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Clinical Report

Long-Term Bioresorption of Bone Fixation Devices Made from Composites of Unsintered Hydroxyapatite Particles and Poly-L-Lactide

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Abstract: Osteosynthetic bone fixation devices made from composites of uncalcined and unsintered hydroxyapatite (u-HA) particles and poly-L-lactide (PLLA) are widely adopted for clinical use because of their bioresorbability and osteoconductive properties. However, how the plate systems constituting these devices change during long-term use *in vivo* is unknown. In this study, we present cases of two patients fitted with u-HA/PLLA devices for >5 years after surgery and evaluate the resorption process on the basis of the residual versus the resorbed material. In both cases, the majority of the degraded plates and screws had been replaced by bone. In post-operative three-dimensional (3D) CT imaging, plate and screws were maintained until two years after surgery, and then they were degraded and replaced to bone in 4-6 years after surgery. Examination of the aggregation of hydroxyapatite and decrease in molecular weight suggested that the residual material was in the final stages of resorption. The plate system examined demonstrated stable degradation without foreign body reactions in vivo. Although complete resorption is a lengthy process, it is possible to follow its progress using CT.

Key words: Resorbable plate, Unsintered hydroxyapatite/poly-L-lactide composite plate, Resorption process

Introduction

Resorbable, rigid internal fixation avoids the necessity for metal internal fixation devices in orthopedic and craniofacial, oral and maxillofacial, and plastic and reconstructive surgeries[1,2)]. The development of resorbable fixation systems has focused on resorption after successful bone fixation and ossification without foreign body reactions[2)]. However, some problems remain such as the slow degradation of high-strength poly-L-lactide (PLLA) devices[3,4)], which is much slower than that of natural cortical bone[5,6)]. Therefore, in recent years, plate systems comprising composites of uncalcined and unsintered hydroxyapatite (u-HA) particles and PLLA have been developed for clinical use. To manufacture these devices, a PLLA matrix containing hydroxyapatite (HA) is reinforced into a composite by compression molding. Raw blocks of this composite are then machined to create internal fixation devices with high mechanical strength, resorbability, bioactivity, bone-bonding capacity, and osteoconductivity[7)]. Forged composites of u-HA and PLLA (u-HA/PLLA) possess greater strength than similar materials used previously. A high u-HA content of the composite leads to faster and more homogeneous hydrolyzation than in PLLA-only devices[7)].

However, the changes that occur in these plate systems during long-term use *in vivo* are unknown. In this study, we evaluated the resorption of internal fixation devices made from u-HA/PLLA by examining the residual versus the resorbed material.

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Materials and Methods

Ethics approval for this study was obtained from the Ethics Committee of Kagawa Prefectural Central Hospital. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Case 1

In August 2009, a 60-year-old man presented with a slow-growing swelling on the median-to-left side of the mandible. He

