ELSEVIER

Biomaterials 21 (2000) 889–898

Biomaterials

Biodegradation behavior of ultra-high-strength hydroxyapatite/poly (L-lactide) composite rods for internal fixation of bone fractures

Taizo Furukawa[a,*], Yoshitaka Matsusue[a], Tsunoru Yasunaga[a], Yasuo Shikinami[b], Masaki Okuno[b], Takashi Nakamura[a]

[a]*Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kawahara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*
[b]*Takiron Co, Ltd., Nagano Yasutomi-cho 405, Shisou-gun, Hyogo 671-2421, Japan*

Received 9 April 1999; accepted 21 October 1999

Abstract

The purpose of this study was to investigate the biodegradation behavior of the ultra-high-strength hydroxyapatite/poly(L-lactide) (HA/PLLA) composite rods for fracture repair. Two kinds of composite materials were used in this study: u-HA/PLLA, which contained 30% by weight of uncalcined HA as reinforcing particles, and c-HA/PLLA, which contained 30% by weight of calcined HA as reinforcing particles. These composite rods were implanted in the subcutis and in the medullary cavities of rabbits. The specimens were removed at specific intervals between 2 and 52 weeks and the mechanical strength was measured for the rods in the subcutis, and the molecular weight and crystallinity were measured for the rods in both the subcutis and medullary cavities. The rod surfaces were examined using a scanning electron microscope (SEM). The specimens were examined histologically by light microscopy. The bending strength of the composites implanted in the subcutis was maintained at more than 200 MPa at 25 weeks and at 150 MPa at 52 weeks. The molecular weight dropped to 45% of the initial values at 8 weeks and to approximately 10% at 52 weeks. Significant differences in the molecular weight were seen between c-HA/PLLA and u-HA/PLLA, with u-HA/PLLA showing a faster rate of decrease than c-HA/PLLA after 8 weeks. SEM demonstrated that HA particles disappeared increasingly from the rod surfaces over time and that the spaces left by these HA particles formed many pores in the composite surfaces at 52 weeks. Histologically, a fibrous tissue layer was formed around the composite rod from 4 weeks in the subcutis and in the diaphyseal area of the medullary canal. This became more mature over time. Bony tissue contact to the composites without fibrous tissue layers was seen in the metaphyseal area of the medullary canal. During the experimental period, there were no inflammatory cells such as mono- or multi-nuclear phagocytes. Although further long-term studies for degradation are needed, the composites have promising mechanical strength and no adverse tissue reaction for use as fracture-fixation devices during the experimental periods.

Keywords: Composite; Hydroxyapatite; Poly(L-lactide); Biodegradable

1. Introduction

Bioabsorbable fracture-fixation devices made of synthetic polymer have been used as an alternative material for internal fixation of human fractures, particularly peri- or intraarticular fractures. Up to the present, the overall results have been favorable [1–7]. There are two advantages to using bioabsorbable fracture-fixation devices. First, there is no need to remove the devices after the fracture heals, as is the case with metal fixation devices. Second, using bioabsorbable implants prevents the stress-shielding atrophy and weakening of the fixed bone that is usually caused by rigid metallic fixation [8,9]. Among bioabsorbable fixation device materials, poly-L-lactide (PLLA) tends to be used more frequently than polyglycolide (PGA) because its slow degradation is believed to result in a lower rate of inflammatory tissue reaction [10,11]. However, several complications have been reported, including late aseptic swelling [12,13], osteolytic change at the implant site [1,11], incomplete restitution of normal bony architecture in the medullary canal after implant resorption [2,14], and early micromovement of the implant [15–17].

We hypothesize that incorporation of bioactive calcium phosphate into the PLLA matrix might have better

* Corresponding author. Tel.: +81-75-751-3652; fax: +81-75-751-8409.
E-mail address: taizo@kuhp.kyoto-u.ac.jp (T. Furukawa)

0142-9612/00/$ - see front matter
PII: S0142-9612(99)00232-X

mechanical properties and bioactivities in the repair of bone fractures. Considerable attention has been paid recently to composite materials that consist of bioactive ceramics as a filler and a polymeric substance as a matrix [18–24]. Some of these composite materials are intended to provide both bone-bonding ability and the desired mechanical properties, including the ductility of a polymer and the stiffness of cortical bone. Several authors have reported animal or clinical studies of composites made from a combination of hydroxyapatite and bioabsorbable polymers [18,19,25,26]. However, their composite materials lacked the required mechanical strength for fracture-fixation devices, and so the application of these materials was limited to use as a filler for bony defects.

Recently, an ultra-high-strength hydroxyapatite/poly-L-lactide (HA/PLLA) composite material has been developed. The detailed basic characteristics of this material were described in previous reports [27,28]. According to these reports, the mechanical strength of these materials included an initial bending strength of 280 MPa and an elastic modulus of 12 GPa. These are the highest mechanical strength values for any bioactive ceramic particle/polymer composites yet reported [18–21]; however, its biodegradation behavior has not been clarified. In the present study, HA/PLLA composite rods were implanted in the subcutaneous tissue and medullary cavities of rabbits. Rod degradation was evaluated histologically by light microscopy and by measuring their bending strength, molecular weight and crystallinity. In addition, the surface morphology of the composite rods was observed by SEM.

2. Materials and methods

2.1. Preparation of hydroxyapatite/poly-L-lactide composite rods

Two types of synthetic hydroxyapatite were used as reinforcing particles in this study: calcined HA (c-HA) and uncalcined HA (u-HA) [29]. U-HA was synthesized by hydrolysis of pure calcium hydrogen phosphate and calcium carbonate by heating their aqueous solution at 90°C. They were matured for 5 h and fully dried for 10 h after filtering. The chemical reaction occurred according to the following equation:

$$6CaHPO_4 + 4CaCO_3 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 4CO_2 + 2H_2O.$$

The 5–50 μm clusters, aggregated with many micro-hexagonal prism crystals whose aspect ratio was about 4–10 (length 2–3 μm/width 0.3–0.5 μm), were milled and sieved off, and the particle size was limited within a range of 0.3–20 μm, with an average of about 3 μm. The molar ratio by chemical analysis was 1.69 in the Ca/P and very close to that of pure hydroxyapatite, which is 1.67. This u-HA was also identified to be apatite-containing carbonate (CO_3^{2-}) and particles with medium crystallinity by means of Fourier transform infrared absorption spectra (FTIR) and X-ray diffraction, respectively.

C-HA was fabricated by granulating and calcining to decarbonate the u-HA at 900°C. The granules were crushed, milled and sieved off to limit their particle size within a distribution range and average similar to that of the u-HA particles. The molar ratio was 1.67, indicating that the c-HA was pure hydroxyapatite.

