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The complete process of bioresorption and bone replacement using devices made of forged composites of raw hydroxyapatite particles/poly L-lactide (F-u-HA/PLLA)

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Abstract

Here we document the complete process of bioresorption and bone replacement of rods made of forged composites of unsintered hydroxyapatite particles/poly L-lactide (F-u-HA/PLLA) implanted in the femoral medullary cavities of rabbits. Bioresorption, osteoconductive bioactivity and bone replacement were compared in three implantation sites. In the first site, the end of the rod was located near the endosteum in the proximal medullary cavity. In the second, the rod was located at the centre of the bone marrow space without contacting the endosteum. In the third, the rod was in direct contact with cancellous bone within the distal femoral condyle. Micro-computerised tomography, scanning electron microscopy and photomicrographs of stained sections were used to document the complete process of bioresorption and bone replacement. At the first implantation site, the rod was completely resorbed and unbound u-HA particles were detected in and around the endosteum 5–6 years after implantation. At the second site, the rod showed significant shrinkage 4–5 years after implantation due to the release of almost all the PLLA, although a contracted cylindrical structure containing a few u-HA persisted even after ~6 years. At the third site, u-HA particles were almost completely replaced with bone after 5–6 years. Conversely, PLLA-only rods showed little bone conduction, and small amounts of degraded PLLA debris and intervening some tissue persisted even after long periods. Namely, the u-HA/PLLA composites were replaced with bone in the distal femoral condyle, where they were in direct contact with the bone and new bone formation was anatomically necessary. By contrast, composite rods were resorbed without replacement in the proximal medullary cavity, in which new bone growth was not required. We therefore conclude that the F-u-HA30/40 composites containing 30 wt%/40 wt% u-HA particles are clinically effective for use in high-strength bioactive, bioresorbable bone-fixation devices with the capacity for total bone replacement.
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Keywords: Bioactivity; Biodegradation; Bioresorption; Bone regeneration; Calcium phosphates; Hydroxyapatite/PLLA composites

1. Introduction

An important requirement of bioresorbable bone-fixation devices is to accomplish total replacement with bone through osteological bioactivity; however, there is no full documentation of this process to date. Ultra-high strength devices made of forged composites of raw hydroxyapatite particles (neither calcined nor sintered) and poly L-lactide (F-u-HA/PLLA) have the potential for total replacement with bone, as they are bioactive and biodegradable. These materials have many potential applications in various clinical fields, including orthopaedic, plastic reconstructive, oral and maxillary/mandibular facial, cranial, thoracic, spinal and traumatic

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surgery. It is essential to fully understand the total degradation process of bone-fixation devices, both to determine their safety in clinical use and to assess their bioactive performance. Here we present photographic images and graphical figures illustrating the distinctive changes that occur in F-u-HA-30 and F-u-HA-40 forged rods (containing 30 or 40 wt% of u-HA particles in composites, respectively) over time in the femoral medullary cavities of rabbits. Changes in mechanical strength and average molecular weight, bone-bonding behavior, histomorphology and tissue reactions are documented throughout the prolonged process, from the implantation of the devices to their replacement with bone.

2. Materials and methods

Round rods as shown in Fig. 1 were used in this study. Rods had the following characteristics: diameter, 3.2 mm; length, 3.0 cm; particle size of u-HA, 0.2–20 μm (average 3–5 μm); Ca/P = 1.69 (mol, ratio) and CO_3^{2-} = 3.8 (mol%) [1]. The rods were experimentally introduced into the femoral medullary cavities of rabbits through the methaphyseal and diaphyseal regions [2,3]. F-c-HA30 and F-PLLA-only rods were compared with F-u-HA30/40 [4] in a certain period of time when the distinctive changes occurred after 4.5 and 5.5 years, respectively. u-HA is calcined to denaturize into c-HA at 900 °C and ground down and passed through the same screen size as u-HA particles [5]. Biodegradation of the composites was accompanied by the following processes: gradual loss of strength; resorption of PLLA, which degraded to low molecular weight (oligomer), and release of unbound u-HA debris and deposition of calcium phosphates (CaPs), which affected osteoconduction.

2.1. Animal tests and measurement methods

The method of implantation into rabbits has been described previously [2,3,6]. Three different implantation sites and states were investigated in the rabbit femoral cavity: the first was close to the endosteum in the proximal medullary cavity; the second was at the centre of the cavity equidistant from the circumferential endosteum in the proximal or middle medulla; and the third was in direct contact with cancellous bone within the distal femoral condyle.

The progression was fully followed up from implantation to replacement with natural bone. Few animals survived beyond the average lifespan. Each animal that lived out a natural life was examined at the later time intervals of 4, 4.5 and 5.5 years after implantation. Each rod sample (length: ~1.5 cm) implanted into the diaphyseal or the methaphyseal regions of the femur was used for further examinations. Decalcified and non-decalcified specimens were prepared as appropriate. All samples were fixed in 10% phosphate-buffered formalin. Non-decalcified specimens were dehydrated with a graded ethanol series, soaked serially in styrene at 50, 70 and 100% (v/v), and embedded in polyester resin before investigation with scanning electron microscopy (SEM), energy-dispersive X-ray analysis (EDX) and X-ray diffraction. Transverse sections (thickness, 500 μm) were cut using a diamond band (BS-3000; EX-AKT cutting system, Norderstedt, Germany).