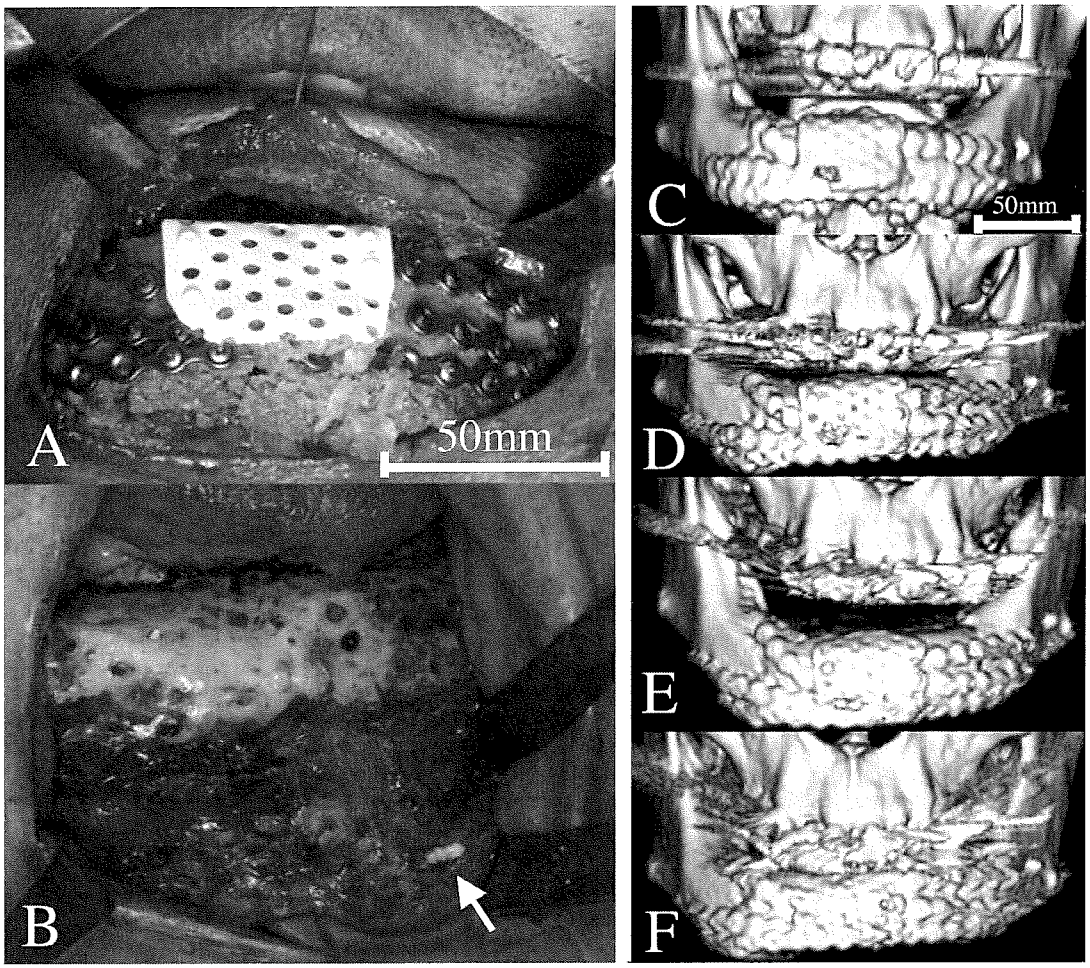

Figure 1. Case 1: Treatment of ameloblastoma: segmental mandibulectomy and mandibular reconstruction using corticocancellous bone block allografts from the iliac crest.
(A) Mandibular reconstruction was secured to an uncalcined and unsintered hydroxyapatite (u-HA)/poly-L-lactide (PLLA) composite resorbable plate and metal plates, forming a corticocancellous block.
(B) At 6 years 1 month after surgery, degradation of the u-HA/PLLA composite resorbable device was almost complete, and it had been replaced with bone except for very small and brittle residual plate and screws (arrow).
Three-dimensional computed tomography imaging.
(C) One month after surgery. (D) Two years after surgery. (E) Four years after surgery, with no remarkable changes. (F) Six years after surgery: the device was degrading rapidly.

had a history of normal pressure hydrocephalus, but the condition was under conservative observation. Panoramic radiography and computed tomography (CT) revealed soap-bubble radiolucency with sclerotic margins in the body of the mandible, approaching the lower border and extending from the median-to-left mandibular body with a pathological fracture. An incisional biopsy was performed, and ameloblastoma was suspected. In October 2009, he underwent segmental mandibulectomy and mandibular reconstruction, using corticocancellous bone block allografts derived from the iliac crest under general anesthesia. The mandibular reconstruction was secured to a u-HA/PLLA composite resorbable plate (Super FIXSORB MX®; Takiron Co., Ltd., Osaka, Japan) and metal plates with fixation screws, forming a corticocancellous block, followed by packing of corticocancellous chips and cancellous marrow around the secured blocks. The blocks were mortised into position with a butt joint to the native mandible. At a follow-up approximately 6 years after surgery, the postoperative course appeared good without recurrence or complications. In post-operative three-dimensional (3D) CT imaging, plate and screws were maintained until two year after surgery, and then they were degraded dramatically in 4-6 years after surgery. In showed 3D-CT at 6 years after surgery, degradation of the u-HA/PLLA composite resorbable device was almost complete, however, it had been shown very small and brittle residual plate and screws in contact with the mandible. Subsequently, the patient underwent occlusal reconstruction with