PLLA was polymerized as described previously [30]. The viscosity-average molecular weight ($\bar{M}_V$) of PLLA powder was 400 kDa before blending with u-HA or c-HA particles. The PLLA powder was purified several times by precipitation from polymer/dichloromethane solution by dropwise addition of ethanol.

The small granules of uniformly distributed u-HA or c-HA microparticles within a PLLA matrix were collected by precipitation polymer solution, that is, drop wise addition of ethanol to PLLA/dichloromethane solution. They were then extruded to make thick billets. The thick billets were forged into thin billets by a new reinforcing process of compression molding at 103°C [28]. The composites were filled with a 30% by weight fraction of u-HA or c-HA particles (u-HA/PLLA, c-HA/PLLA, respectively). Unfilled PLLA rods (100% PLLA) were made by the same method as a control for intramedullary implantation. They were cut into 3.2 mm diameter rods 5 cm long for subcutaneous implantation or 3 cm long for intramedullary implantation.

All the rods used in this study were sterilized with ethylene oxide (EOG, 20% by weight; CO_2 80% by weight) with 50% H_2O at 45°C for 5 h. The remaining gas was removed by aeration for 10 days or more at 45°C until ethylene oxide could no longer be detected by gas chromatography.

The initial mechanical strength and molecular weight of each composite material, as reported previously [27,28], are shown in Table 1.

2.2. In vivo implantation

A total of 40 Japanese white rabbits weighing between 2.8 and 3.4 kg were used for the in vivo implantation of the rods. Rearing of the rabbits and all experiments using them were carried out at the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University. The guidelines for animal experimentation of Kyoto University were carefully observed. The rabbits were anesthetized by an intravenous injection of Nembutal (50 mg/kg body weight) and local administration of 0.5% (w/v) lidocaine. The operations were performed under standard aseptic conditions. Following all surgical procedures, the rabbits were kept in cages and maintained with

Table 1
Mechanical properties of HA/PLLA composites and PLLA[b]

Material	HA/PLLA (wt. ratio)	$\bar{M}_V$ (KDa)	S_b (MPa)	E_b (GPa)
PLLA	0/100	300	259	6.5
u-HA/PLLA	30/70	210	269	7.6
c-HA/PLLA	30/70	250	280	7.8
c-HA/PLLA[a]	50/50	210	214	12.1

[a]Reference data [27].
[b]$\bar{M}_V$ is the viscosity average molecular weight, S_b the bending strength, E_b the bending modulus.

a regular laboratory diet. The rabbits were killed by Nembutal overdose after each follow-up interval.

The first group 12 rabbits was used for subcutaneous implantation. A 4-cm incision was made in shaved skin over the midline of the back, and a subcutaneous pocket was created bluntly. Five rods of u-HA/PLLA were inserted in each of six rabbits. Five rods of c-HA/PLLA were inserted in each of the remaining six rabbits. The skin was closed using 3-0 nylon sutures. The rabbits were killed at 4, 8, 12, 16, 25 and 52 weeks after implantation.

The second group of 28 rabbits was used for the intramedullary implantation of the rods into both knees of the rabbits. A medial parapatellar incision was made and the patella was dislocated laterally. Subsequently, a 3.2-mm diameter longitudinal drill channel was made through the intercondylar area of the femoral condyle with a 3.2-mm dental bur (Morita, Kyoto, Japan). The cavity was washed thoroughly with a sterile physiological saline solution and the rods were inserted into the cavities with a press fit. U-HA/PLLA rods were implanted in the right knee and c-HA/PLLA rods were implanted in the left knee of each rabbit. The capsule and incision were closed with 3-0 nylon sutures. The rabbits were sacrificed in groups of five at 2, 4, 8, and 25 weeks after implantation. PLLA rods were implanted in both knees of the control eight rabbits, and these rabbits were killed in groups of two along with the composites groups.

After sacrifice, a 5-mm portion of each rod was cut off with the surrounding tissues for histological examination. The remaining portions of the rods were carefully removed from the surrounding tissues and the rod surfaces were observed by SEM, after which their bending strength, molecular weight and crystallinity were measured. Bending strength was measured only for the rods taken from the subcutaneous tissue because it was difficult to remove the composite rods from the medullary cavities without damage due to bony contact with them.

2.3. Histological evaluation

Histological examination was performed on decalcified and non-decalcified sections. All samples were fixed in 10% phosphate-buffered formalin. Samples for decalcified sections were immersed in a 10% EDTA solution, dehydrated with a graded ethanol series, soaked serially in xyrol and embedded in paraffin. These sections were stained with hematoxylin and eosin according to standard procedures. Samples for non-decalcified sections were dehydrated with a graded ethanol series, soaked serially with styrene (50, 70 and 100% [v/v]) and embedded in polyester resin. Thin sections (500 μm) were cut with a diamond band saw (BS-3000, EXAKT cutting system, Norderstedt, Germany), then ground down to 60 μm with a speed lap (ML-511, Maruto, Tokyo, Japan) for Giemsa surface staining.

2.4. Strength measurement

The bending strength of composite rods was measured by the three-point bending method according to the Japanese Industrial Standard (JIS) using an Autograph AGS 2000 D (Shimadzu Co., Kyoto, Japan). The support span, radius of the nose, and radius of each support were 25, 6, and 5 mm, respectively. The cross-head speed was 20 mm/min, and the temperature and relative humidity were 23°C and 55%, respectively. The bending strength was determined using the following equation:

$$\delta_{fmax} = \frac{8F_{max}L}{\pi d^3},$$

where δ_{fmax} is the maximum bending strength (MPa). F_{max} the maximum strain (Newton meter), L the support span (mm), and d the diameter of the specimen (mm).

The bending moduli were determined using the following equation:

$$E_f = \frac{4L^3}{3\pi d^4} \times \frac{E}{\gamma},$$

where E_f is the modulus of elasticity in bending (MPa), L the support span (mm), d the diameter of the specimen (mm), and E/γ the gradient of the stress–strain curve of the linear section (MPa).

2.5. Molecular weight measurement

$\bar{M}_V$ of the polymer was determined from the intrinsic viscosity in chloroform at 25°C using the following equation [31]:

$$[\eta] = 5.45 \times 10^{-4} \times \bar{M}_V^{0.73}.$$

2.6. Crystallinity measurement

The crystallinity (%) of the polymer was determined using the following equation:

$$\text{crystallinity}\,(\%) = \frac{\Delta H_{Tm}}{93.7} \times 100,$$

where ΔH_{Tm} is the melting enthalpy (J/g), and 93.7 the melting enthalpy of theoretically 100% crystalline PLLA polymer (J/g) [32].

