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Early tissue response to citric acid-based micro- and nanocomposites

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Abstract

Composites based on calcium phosphates and biodegradable polymers are desirable for orthopaedic applications due to their potential to mimic bone. Herein, we describe the fabrication, characterization, and *in vivo* response of novel citric acid-based microcomposites and nanocomposites. Poly(1,8-octanediol-co-citrate) (POC) was mixed with increasing amounts of HA nanoparticles or microparticles (up to 60 wt%), and the morphology and mechanical properties of the resulting composites were assessed. To investigate tissue response, nanocomposites, microcomposites, POC, and poly(L-lactide) (PLL) were implanted in osteochondral defects in rabbits and harvested at 6 weeks for histological evaluation. SEM confirmed increased surface roughness of microcomposites relative to nanocomposites. The mechanical properties of both types of composites increased with increasing amounts of HA (8–328 MPa), although nanocomposites with 60 wt.% HA displayed the highest strength and stiffness. Based on tissue-implant interfacial assessments, all implants integrated well with the surrounding bone and cartilage with no evidence of inflammation. Both nanocomposites and microcomposites supported bone remodeling; however, nanocomposites induced more trabecular bone formation at the tissue-implant interface. The mechanical properties of citric acid-based composites are within the range of human trabecular bone (1–1524 MPa, 211±78 MPa mean modulus) and tissue response was dependent on the size and content of HA, providing new perspectives of design and fabrication criteria for orthopaedic devices such as interference screws and fixation pins.

Keywords

Bone tissue engineering; Calcium phosphate; Hydroxyapatite; Cartilage tissue engineering; Tissue Interface

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INTRODUCTION

With the growing elderly population and the high incidence of sports-related injuries, orthopaedic surgical procedures are predicted to become more wide-spread and prevalent. Procedures that require substantial bone repair utilize bone autografts as the standard of care. Although fairly successful, donor tissue is often scarce and may not always be practical. Significant morbidity and infection at the donor site are problematic, and the operating time required for harvesting autografts can also be expensive [1,2]. While allografts are readily available, the risk of disease transmission and unpredictable remodeling become impediments to their utilization [3]. Metallic implants are used for orthopaedic implants today, but the lack of integration and the mechanical mismatch of these rigid devices with the surrounding tissue make them permanent, stress shielding implants that ultimately weaken the surrounding bone [4,5]. These implants also release metal ions into the surrounding tissue and interfere with Magnetic Resonance Imaging (MRI) and Computed Tomography (CT), reducing the number of diagnostic tools available to these patients [6,7].

For decades, the use of bioactive ceramics such as hydroxyapatite (HA) have been heavily investigated to fill in bone defects due to their biocompatibility, osteoconductivity, and structural similarity to the mineral content of native bone [8–15]. However, their wide-spread applicability is often hindered by the stringent and complex processing conditions, as well as their inherent brittleness [16]. To overcome these challenges, researchers have combined bioactive ceramics with polymers such as poly(L-lactide) (PLL) to create composite materials with novel properties such as osteoconductivity [8,17–19]. Although PLL is commonly used, these poly(α -hydroxyesters) display various limitations. Drawbacks include slow degradation profiles and susceptibility to bulk degradation which can lead to premature failure of the implant [20–22].

In our earlier studies, the synthesis, mechanical properties, and biocompatibility of a citric acid-based composite containing a broad size distribution of HA microparticles was described [15]. Poly(1,8-octanediol-co-citrate) (POC) was found to be an adequate macrophase polymeric binder for calcium phosphate composites. POC is biocompatible and degrades significantly faster than PLL through surface degradation which potentially allows for faster implant integration within the host environment as polymer is replaced by new bone [23]. In addition, POC is an elastomer that can overcome or modulate the brittle nature of the calcium phosphate component, enhancing the mechanical properties of these composites for orthopaedic applications. Microcomposites with up to 65% HA content were fabricated, closely matching the mineral content of native bone and far exceeding the mineral content reported for PLL-HA composites [15,22,24–26]. Unlike PLL-HA composites which do not undergo degradation after 24 weeks in vitro [27], the mass loss of POC-based HA composites after 20 weeks was 12% in PBS [15]. In addition, these POC-based microcomposites do not elicit inflammation in vivo caused by a low local pH via the release of its degradation products which may be attributable to the basic nature of HA [15,28–30].

