



Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Original Article

Long-term study on the osteogenetic capability and mechanical behavior of a new resorbable biocomposite anchor in a canine model

Xiao-Yan Cao^{a,b}, Cheng Chen^c, Na Tian^d, Xiang Dong^d, Xing Liang^c, Li-Jun Xu^{a,e}, Cheng-Kung Cheng^{b,f,*}^a School of Instrumentation and Optoelectronic Engineering, Beihang University, Beijing, 100191, China^b School of Biological Science and Medical Engineering, Beihang University, Beijing, 100191, China^c State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, 610041, China^d Beijing Engineering Laboratory of Functional Medical Materials and Devices, Beijing Naton Technology Group Co. LTD, Beijing, 100094, China^e Beijing Advanced Innovation Center for Big Data-Based Precision Medicine, Beijing, 100191, China^f Beijing Advanced Innovation Center for Biomedical Engineering, Beihang University, Beijing, 100191, China

ARTICLE INFO

Keywords:

Anchor-bone interaction
Biocomposite anchor
Degradation rate
Mechanical property
Osteogenesis

ABSTRACT

Background: Biodegradable suture anchors are commonly used for repairing torn rotator cuffs, but these biodegradable materials still suffer from low mechanical strength, poor osteointegration, and the generation of acidic degradation byproducts.**Method:** The purpose of this study was to evaluate the long-term mechanical behavior and osteogenetic capabilities of a biocomposite anchor injection molded with 30% β -tricalcium phosphate microparticles blended with 70% poly (L-lactide-co-glycolide) (85/15). This study investigated *in vitro* degradation and *in vivo* bone formation in a canine model. The initial mechanical behavior, mechanical strength retention with degradation time, and degradation features were investigated.**Results:** The results showed that the biocomposite anchor had sufficient initial mechanical stability confirmed by comparing the initial shear load on the anchor with the minimum shear load borne by an ankle fracture fixation screw, which is considered a worst-case implantation site for mechanical loading. The maximum shear load retention of the biocomposite anchor was 83% at 12 weeks, which is desirable, as it aligns with the rate of bone healing. The β -tricalcium phosphate fillers were evenly dispersed in the polymeric matrix and acted to slow the degradation rate and improve the mechanical strength of the anchor. The interface characteristics between the β -tricalcium phosphate particles and the polymeric matrix changed the degradation behavior of the biocomposite. Phosphate buffer saline was shown to diffuse through the interface into the biocomposite to inhibit the core accelerated degradation rate. *In vivo*, the addition of β -tricalcium phosphate induced new bone formation. The biocomposite material developed in this study demonstrated improved osteogenesis in comparison to a plain poly (L-lactide-co-glycolide) material. Neither anchor produced adverse tissue reactions, indicating that the biocomposite had favorable biocompatibility following long-term implantation.**Conclusion:** In summary, the new biocomposite anchor presented in this study had favorable osteogenetic capability, mechanical property, and controlled degradation rate for bone fixation.**Translational potential of this article:** The new biocomposite anchor had sufficient initial and long-term fixation stability and bone formation capability in the canine model. It is indicated that the new biocomposite anchor has a potential for orthopedic application.

Introduction

Fixation with suture anchors is a common and effective method for repairing trauma to the rotator cuff in the shoulder, whereby the anchor is

used to fix a tendon or ligament to the bone at the rotator cuff footprint [1, 2]. The anchor is inserted into the bone, typically using a screw mechanism or interference fit. The anchor may be made of metallic or polymeric materials, including steel or titanium alloys, nonbiodegradable

* Corresponding author. School of Biological Science and Medical Engineering, Beihang University, Beijing, 100191, China.

E-mail address: ckcheng2009@gmail.com (C.-K. Cheng).

<https://doi.org/10.1016/j.jot.2019.12.008>

Received 17 September 2019; Received in revised form 16 December 2019; Accepted 18 December 2019

Available online 14 January 2020

2214-031X/© 2020 The Authors. Published by Elsevier (Singapore) Pte Ltd on behalf of Chinese Speaking Orthopaedic Society. This is an open access article under the

CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

polyetheretherketone (PEEK), and biodegradable poly L-lactide (PLLA) or polyglycolic acid (PGA) copolymers, or other polymers. Metal and nonbiodegradable polymers offer high mechanical stability, which is good for anchoring the fracture but may also result in stress shielding around the healing bone. These nondegradable materials are also typically left permanently implanted, or a second insertion site is often required in the case of revision surgery. In contrast, biodegradable polymers [3] are replaced with bone over time as the implant degrades, and the implant need not be removed after the healing is complete, which also simplifies any revision surgery. These materials also elicit less adverse reactions *in vivo*, and their lower elastic modulus means lesser stress shielding of the healing bone.

However, high failure rates have been reported with the use of biodegradable suture anchors to fix torn rotator cuffs [4–6]. The initial fixation stability and retention of mechanical strength over time need to be improved in order to place biodegradable anchors on par with rigid permanent anchors. Poor initial fixation stability can lead to failure of the bone fixation [1,7]. Failure primarily occurs at the interface between the suture and anchor or between the anchor and bone and is typically caused by the anchor breaking [8]. The mechanical strength of the present generation of biodegradable anchors is not sufficient to withstand the loading placed upon them. Considering the forces borne by ligaments *in situ*, having a stable fixation point is crucial for successful healing [9]. As the anchor degrades, the loss of mechanical strength surpasses the healing rate of the tissue to bone, often leading to failure [1,10]. It has been reported that the typical timeframe for the healing of soft tissue to bone is around 12 weeks, only after which is the tissue able to carry a load [8,11]. Furthermore, as a general rule, about 6–8 weeks is required for good primary healing, and after 8 weeks, the bone is approximately 30% healed [11,12]. Thus the anchor should be able to retain about 70% of its mechanical strength after 8 weeks implantation.

Moreover, another drawback of using currently available biodegradable anchors is the accumulation of acidic byproducts and debris from the degrading material, which can result in adverse patient reactions, especially in sports medicine [7,10,13,14], such as sinus infection, inflammation, and the formation of cysts [15–17].

Immediately after implantation, the anchor will receive the full load normally placed on the tissue and bone, and so the mechanical strength must be sufficient to bear this loading. The decrease in strength associated with material degradation should be slow and predictable, leading to gradual load transfer to encourage the growth of new bone with properties similar to those of native tissue [18]. Biocomposites containing PLA and/or PGA and inorganic calcium phosphate (CaP) have the advantages of high mechanical strength and controlled retention of mechanical strength. These materials are widely used in orthopedics and bone tissue engineering [19,20]. Davide Barbieri [21] reported on the dynamic mechanical properties of composites with different ratios of apatite filler and poly (L-co-D) lactide (PL/DLA) (96L/4D) copolymer in the short term. The results showed that the dynamic elasticity abruptly decreases when the samples are moistened, and this decrease is directly correlated with the apatite content, but the effect of the apatite on the degradation mechanism and the long-term degradable properties are not investigated. Kulkova et al. [22] compared the properties of cone-shaped implants made of β – tricalcium phosphate/poly (L-lactide-co-glycolide) (β -TCP/PLGA) composites with a traditional titanium-alloy implant using a push-out test in a pig. The results showed that the push-out force of the β -TCP/PLGA composites was 35–60% of that obtained with titanium 6 aluminum 4 vanadium (Ti6Al4V) implants. However, the quality of the new bone surrounding the implants was similar. Thus, CaP-reinforced degradable polymer composites may offer greater mechanical strength and improved osteointegration over alternative biodegradable materials.

