

A citric acid-based hydroxyapatite composite for orthopedic implants

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Abstract

We describe a novel approach to process bioceramic microparticles and poly(diols citrates) into bioceramic–elastomer composites for potential use in orthopedic surgery. The composite consists of the biodegradable elastomer poly(1,8-octanediol-citrate) (POC) and the bioceramic hydroxyapatite (HA). The objective of this work was to characterize POC–HA composites and assess the feasibility of fabricating tissue fixation devices using machining and molding techniques. The mechanical properties of POC–HA composites with HA (40, 50, 60, 65 wt.%) were within the range of values reported for tissue fixation devices (for POC–HA 65 wt.%, $S_b = 41.4 \pm 3.1$, $E_b = 501.7 \pm 40.3$, $S_c = 74.6 \pm 9.0$, $E_c = 448.8 \pm 27.0$, $S_t = 9.7 \pm 2.3$, $E_t = 334.8 \pm 73.5$, $S_s = 27.7 \pm 2.4$, $T_s = 27.3 \pm 4.9$, all values in MPa). At 20 weeks, the weight loss of POC–HA composites ranged between 8 and 12 wt.%, with 65 wt.% HA composites degrading the slowest. Exposure of POC–HA to simulated body fluid resulted in extensive mineralization in the form of calcium phosphate with Ca/P of 1.5–1.7 similar to bone. POC–HA supported osteoblast adhesion in vitro and histology results from POC–HA samples that were implanted in rabbit knees for 6 weeks suggest that the composite is biocompatible. Synthesis of POC–HA is easy and inexpensive, does not involve harsh solvents or initiators, and the mechanical properties of POC–HA with 65 wt.% HA are suitable for the fabrication of potentially osteoconductive bone screws.

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1. Introduction

Orthopedic, cranio-facial, and oral-maxillofacial surgeons often use tissue fixation devices such as pins, plates, and screws that are made from poly(L-lactide) (PLLA), a biodegradable polymer [1–7]. Although biodegradable devices can have significant advantages over their metal counterparts, there are concerns with their use. These concerns include slow degradation, which can be as long as 5 years, and their inability to fully integrate with bone, which can be a problem for revision surgeries [8–12]. A strategy to improve the osteointegration capacity of polymers has been to blend them with hydroxyapatite (HA), a bioceramic that can be found in natural bone. Although HA is very brittle and hard to process into fixation devices of sufficient strength and fatigue resistance, it can impart osteoconductivity to polymers [13,14].

Researchers have shown that under certain conditions, addition of HA particles can improve the mechanical properties of the PLLA component when used in a composite blend [15–17]. Therefore, composites of polymers with bioceramics may be a suitable compromise to meet mechanical property requirements and achieve osteointegration of the implant. Nevertheless, the problem of the physical limitation to the amount of HA that can be incorporated in a PLLA–HA composite (~30% to avoid brittleness) together with slow degradation of PLLA (5 years) motivate the search for composite materials that can be easily processed and will fully integrate with the surrounding bone and tissue within a year of implantation.

We have recently described the synthesis and characterization of elastomeric and biodegradable polyesters, referred to as poly(diols citrates), for soft tissue engineering [18,19]. Their mechanical properties and degradation rates can be controlled with synthesis conditions of the polycondensation reaction and choice of diol, and their

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preparation does not involve any harsh solvents or exogenous initiators and catalysts that would not be biocompatible after degradation. Furthermore, poly(diols citrates) can be very inexpensive, relative to poly(α -hydroxy acid) biodegradable polymers. Thus, we hypothesized that a composite of a poly(diols citrate) with HA would have the desired characteristics of a bioceramic suitable for hard tissue engineering but with improved processability, mechanical properties, and degradation characteristics. Poly(1,8-octanediol-co-citrate) (POC) was selected because of its faster degradation rate (a few months to 1 year) than PLLA (3–5 years) and because its mechanical properties can be tailored by simply changing reaction conditions such as reaction temperature and time, and the ratio of 1,8-octanediol to citric acid [18,19]. POC has also been shown to be biocompatible and could potentially enhance the bio-integration of the surrounding soft tissue as in the case of fixation of a ligament graft. Moreover, these materials are inexpensive and easy to synthesize, an additional advantage for clinical application. The objective of this work was to assess the mechanical, degradation, mineralization, and tissue compatibility characteristics of POC–HA composites. The feasibility of fabricating POC–HA composites into bone screws by compression molding and machining was also evaluated.

2. Materials and methods

2.1. Materials

HA [M_w : 502.32, assay >90% (as $\text{Ca}_3(\text{PO}_4)_2$); particle size: >75 μm (0.5%), 45–75 μm (1.4%), <45 μm (98.1%)] was purchased from Fluka (St. Louis, MO, USA). 1,8-octanediol (98%) and citric acid (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used as received. Polytetrafluoroethylene (PTFE) tubes were purchased from McMaster-CARR, Chicago, USA.