While these citric acid-based microcomposites show great promise as biomaterials for bone substitutes, nanocomposites have gained recent recognition for several reasons [17,18,25,31]. Bone itself is an example of a nanocomposite where partially carbonated HA nanocrystals are embedded within a collagen-rich organic matrix [32]. These nanometer-sized inorganic crystallites have also been shown to enhance the mechanical properties of biomaterials intended for bone [33,34].

A plethora of studies describe the utility of polymer-HA composites and many delineate the rationale behind fabricating composites containing nanometer-sized HA and their advantages over microcomposites [17,18]. However, there is a shortage of studies comparing the physiological response of nanocomposites to microcomposites, with all other parameters being the same. *In vivo* characterization will allow for further verification of nanocomposites as ideal, beneficial, and biomimetic bone-substitute materials, and provide material and tissue-response standards for engineering orthopaedic composites. The aim of this work is to describe the fabrication and characterization of novel nano- and microcomposites composed of POC and HA particles (POC-HA) with a narrow size distribution, and assess the body's interfacial response to these composite materials. These studies are relevant to the fabrication of fixation devices such as interference screws and bone pins.

MATERIALS AND METHODS

Materials

Hydroxyapatite nanocrystal (medical grade, mean diameter: 100 nm) and microparticles (medical grade, mean diameter: 20–50 μm) were purchased from Berkeley Advanced Biomaterials, Inc. (Berkeley, CA, USA). 1,8-octanediol (98%) and citric acid (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). JB-4 plus embedding kit was purchased from Polysciences, Inc. (Warrington, PA, USA).

Composite fabrication and characterization

Synthesis and characterization of the POC pre-polymer and the fabrication method of POC-HA composites have been reported elsewhere [35]. Briefly, POC pre-polymer was obtained from a condensation reaction of 1,8-octanediol and citric acid (1 to 1 mole ratio) and then mixed with various amounts of HA particles to obtain composites with 40 and 60 wt. % HA. The resulting clay-like mass was post-polymerized at 80°C for 3 days and 120°C for 1 day under vacuum. The following mechanical properties were measured using a Sintech mechanical tester model 20/G (Triangle Park, NC, USA) according to the Japanese Industrial Standard (JIS) K7208: bending strength (S_b) and modulus (E_b) (three-point bending tests using rods with a diameter of approximately 6 mm and a length of 30 mm) and compression strength (S_c) and modulus (E_c) (using rods with a diameter of approximately 6 mm and a length of 15–30 mm). All rods used for the mechanical tests were polished with sandpaper before testing. For POC, rectangular samples with the dimensions 5 mm $\times$ 10 mm $\times$ 50 mm were used for bending tests and rectangular samples with the dimensions 5 mm $\times$ 10 mm $\times$ 15 mm were used for compression tests. For each mechanical test, at least 6 samples were used and the mean values and standard deviations were calculated. Individual HA nanocrystals were visualized by transmission electron microscopy (TEM, Hitachi-H 4800, EPIC, Northwestern University, Evanston, IL), and individual HA microparticles and surface morphology of all POC-HA composites were observed by scanning electron microscopy (SEM, Hitachi 3500 N, EPIC, Northwestern University, Evanston, IL, USA) to assess the distribution of HA particles within the POC matrix.

Animal Experiments

Non-porous POC-HA (40 and 60 wt. % HA nanocomposites and microcomposites), POC, and PLL plugs with a diameter of 2.7 mm and a length of 4.0 mm were shaped using a mosaicplasty harvester (Smith & Nephew, Memphis, TN, USA). PLL plugs were chosen as a control due to its wide-spread use as fixation devices and were derived from biodegradable interference screws (Arthrex, Inc., Naples, FL, USA). Samples were sterilized using an AN74j/Anprolene ethylene oxide sterilization system that performed a 24 hr degassing step under vacuum after gas exposure (Anderson Sterilization, Inc. Health Science Park, Haw

River, NC, USA). Six experimental groups consisting of skeletally mature male New Zealand rabbits were investigated (Covance, Kalamazoo, MI, USA): 1) 40 wt. % HA nanocomposite (40nano), 2) 40 wt. % HA microcomposite (40micro), 3) 60 wt. % HA nanocomposite (60nano), 4) 60 wt. % HA microcomposite (60micro), 5) POC, and 6) PLL. All animals received implants in both knees and each group consisted of three knees from the same side.