The mechanism of degradation is another critical factor in determining the success of a biodegradable implant. Polymeric implants degrade through surface degradation, bulk degradation, or a combination of both [17]. Degradation is a complex process influenced by a variety of intrinsic factors related to the polymer structure and properties,

as well as the environment in which the degradation takes place [23,24]. Some of the factors that can influence the degradation rate are the structure of the polymer matrix, the composition of CaP filler, fabrication technique, size and dispersity of CaP particles, and shape of the implant. Rapid degradation of the polymer can lead to a build-up of acidic byproducts, while slow degradation can lead to adverse tissue reactions, such as aseptic swelling, osteolysis at the implant site, partial osteointegration, and early micromotion of the implant [25]. Landes [26] reported that although PLGA 85:15 implants showed reliable degradation and biocompatibility, burr holes at the implant site were found to ossify again at 24 months when the PLGA implant degraded within 12 months. CaP fillers incorporated into the biocomposite have been shown to be conducive to new bone formation and also act to buffer the acidic environment [22]. Therefore, incorporating CaP into the PLGA polymers may act to increase the level of ossification and reduce the risk of adverse tissue response due to the build-up of acidic degradation byproducts.

The aim of this study was to investigate whether the β -TCP reinforced PLGA biocomposite anchors can supply enough initial fixation stability and long-term mechanical strength retention, long-term osteogenesis in a large canine model to assess its potential for use in rotator cuff repair by improving the interaction between the anchor and bone. The effect of the β -TCP microparticles on the degradation mechanism of the biocomposites was also considered.

Materials and methods

Materials

The biocomposite containing 70 wt% PLGA (L/G = 85/15) and 30 wt % β -TCP micro-particles was supplied by Arctic Biomaterials Oy Ltd, Tampere, Finland. The β -TCP particles had a mean size of 5.0 μ m, were irregularly shaped, and were distributed uniformly within the biocomposite, which was characterized by scanning electron microscope (SEM) (Fig. 1B–D). The material was injection-molded (Babyplast 6/10P, Cronoplast, S.L.) to form the anchors under the conditions listed in Table 1.

As shown in Fig. 1A, the screw-shaped anchors have a hollow core with a closed, rounded tip, and lateral eyes at the proximal end. The thread has a variable pitch, a maximum outer diameter of 4.5 mm, and a length of 17 mm (*in vitro* study) and 14 mm (*in vivo* study); 3 mm was removed from the tip of the anchors for the *in vivo* investigation to allow the anchors to be implanted vertically into the beagle femur without penetrating the contralateral cortical bone. Plain PLGA anchors acted as a general control to investigate the effects of the addition of the micro β -TCP on the degraded, mechanical, and osteogenetic properties in a long-term study and were molded with the same design as the biocomposite anchors; 144 anchors were created for each group. All anchors were sterilized by ethylene oxide (EtO).

In-vitro degradation

All anchors were first immersed in 20 ml of ‘Sørensen’ buffer solution (pH 7.4 \pm 0.2) at 37 \pm 1 $^{\circ}$ C in a sealed glass vial. The buffer solution consisted of potassium dihydrogenphosphate (KH₂PO₄, 0.0121 mol/l) and disodium hydrogenphosphate (Na₂HPO₄, 0.0545 mol/l) in ultrapure water with a resistivity of 18.2 M Ω cm. During the study period, the solution was changed every two weeks. The ratio of the test sample mass to the buffer volume was about 1:180 (g/ml). The pH and temperature of the solution were measured in at least two different vials chosen randomly at each test time point. Test intervals were 0, 3, 6, 9, 12, 15, 18, 26, 39, 52, 78 and 104 weeks. At each time point, 12 biocomposite anchors and 12 PLGA anchors were required: 6 mechanical test samples, 3 molecular weight test samples, and 3 samples for mass change, thermal properties, SEM, and size change. Changes in the pH level of the buffer solution were also analyzed. Except for the mechanical test, all samples were dried in a vacuum oven at room temperature until

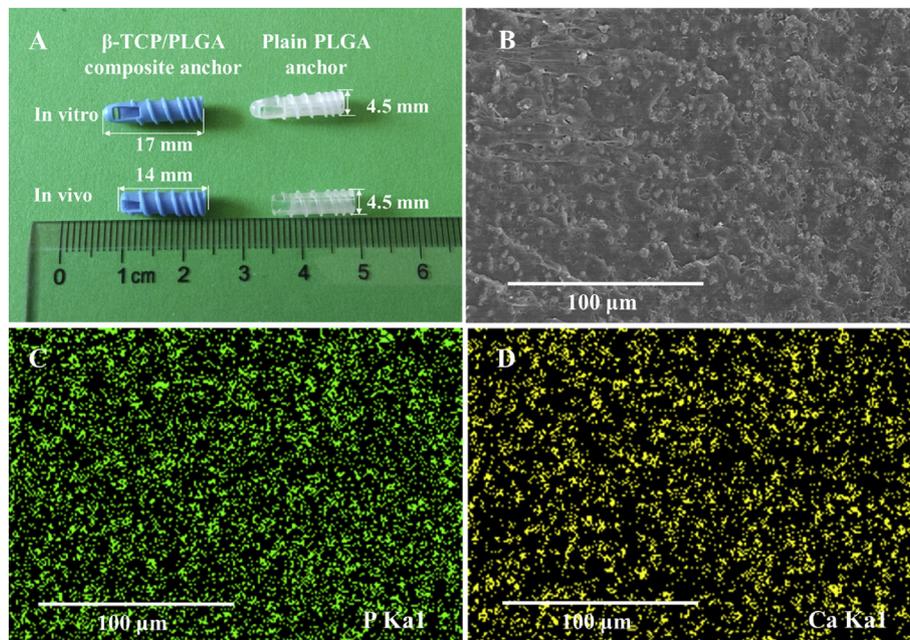


Figure 1. (A) Screw-shaped anchors for *in vitro* and *in vivo* studies. SEM image of the cross-section of the anchor showing the homogenous distribution of (B) β -TCP/PLGA, (C) phosphorus, and (D) calcium.

Table 1

Injection molding conditions.

Injection pressure (MPa)	7
Melt temperature ($^{\circ}$ C)	195–215
Mold temperature ($^{\circ}$ C)	Room temperature
Injection time (s)	2
Cooling time (s)	10

their weight loss reduced to a negligible level. All results are given as a mean and standard deviation.

In vitro characterization

Mechanical properties

The mechanical strength of the anchors was measured through shear testing with an Instron E3000 (Instron Co., USA) following ASTM B769-11. The test was conducted in deionized water at 37° C for samples removed from the ‘Sørensen’ buffer solution at 0, 3, 6, 9, 12, 15, 18, 26, and 39 weeks. The shear test setup is schematically shown in Fig. A.1. The samples were loaded at a constant speed of 5 mm/min until the anchors fractured. Loading was applied using displacement control. The maximum shear load (N) and displacement were determined from the recorded load–displacement curve. The maximum shear load (N) was the ordinate value corresponding to the highest point of the load–displacement curve. The shear stiffness (N/mm) was determined from the slope of the linear segment of the load–displacement curve calculated by the software of origin 8.0 (OriginLab Co. USA). The shear strength was not calculated due to the irregular cross-section of anchors, and the maximum shear load can represent the shear strength due to the same mean cross-section area.

Biocomposite material properties

Differential scanning calorimetry (DSC 200F3, Netzsch-Gerätebau GmbH, German) was used to evaluate the thermal properties of the anchors, including glass transition temperature (T_g), crystallinity temperature (T_c), and melting point (T_m). Three samples were tested at each time point.

The phases and crystallinity of both anchors were characterized by X-ray diffractometry (XRD, D8 advance, Bruker, German).

The mass of the anchor decreased once the implant lost cohesive strength, and the polymer started to fragment into a lower molecular weight polymer. The dry mass (m_{dry}) of the anchors was weighed using a precision balance (BSA250S-CW, Sartorius). The mass ratio of β -TCP (w_{TCP}) was measured with a Thermo Gravimetric Analyzer (TGA 209F1, Netzsch-Gerätebau GmbH, German) from 50° C to 600° C with the temperature increasing by 10° C min^{-1} . Measurements were taken in a nitrogen atmosphere and in an isothermal oxygen atmosphere for 30 min to burn up the residual organic carbon of PLGA. The initial (0 week) dry mass m_0 and β -TCP ratio w_0 were used to calculate the organic mass retention ratio using equation (1).

$$\text{Organic mass retention} = m_{dry}(1 - w_{TCP})/(m_0(1 - w_0)) \times 100\% \quad (1)$$

The diameter of each sample was measured immediately after removal from the buffer solution. The dimensions were measured with a slide caliper (Mitutoyo, 0–200 mm) and recorded in mm to an accuracy of two decimal places.