2.2. Methods

2.2.1. Fabrication of POC–HA composites

POC pre-polymer was synthesized according to published methods [18]. Briefly, 0.05 mol of 1,8-octanediol and 0.05 mol of citric acid were added to a 100 ml round bottom flask and exposed to a constant flow of nitrogen gas. The mixture was melted under vigorous stirring at 160–165 °C. Following melting, the mixture was polymerized at 140 °C for 1 h to create a POC pre-polymer. The POC pre-polymer was mixed with various amounts of HA particles to obtain composites of 40, 50, 60, and 65 wt.% HA by weight. Briefly, POC pre-polymer was mixed with the desired amount of HA powder and placed in PTFE dishes that were pre-warmed to 80 °C. The POC–HA mixture was stirred until it became clay-like, a process that generally took 5–10 h depending on the HA content. The POC–HA mass was then inserted into PTFE tubes to make rods or into other PTFE molds designed to meet the dimensional requirements for sample mechanical testing protocols or in situ formation of bone screws. The POC–HA in the mold was then post-polymerized at 80 °C for 3 days followed by 120 °C under 2 Pa vacuum for 1 day. The surface morphology of various POC–HA composite cross-section was visualized using SEM to quantitatively assess the distribution and size of the HA particles. POC–HA interference screws were fabricated via molding and machining.

2.2.2. Characterization of the mechanical properties of POC–HA composites

The following mechanical properties were measured using a Sintech mechanical tester model 20/G (Triangle Park, NC): (1) bending strength (S_b) and modulus (E_b) according to Japanese industrial standard (JIS) K7203, (2) compression strength (S_c) and modulus (E_c) according to JIS K7208, (3) tensile strength (S_t) and modulus (E_t) according to JIS K7113, (4) shear strength (S_s) [20], and (5) torsional strength (T_s) [21]. All rods used for the mechanical tests were polished with sandpaper before measurement. For all mechanical tests, at least six samples were tested and the mean values and standard deviations (SD) were calculated. The density of POC–HA composites was measured using the Archimedes principle as previously described [22].

2.2.3. In vitro degradation of POC–HA composites

The degradation of POC–HA composite samples (10 mm diameter \times 2 mm thick) with HA percentages of 40, 50, 60, and 65 wt.% was assessed in vitro in phosphate-buffered saline (PBS), pH 7.4, at 37 °C for up to 20 weeks under static conditions. Within the POC–HA composite, only the POC is expected to degrade when incubated in aqueous solution. PBS was changed as necessary to ensure that the pH did not drop below 7. Prior to weighing, samples were extensively rinsed with deionized water and dried. Weight loss was calculated by comparing the initial weight (W_0) with the weight measured at 2, 6, 12 and 20 weeks (W_t), as shown in Eq. (1). The results are presented as mean \pm standard deviation ($n = 4$):

$$\text{Mass loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100. \quad (1)$$

2.2.4. Mineralization of POC–HA composites

Surface mineralization of POC–HA composites was assessed in vitro using modified simulated body fluid (SBF) [23]. The SBF consisted of (mmol): Na^+ (142.0), K^+ (4.0), Mg^{2+} (1.5), Ca^{2+} (5.0), Cl^- (147), HCO_3^- (4.2), HPO_4^{2-} (2.0) and SO_4^{2-} (0.5) with the pH adjusted to 7.2 using tris(hydroxymethyl)aminomethane [24]. Briefly, discs (10 mm diameter \times 2 mm thick) of POC–HA composites with HA percentage of 40, 50, 60 and 65 wt.% were immersed in 10 ml of the SBF at 37 °C for up to 15 days. Fresh SBF was added every other day to maintain the ionic concentration and pH during mineralization. The morphology of deposited calcium phosphate crystals was observed via scanning electron microscopy (SEM) (Hitachi 3500 N, EPIC, Northwestern University). The stoichiometric Ca/P molar ratio was analyzed by energy-dispersive X-ray (EDX) analysis.

2.2.5. In vitro evaluation of the cell compatibility of POC–HA composites

POC and POC–HA discs (7.0 mm diameter \times 2 mm thick, with 40, 50, 60, and 65 wt.% HA) were sterilized by incubation in 70% ethanol for 30 min, washing with sterile PBS (pH 7.4), and UV exposure for 30 min. After sterilization, samples were washed several times with cell culture medium prior to placement in the wells of a 48-well tissue culture plate. A 40 μl volume of a suspension of human osteoblast cells (HOB) (Cambrex, Pittsburgh, PA) (3×10^5 cells ml^{-1}) was added to each well and incubated in osteoblast growth medium (OBM and OGM Single-Quots from Cambrex) at 37 °C in humidified air and 5% CO_2 for up to 14 days. The culture medium was changed every 3 days. Samples were fixed with 2.5% glutaraldehyde in PBS for 24 h at 4 °C. The morphology of the cells on the POC was observed via light microscopy and that of cells on the composite samples, via SEM.

2.2.6. In vivo biocompatibility of POC–HA composites

Six New Zealand white rabbits (Covance, Kalamazoo) weighing between 2.3 and 2.7 kg were used to assess biocompatibility. The animal experiments were carried out in compliance with a protocol approved by Northwestern University's Animal Care and Use Committee (Chicago, IL). The rabbits were anesthetized by ketamine 40 mg kg^{-1} IM, xylazine 5–7 mg kg^{-1} IM supplemented with isoflurane (1–2% inhalation). A 4.0 cm medial parapatellar arthrotomy was created, exposing the

medial femoral condyle. Using a mosaicplasty harvester (Smith & Nephew, Memphis, TN), a bone defect with a 2.7 mm (diameter) \times 4.0 mm (depth) was drilled in the right medial femoral condyle. A POC–HA non-porous plug containing 65 wt.% HA and matching the dimensions of the defect was inserted via press fit. After all surgical procedures, the rabbits were kept in cages and maintained with a regular laboratory diet. The knees were harvested at 6 weeks of implantation and gross examination was documented with a digital camera. POC–HA implants were stored in 10% neutral-buffered formalin. A 5 μ m sections were stained with hematoxylin and eosin for the decalcified samples, and with von Kossa for undecalcified samples. Sections were evaluated via standard light microscopy.