The surgical protocol follows NIH guidelines for the care and use of laboratory animals and was approved by Northwestern University's Animal Care and Use Committee (Chicago, IL, USA). Briefly, anesthesia was induced with an intramuscular injection of ketamine (40 mg/kg) and xylazine (5–7 mg/kg) and maintained with isoflurane (1%–2% inhalation). After shaving and sterilely prepping both lower extremities, a 4-cm medial parapatellar arthrotomy was created in each knee, exposing the medial femoral condyle. Using a mosaicplasty harvester, a bone defect with dimensions 2.7 mm (diameter) × 4.0 mm (depth) was created in bilateral medial femoral condyles. All rabbits were allowed to ambulate freely post operatively.

Characterization of the tissue-implant interface

After 6 weeks, the implant and surrounding tissue were harvested for histology and histomorphometry. Gross examination was documented with digital photography. All explants were immediately fixed in 10% neutral-buffered formalin, dehydrated in graded series of ethanol, and embedded using JB-4. All specimens were sectioned at 5–10 μm longitudinal thickness for histological assessment using a HM 355 S Rotary Microtome (Richard-Allan Scientific, Kalamazoo, MI, USA) and stained with Masson Goldner Trichrome staining. Sections were assessed via standard light microscopy (Nikon Eclipse TE2000-U, Japan) and quantified via histomorphometric analysis using Image-Pro Plus v. 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 ratio of trabecular bone surface area to total tissue area. All tissue-implant interfaces were analyzed at 200 $\times$ magnification with 20 random fields of view of 200 $\times$ 200 μm^2 starting from the edge of the implant, except for the POC group where the thickness of fibrous capsule was significant (Fig. 1). For POC, the 200 $\times$ 200 μm^2 fields of view began after the fibrous capsule. Three sections per sample were analyzed for a total of 9 sections per group. Fibrous capsule widths were measured using image analysis software (Image-Pro Plus v. 5.0).

Statistical analysis

Data are expressed as means $\pm$ standard deviation. The statistical significance between two sets of data was calculated using unpaired Student's t-tests. All analyses were carried out using GraphPad Prism 4.0 and a p-value of 0.05 or less was considered to be significant.

RESULTS

Fabrication and mechanical properties of POC-HA composites

Depending on the percentage of HA, both nanocomposites and microcomposites ranged from flexible to rigid as shown in Figure 2. Nanocomposites could incorporate up to 60 wt. % of HA but beyond this value, a clay-like mass could not be formed. Nanocomposites with 40 and 60 wt. % were successfully placed into molds and easily machined into devices such as interference screws (Fig. 2). Microcomposites with HA contents of 40 and 60 wt. % were too flexible to machine; nevertheless, interference screws were successfully fabricated via molding. The mechanical property measurements are presented in Table 1. The mechanical properties increased with an increase in HA and a decrease in POC for both nanocomposites

and microcomposites, and 60nano was found to have the highest bending modulus, compression modulus, and compression strength. The correlations for bending strength could not be determined due to the lack of a failure point for most samples.

Morphological assessment of individual HA particles and POC-HA composites

Individual HA nanocrystals and microparticles were visualized via TEM and SEM and the surface morphology of all composites types were visualized via SEM (Fig. 3). HA nanocrystals were found to be needle-like while the HA microparticles were more spherical in shape (Fig. 3A, D). The surface of 40nano exhibited a uniform distribution of HA nanoparticles and aggregates throughout the POC macrophase. Qualitative observations suggest that with increasing wt. % of HA, a decrease in the inter-particle and inter-aggregate distance occurred, giving the appearance of a homogenous particle phase with increased packing (Fig. 2B, C). In contrast, the microparticles within the microcomposites were less uniformly distributed and increasing HA content increased the heterogeneity of the surface topography. (Fig. 2E, F)