The average molecular weight (M_n) of the polymer matrix was measured by gel permeation chromatography (GPC) (Agilent 1260, Agilent Technologies, Inc. US). High-performance liquid chromatography (HPLC) grade dimethylformamide (DMF) (Sigma Aldrich) was used as the mobile phase at a flow rate of 0.5 ml min^{-1} at 45° C. The GPC equipment was calibrated against narrow polystyrene standards. Samples were dissolved in DMF, filtered through a $0.22 \mu\text{m}$ filter membrane, and $200 \mu\text{l}$ DMF was added to each sample as an analysis reference.

The surface and cross-sectional morphology of the anchors were characterized by SEM (Hitachi, S4800) at each investigation point. The samples were first removed from the buffer solution and then dried at room temperature under vacuum. The cross-sectional SEM test samples were quenched in liquid nitrogen.

Change in pH level

The test vial was placed in deionized water at 37° C, and the pH was measured daily before week 2 (change cycle of buffer) to determine the buffer baseline. After 2 weeks, the pH level was measured at random times during each change of the buffer. The pH sampling frequency was increased during periods of elevated mass loss. The pH meter (PB-10, Sartorius) was calibrated before use with calibration solutions of pH 4.01

and pH 9.18. The pH level of at least three different vials chosen randomly was recorded.

In vivo study

Long-term tissue reactions and bone in-growth with the new biocomposite anchor were evaluated *in vivo* at 52, 78, and 104 weeks according to ISO 10993.6–2016 and ISO 15814–1999, which defined the long-term time point for absorbable implants. The study protocol was approved by the Ethics Committee for Animal Research of Sichuan University (reference number SCXK (Chuan) 2013-185), China. The study was performed on 6 adult beagle dogs (10–12 months of age and weighing between 10 and 18 kg), each of which was clinically examined by a veterinarian to confirm good health before initiating the study. The animals were fed separately in the laboratory animal center of Sichuan University, which was maintained at 25 °C and 55% humidity, with 12 h of light and 12 h of darkness per day. The animals were quarantined a week ahead of the experiment.

The preparation of the animals and implantation of the anchors was performed by a veterinarian. The beagles were anesthetized, and both hind legs were shaved, disinfected, and draped. A longitudinal incision in the skin was made above the femur to allow for dissection of the muscles and fascia. The femur was exposed by stripping the periosteum, and four 4.5 mm holes were drilled towards the center of the bone. The 14 mm long β -TCP/PLGA and PLGA anchors were randomly assigned for implantation in the four holes in each femur. An absorbable suture was used to close the wound.

The beagles were housed separately after the procedure. Two beagles were sacrificed at each checkpoint (52, 78 and 104 weeks). The section of the femur containing the anchors was resected, and the anchors were not removed from the bone so as to keep them intact for further examination. The bone specimens were preserved with 4% paraformaldehyde.

Histological analysis

The bone specimens with anchors were decalcified in ethylene diamine tetraacetic acid (EDTA) for 24 weeks, trimmed and dehydrated using standard alcohol, and then embedded in paraffin. The samples were then sectioned with a saw microtome. The decalcified sections were cut parallel to the longitudinal axis of the anchors and then stained with hematoxylin and eosin (H&E) and Masson. At each checkpoint, three H&E and Masson stained images were analyzed in Image-Pro Plus 6.0 (Media Cybernetics, Inc) to assess the osteogenetic and collagenous fiber quantity. Integrated optical density (IOD, a unitless measurement of the product of mean density and area) characterized the quantity of new bone formation. Since the regions of bone selected for quantifying osteogenesis were different for different images stained by HE and Masson, the relative osteogenetic and collagenous fiber quantity was calculated using equations (2) and (3), where the $IOD_{H\&E}$ and IOD_M was the integrated optical density of the selected area ($Area_{H\&E}$ and $Area_M$).

$$\text{Relative osteogenic quantity} = IOD_{H\&E}/Area_{H\&E} \quad (2)$$

$$\text{Relative collagenous fiber quantity} = IOD_M/Area_M \quad (3)$$

Tissue reactions were also analyzed under a microscope, as well as any remaining implant debris and/or residuals.

The statistical analysis

The results are given as a mean and standard deviation. The maximum shear load of two groups' anchors at 3, 6, 9, 12, 15, 18, 26 weeks was statistically analyzed, and the maximum shear load of each group's anchors at 12 weeks was statistically analyzed in comparison to that of anchors at 0.14 weeks. The Relative osteogenetic quantity and relative collagenous fiber quantity of the two groups were also respectively statistically analyzed. The statistical software was SPSS 21.0 (SPSS,

Chicago, USA). A *p*-value of less than 0.05 was defined as statistically significant. A two-tailed heteroscedasticity unpaired *t*-test was conducted to compare the test results at defined points.

Results

Mechanical properties in vitro

The initial shear load of the β -TCP/PLGA composite anchor was 235.3 N, which was lower than that of the plain PLGA anchor, 301.1 N (Fig. 2A and C). Both anchors failed by ductile fracture (Fig. 2A and B). The shear stiffness of both anchors decreased with degradation time. However, the stiffness of the biocomposite was slow to change and stayed relatively constant until 12 weeks (Fig. 2D). The maximum shear load of the β -TCP/PLGA anchor gradually declined up to 15 weeks, but after 18 weeks, the maximum shear load exhibited a near-linear decline. In contrast, the maximum shear load of the plain PLGA anchor decreased sharply after week 3 (Fig. 2C). At week 12 (typical timeframe for the healing of soft tissue to bone), the β -TCP/PLGA anchors retained 83% of their initial maximum shear load, while the plain PLGA anchors only retained 33% of their initial maximum shear load (Fig. 2E). Both materials showed a significant loss (from *t*-test) in maximum shear load retention at week 12 (Fig. 2E).

Biocomposite material properties in vitro

A significant finding was that at 0.14 weeks, the DSC curve of the β -TCP/PLGA composite included a melting point, while this peak was absent in plain PLGA (Fig. 3A and B). The recrystallization peaks of both anchors appeared after 0.14 weeks. The T_g , T_c , and T_m for the biocomposite at each checkpoint were higher than those of plain PLGA. Each group of characteristic temperature peaks and glass transition platforms shifted to left with degradation time, except the melting peaks of the biocomposite anchors. The recrystallization peaks of both materials gradually increased and became narrower with degradation time. However, the rate of change of T_g , T_c , and T_m of the biocomposite was slower than that of plain PLGA, which is evident by the melting and recrystallization peaks of the plain PLGA being distinctly lower at 52 weeks.

The XRD spectra of both anchors are shown in Fig. 3C and D. According to the PDF card no. 09-0169 from MDI Jade 5.0 (Materials Data Inc.) for β -TCP, the peaks at 28, 31, and 34 of 2 theta observed in this study were characteristic peaks for β -TCP (Fig. 3C). The peak at 17 of 2theta was a characteristic peak of PLLA chains in PLGA, and its intensity increased with degradation time, while the crystallization peak at 17 for the composite anchor did not appear until week 26 (Fig. 3D).

The retention of mass in the plain PLGA and β -TCP/PLGA composite is shown in Fig. 4A. The β -TCP/PLGA composite anchors essentially maintained their organic mass for 39 weeks, but the plain PLGA anchors showed a significant decrease in mass after 18 weeks. The β -TCP content in the biocomposite anchors remained basically unchanged up to 39 weeks but increased significantly after 52 weeks (Fig. 4A).

The initial molecular weight of plain PLGA (1.038×10^5 g/mol) was greater than the β -TCP/PLGA composite (4.970×10^4 g/mol) but decreased sharply after week 3. The composite had a slower rate of degradation, and the change in molecular weight was relatively flat (Fig. 4B).