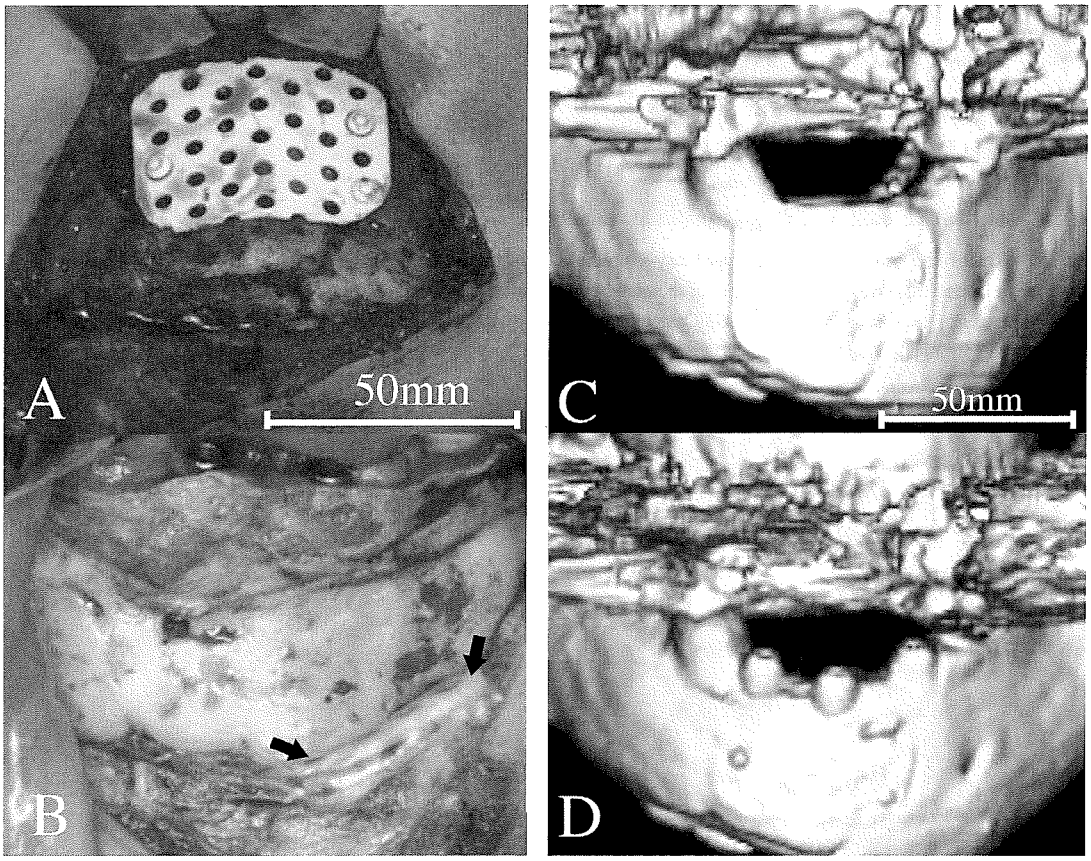

Figure 2. Case 2: Treatment of mandibular osteonecrosis: segmental mandibulectomy and mandibular reconstruction using corticocancellous bone-block allografts from the iliac crest.
(A) Mandibular reconstruction was secured to an uncalcined and unsintered hydroxyapatite (u-HA)/poly-L-lactide (PLLA) composite resorbable plate and metal plates, forming a corticocancellous block.
(B) At 5 years 3 month after surgery, the u-HA/PLLA composite resorbable device was almost completely degraded, and it had been replaced with bone except for soft remnants of the resorbable plates and screws (arrow).
Three-dimensional computed tomography imaging.
(C) Two years after surgery. (D) Five years after surgery: the device was almost completely absorbed.

dental implants under general anesthesia. At that time, we simultaneously removed the now small and brittle residual plate and screws. The u-HA/PLLA composite resorbable device was almost completely degraded and was replaced with bone (Fig. 1).