Macroscopic, histological, and fibrous capsule analysis

At 6 weeks, there was no evidence of wound infection at the implant site and all rabbits recovered well without any signs of erythema, swelling, or sinus tract formation upon gross examination. After exposing the medial femoral condyle, a thin cartilaginous tissue layer covering the surface of the implants was observed (Fig. 4A, B). The meniscus opposite the implant was intact without any degeneration and the implant did not show any signs of loosening. Moreover, the surrounding articular cartilage recovered completely (Fig. 4C, D). Representative histological images of the bone-implant interface are displayed in Figure 5 and Figure 6. No macrophages or giant cells were observed around the implants and degradation for all implants was negligible although some cell migration was demonstrated within the periphery of the nanocomposites. Although all POC-HA composite implants and PLL had negligible and discontinuous fibrous tissue around the implant, POC samples had a continuous fibrous capsule with an average thickness of 130.2 μm (Table 2). Except for implants that consisted of only POC, all implants integrated well with bone at the tissue-implant interface with the presence of osteoid and trabecular bone. However, tissue after the fibrous capsule for pure POC implants appeared healthy and normal (Fig. 6).

Histomorphometric analysis

Three parameters were measured for histomorphometric analysis among the POC-HA composites, POC, and PLL implants within the first 200 μm from the edge of implant: trabecular surface area, osteoid surface area, and active osteoblast surface area (Fig. 7–9). Due to the presence of a relatively large fibrous capsule, tissue response measurements for POC implants were taken from 200 μm perpendicular to the edge of the fibrous capsule as well as 200 μm perpendicular to the edge of the implant (Table S1). Both POC-HA microcomposites (40 and 60 wt. % HA) were found to be significantly different in trabecular bone surface area in comparison to the PLL control, and composite types (nano or micro) of the same HA wt. % were also found to be significantly different from each other (Fig. 7, $p < 0.05$). Citric acid-based composites had no significant differences in osteoid surface area when compared to PLL, but again, microcomposites and nanocomposites of the same HA wt. % exhibited significant differences (Fig. 8). For osteoblast activity, only microcomposites with 60 wt. % HA displayed significant differences relative to PLL, but no differences were evident among the groups with the same HA percentage (Fig. 9).

DISCUSSION

Bone is a natural composite material where 65% of the total bone mass is composed of an inorganic solid, carbonate HA [37]. Hydroxyapatite has been previously reported to provide bioactivity and osteoconductivity allowing cell attachment, bone bonding, tissue ingrowth, osteoprogenitor cell growth, and ultimately the development of new bone [15,38–42]. In order to incorporate these benefits, the combination of polymers and calcium phosphate ceramics has been studied extensively for orthopaedic applications [15,17,31,43].

The mechanical and biological properties of bone depend in large part on nanoscale features of its ceramic components. Hence, synthetic bone substitutes and devices built to integrate or coexist with bone such as fixation devices should take these nanoscale features into consideration. Awareness of this issue has led to the development of various types of nanocomposites [44–46]. In this article, we describe the fabrication of osteoconductive POC-HA nanocomposites and microcomposites using particles with a narrow size distribution. We report the effect of HA particle size and content on the mechanical properties and interfacial tissue response of composites thereof.

Many studies have shown that the Young's modulus can readily be improved by the addition of rigid inorganic particulates, which generally have much higher stiffness than the macrophase polymer [47–50]. Furthermore, nanocomposites were found to have significantly higher stiffness and strength relative to their corresponding microcomposite counterparts (Table S2). Although both microcomposite and nanocomposite elastic moduli were in range with those reported for human trabecular bone (1–1524 MPa), 60nano displayed a stiffness most similar to the mean modulus of human trabecular bone (211 ± 78 MPa) [4,5]. The strength of particulate-filled polymers is highly dependent on stress transfer between the particles and the matrix, and well-bonded particles will efficiently transfer stress from the matrix to the particles improving the overall strength [33,51,52]. The mechanical properties of the nanocomposites described in our study are likely a result of increased physical bonding or adhesion between the spindled HA nanocrystals and the POC macrophase as reported by Jayabalan et al [53]. The spherical HA microparticles have reduced mechanical interlocking with the POC macrophase, resulting in lower values relative to nanocomposites. In addition, smaller particles contribute to an increase in toughness [47,54].

The mechanical properties reported for microcomposites in this work differ from those previously reported by our group because the size distribution of the microparticles is significantly different (25–75 μm vs. 20–50 μm), emphasizing the importance of particle size and homogeneity on the design of a composite [15]. In addition, while other polymer-HA composites have a capacity for HA far less than 65% by weight, here we highlight the unique polymeric properties of the elastomeric POC matrix that when combined with HA, can enhance processability and incorporate biomimetic characteristics of the mineral phase of bone.