2.2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed on three or more means with a Newman–Keuls multiple comparison test applied within groups. A $P < 0.05$ was considered to be significant.

3. Results

3.1. Fabrication of POC–HA composites and characterization of their mechanical properties

The fabrication process revealed that below 40 wt.% the composites were too rubber-like to allow machining and at 70 wt.% a clay-like mass could not be made so that the resulting sample was brittle and difficult to mold. However, POC–HA composite with HA percentage of 40–65 wt.% were successfully fabricated using molds and could be easily machined into interference screws (Fig. 1). SEM images of cross-sections of composites with 40 wt.% HA revealed an even distribution of particles and aggregates surrounded by POC (Fig. 2A). Increasing HA wt.% lead to a decrease in

the inter-particle and aggregate distance, giving the appearance of a more homogenous particulate phase (Fig. 2(B–D)). The mechanical property measurements are summarized in Table 1. According to the statistical analysis, the POC–HA composites with 65 wt.% HA had the highest bending strength and modulus, compression strength and modulus, tensile strength and modulus, shear strength and torsional strength. Overall, there is a trend towards increased mechanical properties with increasing HA content. The density of samples increased with increasing percentage of HA.

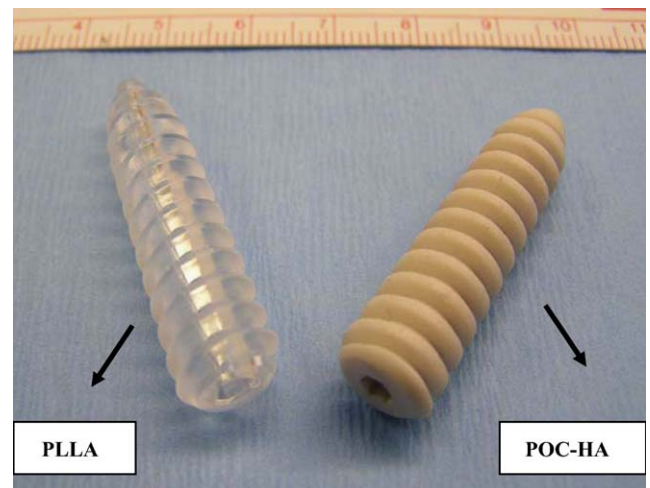


Fig. 1. Digital image of PLLA and POC–HA ligament graft interference screws. Composites were prepared by an in situ post-polymerization of a POC–HA blend (65 wt.% HA) at 80 °C for 3 days and 120 °C for 1 day.

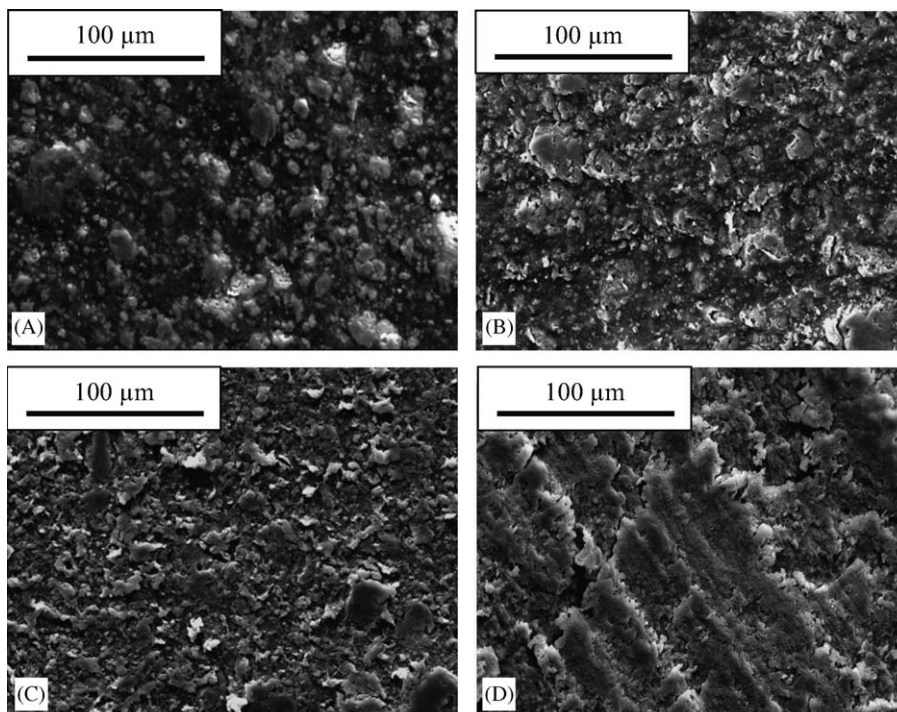


Fig. 2. SEM images of POC–HA composites with (A) 40 wt.% HA, (B) 50 wt.% HA, (C) 60 wt.% and (D) 65 wt.% HA.