There was little similarity in the change in diameter over time between the two materials. The PLGA showed a rapid initial swell, but after 26 weeks, the swelling plateaued, while the β -TCP/PLGA anchor did not reach its swelling equilibrium even by the final checkpoint at week 52 (Fig. 4C). Thus, both anchors swelled throughout the degradation process. Fig. 4D shows the appearance of both anchors after drying at each checkpoint. Both materials turned a whitish color during degradation. At week 52, there were obvious signs of degradation on the surface of the composite anchor, while the PLGA anchor had fragmented into particles. Although the composite anchors fragmented at 78 weeks, the screw was

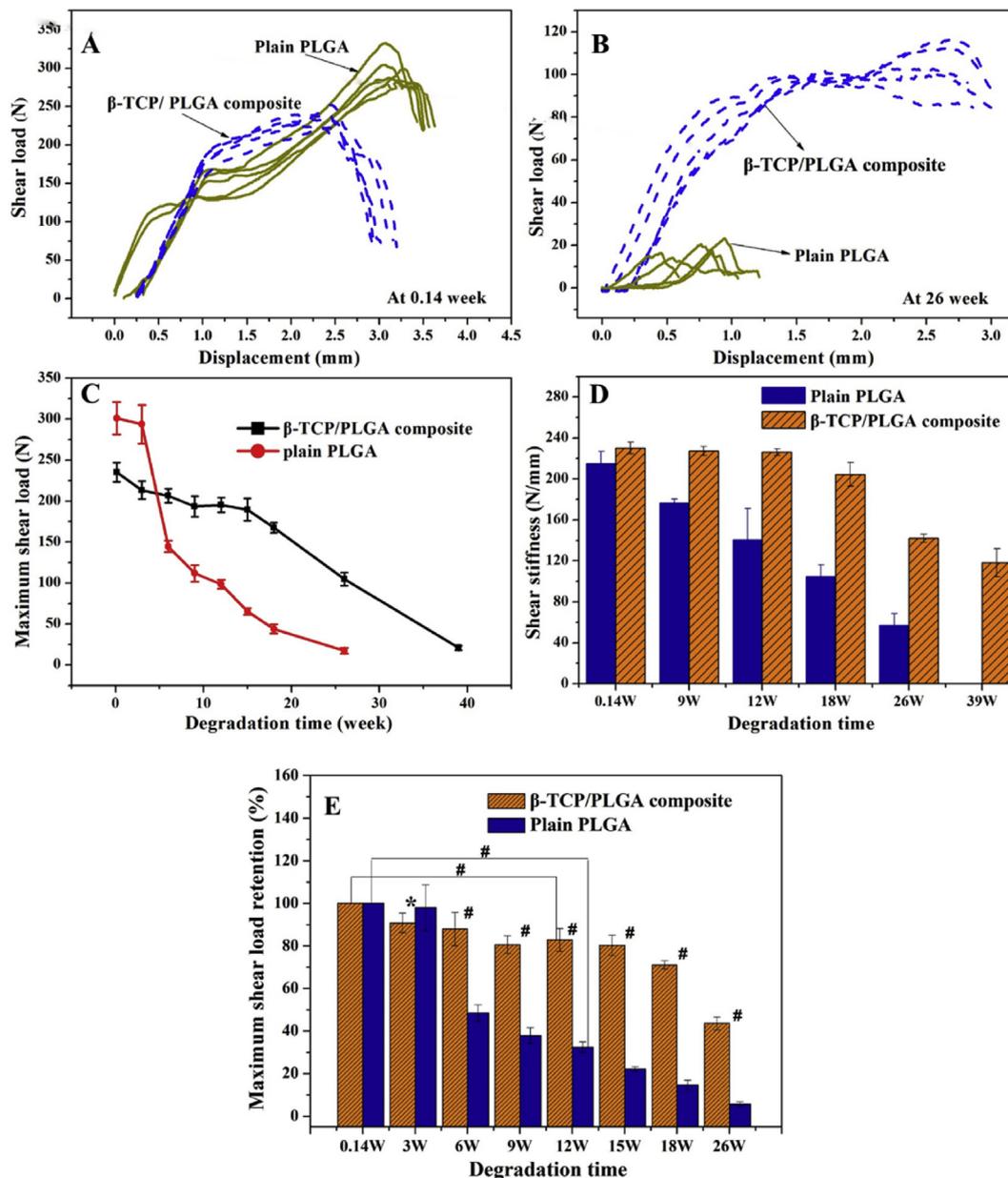


Figure 2. (A–B) Plots of shear load versus displacement for both anchors at 0.14 weeks (24 h) and 26 weeks. (C) Change in maximum shear load with degradation time; (D) Bar chart of shear stiffness with degradation time; (E) Shear load retention with degradation time: # $p < 0.05$ is considered to be statistically significant in t -test; * $p > 0.05$ is considered to show no significant difference in t -test.

still intact, and the thread structure visible. At 104 weeks, the composite anchors fragmented into particles (Fig. 4D).

During the entire degradation time, neither material showed signs of porosity on the surface (Fig. 5A–F and a–g). Due to the uniformly dispersed β -TCP particles (Fig. 5a–g), the composite anchor had a rougher surface than the plain PLGA anchor. By week 26, the PLGA anchor showed considerable surface cracking (Fig. 5E), which appeared to organize into two larger vertical cracks by week 52 (Fig. 5F). Thus, the PLGA anchor turned into fragments by week 52 (Fig. 5D). In contrast, there were no cracks visible on the surface of composite anchors at week 26 (Fig. 5e), but cracks were apparent by week 52 (Fig. 5f). For both materials, the majority of cracks were oriented in the direction of the melt flow, which is important as it indicates the manufacturing process had a demonstrable effect on the structural properties.

The cross-sectional morphology of both materials showed a connected porous structure (Fig. 5G–L and h–n). In the composite anchor group, the β -TCP particles were dispersed uniformly, and the nano-sized

crevices first appeared at week 3 in the region of the β -TCP particles (Fig. 5i). By week 12, the β -TCP particles began to fragment and dissolve in PBS (Fig. 5j), and by week 18, the porous structure first appeared in the polymer walls surrounding the β -TCP particles (Fig. 5k). During degradation, the polymer wall thinned considerably in both groups, and in the composite group, the β -TCP particles dissolved gradually in the PBS. The anchor walls became flocculent by week 52 in the PLGA anchor and by week 78 in the composite anchor (Fig. 5L and n).

pH changes

The pH levels in both groups fluctuated throughout the study. After week 18, the pH level of the solution containing the plain PLGA anchors declined considerably (Fig. 6, window A), while the pH level of the solution containing the β -TCP/PLGA anchors remained relatively constant. By week 39, the pH level of the β -TCP/PLGA solution decreased considerably (Fig. 6, window B), while the other pH values recorded