After 6 weeks post-implantation, histology results confirmed that the osteocyte morphology and arrangement surrounding the implant appeared normal without any inflammation for all implants. Little to no fibrous tissue was observed for all POC-HA composites. Although PLL had a larger average fibrous capsule thickness of 10.2 μm , this was not significantly different from the POC-HA composites. POC samples revealed a relatively large fibrous capsule with an average thickness of 130.2 μm (Table 2). This value is in contrast to our biocompatibility evaluation in a murine subcutaneous model in which a thickness of 45 μm was reported. The discrepancy may be explained by the mechanical insufficiency of an elastomer within a hard tissue environment as well as the difference in animal models

[23,55]. Moreover, a local pH change caused by a faster release of acidic degradation components of POC and the lack of basic HA nanoparticles buffering the local surrounding may also contribute to this tissue response [30]. Nonetheless, the faster degradation rate of POC-HA composites in comparison to PLLA may induce an incremental local pH change as well. Interestingly, full osteochondral repair was demonstrated for POC-HA composites (Figure 4). A continuous layer of trabecular bone was formed in the subchondral area below the cartilage tissue suggesting that both POC-HA nanocomposites and microcomposites are useful materials for applications such as osteochondral healing in which both soft and hard tissue must be repaired.

Histomorphometric analyses identified differences in bone response between the nanocomposites and microcomposites at the implant-tissue interface. Microcomposites had a lower content of trabecular bone in comparison to PLL and were also found to be lower in trabecular bone in comparison to their nanocomposite counterparts (Fig. 7). This result emphasizes the enhancement of bone formation by nanocomposites as previously reported [56]. In contrast, all osteoid surface fractions were comparable to that of PLL but microcomposites had significantly higher levels of osteoid in comparison to their nanocomposite counterpart (Fig. 8). These findings may support a kinetics-based phenomenon in which nanocomposites stimulate osteoblast recruitment and hence osteoid deposition earlier. Eventually, osteoid is mineralized and incorporated into trabecular bone at a faster rate than microcomposites. The process of bone formation is known to take anywhere between 4 and 8 weeks, and an earlier time point may have captured and elucidated this outcome more accurately [57]. In contrast, the increased presence of osteoid may be indicative of two trends for microcomposites: a slower rate of bone formation as proposed above or a slower rate of mineralization. These hypotheses will be confirmed through long-term studies.

Regarding osteoblast activity, only microcomposites with 60 wt. % of HA were found to be significantly higher than PLL (Fig. 9). These data suggest that bone response to citric acid-based nanocomposites is similar to PLL implants that are currently in clinical use [58,59]. Notably, there were no significant differences between high and low content (60 and 40 wt. %) within composites of equally sized HA for all three parameters which suggests that particle size may be more important than HA content during bone formation. Therefore, the size of the HA particles and the distribution of these particles at the surface of an implant may modulate cellular adhesion and growth by modulating protein absorption and osteoblast recruitment [54,60–62].

The contributing factors of bone formation are multifaceted and further investigation to obtain the optimal properties is needed. Moreover, the current study investigated only the short-term tissue response to POC-HA composites. Ongoing long-term studies aim to further describe the integration and degradation of these composites and the tissue ingrowth and maturation of the surrounding bone. These studies will provide insight into the use of citric acid-based composites in orthopaedic devices.

CONCLUSIONS

Osteoconductive nanocomposites and microcomposites based on citric acid were successfully engineered with an HA content similar to that of native bone tissue. The mechanical properties of the composite ranged from flexible to stiff and the biocompatibility at 6 weeks in an osteochondral defect in rabbits was comparable to PLL implants that are in clinical use today. Histomorphometric analysis at the tissue-implant interface revealed higher trabecular bone surface fractions for nanocomposites in comparison to their microcomposite counterparts, and osteoid, osteoblast activity, and trabecular bone fractions

for nanocomposites were found to be comparable to PLL. Particle size and distribution are important parameters to consider when fabricating biomaterials for bone regeneration and fixation devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cartoon depiction of a single field analyzed by histomorphometry. The surface area of osteoid was measured and is shown by the area within the solid line, osteoblast surface area within the dotted line, and the trabecular bone surface area within the dashed line. T: Trabecular bone, Ostd: Osteoid, OBst: Osteoblast, I: Implant.