Table 1
Effect of HA fraction on mechanical properties of POC–HA composites

T_s (Nm)	Composite	HA/POC (wt.%)	ρ (g cm^{-3})	S_b (MPa)	E_b (MPa)	S_c (MPa)	E_c (MPa)	S_t (MPa)	E_t (MPa)	S_s (MPa)
HA40	40/60	1.609 (± 0.016)	33.9 (± 5.7)	274.5 (± 79.9)	32.0 (± 13.0)	188.8 (± 21.1)	7.8 (± 0.5)	21.4 (± 1.8)	23.3 (± 1.6)	22.9 (± 1.6)
HA50	50/50	1.653 (± 0.014)	37.7 (± 4.6)	323.4 (± 62.5)	64.0 (± 9.4)	263.6 (± 13.7)	7.1 (± 0.3)	30.2 (± 2.2)	25.1 (± 1.7)	24.2 (± 2.0)
HA60	60/40	1.734 (± 0.061)	34.7 (± 3.0)	314.3 (± 53.9)	52.6 (± 11.5)	296.9 (± 40.5)	6.4 (± 2.0)	85.4 (± 8.8)	25.9 (± 1.6)	21.4 (± 1.9)
HA65	65/35	1.885 (± 0.072)	41.4 (± 3.1)	501.7 (± 40.3)	74.6 (± 9.0)	448.8 (± 27.0)	9.7 (± 2.3)	334.8 (± 73.5)	27.7 (± 2.4)	27.3 (± 4.9)

Note: ρ : density; S_b : bending strength, E_b : bending modulus, S_s : shear strength, S_c : compression strength, E_c : compression modulus, S_t : tensile strength (rectangular specimens), E_t : tensile modulus, T_s : torsional strength. Statistical significance is presented in Table A1 in Appendix A.

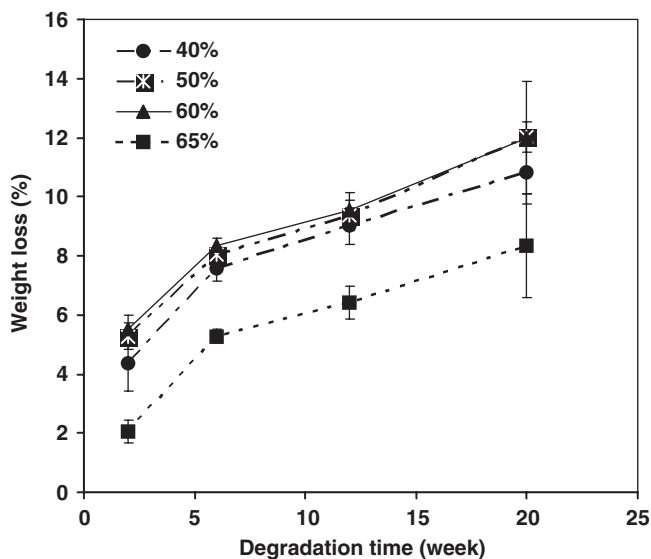


Fig. 3. In vitro weight loss of POC–HA composites with HA mass content of 40, 50, 60, and 65 wt.% (PBS at 37°C) at 2, 6, 12 and 20 weeks. POC–HA composites were prepared at 80°C for 3 days and 120°C for 1 day. Statistical significance is presented in Table A2 and Table A3 in Appendix A.

3.2. In vitro degradation of POC–HA composites

The weight loss over time profiles for POC–HA composites incubated in PBS at 37°C are shown in Fig 3. Weight loss is due to the aqueous hydrolytic degradation of POC within the composite. The degradation of the POC–HA composite discs with HA weight percentages of 40, 50, and 60 was very similar at all time points with a total weight loss of approximately 12 wt.% (± 1.1 , 0.5, 1.9, respectively) at 20 weeks. POC–HA composite with 65 wt.% HA had a weight loss of approximately 8.4 wt.% (± 1.7) at 20 weeks.

3.3. Mineralization and cell compatibility of POC and POC–HA composites

Mineralization was not observed on the surface of POC incubated in SBF (Fig. 4A and B). However, mineral nodules began to form at 3 days and aggregated on the surface of composites throughout the 15 days of incubation in SBF at 37°C (Fig. 4C–F). Mineral nodules merged into

a continuous covering on most of the sample's surface at 15 days. The composition of the mineral, in terms of the molar ratio of Ca/P, was confirmed by the EDX analysis to be 1.63 ± 0.98 . Light microscopy confirmed that HOB cells can attach, spread, and proliferate on the surface of POC (Fig. 5A and B). SEM confirmed that POC–HA composites also supported cell attachment and spreading (Fig. 5C and E). At 14 days, POC–HA composites were almost completely covered by layers of cells (Fig. 5D and F).

3.4. In vivo biocompatibility of POC–HA composites

On gross examination, the implant seemed well integrated with surrounding cartilage with the implants having a similar color and consistency (Fig 6A). Implants were firm to touch and flush to surrounding cartilage. There was no evidence of inflammation or degenerative changes around the implant or the articulating tibial plateau. The bone–implant interface was well integrated with very little fibrous tissue encapsulation (thickness of the fibrous capsule was less than 50 μm for all samples). Osteoblasts, histiocytes and demineralized bone was present at the implant/tissue interface (Fig. 6B and C). The morphology of the bone surrounding the implant was essentially similar to that of normal bone (Fig. 6D). Inflammation was minimal and several locations along the implant were devoid of a fibrous capsule. The implant is well preserved as no inflammatory reaction and bone resorption are observed at the interface for the undecalcified bone (Fig. 6E). New osteoids and mineralized chondrocytes are present immediately adjacent to the implant's interface, confirming normal bone remodeling (arrow of Fig. 6F).