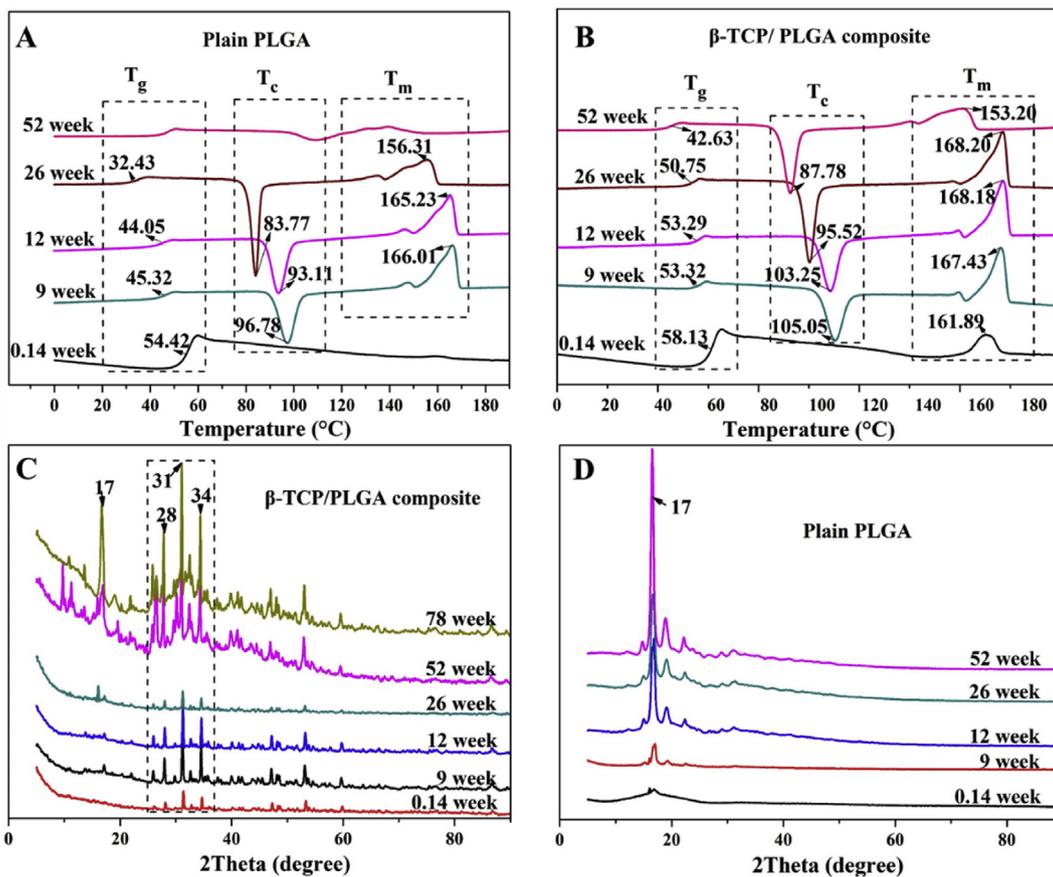


Figure 3. The DSC curves (A–B) and XRD spectra (C–D) of both anchors with degradation time.

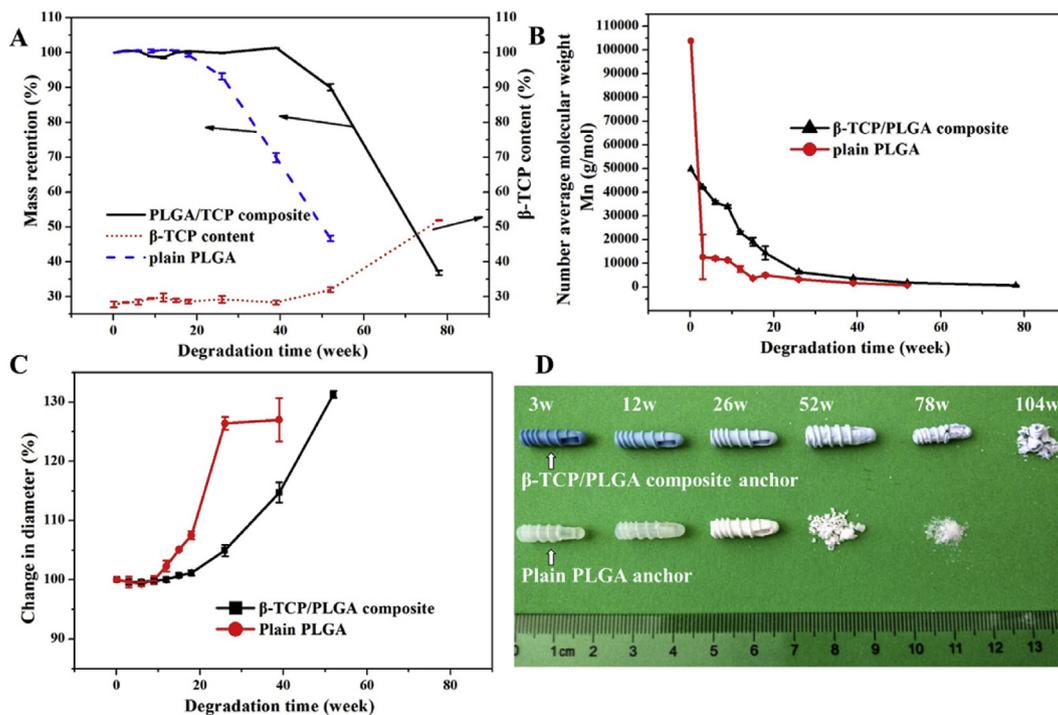


Figure 4. A) Mass retention and β-TCP content in both groups, (B) Change in molecular weight of both groups, (C) Diameter retention of both anchors, and (D) Images of dry anchors following *in vitro* degradation after 3, 12, 26, 52, 78, 104 weeks.

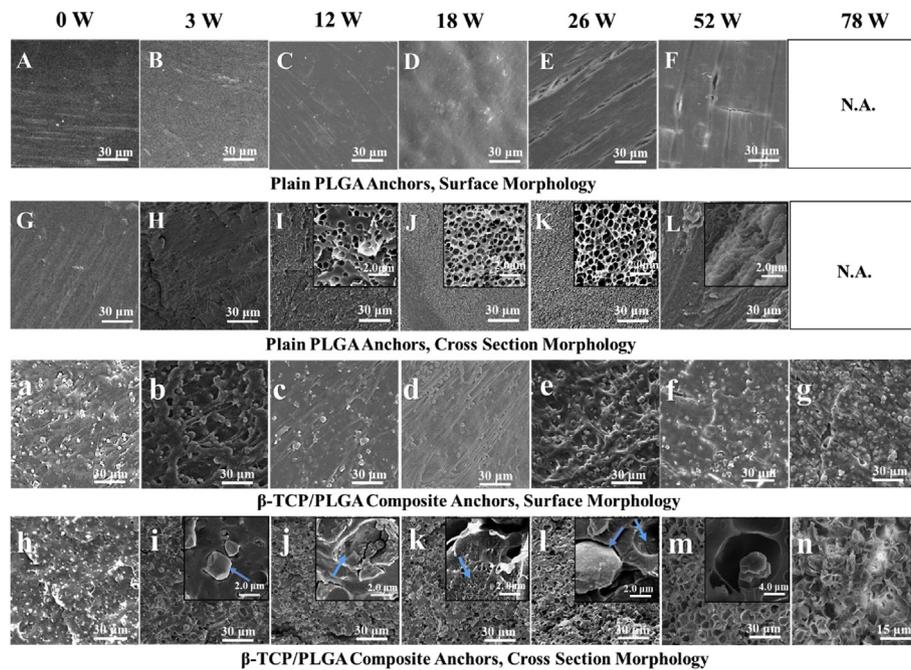


Figure 5. SEM images of the surface and cross-section morphology of the plain PLGA and β -TCP/PLGA composite anchors. Note: N.A. = Not Available.

throughout the degradation time were relatively evenly distributed around the baseline. All pH values were within 7.2–7.6, demonstrating that the PBS buffer was capable of maintaining a constant pH.

Histological analysis

Decalcified bone tissue sections with H&E and Masson staining were investigated at 52, 78, and 104 weeks. New bone formation was evident at the bone-screw interface and in areas where the screw degraded, allowing osseointegration to occur. The new bone matured with implantation time for both anchors (Fig. 7A–L and a–l). However, with implantation time, the biocomposite anchors showed greater osteointegration than the plain PLGA anchors into the core of the screw (Fig. 7, comparing images A–C against D–F, and comparing images a–c against d–f) and around the screw–bone interface (Fig. 7, comparing images G–I against J–L, and comparing images g–i against j–l). At 52 weeks, the

biocomposite anchors demonstrated more mature bone tissue, which was evident by the typical central canals in the bone (Fig. 7, comparing images A against D, and comparing images a against d, black arrows). At 104 weeks, mature bone tissue and fibrocartilage had developed at the screw interface (Fig. 7I, L and i, l) and at the implant site for both anchors (Fig. 7C, F and c, f).