2.2. HE and Villanueva Goldner staining

Samples were decalcified by immersion in 10% EDTA solution, dehydrated with a graded ethanol series, soaked serially in xylene and embedded in paraffin wax. Sections (thickness, ~4 μm) were prepared from each rod using a grinding machine with a diamond lap disc (Maruto Co. Ltd., Tokyo, Japan), and stained with haematoxylin and eosin (HE) according to standard procedures. The sections were then examined using light microscopy. Villanueva Goldner staining to non-decalcified specimens was also carried out, using the method described previously [7].

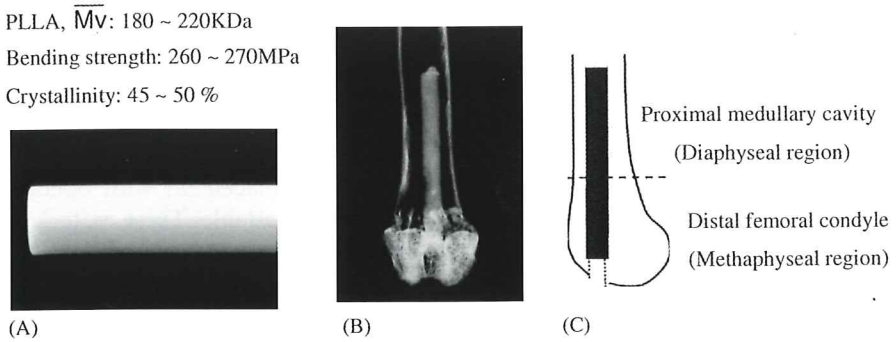


Fig. 1. Common initial properties, F-u (c)-HA-30, 40, PLLA: (A) An implanted round rod; (B) A radiograph of a rod in the femur of rabbit; (C) Two sections of a rod removed for histological and morphological examinations.

2.3. SEM, EDX

Slices were polished and the bone–implant interface was examined using an SEM (S-2460N; Hitachi, Japan) connected to an Electron-Probe Microanalyzer (EPMA) system (Horiba EMAX, Japan).

2.4. X-ray diffraction

Powder X-ray diffraction analysis was conducted with Cu-Kα radiation generated at a voltage and current of 40 kV and 30 mA, respectively, using a RINT RAPID system (Rigaku, Japan). This method was used to determine the origin and identity of c-HA particles and aggregates of white crystalline particles deposited on the surface of reactive bone after 4.5 years. Reactive bone was defined in this study as any intramembranous bone that forms on other hard or soft tissues as a response to stress [8]. This is distinct from the process of heterotropic calcification, which refers to the deposition of acellular minerals in soft tissue. Reactive bone can be woven or lamellar, depending on the rate of deposition. It is sometimes mineralised, and often has a specular or traveculated pattern.

2.5. μCT analysis

A micro-computerised tomography (μCT) system (Desktop Micro CT1072; SkyScan, Aartselaar, Belgium) was used to non-destructively determine the extent of biodegradation and bioresorption, accompanied by bone formation. Samples were scanned with a cone-beam configuration and X-ray-tube settings of 100 kV and 98 μA. Each sample was X-ray irradiated through an aluminium filter (thickness, 1 mm) and scanned with an isotropic voxel size of 3.9 μm, an exposure time of 5.5 s and a rotational step of 0.90°. This produced serial cross-sectional images (1024 × 1024 pixels). Three-dimensional μCT images were produced using VG Studio 1.1 software (Volume Graphics; GmbH, Heidelberg, Germany).

2.6. Ca/P ratios and fractions of residual u-HA particles in composites

The stoichiometric Ca/P molar ratios of deposited crystals on the surfaces of rods and reactive bone were analysed using SEM and EDX. The residual fraction of PLLA in each composite was measured by leaching PLLA from the rods in dichloromethane.

3. Results

Bioresorption, osteoconductive behaviour and the time taken for the total replacement of the F-u-HA30/40 devices with bone were all significantly influenced by the site and the fitting state at the time of implantation, whether the rod was in direct contact with the bone and the endosteum or not, as described later. For the purposes of our discussion, we have divided the in vivo degradation process into separate stages on the basis of the changes occurring within the composite rods.