4. Discussion

The vast majority of orthopedic implants are made from metals, which can cause unwanted tissue reactions, and lead to significant bone removal if a secondary intervention is required [25–27]. Alternatively, biodegradable polymers such as PLLA have been used for the fabrication of some fixation devices where significant weight bearing is not an issue for the proper function of the device. Unfortunately, these implants are not osteoconductive, have a slow degradation rate (3–5 years), and are significantly more expensive than the metal counterparts [20,28–31]. One way

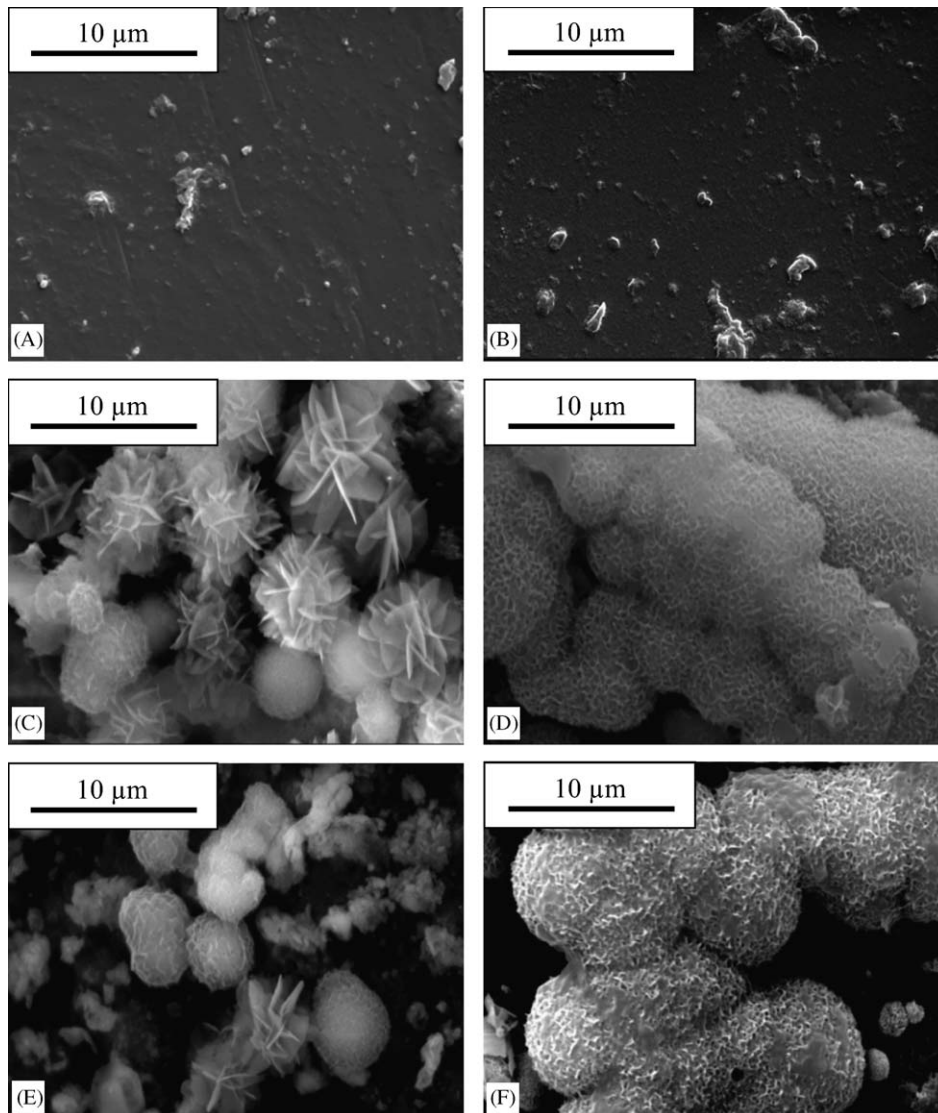


Fig. 4. Mineralization in SBF for POC at (A) 3 days and (B) 15 days; POC–HA with 40 wt.% HA at (C) 3 days and (D) 15 days; POC–HA with 65 wt.% HA at (E) 3 days and (F) 15 days. Magnification of all images: $\times 4.5K$.

to deal with the osteointegration deficiencies of polymers has been to blend them with bioceramics such as HA and tricalcium phosphate (TCP) [32–36]. Several researchers have developed and investigated composites of HA or TCP with poly(α -hydroxy acids) such as PLLA [15–17,34,37,38]. Studies have found such composites to osteointegrate more readily than the pure polymer, supporting the further study of HA/polymer composites [17,33,34,39]. Nevertheless, the polymer component remains a relatively large percentage of the composites, typically 70 wt.%, and in the case of PLLA that is used commercially, the time to total degradation after its function has been completed is still too long.

Herein we describe for the first time a process that produces a bioceramic–elastomer composite with a wide range of mechanical properties and a HA content that is similar to that of bone. Novel bioceramic composites, based on the biodegradable elastomer poly(diols citrate),

were engineered with the expectation that they would have enhanced osteointegration potential relative to current biodegradable fixation implants. The rationale was to design a fixation implant that would consist mostly of the bioceramic component to maximize osteointegration while employing a degradable and relatively inexpensive elastomer as the macrophase binder. We hypothesized that POC would be a good candidate to improve the processability and mechanical properties of bioceramic implants due to its biocompatibility, adjustable mechanical properties, controllable degradation rates (a few months to 1 year), and mild synthesis conditions [18]. An important criterion for the POC–HA composites was the ability to process samples via molding and machining methods. Here we report the successful synthesis of POC–HA composites with HA compositions of 40, 50, 60, and 65 wt.% that were able to be molded and machined into bone screws (Fig. 1). An HA composition of 60–65 wt.% is similar to that found