Case 2

In December 2008, a 20-year-old woman presented with failed bone healing of a complex mandibular fracture. Schizophrenia was present in her medical history; however, she had no immunodeficiency or other diseases. In May 2009, we performed resection of mandibular osteonecrosis and mandibular reconstruction, using corticocancellous bone block allografts derived from the iliac crest, under general anesthesia. The mandibular reconstruction was secured to a Super FIXSORB MX® resorbable plate and metal plates, forming a corticocancellous block, followed by packing of corticocancellous chips and cancellous marrow around the secured blocks. These materials were maintained until two years after surgery in 3D-CT. At 5 years after surgery, degradation of the u-HA/PLLA composite resorbable device was almost complete, the device almost integrated with the surrounding bones. At a follow-up, she complained of facial asymmetry in the area of the iliac bone graft. Subsequently, we performed mandibular osteoplasty. During this procedure, we simultaneously removed soft remnants of the resorbable plates and screws. Almost total degradation of the resorbable plates and screws was confirmed (Fig. 2). The removed plates and screws, and adherent tissue were subjected to evaluation on their unexposed side as detailed in the following sections.

Scanning electron microscopy

Surface of the sample was observed with a scanning electron microscope (SEM; S-2600N; Hitachi, Ltd., Tokyo, Japan).

Measurement of weight-average molecular weight

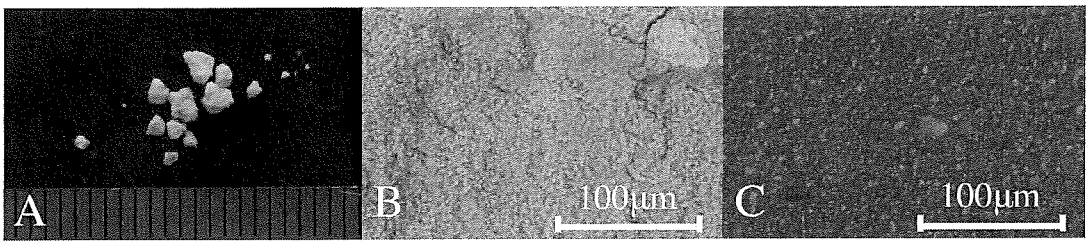

Figure 3.(A) Removed specimens were brittle and easy to crumble, demonstrated here by the specimen removed from Case 1.
(B) Scanning electron microscopy (SEM) image of the plate removed from Case 1. (C) SEM image of a new plate.
In the SEM images of the specimen removed from Case 1, a remarkable quantity of superficial hydroxyapatite is evident on the removed plate compared with the new plate.