3.1. Total bone replacement process in the methaphyseal region [6]

The direct bonding of the composite devices to bone was supported by exposed u-HA particles on the surface. This persisted until the mechanical strength was lost due to uniform hydrolysis throughout the PLLA matrix. The rods decreased gradually in size as low PLLA molecules and unbound u-HA particles were steadily released over the first ~2.5 years after implantation. $\overline{Mv}$ of PLLA had drastically decreased and the u-HA fraction in the composites critically increased after ~2–2.5 years. The PLLA matrix was completely absent from the composites after ~4.5–5.0 years, and holes created during the insertion procedure in the distal femoral condyle were almost filled with new bone. This has not previously been achieved with PLLA-only devices. Fig. 2 illustrates the changes in the distal femoral condyle (detected by SEM) that had occurred 2–5.5 years after implantation, along HE-stained images taken after 5.5 years comparing with F-PLLA-only one. Fig. 3 summarises the morphological, Mv and u-HA content changes in this site that were observed in the F-u-HA30/40 rods. After ~2.5 years, $\overline{Mv}$ decreased to <50 KDa and mechanical strength was almost lost. The rod size remarkably contracted after ~3.5 years. No residual PLLA was detected in the composites after ~4.5 years. After ~5 years, the u-HA fraction reached levels of >70 w%. However, a level of 100 wt% was not achieved, probably due to the interposition of collagenous materials and the technical impossibility of total extraction. Almost all of the u-HA particles were replaced with natural bone after 5.5 years without any significant foreign body reaction or, alternatively, the rod hole is fully replaced with natural bone remaining few small aggregates of u-HA particles of which releasing out was obstructed by the newly formed surrounding bone as shown in Fig. 2, which is consistent with the total bone replacement. No inflammatory reaction was observed because the tissue reactions remained mild throughout the biodegradation and replacement processes. By contrast, in PLLA-only, few degraded PLLA debris still remain including fat cells in the rod hole, which is unfilled with natural bone.

3.2. Total resorption in the diaphyseal region

Although the bone marrow itself has osteogenic activity, it is significantly lower than that of the

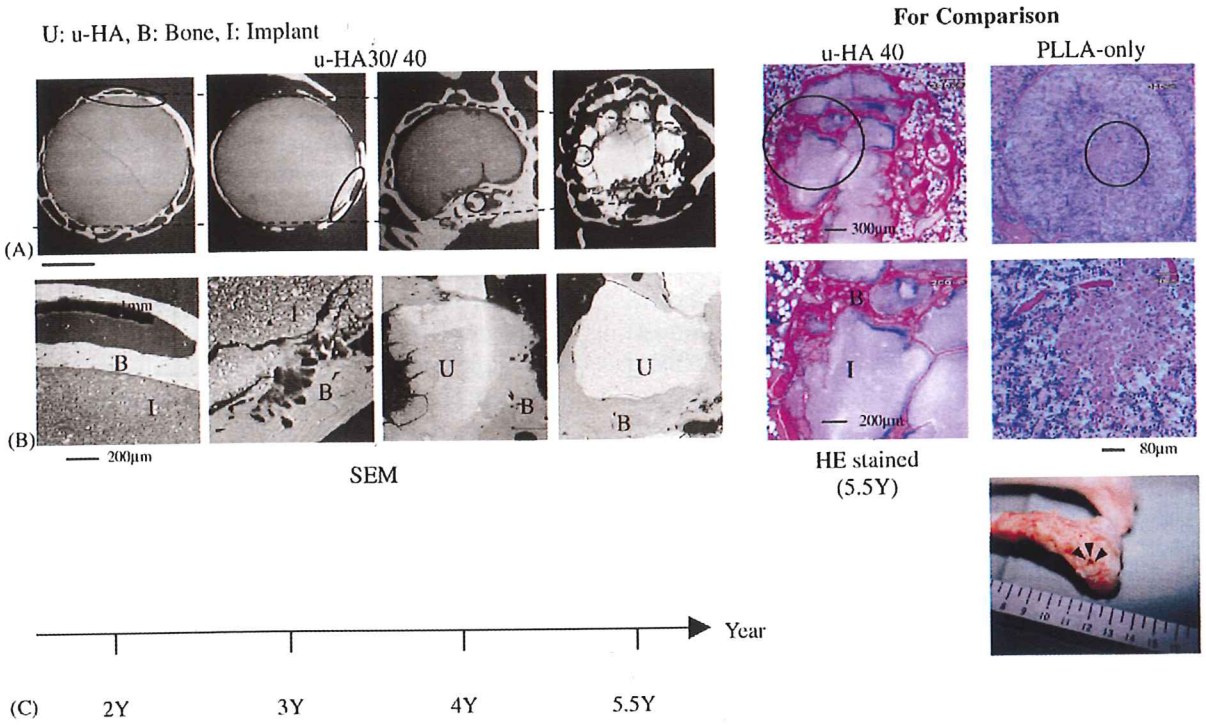


Fig. 2. Cross-sectional morphological change of F-u-HA 30/40 rods over time in the distal femoral condyle: (B) The magnified views in the circular area of (A); (C) An unfilled hole implanted with a F-PLLA-only rod in the lateral distal femoral condyle of a dog 5.5 years after implantation. For comparison micrographs of u-HA, and PLLA-only are stained with Haematoxylin and Eosin (HE).