At each checkpoint, both the relative osteogenetic and collagenous fiber quantity of the biocomposite anchor was higher than that of the plain PLGA anchor (Fig. 8A and B). The statistically analyzed results showed there was no statistical significance of the relative osteogenetic quantity and relative collagenous fiber quantity of two groups ($p > 0.05$). It might be due to the small sample size and obvious individual differences in animals. However, at 104 weeks, the relative collagenous fiber quantity was higher than the relative osteogenetic quantity for both anchors. H&E and Masson (M) stained images at 104 weeks demonstrated the presence of immature fibrous cartilage (Fig. 7C, F and c, f). There were no late obvious inflammatory reactions with the use of both anchors, as macrophages, giant cells, PMNs, and eosinophils were not observed (Fig. 7A–L). This indicated that both anchors had favorable biocompatibility.

Discussion

To ensure sustained long-term bone formation, the implant material chosen must have a degradation rate that closely matches the in-growth rate of new bone. It is known that the *in vivo* hydrolytic degradation properties of bioresorbable medical devices made of poly(α -hydroxy acids) can be predicted from *in vitro* degradation studies [27], but such *in vitro* methods cannot accurately simulate tissue reactions and the propensity for new bone formation. Thus, this present study investigated the *in vitro* degradation behavior of biodegradable anchors in a PBS solution and concurrently investigated the *in vivo* biological response after implantation in canines for 104 weeks. Strength retention and material degradation were investigated.

Initial mechanical stability and strength retention of anchors

The initial maximum shear load and the retention of the β -TCP/PLGA composite anchors were demonstrated to closely match the healing rate

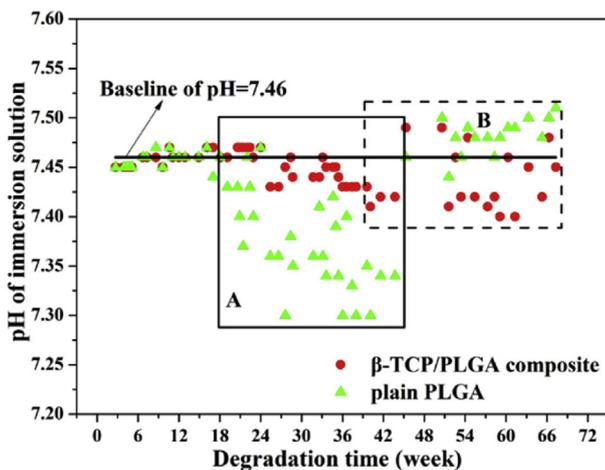


Figure 6. pH levels: (A) pH fluctuation zone of the solution containing plain PLGA anchors. (B) pH fluctuation zone of the solution containing β -TCP/PLGA composite anchors.

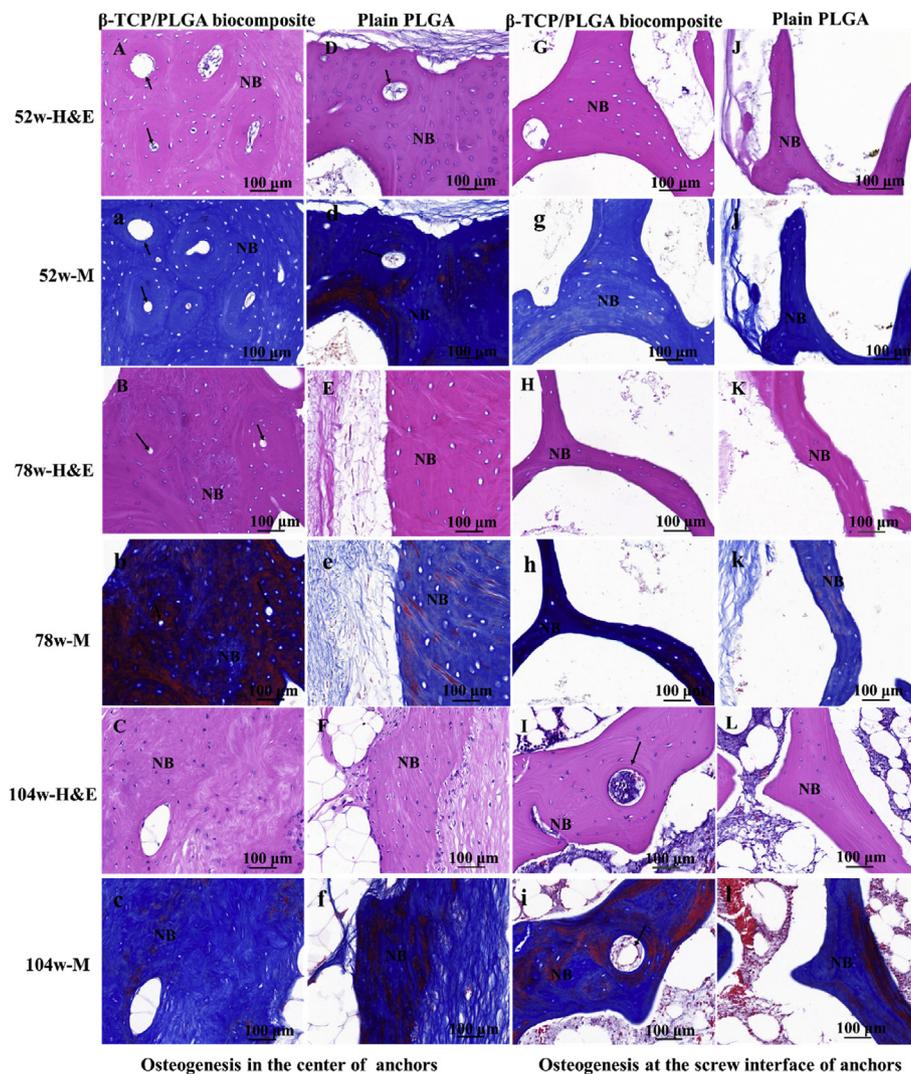


Figure 7. Digital images of β -TCP/PLGA composite and plain PLGA anchors stained with H&E and Masson at 52 weeks, 78 weeks, and 104 weeks. (A–L) show osteogenesis in the center of the anchors. (a–l) show osteogenesis at the screw interface. NB shows the new bone. Black arrows show central canals.

of bone [11,27]. The initial maximum shear load of the β -TCP/PLGA anchor was greater than the minimum shear load borne by an ankle fracture fixation screw, which is considered a worst-case implantation site for mechanical loading (Fig. 2C). It has been reported that 100N is

the minimum initial load borne by bone anchors used for securing ankle fractures [28]. The optimal strength and rate of strength retention (i.e. degradation rate) for any implant depend on the application, as the degradation rate should be closely aligned with the healing rate of the

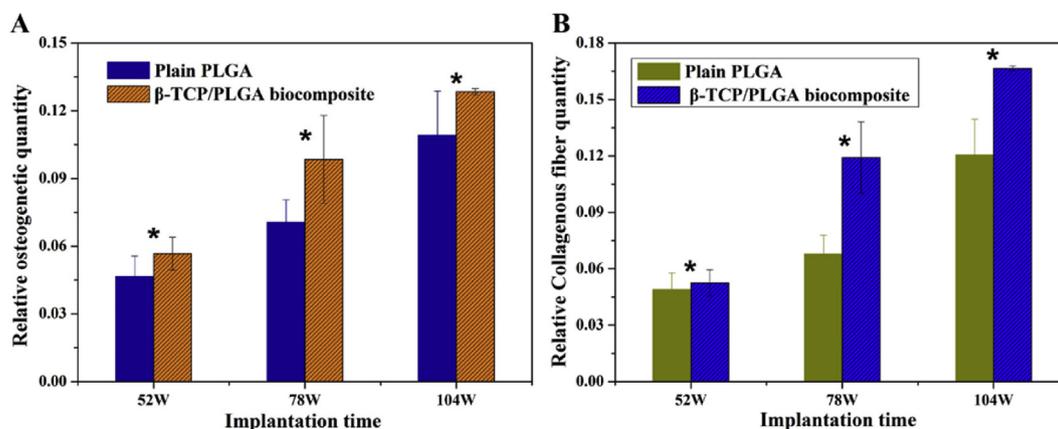


Figure 8. (A) relative osteogenic quantity; (B) relative collagenous quantity; * $p < 0.05$ is considered to show no significant difference in t -test.

bone and the load placed on the implant [27]. For implants used in sports medicine, a strength retention time of 8–12 weeks (strength retention $\geq 70\%$ [11] is typically needed to ensure a stable fixation [29]. For the new composite anchors introduced in this present study, the anchors were able to retain 70% of their initial shear strength after 12 weeks ($p < 0.05$), which has been demonstrated to be a suitable rate for biological union and the transfer of loading [11] (Fig. 2C and E).