2.7. Surface morphology

The surface morphology of the rods without the surrounding tissues was observed by SEM (Hitachi S-2460N, Tokyo) connected to an energy-dispersive X-ray microanalyzer (Horiba EMAX-5770, Kyoto). X-ray intensities for calcium, phosphorus, and carbon were analyzed across the implant surface.

2.8. Statistical analysis

Values were compared using two-way ANOVA. Subsequently, possible differences were investigated in a post hoc test using Fischer's protected least significant difference (StatView, Version 4.5, Abacus Concepts, Inc., North Carolina). Differences at $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Strength retention

Changes in the bending strength of the composite rods over time are shown in Fig. 1. In subcutis, both u- and c-HA/PLLA rods maintained 85% of their initial values at 8 weeks and 80% at 25 weeks. However, at 52 weeks the u-HA/PLLA rods maintained only 45% their initial bending strength, while the c-HA/PLLA rods maintained 55%. There was a significant difference between the u-HA/PLLA and c-HA/PLLA rods (Table 2).

3.2. Change in molecular weight over time

Changes in molecular weight over time are shown in Fig. 2 (a, b). Details of statistical analysis are shown in Table 3. In the subcutis, although the bending strength was 80% of the initial value at 25 weeks, the molecular weight of the rods had dropped to approximately 20% of their initial value. The molecular weights of the u-HA/PLLA rods at 8, 16, 25, and 52 weeks after implantation were 55, 30, 20 and 9% of their initial values, respectively, while those of the c-HA/PLLA rods were 65, 40, 20 and 15%, respectively. Thus, the u-HA/PLLA rods demonstrated a significantly greater decrease in molecular weight than the c-HA/PLLA rods. In the medullary cavity, the changes in molecular weight over time demonstrated a pattern similar to those in subcutis. The u-HA/PLLA showed a greater decrease of molecular weight than the c-HA rods, although the difference was significant only at 25 weeks. The molecular weight of the composites began to decrease at 2 weeks and decreased at a significantly faster rate than the unfilled PLLA rods. In unfilled PLLA rods, the molecular weight began to decrease at 4 weeks.

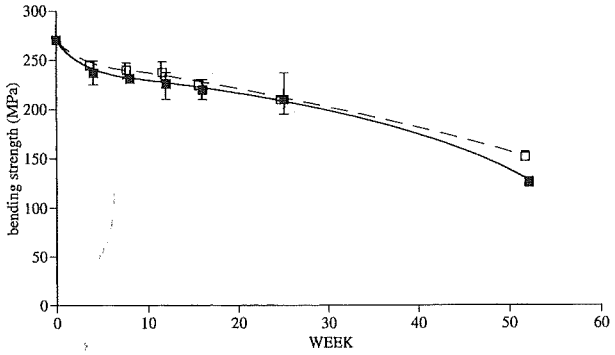

Fig. 1. Changes of the bending strength of HA/PLLA composite rods (∅ 3.2 mm) with time in subcutis. The vertical bar represents the maximum and minimum values. (■) u-HA/PLLA, (□) c-HA/PLLA.

Table 2
Statistical analysis of the data shown in Fig. 1: change in bending strength of HA/PLLA rods with time[b]

	Weeks after implantation					
	4	8	12	16	25	52
c-HA/PLLA vs. u-HA/PLLA	n.s	n.s	n.s	n.s	n.s	[a]

[a] With a significant difference at $P < 0.005$.
[b] n.s. = not significant.

There were no significant differences in the molecular weight retentions between composite rods in the medullary cavities and those in the subcutis except in u-HA/PLLA rods at 25 weeks, when u-HA/PLLA showed a faster rate of decrease in the medullary cavities than in the subcutis ($P < 0.05$).

3.3. Change in crystallinity with time

The initial values of the crystallinity of both c- and u-Ha/PLLA were 45%. The change in crystallinity of the composites with time are shown in Fig. 3 (a, b). Details of statistical analysis are shown in Table 4. In the subcutis, c- and u-HA/PLLA rods showed similar patterns of increasing crystallinity at 16 weeks after implantation; however, u-HA/PLLA showed higher values than c-HA/PLLA after that time. At 52 weeks, the difference in these values was 5%. A similar trend was seen in the medullary cavity; however, there was no significant difference between u- and c-HA/PLLA. Unfilled PLLA rods demonstrated a significantly lower rate of increase in crystallinity with time than either composite.

No significant differences were observed in the changes in crystallinity between subcutis composites and those from the medullary cavities.

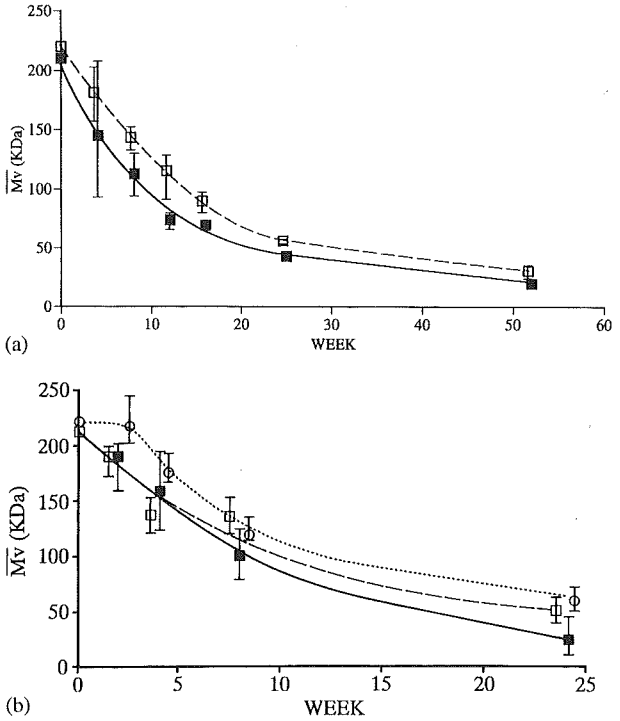

Fig. 2. Changes in $\bar{M}_V$ of HA/PLLA composite and PLLA rods (∅ 3.2 mm) with time in: (a) subcutis; (b) medullary cavity. (■) u-HA/PLLA, (□) c-HA/PLLA, (○) PLLA.