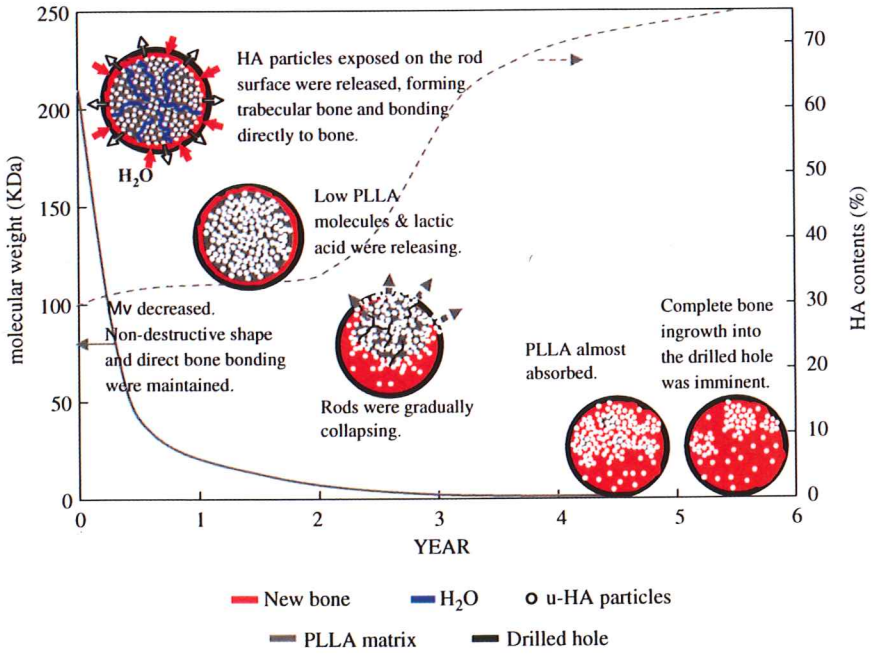


Fig. 3. Illustration of morphological, Mv and u-HA content changes in F-u-HA30/40 rods in the distal femoral condyle.

periosteum or endosteum. Bioresorption and bioactivity levels therefore differed depending on the proximity of the implants to the endosteum. The morphological changes of rods that occurred during biodegradation are shown in greater detail through the total course in Fig. 4. Predictably, around 4.5 years after implantation of the F-c-HA rods, white granules were observed on the reactive bone surfaces as shown in for comparison. EDX and X-ray diffraction identified these as c-HA particles. This result is consistent with the findings of

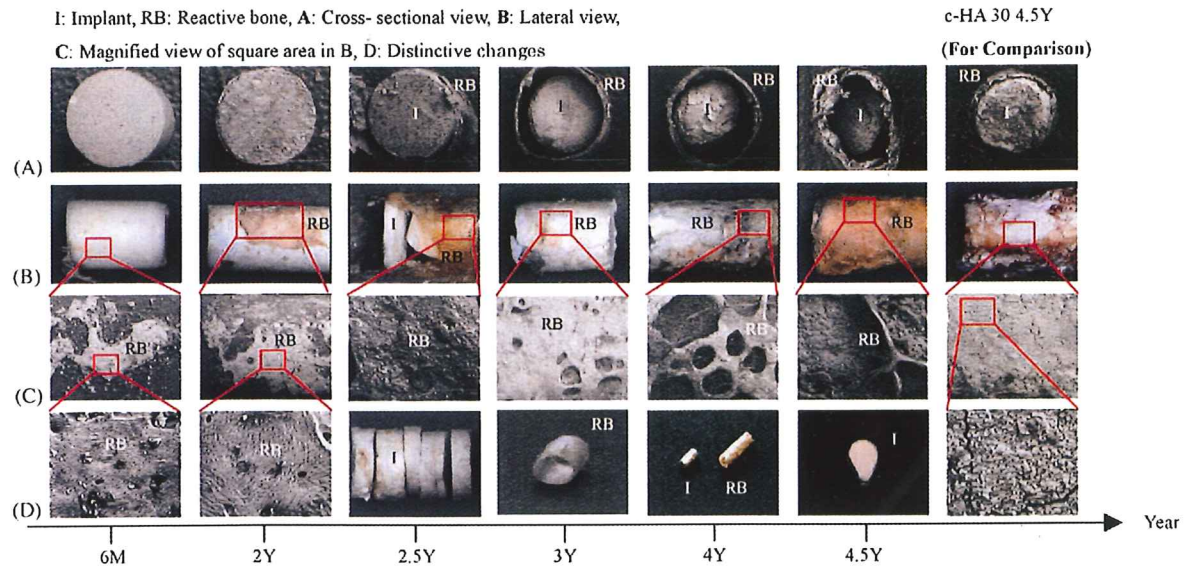


Fig. 4. Morphological changes during biodegradation and bone replacement in the proximal medullary cavity up to 4.5 years:

(After 6 months): $\overline{Mv}$ and the bending strength had decreased to ~50 KDa and 200 MPa, respectively, although the rod size remained unchanged (A). The thin collagenous reactive bone was deposited sporadically on the rod surface(C), (D).

(After two years): $\overline{Mv}$ had decreased to ~10 KDa. There was no reduction in the rod size, although several large cracks were present. Thick lumps of reactive bone were macroscopically visible on the rod surface (A), (B), with many collagenous fibers (C). $\overline{Mv}$ decreased to <10 KDa and low PLLA molecules at an oligomer level were subsequently released from the composite rod.

(After 2.5 years) $\overline{Mv}$ had decreased to between 5 and 7 KDa. Many cracks were present and the rod had split into several sections (B), (D) as a result of the excessively high loads endured during shrinkage. The thickness of the reactive bone around the rod increased as the u-HA fraction increased, creating a cylinder (A), (B). The u-HA fraction had reached ~55% at this stage.

(After 3 years) Even when $\overline{Mv}$ decreased to <1 KDa, the thick tubular reactive bone (A), (D) remained covering the rod, which was highly fragile. This restricted the dispersal of the debris. (A) shows the tubular reactive bone (diameter, 3.2 mm) containing a degraded rod (diameter, ~2.7 mm). (D) shows the thick tubular reactive bone without a contracted rod.