Figure 2.

Digital images of POC-HA nanocomposites. A) Flexible rods made with POC-HA with 40 wt. % HA nanoparticles were made via molding and B) a machined interference screw made from POC-HA with 60 wt. % HA nanoparticles (right) is compared to a commercial interference screw made from PLL (left).

Figure 3.

Electron microscopy images of individual HA particles and POC-HA composites. A) TEM image of needle-like HA nanocrystals (SB=100 nm) and D) round HA microparticles, and SEM images of B) 40nano, C) 60nano, E) 40micro and F) 60micro. An increase in nanoparticle content increased surface homogeneity, while an increase in microparticle content increased heterogeneity. SB for all POC-HA composites (B,C,E,F)=50 μ m.

Figure 4. Representative digital images and Masson Goldner Trichrome stains of POC-HA composites implanted in rabbit knees at 6 weeks. Digital images displayed new cartilage formation over A) POC-HA nanocomposites and B) POC-HA microcomposites. Masson Goldner Trichrome staining revealed osteochondral repair in both C) POC-HA nanocomposites and D) POC-HA microcomposites. I: Implant, SB=500 μ m.

Figure 5. Representative hematoxylin and eosin images at 6 weeks of A) 40nano, B) 60nano, C) 40micro, and D) 60micro. Samples demonstrated good integration and biocompatibility. I: Implant, SB=50 μ m.

Figure 6.

Representative images with Masson Goldner Trichrome staining at 6 weeks of A) nano40, B) micro40 C) PLL, D) nano60, E) micro60, and F) POC. Except for POC, composite samples did not elicit a large fibrous capsule relative to PLL. I: Implant, SB=100 μ m. Black arrows point to osteoblasts lining osteoid, yellow arrows point to trabecular bone, and white arrows point to osteoid.

Figure 7.

Total trabecular bone surface area divided by the total tissue surface area (%) of implants at 6 weeks. Unlike their nanocomposite counterparts, microcomposites elicited a lower content of trabecular bone when compared to PLL. POC samples were analyzed 200 μ m from the fibrous capsule. Asterisk notes significance with PLL. # notes significance with microcomposite counterpart of equal HA percentage, $p < 0.05$.

Figure 8.

Total osteoid surface area divided by the total trabecular surface area (%) of implants at 6 weeks. Microcomposites displayed higher osteoid content, or pre-bone, in comparison to their nanocomposite counterparts. POC samples were analyzed 200 μ m from the fibrous capsule. ≠ notes significance with microcomposite counterpart of equal HA percentage, $p < 0.05$.

Figure 9.

Active osteoblasts surface area divided by the total trabecular surface area (%) of implants. All samples, except for 60micro, demonstrated similar results when compared to PLL. POC samples were analyzed 200 μ m from the fibrous capsule. Asterisk notes significance with PLL, $p < 0.05$.

Table 1

Strength and elastic modulus for bending and compression of POC, POC-HA nanocomposites, POC-HA microcomposites, and PLL.

	Eb (MPa)	Sb (MPa)	Ec (MPa)	Sc (MPa)
40nano	27±3	NB	45±9	8±3
60nano	322±19	32±2	328±20	47±4
40micro	9±2	NB	8±1	1.3±0.4
60micro	24±1	NB	25±2	5±1
POC	2.57 ± 0.87	NB	0.38 ± 0.35	NB
PLL ^a [36]	3600–3650	64–106	--	--

Note: Sb: bending strength; Eb: bending modulus; Sc: compression strength; Ec: compression modulus; NB: no break.

^a Molecular weight ranges from 23,000–67,000. Human trabecular bone compression modulus range 1–1524 MPa and average 211±78 MPa [4,5].

Table 2

Average fibrous capsule thicknesses of all implants at 6 weeks.

Implant	Fibrous Capsule Thickness (μm)
nano40	4.0 $\pm$ 1.8
micro40	2.8 $\pm$ 0.3
nano60	1.9 $\pm$ 2.1
micro60	7.6 $\pm$ 5.4
POC	130.2 $\pm$ 28.0
PLL	10.2 $\pm$ 7.4