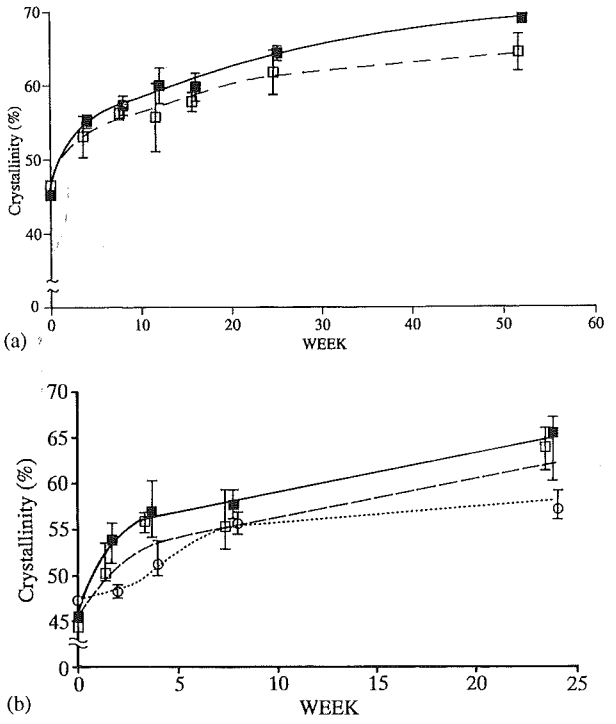

Fig. 3. Changes in the crystallinity of HA/PLLA composite and PLLA rods (∅ 3.2 mm) with time in: (a) subcutis; (b) medullary cavity. (■) u-HA/PLLA, (□) c-HA/PLLA, (○) PLLA.

Table 3
Statistical analysis of the data shown in Fig. 2: change in molecular weight of HA/PLLA and PLLA rods with time[g]

	Weeks after implantation						
	2	4	8	12	16	25	52
c-HA/PLLA[a] vs. u-HA/PLLA[a]	—	n.s	[b]	[b]	[c]	[d]	[d]
c-HA/PLLA[c] vs. u-HA/PLLA[c]	n.s	n.s	n.s	—	—	[f]	—
u-HA/PLLA[c] vs. PLLA[c]	[c]	[f]	[f]	—	—	[b]	—
c-HA/PLLA[c] vs. PLLA[c]	[c]	[f]	[f]	—	—	[b]	—

[a]In subcutis.
[b]With a significant difference at $P < 0.02$.
[c]With a significant difference at $P < 0.01$.
[d]With a significant difference at $P < 0.005$.
[e]In medullary canal.
[f]With a significant difference at $P < 0.05$.
[g]n.s. = not significant.

Table 4
Statistical analysis of the data shown in Fig. 3: change in crystallinity of HA/PLLA and PLLA rods with time[f]

	Weeks after implantation						
	2	4	8	12	16	25	52
c-HA/PLLA[a] vs. u-HA/PLLA[a]	—	n.s	n.s	n.s	n.s	[b]	[b]
c-HA/PLLA[c] vs. u-HA/PLLA[c]	n.s	n.s	n.s	—	—	n.s	—
u-HA/PLLA[c] vs. PLLA[c]	[d]	[e]	[b]	—	—	[e]	—
c-HA/PLLA[c] vs. PLLA[c]	[b]	[b]	[b]	—	—	[e]	—

[a]In subcutis.
[b]With a significant difference at $P < 0.05$.
[c]In medullary canal.
[d]With a significant difference at $P < 0.01$.
[e]With a significant difference at $P < 0.02$.
[f]n.s. = not significant.

3.4. Histological results

Histological examination by light microscopy revealed no distinct differences between the two composite types during the experimental periods.

There were no inflammatory cells, such as neutrophils or macrophages, in the subcutaneous tissue during the experimental period. However, there was a fibrous tissue layer, which became thicker over time, surrounding the composite rods. At 4 weeks, a fibrous tissue layer was seen around the composite rods, with evenly distributed fibrocyte and collagen fibres running parallel to the rod surface (Fig. 4a). At 52 weeks, the matured fibrous tissue layer was approximately 130 μm thick, with spindle-shaped fibrocytes in close contact with the composite rods (Fig. 4b). Signs of rod degradation, such as swelling, deformity, or cracking, were slightly observed after 25 weeks; however, there was no fragmentation, even at 52 weeks (Fig. 5).

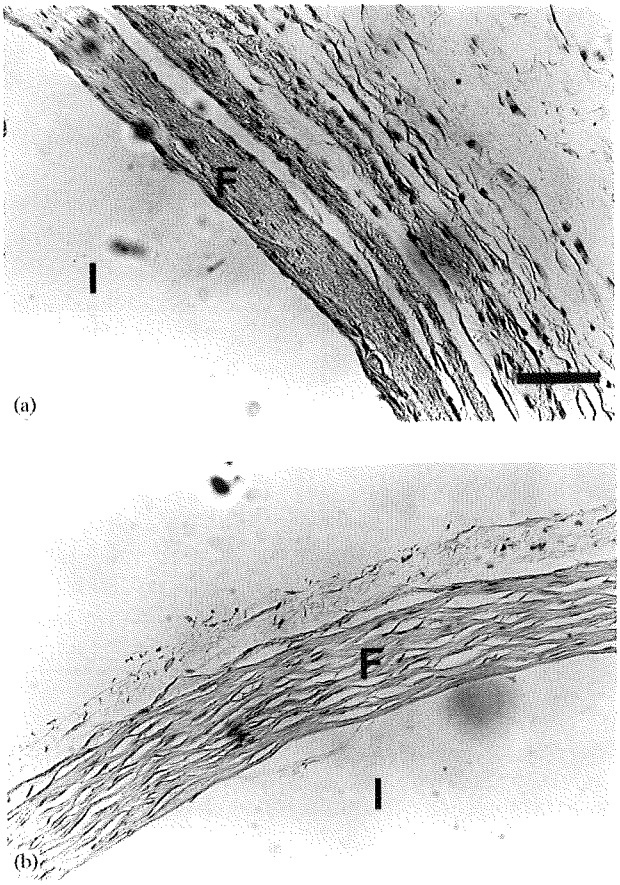

Fig. 4. Photomicrograph of a decalcified section of u-HA/PLLA rod after subcutaneous implantation. (a) 4 weeks: dense fibrous tissue layer directly facing u-HA/PLLA rod. (b) 52 weeks: mature dense fibrous capsule (130 μm thickness) in contact with u-HA/PLLA rod. F: fibrous tissue, I: implant. Hematoxylin and eosin staining (original magnification × 200; scale = 100 μm).