(After 4 years) $\overline{Mv}$ decreased to levels that were too low to be measured. The release of the PLLA matrix appeared to be almost complete by this stage. The fraction of u-HA particles reached ~70% and the diameter of the rod decreased to <2.0 mm.

(After 4.5 years) $\overline{Mv}$ was no longer measurable by this stage and no residual PLLA was detected using strong solvents. This suggested that the complete degradation and resorption of PLLA had occurred. The rod itself had become quite soft and fragile with numerous pores and micro cracks, however the u-HA particles had not been totally resorbed at this stage.

(For Comparison) Particulate c-HA crystals could be macroscopically actualized on the surface of the reactive bone as a result of unknown cell reaction.

Oonishi et al. [10,11], who reported that surface-only bioactive ceramic granules [both sintered HA (s-HA) and c-HA] <10 μm in size were initially resorbed into the trabecular bone but were subsequently expelled.

The two or three dimensional μCT images and micrographs, stained with the Villanueva and Goldner methods that presented in Fig. 5, detail the processes of degradation and bioresorption towards the end of the process (5.5 years after), when the rods were extremely fragile.

3.3. Comparison of bioresorbability, bone formation and tissue reaction in F-u-HA/PLLA and F-PLLA-only devices

Fig. 2C, for comparison shows unfilled holes in F-PLLA-only rods 5.5 years after implantation into the lateral distal femoral condyle of a dog. Fig. 2B, for comparison shows a magnified HE staining of F-PLLA-only revealed the presence of osteoclast cells in the drilled holes, which were still interposed with few crystalline debris of degraded PLLA. Similar results were previously confirmed radiographically in the lateral distal femoral condyle of humans up to 9 years after implantation [12]. Total replacement of the biodegraded device with bone was not observed, and was unlikely to occur subsequently.

Our results demonstrated clear differences between F-u-HA and PLLA-only devices. Bioactive, bioresorbable u-HA particles were resorbed into the surrounding natural bone and showed strong osteoconductivity without causing physical irritation. By contrast, c-HA particles that had been released into the proximal medullary cavity were present on the outer surface of reactive bone as aggregates of the original crystalline particles ~4.5 years after implantation. This demonstrates that c-HA particles are non-resorbable surface-only bioactive bioceramics, which may cause physical irritation leading to inflammation or other complications after dispersal into the soft tissues [10,11]. The biodegradable PLLA-only devices cause only temporary tissue reaction and physical irritation during bursts of

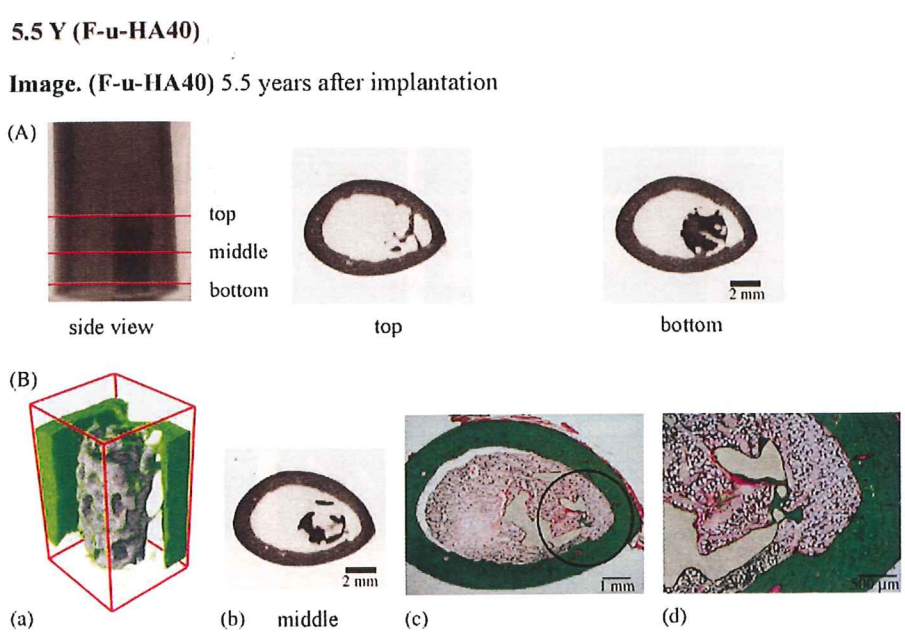


Fig. 5. Changes in two dimensional μCT images (A) and (B) (b), a three dimensional μCT images (B) (a) and Villanueva Goldner staining (B) (c, d) immediately before the complete resorption of the composite rod 5.5 years after implantation in the proximal medullary cavity. (a) A three dimensional μCT image of a composite rod. (b) A cross-sectional μCT image at the middle position in (A) of the proximal femoral cavity. (c) A magnified image of (b) stained with Villanueva Goldner. (d) A magnified image of the circled section in (c). After 5.5 years, the F-u-HA was close to the point of total resorption, particularly at the top of the rod. Osteological condition was detected in areas close to the endosteum of the medullary cavity. The remaining small aggregates of u-HA debris were subsequently resorbed into the endosteum accompanied by green staining of the osteoid tissues, which demonstrated the bioactivity of u-HA.

macro-PLLA fragments release, which are the result of irregular heterogeneous hydrolytic reactions. The longest observation in this experiment continued up until ~7.3 years (88 months) after which the last rabbit died of old age. However, it is unnecessary to examine the process from substantial bone replacement (~66 months after implantation) to naturally occurring death of old rabbits (~76–88 months after), because bone marrow cells are extensively replaced with fat cells, coinciding with a declining haematopoietic capacity, due to the final aging process.