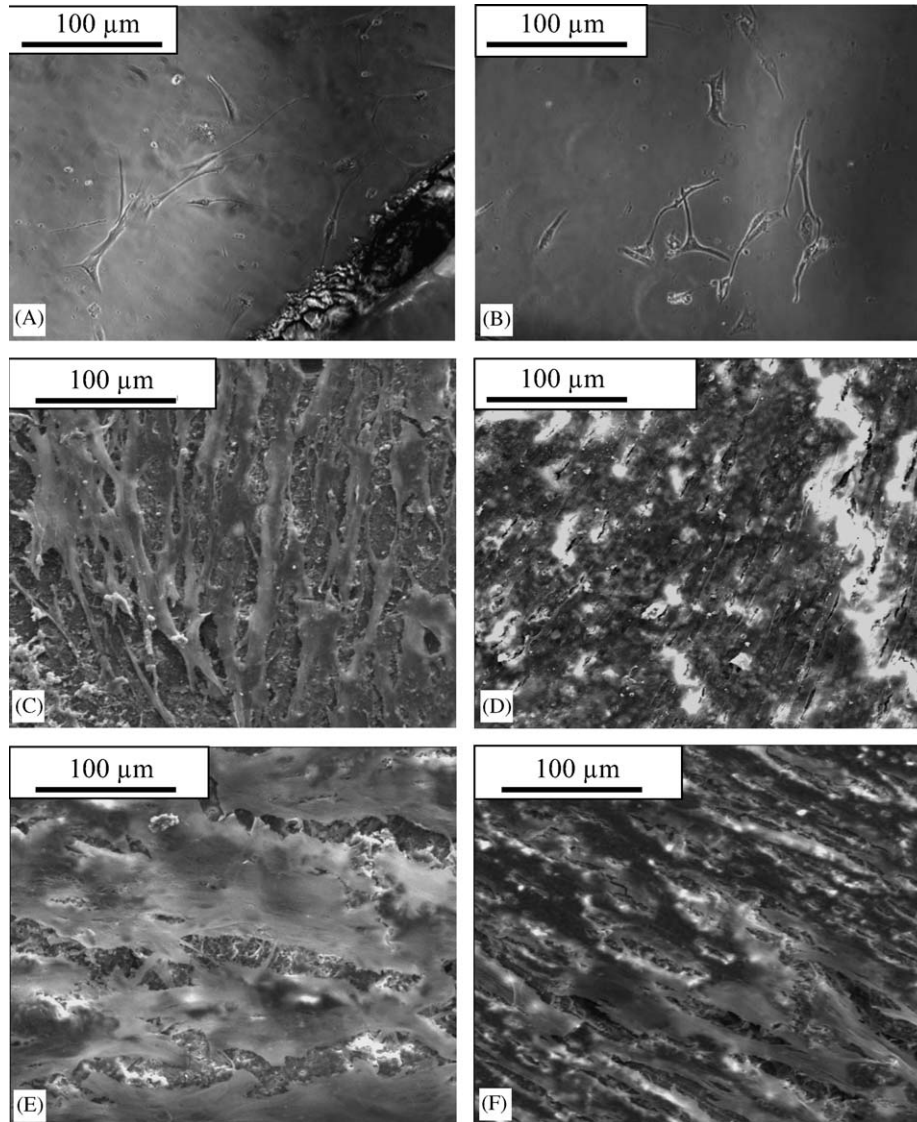


Fig. 5. Light microscopy image of POC seeded with human osteoblast in vitro for (A) 3 days and (B) 14 days; and SEM images of POC–HA composites with various percentages of HA seeded with human osteoblasts: HA 40 wt.% at (C) 3 days and (D) 14 days and HA 65 wt.% at (E) 3 days and (F) 14 days.

in bone and is expected to improve osteointegration. HA percentages of 70 or higher that were fabricated under the reaction conditions used for this study could not be molded or machined. An HA content below 40 wt.% resulted in composites that were too rubber-like and difficult to machine.

The mechanical property measurements of the POC–HA composites evaluated in this study were within the range of values reported for biodegradable polymers and composites used or proposed for bone fixation devices [40]. Reported mechanical properties for polymers and composites have included bending, compression, tensile strengths, and shear strengths, whose values were in the range 40–412, 78–130, 0.6–290, and 19–250 MPa, respectively. Reported values for bending, compression, and tensile moduli were in the range 1.6–124.4, 4.8–8.0, and 0.01–29.9 GPa, respectively [15,40]. The POC–HA composites tested in this study had bending,

compression, tensile, and shear strengths that were in the range 34–41, 32–75, 6–10, and 23–28 MPa, respectively. Bending, compression, and tensile moduli for POC–HA composites were in the range 0.275–0.502, 0.19–0.45, and 0.02–0.34 GPa, respectively. Except for the bending and compression moduli, the mechanical properties of POC–HA are comparable to those of other biomaterials proposed for tissue fixation. In this study, the mechanical properties of the POC–HA composites were increased by increasing the HA component. It is also possible to modulate the mechanical properties with the reaction conditions, i.e. reaction temperature and time, and choice of diol for the polycondensation reaction. Ultimately, the utility of these composites will have to be evaluated in vivo in the intended application.