The main parameters affecting the initial strength and mechanical retention are materials properties, structure of the anchors, and manufacturing process. Prior to implantation, the initial molecular weight (Mn) of the plain PLGA anchor was approximately twice that of the β -TCP/PLGA composite anchor (Fig. 4B). The thermal history and shear stress induced by processing promote the breakage of polymer chains, causing the initial Mn to decline [21,30]. The higher the molecular weight, the greater the number of entanglement points in the polymer. This situation makes a slip between molecular chains more difficult, which acts to increase the mechanical strength of the material [29]. There were more entanglement points between molecular chains in the plain PLGA anchors than the β -TCP/PLGA composite. The micro-sized interfacial cracks in the β -TCP/PLGA anchors also acted to lower the initial shear strength (Fig. 5f). However, the maximum shear load retention time of the new biocomposite anchor was longer than that of the plain PLGA anchor ($p < 0.05$) (Fig. 2E). This was due to the addition of β -TCP particles, which caused the PLGA to crystallize, resulting in delayed degradation of the material (Fig. 4). The micro-structure also improved the dispersity of the β -TCP particles in the PLGA matrix, strengthening the biocomposite anchor during degradation (Figs. 1 and 3C). In contrast, the rapid degradation of the amorphous PLGA anchor leads to a rapid decline in Mn and a significant decrease in the maximum shear load retention after 3 weeks (Figs. 3C and 4B).

Tissue reaction and bone formation

With longer implantation, the capability for osteogenesis was strengthened by the addition of the β -TCP particles (Figs. 7 and 8), indicating that β -TCP promotes osteointegration [22,31]. Bone integration of the screw interface can improve the fixation stability (Kulkova et al., 2014), and bone formation at sites where the implant degraded can reduce adverse reactions caused by holes at the implanted site [7].

This study did not find any obvious late inflammatory reactions with the use of the biocomposite anchors (Fig. 7). This may be due to the alkali degradation products of β -TCP buffering the pH level at the implantation site [31–33], which decreased the accumulation of the acidic byproducts released by PLGA (Fig. 6). Therefore, the addition of β -TCP particles delayed the degradation of the composite anchors and buffered the pH value of the local environment [21]. The slower degradation rate of the new composite anchor may reduce the likelihood of adverse reactions to acidic degradation byproducts. Thus, the β -TCP/PLGA composite anchors have the potential to avoid adverse reactions during implant degradation [34–36].

Degradation mechanism

A significant finding of this study was that the biocomposite degradation began at the interface between the β -TCP and PLGA particles, which was evident by nano-cracking in these regions (Fig. 5i), where the buffer diffused into the anchors and further weakened the interface [37].

Appendix A

The shear test setup is shown schematically in Fig. A.1 where samples were loaded at a constant speed of 5 mm/min until the anchors fractured. Loading was applied using displacement control. The maximum shear load (N) and displacement were determined from the recorded load–displacement curve, and the shear stiffness (N/mm) was determined from the slope of the linear segment of load–displacement curve calculated by the software of origin 8.0 (OriginLab Co. USA).

There was an obvious increase in the porous structure (Fig. 5i–n). However, the addition of β -TCP slowed down the hydrolysis of PLGA, since the β -TCP particles hindered the diffusion of the buffer. Both anchor materials, plain PLGA and β -TCP/PLGA, degraded through core accelerated bulk degradation (Fig. 5l–L and l–n, respectively) [17,27]. Cracks initially appeared on the surface of the composite anchors and were aligned with the flow direction of the injected melt. This indicated that the manufacturing process had a demonstrable effect on the structural and degradation properties. The porous structure of the biocomposite anchors can induce osteoblast growth and promote osteogenesis, strengthening the fixation at the anchor–bone interface.

A limitation of this study is that the canine implant model used and the detailed analysis of mechanical properties of the anchor cannot verify the effectiveness of the biocomposite anchor for use in rotator cuff repair. However, the methods used do verify the fixation stability of the anchor and the strong interaction with bone, which are key factors for successful repair of a torn rotator cuff. Further studies may consider a functional verification of the biocomposite anchor in an animal model.

There is another potential limitation that the biocomposite anchors cannot substitute steel or alloy anchors in all situations. For the patients with osteoporosis, the higher mechanical strength of steel or alloy anchors are needed to supply the stable fixation of musculoskeletal injury in sports medicine [1]. However, in the future, the favorable bone-fixation implants would have osteoinductive or osteoconductive capacity, supply appropriate mechanical strength, completely be absorbed when new bone forms at the implantation site, and be used especially for the secondary procedure [35]. The bioabsorbable composite anchors can deal with this demand.

Conclusion

The new β -TCP/PLGA biocomposite anchor presented in this study has sufficient initial strength and mechanical retention to provide a stable fixation. The addition of uniformly distributed β -TCP particles prolonged the strength retention time over a conventional PLGA anchor. The weak interface between particles in the composite acted to slow the material degradation through core accelerated bulk degradation. The mode of degradation and addition of β -TCP allowed for rapid and efficient bone ingrowth. There were no late inflammatory reactions with the use of the biocomposite material. The new composite anchor has the potential for improving the fixation of anchors in bone in orthopedic applications.

Conflict of interest

The authors have no conflicts of interest to disclose in relation to this article.

Acknowledgements

This work was supported by the Chinese National Science and Technology Program (National Key Research and Development Plan) (Grant number: 210YBXM2016110002). The authors would like to thank Mr. Colin McClean for his assistance with proofreading this manuscript. The authors would also like to thank Professor Li Chuan of National Yang-Ming University for his suggestions on manuscript preparation.

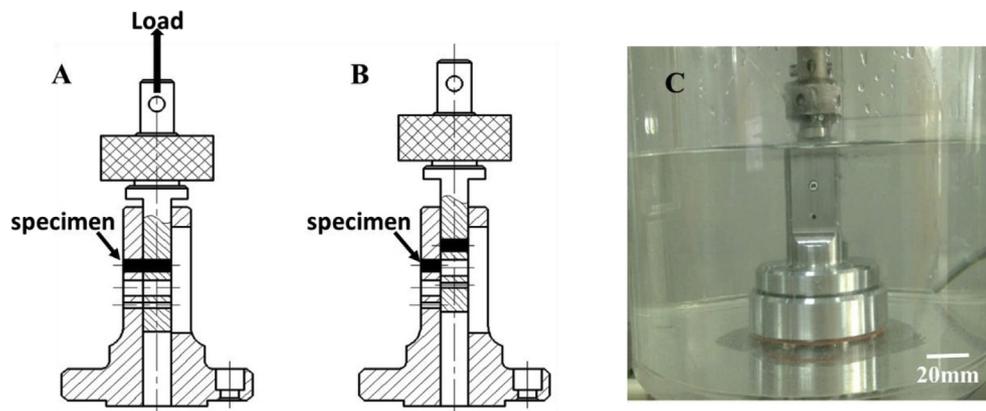


Fig. A.1. A) Schematic representation of the shear test adapted from ASTM B769-11: A) before shear test (B) after shear test; (C) Fixture for the shear test in deionized water medium.