4. Discussion

The use of non-resorbable particulate bioceramics in bone-fixation surgery can lead to undesirable tissue responses due to the release of non-resorbable debris. In contrast, our detailed investigation of the complete process of biodegradation and bioresorption clearly demonstrates the effectiveness of u-HA/PLLA composite materials for clinical use in bone-fixation devices.

4.1. Differences in hydrolytic degradation between F-PLLA-only and F-u-HA30/40 materials

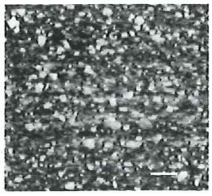
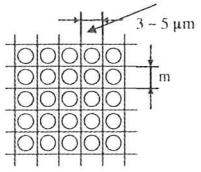
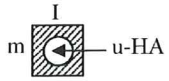
F-PLLA-only devices have the potential to release uneven PLLA fragments at irregular time intervals during the degradation process. This, in turn, can induce physical inflammatory responses, although the occurrence of this is rare.

By contrast, uniform hydrolysis occurs throughout the thin PLLA film matrices in F-u-HA/PLLA composite devices (Table 1, Fig. 2, 2Y and 3Y). This results in the steady release of small amounts of debris as the materials degrade, which does not provoke adverse tissue responses in vivo. The F-u-HA/PLLA composite structure is predicted to consist of cubes with sides of average length 5.8–7.3 μm (F-u-HA30–50) involving u-HA cubic particles averaging ~5 μm in diameter, as illustrated in Table 1. A thin PLLA film is present between the HA particles, and the thickness of which depends on the particle content. This film provides an interface with invading water molecules, which allows homogeneous hydrolysis and steady degradation of the PLLA. This results in the release of an even flow of fine debris, as illustrated in Fig. 3.

4.2. Bioactivity and bioresorbability are dependent upon implantation site

The tissue responses to u-HA/PLLA composites depend upon their bioactivity and bioresorbability. Particulate u-HA is a bioactive bioresorbable material that contains carbonate hydroxyapatite (CO_3HA).

Table 1
Theoretical structure of a PLLA matrix involving u-HA particles (Structure of a PLLA matrix subdivided by u-HA particles)

	 SEM showing dispersion of particulate u-HA in F-u-HA40 (a)		 Schematic illustration of PLLA matrix calculatedly subdivided by u-HA particles with average diameter of 3-5 μm (b)	
l and m (length)		u-HA/PLLA (vol. ratio)	u-HA penetrating capability to the opposite surface (PLLA matrix)	Thickness of PLLA cellular matrix
	7.3 μm/u-HA30	17/83	No (Continuous)	Thick
	6.4 μm/u-HA40	24/76	No (Continuous)	Thin
	5.8 μm/u-HA50	32/68	Local (Discontinuous)	Thinner

This is a theoretical reason for the uniform degradation of F-u-HA 30, 40 and 50 materials.

The osteogenic activity of bone marrow is significantly weaker than that of the circumferential periosteum and endosteum. This has a greater influence on bone formation than does the osteoconductivity of the implant. However, the type of material used, its surface properties and its anatomical location all have significant effects on bone formation and remodelling around an implant. In particular, new bone formation is highly dependent upon whether the material is in direct contact with the endosteum when it is implanted transcortically into the medullary cavity [14]. The osteoconductive behaviour of the implant can therefore alter depending on whether it is located in the distal femoral condyle or the proximal medullary cavity, and whether it is in contact with the endosteum.

The μCT images illustrated the differences in behaviour at the three implantation sites investigated. At the first site, the rod was completely resorbed and unbound u-HA particles were dispersed into the endosteum 5–6 years after implantation. At the second site, the rod showed significant contraction, and low molecular weight PLLA molecules had all been released 4–5 years after implantation; however, a rod-shaped structure consisting of a few u-HA particles was still visible after ~5.5 years (Fig. 5 image). Noticeably aggregates of c-HA particles, which initially appeared to have been absorbed, subsequently reappeared on the surface of reactive bone after ~4.5 years (Fig. 4 for comparison). Finally, at the third site, the majority of the u-HA particles were replaced with new bone after 4–5 years without provoking a foreign-body reaction (Fig. 2).

Bioactive bioresorbable u-HA/PLLA composites were capable of being replaced with new bone in the distal femoral condyle, where the implant was in direct contact with cancellous bone and new bone formation was anatomically required. In addition, these composites were totally resorbed without bone replacement in the proximal medullary cavity where bone formation was not essential. We therefore propose that F-u-HA/PLLA composites are highly effective materials for bioactive bioresorbable bone-fixation devices with the potential for total replacement with new bone.