Although polymer–HA composites are expected to integrate with bone, the weight percent and rate of

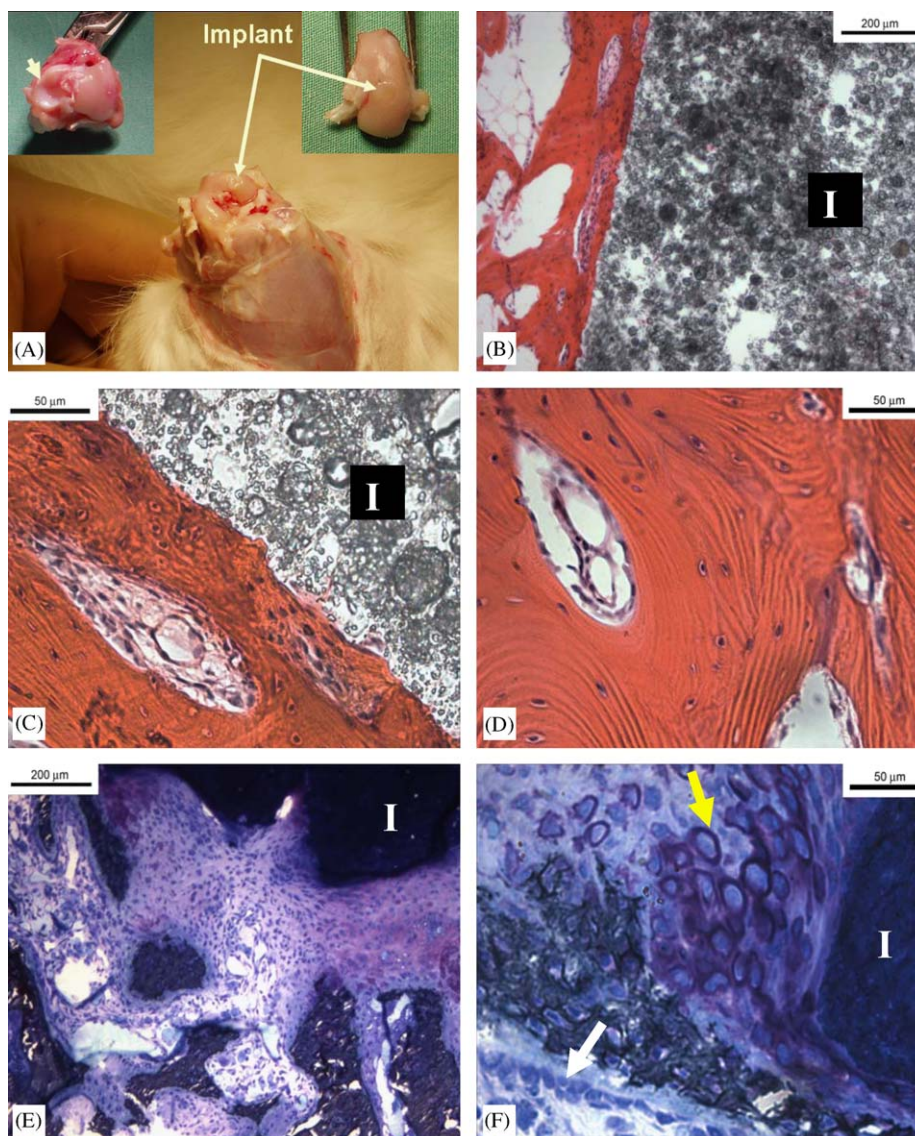


Fig. 6. Biocompatibility evaluation of POC-HA with 65 wt.% HA after 6 weeks of implantation in a rabbit knee. (A) Digital image of the implant and surrounding cartilage of the medial chondyle. Left insert shows an intact meniscus (arrowhead) and tibia opposite the implant. (B) and (C) Low and high magnification, respectively, of hematoxylin and eosin (H&E) stains of decalcified tissue containing the POC-HA implant. (D) H&E stain of an area of normal rabbit femur bone for comparison. (E) and (F) Low and high magnification, respectively, of von Kossa stain of the implant and surrounding bone (I: implant; yellow arrow: chondrocytic cells; white arrow: layer of osteoblasts depositing new bone).

degradation of the polymer component are parameters that may influence the function and in vivo integration of the composite. We have previously reported the complete degradation of POC within 6 months when incubated in PBS at 37 °C [18]. The lower degradation rates reported for the POC-HA composites likely reflect differing extents of the polycondensation reaction due to the lower weight percentages of polymer and the presence of thermally conductive HA particles. Both of those parameters are expected to affect the degree of cross-linking relative to pure POC for the same reaction temperature and time. Furthermore, it is also possible for the POC to covalently react with OH groups on the HA particles effectively cross-linking the POC-HA matrix [41–43]. HA would also serve

as a buffer to the acidic functional groups and products generated from POC degradation, minimizing any auto-hydrolytic effect on degradation. More detailed studies would be required to fully understand the mechanisms of hydrolytic degradation of POC-HA composites. Noteworthy, the degradation of the POC component can be significantly increased by “doping” with glycerol or *N*-methyl-diethanolamine (MDEA) [19].

Regarding the potential bioactivity of POC-HA in vivo, HA has been shown to induce the deposition of calcium phosphate mineral on the surface of ceramic implants and bond to bone [44,45]. The capacity of POC and POC-HA to mineralize was assessed in vitro using a modified simulated body fluid (SBF) solution. Based on the SEM

and EDX analysis, POC–HA composites with 40–65 wt.% HA in SBF successfully induced surface mineralization. The mineralization process involved a nucleation phase and a growth phase as evidenced by the complete coverage of the samples after 15 days of incubation in SBF [23]. However, POC was not conducive to mineralization within the time frame studied. The apatite or calcium phosphate mineral deposition is expected to contribute to improved bone bonding in vivo and help fill in any void volumes or pores left behind by degraded POC [46]. Depending on which bioceramic is chosen, most of the mass of the implant is expected to be integrated (HA) or remodeled (TCP) by bone tissue and the remaining POC should be totally degraded within 2 years of implantation.