In the medullary cavities, there were different findings between diaphyseal and metaphyseal regions. In the diaphyseal region, where cancellous bone tissue was rarely seen, a fibrous tissue layer with a thickness similar to that seen in subcutaneous implantation was observed and new bone formation was not seen (Fig. 6a). In the metaphyseal region, new bone formation was observed and osteoblasts and osteoclasts were seen between the surface of composite rods and new bone, even from the early stage (Fig. 6b). In the medullary cavities, no inflammatory cells such as macrophages or multinucleated giant cells were observed at any interval.

3.5. Changes in surface morphology over time

No apparent macroscopic changes were noted in the surface of the composite rods removed from the surrounding tissues during the experimental periods.

In SEM, HA particles were exposed and distributed homogenously in the surface of the composite rod before

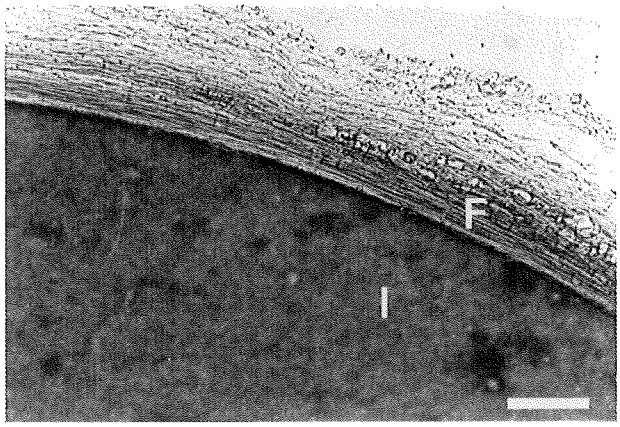

Fig. 5. Photomicrograph of an undecalcified section of u-HA/PLLA rod 52 weeks after subcutaneous implantation. F; fibrous tissue layer; I; implant. The gross shape of the rod was still maintained. Giemsa surface staining (original magnification × 200; scale = 100 μm).

implantation (Fig. 7a). In contrast, after subcutaneous and intramedullary implantation. HA particles disappeared increasingly from the rod surfaces over time and the spaces left by these HA particles formed many pores in the composite surfaces (Fig. 7b). These findings were observed both in u- and c-HA/PLLA rods, with the loss of HA particles slightly greater in the u-HA/PLLA than in the c-HA/PLLA (Fig. 7c, d).

4. Discussion

Verheyen et al. [25] tried to produce HA/PLLA composites with high mechanical strength by the as-polymerized method. However, the initial flexural strength (Sf) of their composites was low (about 70–90 MPa), the retention time was short (loss of 50% of Sf within 3 weeks), and a faster decline of Sf in PLLA than that in composites was observed, with significant differences between in vivo and in vitro tests. Our composites were produced by a forging process, which was a unique compression molding and a reinforcing method. Rather than a simple system of particle-reinforced composites, the matrix polymer is simultaneously reinforced by a complex crystal orientation so that the filling of composites is very compact and composites have the theoretical densities, which was calculated from the proportional allotment of specific gravities of HA and PLLA used in composites. Consequently, our composites have a maximum bending strength of 280 MPa in a c-HA content of 30% by weight, and an ideal balance of ductility of polymer and stiffness of HA particles. The bending strength somewhat decreased when the fraction surpassed 40% by weight [27,28]. The high mechanical strength is indispensable for fracture-fixation devices under highly loaded conditions. Therefore, in this work we chose HA30/PLLA rods for in vivo experiments.

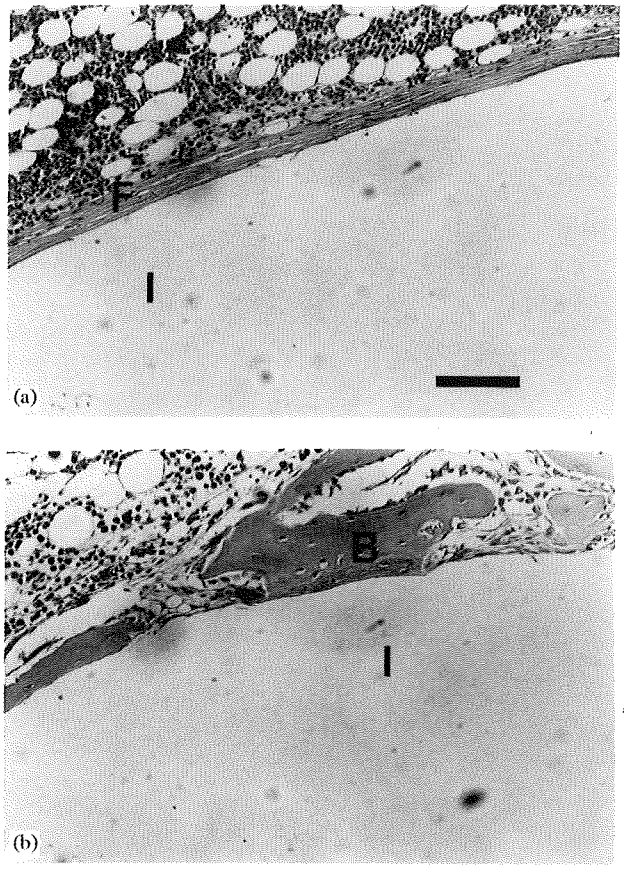

Fig. 6. Photomicrograph of a decalcified section of u-HA/PLLA rod 4 weeks after intramedullary implantation. In diaphysial region (a), dense fibrous tissue layer surrounding u-HA/PLLA rod as seen in subcutaneous implantation. In metaphysial region (b), new born formation was observed. Osteoblasts and osteoclasts were seen between the implant and new bone. I: implant, B: bone. Hematoxylin and eosin staining (original magnification $\times 200$; scale = 100 μm).

In our study, the rods had a bending strength of more than 200 MPa, which exceeds the bending strength of human cortical bone (120–210 MPa). At 25 weeks, the bending strength of rods in the subcutaneous tissue was 80% of the initial bending strength. This retention of mechanical strength is longer than that of absorbable implants that are used currently as fracture-fixation devices [33–35]. Generally, it takes more than 8–12 weeks for the union of fractured human bone, although many factors such as fracture site, stability, and patient's age influence the healing period. During this period, fixation stability is needed for the replaced bone fragment. However, Ahl et al. [15], reporting on the fixation of ankle fractures with a PGA device, observed small movements in the ankle mortise during fracture healing using roentgen stereogrammetric analysis. Thus, the mechanical weakness of an absorbable implant occasionally causes a less stable fixation. Our in vivo results suggest that our composites maintain the required mechanical strength for the healing period of fractured bone and would be useful as fixation devices for human fractures.