4.3. Additional advantages of particulate u-HA

The resorption characteristics of particulate calcium phosphate (CaPs) bioceramics should be evaluated from two perspectives, both of which are particularly important in bone-replacing materials [15,16]: first, with respect to in vivo solution-mediated dissolution; and second, in terms of cell-mediated phagocytosis and/or resorption. The first process should be more important during the early stages after implantation, although cell-mediated degradation may also occur with certain CaPs. LeGeros et al. investigated the ultrastructural changes in CaPs materials in cell culture and in vivo [17,18]. The following sequence of events was observed on surfaces and at the biomaterial–bone interface: first, partial dissolution of the CaPs ceramic macro-crystals, which increased the concentrations of calcium and phosphate ions in the local environment; second, carbonate (CO_3) apatite formation, which was intimately associated with an organic matrix; and third, the incorporation of CaPs micro- and macro-crystals into the collagenous matrix during the formation of new bone in osseous defects.

CO_3HAs are among the most bioactive ceramics known [19,20]. The bioactive u-HA particles in the present study contained a relatively high proportion of

CO_3^{2-} ions (3.8 mol%) and significant bioactivity was detected at implantation sites.

α-Tri-calcium phosphate (TCP), tetra-calcium phosphate (TeCP), tetra, di-calcium phosphate anhydride (Te-DCPA), tetra, di-calcium phosphate dehydrate (Te-DCPD), octa-calcium phosphate (OCP), calcite ($CaCO_3$) and low crystalline HA particles are all classified as bioactive and bioresorbable CaPs [12]. These form carbonate apatite or amorphous CaPs and are digested by osteoclasts or macrophages [21]. When designing PLLA-containing composites of these materials, it is essential to recognise that their chemical composition, crystal structure, crystal and grain size, neck geometry, neck-dissolution rates and compactness govern their degradation characteristics and have reinforcing effects on their mechanical properties. In addition, the pH value determines the storage stability of composites. The raw HA particles as synthesised used in this study had a neutral pH (6.9) in an aqueous solution of 10 wt% u-HA. This compares with the following pH values for other bioceramic materials: c-HA, 6.6; s-HA, 11.1; OCP, 5.7; α-TCP, 8.7; β-TCP, 9.7; TeCP, 11.6; DCPA, 6.5; and DCPD, 6.9. u-HA is one of few neutral particulate bioceramics suitable for use in bioactive and bioresorbable composite products.

4.4. *u-HA particles and the composite [22]*

4.4.1. *Preparation*

u-HA particles were synthesised by hydrolysis of calcium hydrogen-phosphate anhydrate ($CaHPO_4$) in the presence of calcium carbonate ($CaCO_3$). The resulting column crystals (average diameter, ~0.2–3 μm; surface area, $2\,m^2/g$) aggregated to form secondary particles (average diameter, 5–20 μm). These crystals, used in the composites, were ground down to an average diameter of 3–5 μm. The size range of u-HA particles was suitable for triggering phagocytosis [1] and for incorporation into the PLLA matrix, to exhibit effective bioactivity and for reinforcing the composite.

4.4.2. *Chemical composition*

Hydrolysis of $CaHPO_4$ in the presence of $CaCO_3$ (DCPA + $CaCO_3$) produces A · B-type CO_3HA (A-type = 1.27%; B-type = 2.53%; B/A = 1.99). In the A-type crystal, the CO_3^{2-} ion was substituted with an OH site. In the B-type crystal, the CO_3^{2-} ion was substituted with a PO_4 site. The chemical composition was: $Ca_{9.80}(PO_4)_{5.80}(CO_3)_{0.20}(OH)_{1.00}(CO_3)_{0.40} \cdot 1.2\,H_2O$ [Ca/P = 1.69 (mol); CO_3^{2-} = 3.8%]. A significant loss of CO_3^{2-} occurred at ~800–1000 °C, resulting in the formation of c-HA ($Ca_{9.67}(PO4)_{5.80}(OH)_{1.94}$).

Impure CO_3HA exists in natural bone; tooth enamel contains 2% CO_3^{2-} and other apatite-containing tissues contain 10–20% CO_3^{2-}. Therefore, CO_3HAs should be selected on the basis of their bioactivity, bioresorbability and biocompatibility; for example, low crystalline HA is reported to be significantly more bioresorbable than the bioceramic materials TeCP and Te-DCPD [10].

4.4.3. *Dissolution rate*

Several reports have described the relative order of dissolution of CaPs under various conditions. For example, amorphous CaPs show relatively high dissolution rates when immersed in acid-buffer solution (saline). Oonishi et al. [10,11] showed that the minimum time taken for the formation of measurable precipitation in a simulated physiological solution in vitro increased as follows: low-crystalline HA < well-crystallised HA < sintered HA < α-TCP < TeCP < β-TCP. Ducheyne et al. [23,24] reported that the dissolution rate of CaPs in a calcium- and phosphate-free buffer solution at pH 7.3 increased in the following order: HA < calcium–deficient HA < β-TCP < α-TCP < TeCP. Furthermore, bioactive bioresorbable bioceramics form carbonate–apatite or amorphous CaPs, including carbonate, on particle surfaces remodelled by osteoclasts or macrophages. The slight pH decrease associated with PLLA degradation might enhance the dissolution rate of u-HA particles. However, these particles do not substantially influence the hydrolytic-reaction rate that allows the continuous steady release of PLLA particles, resulting in mild tissue reactions throughout the degradation process. Therefore, u-HA particles are highly suitable for use in bone-fixation devices from the perspective of solution-mediated dissolution.