POC has been shown to be compatible (i.e., as per cell adhesion, proliferation, and differentiation assays) with several cell types including human and pig endothelial cells, human and pig smooth muscle cells, bovine chondrocytes, and bovine fibroblasts [19,46]. It was also shown to be biocompatible in vivo in a rat subcutaneous implantation model [18]. In this study, the favorable cell adhesion and spreading characteristics of POC and POC–HA composites were confirmed in vitro with the use of primary human osteoblasts (Fig. 5). Furthermore, preliminary biocompatibility studies based on an osteochondral defect created in the rabbit femoral chondyle suggest that the POC–HA composite does not induce a chronic inflammatory response and that surrounding bone is in close contact with the implant (Fig. 6).

5. Conclusions

Herein we describe the development of biodegradable composites based on an elastomeric biodegradable polyester. The feasibility of processing POC–HA composites into useful forms was confirmed as interference screws (bone screws) were successfully engineered using both, molding and machining methods. Advantages of POC–HA or POC–bioceramic composites include: (1) simple synthesis and in-situ cross-linking polymerization at relatively

mild temperatures while avoiding the use of exogenous catalysts and toxic solvents, (2) incorporation of a high percentage of the bioceramic component, (3) increased osteoblast adhesion and mineralization, potentially enhancing osteointegration, (4) use of a polymer component that should degrade completely within 2 years rather than 3–5 years as in the case with PLLA, and (5) decreased cost relative to the use of poly(α -hydroxyl acids) such as PLLA. The mechanical properties of POC–HA composites can be adjusted with the percent of HA in the composite. Future work will assess the effect of bioceramic particle size on mechanical properties and in vivo bone integration characteristics of the composite.

Appendix A. Mechanical measurements

- (1) Bending strength (S_b) and modulus (E_b) (Japanese Industrial Standard (JIS) K7203 three-point bending test using rods with a range of diameter from 5.0 to 6.5 mm and a length of 30 mm).
- (2) Compression strength (S_c) and modulus (E_c) (Japanese Industrial Standard (JIS) K7208 using rods with a range of diameter from 5.0 to 6.5 mm and a length of 15–30 mm).
- (3) Shear strength (S_s) (measured by Suuronen's method [20] at a testing speed of 10 mm min⁻¹) using rods with a range of diameter from 5.0 to 6.5 mm and a length of 20 mm.
- (4) Tensile strength (S_t) and modulus (E_t) (Japanese Industrial Standard (JIS) K7113 using dog bone-shaped samples (26 mm × 4 mm × 1.6 mm) at testing speed of 10 mm min⁻¹).
- (5) Torsional strength (T_s). The test rod was installed to Sintech 20/G Materials Testing Machine, using the dumbbell-shaped samples having an average diameter of 4.7 mm and length of 16.5 mm. The rotating wheel was turned at a rate of 0.4 rev min⁻¹ by means of a chain attached to the load cell. The load pulling the chain was recorded and used for the calculation of the torque strength (see Tables A1–A3).

Table A1
Results of ANOVA on mechanical properties

	P-values							
	S_b (MPa)	E_b (MPa)	S_c (MPa)	E_c (MPa)	S_t (MPa)	E_t (MPa)	S_s (MPa)	T_s (N m)
HA40 vs. HA50	NS	NS	$P < 0.001$	NS	NS	NS	NS	NS
HA40 vs. HA60	NS	NS	$P < 0.05$	NS	NS	$P < 0.001$	NS	NS
HA40 vs. HA65	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS	$P < 0.001$	$P < 0.01$	$P < 0.05$
HA50 vs. HA60	NS	NS	$P < 0.05$	NS	NS	$P < 0.001$	NS	NS
HA50 vs. HA65	NS	$P < 0.001$	NS	NS	$P < 0.05$	$P < 0.001$	NS	NS
HA60 vs. HA65	$P < 0.05$	$P < 0.001$	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.001$	NS	$P < 0.05$

Note: S_b : bending strength, E_b : bending modulus, S_s : shear strength, S_c : compression strength, E_c : compression modulus, S_t : tensile strength (rectangular specimens), E_t : tensile modulus, T_s : torsional strength. Tensile strength POC vs. HA(40–65 wt.%): $P < 0.001$; tensile modulus POC vs. HA(60–65 wt.%): $P < 0.001$; and POC vs. HA(40–50 wt.%): NS.

Table A2

Results of ANOVA on degradation between POC–HA(40–65 wt.%) composites, respectively, in 2 and 20 weeks

Time (weeks)	P-values					
	HA40 vs. HA50	HA40 vs. HA60	HA50 vs. HA60	HA40 vs. HA65	HA50 vs. HA65	HA60 vs. HA65
2	NS	NS	NS	$P < 0.01$	$P < 0.001$	$P < 0.001$
20	NS	NS	NS	$P < 0.05$	$P < 0.05$	$P < 0.01$

Table A3

Results of ANOVA on degradation of POC–HA(40–65 wt.%) composites

Time (weeks)	P-values			
	HA40	HA50	HA60	HA65
2 vs. 6	$P < 0.001$	$P < 0.001$	NS	NS
2 vs. 12	$P < 0.001$	$P < 0.001$	NS	NS
2 vs. 20	$P < 0.001$	$P < 0.001$	NS	$P < 0.01$
6 vs. 12	$P < 0.05$	$P < 0.01$	NS	NS
6 vs. 20	$P < 0.001$	$P < 0.001$	NS	$P < 0.01$
12 vs. 20	$P < 0.01$	$P < 0.05$	$P < 0.05$	$P < 0.05$

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