References

- [1] Chaudhry S, Dehne K, Hussain F. A review of suture anchors. *Orthop Traumatol* 2019;33(4):263–70.
- [2] Tang C, Chen Y, Huang J, Zhao K, Chen X, Yin Z, et al. The roles of inflammatory mediators and immunocytes in tendinopathy. *J Orthop Translat* 2018;14:23–33.
- [3] Deng C, Chang J, Wu C. Bioactive scaffolds for osteochondral regeneration. *J Orthop Translat* 2019;17:15–25.
- [4] Bishop J, Klepps S, Lo IK, Bird J, Gladstone JN, Flatow EL. Cuff integrity after arthroscopic versus open rotator cuff repair: a prospective study. *J Shoulder Elb Surg* 2006;15(3):290–9.
- [5] Ricchetti ET, Aurora A, Iannotti JP, Derwin KA. Scaffold devices for rotator cuff repair. *J Shoulder Elb Surg* 2012;21(2):251–65.
- [6] Zhao S, Su W, Shah V, Hobson D, Yildirim L, Yeung KWK, et al. Biomaterials based strategies for rotator cuff repair. *Colloids Surf B Biointerfaces* 2017;157:407–16.
- [7] Konan S, Haddad FS. A clinical review of bioabsorbable interference screws and their adverse effects in anterior cruciate ligament reconstruction surgery. *The Knee* 2009;16(1):6–13.
- [8] Burkhart SS. *Burkhart's view of the shoulder*. 1 ed. Lippincott Williams & Wilki; 2006. p. 193–202.
- [9] Li H, Chen Y, Chen S. Enhancement of rotator cuff tendon-bone healing using bone marrow-stimulating technique along with hyaluronic acid. *J Orthop Translat* 2019;17:96–102.
- [10] Prokop A, Jubel A, Helling HJ, Eibach T, Peters C, Baldus SE, et al. Soft tissue reactions of different biodegradable polylactide implants. *Biomaterials* 2004;25(2):259–67.
- [11] Wall A, Board T. Tendon healing in a bone tunnel: a biomechanical and histological study in the dog. In: Banaszkiwicz PA, Kader DF, editors. *Classic papers in orthopaedics*. London: Springer-Verlag; 2014. p. 453–6.
- [12] An YH, Woolf SK, Friedman RJ. Pre-clinical *in vivo* evaluation of orthopaedic bioabsorbable devices. *Biomaterials* 2000;21(24):2635–52.
- [13] Weiler A, Hoffmann RFG, Stähelin AC, Helling HJ, Südkamp NP. Biodegradable implants in sports medicine: the biological base. *Arthroscopy* 2000;16(3):305–21.
- [14] Cobaleda Aristizabal AF, Sanders EJ, Barber FA. Adverse events associated with biodegradable lactide-containing suture anchors. *Arthroscopy* 2014;30(5):555–60.
- [15] Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials* 2000;21(23):2335–46.
- [16] Meyer F, Wardale J, Best S, Cameron R, Rushton N, Brooks R. Effects of lactic acid and glycolic acid on human osteoblasts: a way to understand PLGA involvement in PLGA/calcium phosphate composite failure. *J Orthop Res* 2012;30(6):864–71.
- [17] Gijjupalli K, Shavi GV, Averineni RK, Bhat M, Udupa N, Nagaraja Upadhya P. Poly(α -hydroxy acid) based polymers: a review on material and degradation aspects. *Polym Degrad Stab* 2017;144:520–35.
- [18] Ahsan T, Sah RL. Biomechanics of integrative cartilage repair. *Osteoarthritis Cartil* 1999;7(1):29.
- [19] Duan P, Pan Z, Cao L, Gao J, Yao H, Liu X, et al. Restoration of osteochondral defects by implanting bilayered poly(lactide-co-glycolide) porous scaffolds in rabbit joints for 12 and 24 weeks. *J Orthop Translat* 2019;19:68–80.
- [20] Yu W, Li R, Long J, Chen P, Hou A, Li L, et al. Use of a three-dimensional printed polylactide-coglycolide/tricalcium phosphate composite scaffold incorporating magnesium powder to enhance bone defect repair in rabbits. *J Orthop Translat* 2019;16:62–70.
- [21] Barbieri D, de Bruijn JD, Luo X, Fare S, Grijpma DW, Yuan H. Controlling dynamic mechanical properties and degradation of composites for bone regeneration by means of filler content. *J Mech Behav Biomed Mater* 2013;20:162–72.
- [22] Kulkova J, Moritz N, Suokas EO, Strandberg N, Leino KA, Laitio TT, et al. Osteointegration of PLGA implants with nanostructured or microsized beta-TCP particles in a minipig model. *J Mech Behav Biomed Mater* 2014;40:190–200.
- [23] Wiggins JS, Hassan MK, Mauritz KA, Storey RF. Hydrolytic degradation of poly(D,L-lactide) as a function of end group: carboxylic acid vs. hydroxyl. *Polymer* 2006;47(6):1960–9.
- [24] Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 2011;3(3):1377–97.
- [25] Furukawa T, Matsusue Y, Yasunaga T, Shikami Y, Okuno M, Nakamura T. Biodegradation behavior of ultra-high-strength hydroxyapatite/poly (L-lactide) composite rods for internal fixation of bone fractures. *Biomaterials* 2000;21(9):889–98.
- [26] Landes CA, Ballon A, Roth C. Maxillary and mandibular osteosyntheses with PLGA and PL(DL)LA implants: a 5-year inpatient biocompatibility and degradation experience. *Plast Reconstr Surg* 2006;117(7):2347–60.
- [27] Lehtonen TJ, Tuominen JU, Hiekkanen E. Resorbable composites with bioresorbable glass fibers for load-bearing applications. *In vitro* degradation and degradation mechanism. *Acta Biomater* 2013;9(1):4868–77.
- [28] Yablon IG, Segal D, Leach RE. *Ankle injuries*. 1 ed. Churchill Livingstone; 1983. p. 18.
- [29] Nieminen T, Rantala I, Hiidenheimo I, Keranen J, Kainulainen H, Wuolijoki E, et al. Degradative and mechanical properties of a novel resorbable plating system during a 3-year follow-up *in vivo* and *in vitro*. *J Mater Sci Mater Med* 2008;19(3):1155–63.
- [30] Abert J, Amella A, Weigelt S, Fischer H. Degradation and swelling issues of poly-(D,L-lactide)/beta-tricalcium phosphate/calcium carbonate composites for bone replacement. *J Mech Behav Biomed Mater* 2016;54:82–92.
- [31] Agrawal CM, Athanasios KA. Technique to control pH in vicinity of biodegrading PLA-PGA implants. *J Biomed Mater Res A* 2015;38(2):105–14.
- [32] Hile DD, Doherty SA, Trantolo DJ. Prediction of resorption rates for composite polylactide/hydroxylapatite internal fixation devices based on initial degradation profiles. *J Biomed Mater Res B Appl Biomater* 2004;71(1):201–5.
- [33] Naik A, Shepherd DV, Shepherd JH, Best SM, Cameron RE. The effect of the type of HA on the degradation of PLGA/HA composites. *Mater Sci Eng C Mater Biol Appl* 2017;70(Pt 1):824–31.
- [34] Randelli P, Compagnoni R, Aliprandi A, Cannao PM, Ragone V, Tassi A, et al. Long-term degradation of poly-lactic co-glycolide/beta-tricalcium phosphate biocomposite anchors in arthroscopic bankart repair: a prospective study. *Arthroscopy* 2014;30(2):165–71.
- [35] Barber FA, Spenciner DB, Bhattacharyya S, Miller LE. Biocomposite implants composed of poly(lactide-co-glycolide)/beta-tricalcium phosphate: systematic review of imaging, complication, and performance outcomes. *Arthroscopy* 2017;33(3):683–9.
- [36] Jeong JH, Jin ES, Kim JY, Lee B, Min J, Jeon SR, et al. The effect of biocomposite screws on bone regeneration in a rat osteoporosis model. *World Neurosurg* 2017;106:964–72.
- [37] Kobayashi S, Yamada S. Strain rate dependency of mechanical properties of TCP/PLLA composites after immersion in simulated body environments. *Compos Sci Technol* 2010;70(13):1820–5.