymer composites produced by conventional methods have been investigated as bone substitutes based on both natural and synthetic polymers. We have investigated a novel class of biodegradable polymers, polyphosphazenes as potential candidates to form self-setting calcium phosphate–polymer composites due to their biocompatibility, highly flexible backbone, biodegradability, and the ability of the side groups to interact with hydroxyapatite (HA) thereby reinforcing the composite\(^2\). The aim of the present study was to evaluate the feasibility of forming amino acid ester based polyphosphazenes and calcium deficient HA composites at physiological temperature.

Materials & Methods

Synthesis of Polyphosphazene-Calcium Deficient Hydroxyapatite Precursors: The polyphosphazene used in this study was poly[[50%ethyl alanine] (50%phenyl phenoxypolypepsphazene] (PNEA\(_{50}\)PhPh\(_{50}\)). The hydroxyapatite precursors used were tetra calcium phosphate and dicalcium phosphate. The composite precursors were prepared by an emulsion technique. Two different calcium deficient hydroxyapatite-polyphosphazene composites with varying Ca/P ratio (PNEA\(_{50}\)PhPh\(_{50}\)-CDHA – Ca/P: 1.5; PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA – Ca/P: 1.6) were synthesized from the precursors.

Preparation of Composites from the Precursors: The polyphosphazene–calcium deficient HA precursor powders were finely ground using a mortar and pestle (Fisher, USA). The composite precursor powders were mixed with 0.5% phosphoric acid (Acros 201140010) in the ratio 1:1 (w/v) to form a paste. The pastes were allowed to set for 24 hours at 37°C in a humidified atmosphere to form the composites.

X-ray Diffraction: X-ray diffraction (XRD) measurements were performed on a Seimens D5000 diffractometer to confirm the formation of calcium deficient hydroxyapatite composites under the current fabrication conditions. The composites were finely ground and mounted on a glass slide and analyzed between 20° and 50° (20) at an angular sweeping rate of 2° (20/min) with a step size of 0.05 degrees.

Mechanical Property Evaluation of the Composites: The mechanical properties of the six composites were evaluated according to an ASTM standard\(^3\). The precursors of composites were mixed with 0.5% phosphoric acid (Acros 201140010) in the ratio 1:1 (w/v) to form a paste and were injected into a mold with a diameter of 6mm and height of 12mm. The mold was maintained at 37°C for 24 hours in a humidified atmosphere and the compressive strength, maximum load, and the amount of mercury that penetrated into the sample at different pressures was varied from 0.1 to 50psi with an equilibration time of 60 seconds for each intermediate data point. The pore size, and porosity of the scaffolds were determined from the amount of mercury that penetrated into the sample at different pressures.

Porosity of the Composites: The porosities of the composites were evaluated using a mercury porosimeter (Micromeritics, Autopore III, USA). The samples were prepared similar to mechanical testing. The pressure was varied from 0.1 to 50psi with an equilibration time of 60 seconds for each intermediate data point. The pore size, and porosity of the scaffolds were determined from the amount of mercury that penetrated into the sample at different pressures.

Scanning Electron Microscopy: The microstructures of the composites were analyzed using a scanning electron microscope, SEM (JEOL 6700F, USA). The composites were prepared and incubated as explained previously. The samples were coated with Gold/ Palladium and were viewed under the SEM.

Results & Discussion

Fig 1 shows the X-ray diffraction pattern of PNEA\(_{50}\)PhPh\(_{50}\)-CDHA and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA formed at 37°C after 24 hours of incubation. The composites formed have broad peaks from 31° to 34° (20). Thus the X-ray diffraction analyses showed the formation of poorly crystalline hydroxyapatite at physiological temperature (Fig 1).

Fig 2 shows the compressive modulus of PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA prepared under the current fabrication technique. PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA exhibit compressive moduli in the range of trabecular bone.

Fig 1. X-ray diffraction analysis of PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA formed at physiological temperature.

Fig 2. Compressive modulus of PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA formed at physiological temperature.

<table>
<thead>
<tr>
<th>Composites</th>
<th>Mean Pore Diameter (µm)</th>
<th>Porosity (%)</th>
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<tbody>
<tr>
<td>PNEA(<em>{50})PhPh(</em>{50})-CDHA</td>
<td>8.57 ± 0.91</td>
<td>17.14 ± 12.19</td>
</tr>
<tr>
<td>PNEA(<em>{50})PhPh(</em>{50})-CDSHA</td>
<td>10.02 ± 0.99</td>
<td>20.62 ± 3.20</td>
</tr>
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</table>

Table 1. Mean pore diameters and percentage of porosities in PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA composites formed from the precursors at physiological temperature. Values expressed as mean ± standard deviation (SD) (n=3).

Porosity is an important factor for developing composites as scaffolds for bone tissue engineering because the vascularization, and cellular infiltration of the scaffold depends on the porosity. The mean pore diameters of the composites varied from 7.54µm to 16.64µm and the percentage porosity ranged from 5.93% to 20.62% (Table 1).

Fig 3A, B show the scanning electron micrograph of the surface microstructures of PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA composites formed at low temperature.

Fig 3A, B show the scanning electron micrograph of the surface microstructures of PNEA\(_{50}\)PhPh\(_{50}\)-CDHA, and PNEA\(_{50}\)PhPh\(_{50}\)-CDSHA respectively. Thus the gross morphologies of the composite surfaces were similar and present a micro porous structure.

Conclusions

Composition and physical-chemical characteristics of calcium phosphate cement composites play a critical role in the in vitro and in vivo behavior. Poorly crystalline hydroxyapatite that resembles the mineral component of bone formed from its precursors in the presence of biodegradable polyphosphazenes and the surface morphologies of the composites showed a micro porous structure. The compressive moduli of the composites were in the range of trabecular bone.

References


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