The most important degradation mechanism of biodegradable polymer is chemical degradation via hydrolysis [36] in which the uptake of water is especially important for the degradation of the material. In the present study, the molecular weight of the composites decreased faster from the early period than the unfilled PLLA. This is possibly because the body fluid could diffuse more easily into the composite than into the unfilled PLLA due to the absence of chemical bonding between HA particles and the PLLA matrix in the composite and to the surface porosity of the composite as shown by SEM. Consequently, it is supposed that the composites demonstrated a faster degradation than the unfilled PLLA made by the same method. In comparing the results of the present in vivo study with an in vitro study [28], there was no evident difference in degradation of the composites between the studies. This suggests that our composites might be degraded predominantly by hydrolysis in the in vivo environment during the experimental periods, as is the case with unfilled PLLA.

In the present study, for reinforcing particles, we used c- and u-HA particles, which have properties different from those of sintered HA, for two reasons. First, the composites used in the present study were expected to be totally resorbed as a bioabsorbable implant; therefore, u- and c-HA particles did not undergo the high-temperature sintering process and so were considered to be more degradable than sintered HA [37]. Second, recent studies have suggested that particulate debris around orthopedic implants has an adverse effect on the surrounding tissues, although there is not yet a general understanding of the mechanisms involved. Osteolysis and aseptic loosening of implants in total hip arthroplasty using HA-coated implants have been reported by several authors, and HA ceramic particles as well as polyethylene particles were seen at the interface between the non-cemented stem and the femur [38,39]. In an in vitro study, Sun et al. [40] showed the inhibitory effects of sintered HA particles on osteoblast cell cultures mediated by the increased synthesis of PGE_2. Thus, sintered HA that is considered to be biocompatible in bulk form is capable of eliciting inflammatory reactions in particulate form. Therefore, we avoided using sintered HA particles that were considered biostable [37] as reinforcing particles.

In the present study, u-HA/PLLA demonstrated a significantly faster decrease in molecular weight than c-HA/PLLA. This might have been due to the difference in the biodegradability between c- and u-HA particles. The u-HA particles [29,37] were considered to be more degradable and had a lower crystallinity than c-HA, which was made by calcining u-HA at 900°C. In addition, SEM demonstrated that the porosity of the u-HA/PLLA increased more rapidly than that of the c-HA/PLLA. The invasion of body fluid into the inner site of the rod might

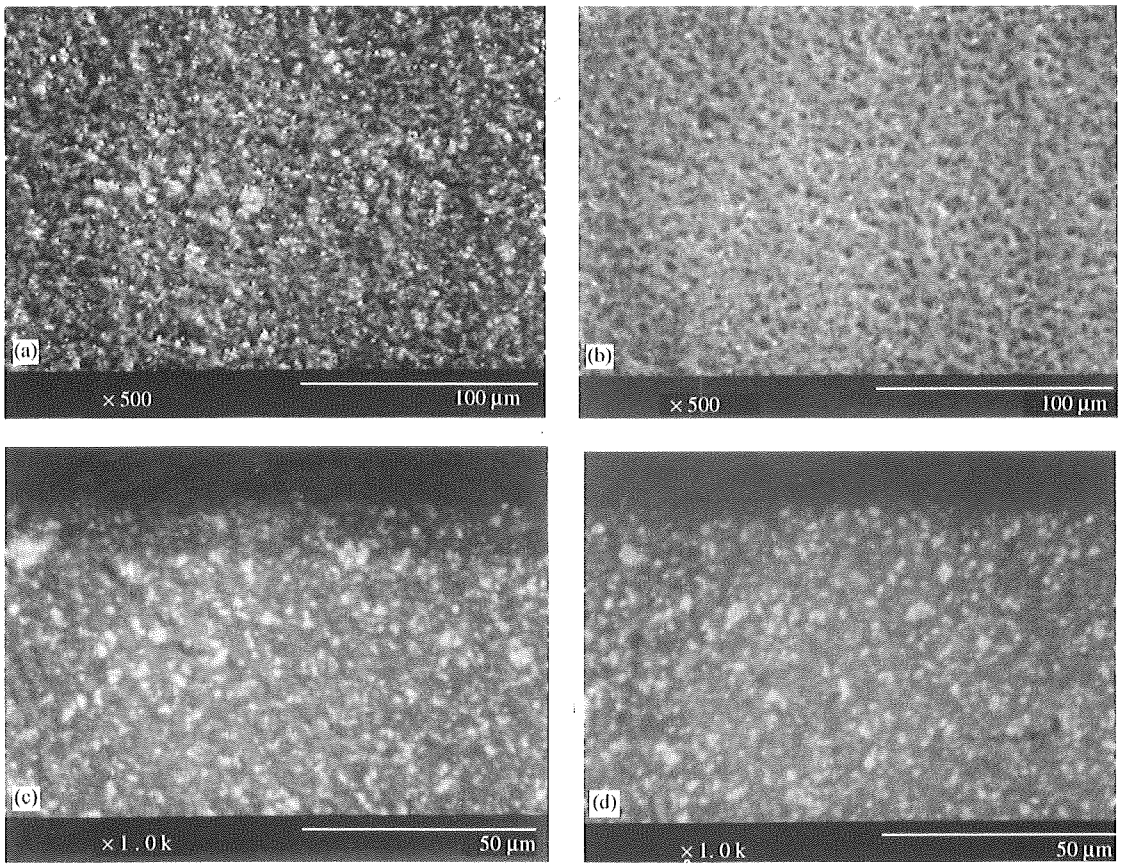

Fig. 7. SEM photographs of the surfaces and the cross-sections of composite rods. (a) The surface of u-HA/PLLA rod before implantation: HA particles were exposed and distributed homogeneously in the surface of rod. (b) The surface of u-HA/PLLA rod 52 weeks after subcutaneous implantation: loss of HA particles from the rod surface was observed. (c) The cross-section of (b). (d) The cross section of c-HA/PLLA 52 weeks after subcutaneous implantation: loss of surface HA particles in u-HA/PLLA was slightly greater than in c-HA/PLLA.

become easier by resorption of particles in u-HA/PLLA than in c-HA/PLLA.

In general, increasing crystallinity is considered to be correlated to decreasing molecular weight. Our results were consistent with this fact; that is, contrary to the molecular weight, the u-HA/PLLA showed higher crystallinity than the c-HA/PLLA, especially from 25 weeks, when the difference was significant. This result also suggests a faster degradation rate for the u-HA/PLLA than for c-HA/PLLA. In order to facilitate resorption of the composite material, it would be advantageous to use u-HA/PLLA composite.