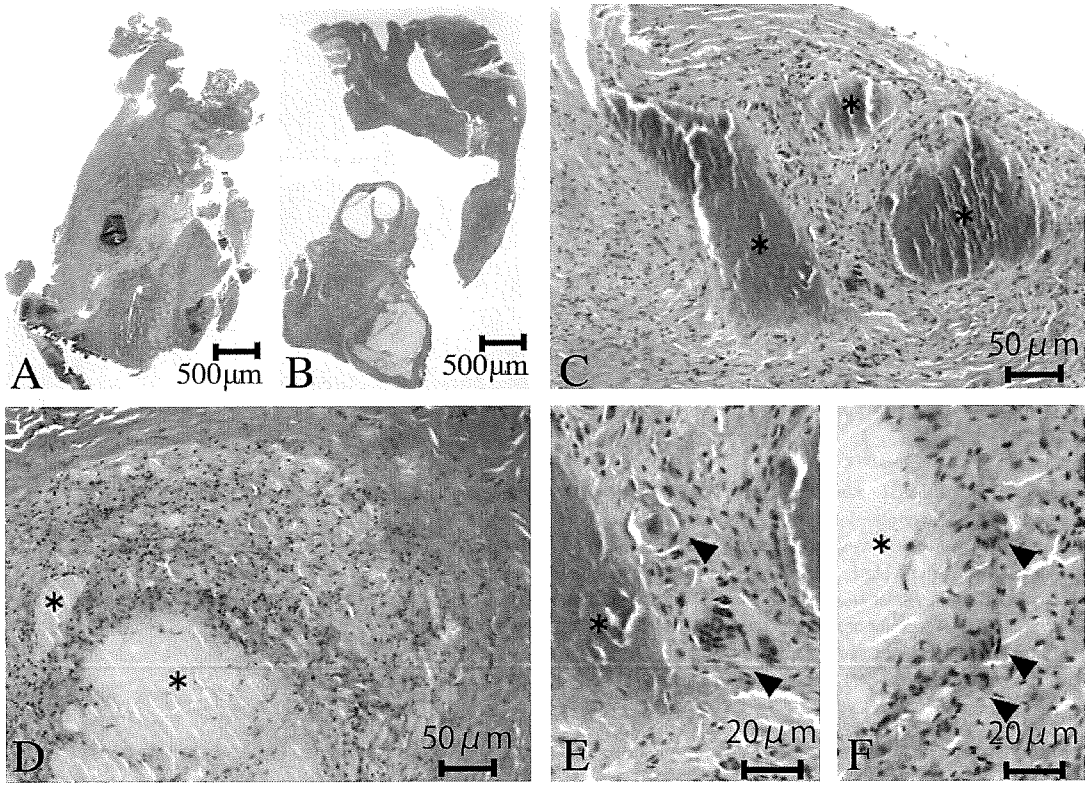

Figure 4. (A) Loupe image of Case 1 (B) Loupe image of Case 2 (C) The removed plates (–) surrounded by dense fibrous connective tissue. And inflammation of surrounded foreign bodys is mild. This situation indicates of encapsulation (Case1; HE). (D) The removed plates (–) surrounded by dense fibrous connective tissue. This situation like Case 1. And many form cells were observated in fibrous tissue (Case 2; HE). (E.F) Giant cells (arrow head) were observated around the removed plate This is evident of mild biodegradation and phagocytosis. (Case 1, Case 2; HE).

To measure the weight-average molecular weight (Mw) of the extracted plates and screws, they were placed in chloroform and PLLA was dissolved. HA was removed by filtration before measurement. Gel permeation chromatography was performed using a HLC-8320GPC EcoSEC (TOSOH Corp., Tokyo, Japan) equipped with a column of TSKgel superMulitiporeHZ-H and two columns of TSKgel superMulitiporeHZ-M (TOSOH Corp.; 3 columns being joined tandemly). Tetrahydrofuran was used as the mobile phase and the Mw was estimated using polystyrene standard (TOSOH Corp.).

Measurement of weight ratio of u-HA

The weight ratio (wt%) of u-HA was determined using a ashing method in which PLLA was volatilized in high temperature and HA was remained as ash.

weight ratio (wt%) of u-HA = weight of ash/ weight of sample × 100,

Histological evaluation

We conducted a histological examination of tissue adherent

to a plate that required removal from a patient experiencing swelling and discomfort. The tissue sample was fixed in 15 % phosphate-buffered formalin, dehydrated with a graded ethanol series, soaked in xylol, embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin (HE) according to standard procedures.

Results

Regarding Case 1, from whom the residual plate and screws were removed approximately 6 years after surgery, the Mw and weight ratio of u-HA of the plate were 3.7 kDa and 61.5 %, respectively. The Mw of the screw was 4.0 kDa. Regarding Case 2, from whom the residual plate and screws were removed approximately 5 years after surgery, the Mw and weight ratio of u-HA of the plate were 3.3 kDa and 60.2 %, respectively. In the SEM image of the residual plate and screws removed from Case 1, a remarkable quantity of superficial hydroxyapatite was observed on the plate compared with a new plate (Fig. 3). Regarding the pathological findings in both cases, the periphery of the removed plates was surrounded by fibrous connective tissue, indicative of encapsulation. Furthermore, phagocytosis of foreign particles was evident in the small number of giant cells around the removed plates, indicative of mild biodegradation (Fig. 4).

Discussion

In this study, we evaluated the long-term degradation and biotransformation of a u-HA/PLLA bioresorbable plate system. We were unable to determine the degree of degradation and resorption using X-ray imaging techniques like panoramic radiography in spite of the characteristics of resorbable plate systems, i.e.,the devices u-HA content make them radiopaque[8]. Also, they cannot be removed postoperatively to conduct a pathological inspection because they are resorbable. Observations from both *in vivo* [9-11] and *in vitro* [7,12] studies have observed that is extremely rare to have knowledge of the condition of these plate systems in clinical use in patients without complications.