4.5. *Bone ingrowth and replacement*

The remodelling of hard tissues involves the reorganisation and growth of HA crystals through osteoblast cells, along with resorption and metabolism. It is conceivable that the physicochemical contraction of particles by dissolution before phagocytosis is essential during bone replacement. Our results show that u-HA particles satisfy these requirements.

4.6. *Suppressing bursts of u-HA particles*

Several factors prevented the release of bursts of u-HA particles into the surrounding tissues. First, during the early stages of degradation, the high PLLA molecular matrix tightly bound the u-HA particles due to effective compression forces during forging; therefore, only u-HA particles that were exposed on the surface of the material were released. Second, even when the PLLA matrix had been degraded < 10–50 KDa, PLLA acted as a binder; it was still able to constrain the residual u-HA particles and retain the shape of the device. Third, when the PLLA matrix lost its binding force ($\overline{Mv}$; ~10 KDa) after 2.0–2.5 years, reactive bone began to form around devices in the proximal medullary

cavity, and suppressed u-HA particle dispersal. Fourth, in the distal femoral condyle, where the devices were in close contact with the cancellous bone, the direct bonds at the bone–implant interface persisted until total bone replacement had occurred. Debris from the degraded PLLA and u-HA particles was therefore confined within this space. No debris was dispersed from the perforations resulting from implantation.

The events described above allay concerns that a burst of dispersion could cause transient but severe inflammatory tissue responses.

4.7. Time course of PLLA absorption and u-HA replacement with new bone

The PLLA matrix in a composite rod (diameter, 3.2 mm; length, 3.0 cm) was completely degraded and totally absorbed in the rabbit femoral cavity after 4–5 years, by which point natural new bone had replaced the majority of the u-HA particles. This is a suitable period of time, as it allows temporary inflammation to be minimised through the gradual release of debris. It is well known that the total process varies depending upon the size of the device and the implantation site. Although the rod may be too large and too long to be totally absorbed considering the body size and the average life span of rabbits, this long-term animal experiment is reasonable for estimating the clinical suitability in humans, with a longer life span and larger body size. During the final stages of the process, when the complete disappearance of the PLLA matrix was imminent, the unbound u-HA particles conducted large amounts of new bone and deposited CaPs into the spaces formed by the release of the remaining low molecular weight PLLA molecules and u-HA particles. The radiopaque density of composite devices increased with time since implantation [25]. These findings supported the theory that replacement with new bone occurred while some u-HA particles still remained in the device.

The suitability of the time taken for the total replacement of a device with new bone should be assessed according to two criteria. First, sufficient mechanical strength should be retained to cope with external loads during the process. Suitability must therefore be judged according to the physical requirements of the region of implantation. Second, the release of debris should not provoke foreign-body reactions.

The apparent mechanical strength naturally depends on the size and thickness of a device, which in turn is dependent upon the area of bone to be fixed. The time taken for complete resorption is proportional to the thickness of the device. Generally, small composite devices intended for use in oral and cranial–facial regions (thickness, <2–3 mm) take 2–3 years or more for total resorption of PLLA [26]. By contrast, larger devices for use in knee, hip joint and lumber regions (thickness, ~4–5 mm) take 4–5 years or more. However, PLLA-only devices require longer periods (an additional 1–3 years) than composite devices for complete resorption. This is because heterogeneous hydrolytic reactions take longer to progress to the centre of PLLA-only devices, and they also tend to have a larger volume per site relative to composite devices. A 4–5 year period is appropriate in light of the phagocytic abilities of living tissues and the longevity of human life. In addition, the steady release of small amounts of debris is particularly important in cases when several large devices are used in combination to treat relatively narrow areas.

4.8. Bone replacement of plates fixed on the surface of cortical bone

From the present documentation, F-u-HA40 plates fixed on the surface of cortical bone could be expected to bury in and unified with the bone in addition to bioresorption and bone replacement. In our clinical experience, we confirmed some such plates used were impalpable and buried in ~4–5 years after implantation, and presented no noticeable foreign body reaction. These results will be reported in detail in the near future.

4.9. Potential applications of bioactive bioresorbable materials

The physical stiffness of the composite devices described here was significantly greater than that of the PLLA-only devices [27], although it was lower than that of the metallic devices currently in use. However, the bioresorbability, osteoconductive bioactivity and bone-replacement potential of composite materials are all distinct advantages. These materials are therefore suitable for use as substitutes for artificial and allograft bone, unlike bioactive ceramics, which have poor bone-replacement ability and lack flexibility.

5. Conclusions

In this report, we have documented the entire process from the implantation of bioactive bioresorbable F-u-HA/PLLA bone-fixation rods in rabbit femoral cavities through their replacement with new bone. Forged reinforced composites of u-HA particles and PLLA are highly suitable for use in bone fixation devices due to their bioactivity, bioresorbability and retention of high mechanical strength, as well as the favourable tissue responses in vivo. These composites are superior to PLLA-only materials and are used clinically in a range of surgical fields.

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