The bending strength of the composites decreased more slowly than the molecular weight. This finding is compatible with those in the literature on PLLA implants [33,41]. In our study, the bending strengths of the composites were maintained at 50–60% of their initial values, even after 52 weeks, but significant difference between u- and c-HA/PLLA was not confirmed for up to 52 weeks. Only at 52 weeks did u-HA/PLLA show significantly lower mechanical strength than c-HA/PLLA. These findings suggests that the degradation rate of these composites was slow, and, from 52 weeks onward, the degradation of the composites would progress and be actualized in macroscopical morphology. Nevertheless, the degradation rate could be enhanced by increasing the residual monomer [42]. The present residual lactide is low, at 0.05% by weight.

There was no significant difference in molecular weight and crystallinity between rods in subcutis and in the medullary cavities except in u-HA/PLLA at 25 weeks. In rabbit femurs, cancellous bone does not exist in the diaphysial area, but in the epi- or metaphysical areas. Therefore, these areas in the rabbit femur should be considered as a different environment for mechanical evaluation of biomaterials [43]. Molecular weight and crystallinity were measured using the diaphysial parts of the rods in the present study. Although removing the implants from the cancellous bone would be difficult due to their bonding, use of the distal part of the rod might have given different results.

Histologically, neither macrophages nor inflammatory giant cells were seen over time, indicating that these composites would be biocompatible, at least during the

experimental periods. Degradation of PLLA was not observed evidently, but the disappearance of HA particles on the surface was observed by SEM. No convincing conclusion has yet been reached as to whether HA is bioresorbed [44]. In the histological specimens, there were no findings that the dissociated u- and c-HA particles from the rods were scattered into the tissues around the rods. In addition, there were no inflammatory cells indicating phagocytosis of these HA particles, which suggests that the HA particles might have been subjected mainly to a chemical dissolution process. Accordingly, these HA particles did not induce an apparent tissue reaction during the experimental period.

Thus, no signs of degradation were seen macroscopically. No disintegration of rods or polymer debris, which could elicit inflammatory tissue reactions, was observed even at 52 weeks. Actually, the formation of crystalline particles could be predictable and might generate the same problems as those already mentioned in the literature [11,13]. Accordingly, we are now conducting a long-term study to evaluate the degradation behavior and the tissue response to HA/PLLA composites, including composites with an HA content 40% by weight as well as an HA content of 30% by weight.

5. Conclusions

The biodegradation of HA/PLLA composite rods in subcutis and the medullary cavities of rabbits were evaluated mechanically and histologically. A bending strength of more than 200 MPa was maintained at 25 weeks in the subcutis. Histologically, there were no inflammatory cells around the implants, suggesting that these composites had biocompatibility. Of the two types of HA/PLLA composites, u-HA/PLLA showed faster degradation rates than c-HA/PLLA in terms of molecular weight and bending strength. To facilitate resorption of the composite material, it would be advantageous to use u-HA/PLLA composite. Although further long-term studies for degradation of the composite material are needed, the results obtained from this in vivo study encourage the clinical use of this material in the repair of human bone fractures.

References

[1] Matsusue Y, Nakamura T, Iida H, Shimizu K. A long-term clinical study on drawn poly-L-lactide implants in orthopaedic surgery. J Long-Term Effects Med Implants 1997;7:119–37.

[2] Pihlajamäki H, Böstman O, Hirvensalo E, Törmälä P, Rokkanen P. Absorbable pins of self-reinforced poly-L-lactic acid for fixation of fractures and osteotomies. J Bone Jt Surg Br 1992;74:853–7.

[3] Bucholz RW, Henry S, Henley MB. Fixation with bioabsorbable screws for the treatment of fractures of the ankle. J Bone Jt Surg Am 1994;76:319–24.

[4] Thanner J, Karrholm J, Malchau H, Wallinder L, Herberts P. Migration of press-fit cups fixed with poly-L-lactic acid or titanium screws: a randomized study using radiostereometry. J Orthop Res 1996;14:895–900.

[5] Nakamura S, Ninomiya S, Takatori Y, Morimoto S, Kusaba I, Kurokawa T. Polylactide screws in acetabular osteotomy. 28 dysplastic hips followed for 1 year. Acta Orthop Scand 1993; 64:301–2.

[6] Rokkanen P, Böstman O, Vainionpää S, Makela EA, Hirvensalo E, Partio EK, Vihtonen K, Patiala H, Törmälä P. Absorbable devices in the fixation of fractures. J Trauma 1996;40:123–7.

[7] Yamamuro T, Matsusue Y, Uchida A, Shimada K, Shimozaki E, Kitaoka K. Bioabsorbable osteosynthetic implants of ultra high strength poly-L-lactide: a clinical study. Int Orthop 1994;18:332–40.

[8] Hanafusa S, Matsuse Y, Yasunaga T, Yamamuro T, Oka M, Shikinami Y, Ikada Y. Biodegradable plate fixation of rabbit femoral shaft osteotomies: a comparative study. Clin Orthop 1995;315:262–71.

[9] Akeson WH, Woo SL, Rutherford L, Coutts RD, Gonsalves M, Amiel D. The effects of rigidity of internal fixation plates on long bone remodeling: a biomechanical and quantitative histological study. Acta Orthop Scand 1976;47:241–9.

[10] Böstman OM. Absorbable implants for the fixation of fracture. J Bone Jt Surg 1991;73-A(1):148–53.

[11] Böstman OM. Osteoarthritis of the ankle after foreign-body reaction to absorbable pins and screws. J Bone Jt Surg Br 1998; 80-B(2):333–8.

[12] Tegnander A, Engebretsen L, Bergh K, Eide E, Holen KJ, Iversen OJ. Activation of the complement system and adverse effects of biodegradable pins of polylactic acid (Biofix) in osteochondritis dissecans. Acta Orthop Scand 1994;26:472–5.

[13] Bergsma EJ, Rozema FR, Bos RR, de Bruijn WC. Foreign body reactions to resorbable poly(L-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures. J Oral Maxillofac Surg 1993;51:666–70.

[14] Matsusue Y, Nakamura T, Suzuki S, Iwasaki R. Biodegradable pin fixation of osteochondral fragments of the knee. Clin Orthop 1996;322:166–73.

[15] Ahl T, Dalen N, Lundberg A, Wykman A. Biodegradable fixation of ankle fracture. Acta Orthop Scand 1994;65:166–70.

[16] Donigian AM, Plaga BR, Caskey PM. Biodegradable fixation of physeal fractures in goat distal femur. J Pediatr Orthop 1993;13:349–54.