In both cases examined in this study, the majority of the degraded plate and screws were ossified. However, we were able to identify the residual plate and screws in both cases. In Case 1, although some plate- and screw-like fragments remained, they were almost completely degraded. In Case 2, we observed very soft and brittle remnants with a white appearance. The Mw of the residual plates removed from both cases was only about 4 kDa. According to the manufacturer's experience in molecular weight measurement of PLLA, the Mw of 4 kDa approximately corresponds to the Mv of 1 kDa. The weight ratio of u-HA in the plates was 61.5 % in Case 1 and 60.2 % in Case 2. In an *in vivo* study involving rabbits, Shikinami et al. reported that these devices gradually decrease in size as low-Mv PLLA molecules and unbound u-HA particles are steadily released over the first 2.5 years after implantation. In their study, after 2–2.5 years, the Mvof PLLA had drastically decreased and critically, the u-HA fraction of the composite had markedly increased; the u-HA fraction exceeded 70 % within 5 years, and almost all u-HA particles were replaced with natural bone after 5.5 years without any significant foreign body reaction. The initial Mv of their u-HA/PLLA composite was about 200 kDa, and the ratio of u-HA was 40%. In our cases, degradation was advanced, and resorption appeared to be in its final stages. However, regarding the resorption process, Case 1 was decreasing dramatically from 4-6 years after surgery according to postoperative 3D-CT imaging. In the experimental conditions detailed in Shikinami et al.[11], u-HA/PLLA materials were implanted in the femoral medullary cavities of rabbits and were always in contact with the natural bone. In different situations, the interval before initial PLLA degradation may be longer.

Analysis of the debris adherent to the removed plates revealed a remarkable quantity of superficial hydroxyapatite compared with new plates. During the final stages of the process, when complete disappearance of the PLLA matrix is imminent, unbound u-HA particles conduct large amounts of new bone and CaPs into the spaces formed by the release of the remaining low-Mv PLLA molecules and u-HA particles[11]. Therefore, the radiopacity of the device was in part maintained, despite the degree of degradation[13]. These findings suggest that replacement with new bone occurs while some u-HA particles remain in the device. Indeed, despite the decomposition of the device and its replacement with new bone, the plates and screws appeared intact around the mandibular bone in 3D-CT images. This may be due to the condensation of agglomerated HA. In the middle of the resorption process, the PLLA matrix loses its binding force after approximately 2–2.5 years, causing reactive bone to form around devices and suppressing u-HA-particle dispersal. In the final stages of resorption, when devices are in close contact with the bone, direct bonds at the bone-implant interface persist until total bone replacement has occurred. Debris from degraded u-HA particles agglomerates and is confined within the bone-implant interface. These events allay concerns that a burst of dispersion could cause transient but severe inflammatory responses in neighboring tissues[11]. Bioactive, bioresorbable u-HA particles are resorbed into the surrounding natural bone and show strong osteoconductivity without causing physical irritation[11]. In particular, new bone formation is highly dependent upon direct contact between the device and the endosteum[14]. However, during maxillofacial treatments, many osteosynthesis devices are fixed to the periosteum to apply the device's surface to the bone. In these circumstances, some devices need conditions that differ from experimental conditions to undergo complete resorption. Although a less confined space than, for instance, a bone marrow cavity, degraded u-HA particles in the bone–implant interface are prevented from diffusing into and aggregating in surrounding

tissues.

Histopathological examination revealed that the mild degradation was performed by giant cells. However, degradation appeared to progress without foreign body reactions.

In this study, we evaluated the resorption of internal fixation devices made of u-HA/PLLA composite in long-term clinical use by examining the residual versus the resorbed material. The plate system examined demonstrated stable degradation without foreign body reactions in vivo. Although complete resorption is a lengthy process, it is possible to follow its progress using CT. In future, further investigation is necessary with a greater number of cases.

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