[17] Böstman O, Hirvensalo E, Vainionpää S, Makela A, Vihtonen K, Törmälä P, Rokkanen P. Ankle fractures treated using biodegradable internal fixation. Clin Orthop 1989;238:195–203.

[18] Higashi S, Yamamuro T, Nakamura T, Ikada Y, Hyon SH, Jamshidi K. Polymer–hydroxyapatite composites for biodegradable bone fillers. Biomaterials 1986;7:183–7.

[19] Verheyen CC, de Wijn JR, van Blitterswijk CA, de Groot K. Evaluation of hydroxylapatite/poly(L-lactide) composites: mechanical behavior. J Biomed Mater Res 1992;26:1277–96.

[20] Flahiff CM, Blackwell AS, Hollis JM, Feldman DS. Analysis of a biodegradable composite for bone healing. J Biomed Mater Res 1996;32:419–24.

[21] Devin JE, Attawia MA, Laurencin CT. Three-dimensional degradable porous polymer-ceramic matrices for use in bone repair. J Biomater Sci Polym Ed 1996;7:661–9.

[22] Acuna V, Jianguo L, Söremark R. Composites of lactic acid polymer and calcium phosphate or calcium carbonate as degradable bone fillers. In: Doherty PJ et al., editors. Biomaterial–tissue interface. Advances in biomaterials 10. Oxford: Elsevier Science Publisher, 1992. p. 391–8.

[23] Doyle C, Tanner ET, Bonfield W. In vitro and in vivo evaluation of polyhydroxybutyrate and of polyhydroxybutyrate reinforced with hydroxyapatite. Biomaterials 1991;12:841–7.

[24] Bonfield W, Grynpas MD, Tully AE, Bowman J, Abram J. Hydroxyapatite reinforced polyethylene—a mechanically compatible implant material for bone replacement. Biomaterials 1981;12:185–6.
[25] Verheyen CC, de Wijn JR, van Blitterswijk CA, de Groot K, Rozing PM. Hydroxylapatite/poly(L-lactide) composites: an animal study on push-out strengths and interface histology. J Biomed Mater Res 1993;27:433–44.
[26] Watanabe S, Nakamura T, Shimizu Y, Hitomi S, Ikada Y. Traumatic sternal segment dislocation in a child. Chest 1989; 96:684–6.
[27] Shikinami Y, Hata K, Okuno M. Ultra-high-strength resorbable implants made from bioactive ceramic particle/polylactide composites. In: Kokubo T, Nakamura T, Miyaji F, editors. Bioceramics, vol. 9. Tokyo: Elsevier Science, 1996. p. 391–4.
[28] Shikinami Y, Okuno M. Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles/poly-L-lactide (PLLA): Part I. Basic characteristics. Biomaterials 1999; 20:859–77.
[29] Matsuda N, Kaji F. Form control of crystals and aggregation of hydroxyapatites. In: Kokubo T, Nakamura T, Miyaji F, editors. Bioceramics, vol. 9. Tokyo: Elsevier Science, 1996. p. 213–6.
[30] Hyon SH, Jamshidi K, Ikada Y. Melt spinning of poly-L-lactide and hydrolysis of the fiber in vitro. In: Shalaby SW, Hoffman AS, Ranter B, Horbert TA, editors. Polymers as biomaterials. New York: Plenum Press, 1984. p. 51–65.
[31] Schindler A, Harper D, Polylactide. II. Viscosity-molecular weight relationships and unperturbed chain dimensions. J Polym Sci 1979:2593–99.
[32] Fischer EW, Sterzel HJ, Wegner G. Investigation of the structure of solution grown crystals of lactide copolymers by means of chemical reactions. Kolloid-Z und Z polymere 1973;251:980–90.
[33] Matsusue Y, Yamamuro T, Oka M, Shikinami Y, Hyon SH, Ikada Y. In vitro and in vivo studies on bioabsorbable ultra-high-strength poly(L-lactide) rods. J Biomed Mater Res 1992; 26:1553–67.
[34] Törmälä P, Vasenius J, Vainionpää S, Laiho J, Pohjonen T, Rokkanen P. Ultra-high-strength absorbable self-reinforced polyglycolide (SR-PGA) composite rods for internal fixation of bone fractures: in vitro and in vivo study. J Biomed Mater Res 1991;25:1–22.
[35] Majola A, Vainionpää S, Mikkola HM, Törmälä P, Rokkanen P. Absorbable self-reinforced polylactide (SR-PLLA) composite rods for fracture fixation: strength and strength retention in the bone and subcutaneus tissue of rabbits. J Mater Sci: Mater Med 1992;3:43–7.
[36] Gopferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996;17:103–14.
[37] Oonishi H, Hench LL, Wilson J, Sugihara F, Tsuji E, Kushitani S, Iwaki H. Comparative bone growth behavior in granules of bioceramic materials of various sizes. J Biomed Mater Res 1999;44:31–43.
[38] Morscher EW, Hefti A, Aebi U. Severe osteolysis after third-body wear due to hydroxyapatite particles from acetabular cup coating. J Bone Jt Surg 1998;80-B(2):267–72.
[39] Bauer TW, Geesink RCT, Zimmerman R, McMahon JT. Hydroxyapatite-coated femoral stems: histological analysis of components retrieved at autopsy. J Bone Jt Surg 1991;73-A:1439–52.
[40] Sun JS, Liu HC, Chang WHS, Li J, Lin FH, Tai HC. Influence of hydroxyapatite particle size on bone cell activities: an in vitro study. J Biomed Mater Res 1998;39:390–7.
[41] Mainil-Varlet P, Curtis R, Gogolewski S. Effect of in vivo and in vitro degradation on molecular and mechanical properties of various low-molecular-weight polylactice. J Biomed Mater Res 1997;36:360–80.
[42] Nakamura T, Hitomi S, Watanabe S, Shimizu Y, Jamshidi K, Hyon SH, Ikada Y. Bioabsorption of polylactide with different molecular properties. J Biomed Mater Res 1989;23:1115–30.
[43] Lu JX, Gallur A, Flautre B, Anselme K, Descamps M, Thierry B, Hardouin P. Comparative study of tissue reactions to calcium phosphate ceramics among cancellous, cortical, and medullar bone sites in rabbits. J Biomed Mater Res 1998;42:357–67.
[44] Oonishi H, Oomamiuda K. Degradation/resorption in bioactive ceramics in orthopaedics. In: Black J, Hasting G, editors. Handbook of biomaterial properties. London: Chapman & Hall, 1998. p. 406–19.