Causes of titanium release from plate and screws implanted in rabbits

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To investigate the mechanism behind the release of metal from titanium implants *in vivo*, bone-plate-screw sets consisting of pure titanium were implanted into the legs of rabbits for 48 weeks. Four groups of experiments containing control were conducted: (1) The tibia cut artificially was fixed by one set of bone plate and screws, (2) the same set was implanted separately into muscles in the leg, (3) the set was fixed on the tibia and immediately retrieved, and (4) no implantation was performed. The amounts of titanium in all tissues from knee to ankle were quantified using atomic adsorption spectrometry. The ratio of amounts of titanium detected in the groups (1), (2), and (3) was 100 : 10 : 43. No titanium was detected in the group (4). Causes of the release of titanium in the group (1) include that in the groups (2) and (3). Major causes of titanium release were surgical handling in implantation and wear and/or fretting during experimental-term for 48 weeks. Titanium was also released in the absence of wear. No morphological abnormality was observed around tissue of the implant by biopsy at post-operation week 48.

1. Introduction

Titanium and its alloys are widely used in orthopedic, dental, and other implants because of their excellent corrosion resistance and biocompatibility with high specific strength. However, titanium and other elements released from titanium implants are sometimes detected in tissues near the implants and organs [1–7] when implanted *in vivo*. Some of them are also detected in the absence of wear [4–7]. In the presence of wear, the released metallic element may exist as wear debris, oxides, hydroxides, salts, and/or complexes and some of them combine with biomolecules in living bodies. However, the chemical states of metallic elements are indistinguishable *in vivo*. The causes of the release of metallic element from implants are also unclear *in vivo*. Bone plates and screws may be mechanically damaged by wear and/or fretting during implantation, forming metal ions and debris.

In this study, bone-plate-screw sets consisting of commercially pure titanium were implanted into the legs of rabbits for 48 weeks according to an experimental plan containing four groups with control, to investigate causes of the release of titanium element from titanium implant into tissues *in vivo*. Then, titanium element in all tissues from knee to ankle in which the sets were implanted was quantified.

2. Materials and methods

2.1. Implants

Medical-grade regular and straight miniplates with four holes and four self-tapping miniscrews of commercially pure titanium (JIS H 4600, TP 340 C, Keisei Instruments, Tokyo, Japan) were used as an implant set. Surface roughness of the plate was determined as $Ra = 0.2\,\mu m$. The surface of the implant was not coated.

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2.2. Animals

Eighteen Japanese white rabbits (JW/CSK, male, three months, 2.8–3.2 kg in weight) were used. The breeding room was maintained at $22 \pm 5\,^{\circ}C$, a humidity of $50 \pm 10\%$, and a light cycle from 5:00 am to 7:00 pm. Rabbits were bred isolatedly and given 120 g of radiation-sterilized feed each day and unlimited access to water. NIH guidelines for the care and use of laboratory animals were employed.

2.3. Implantation

Implantation was conducted based on a method consisting of four groups (Fig. 1).

2.3.1. Osteotomy group (Osteotomies, n = 7)

Rabbits taken a supine position were anesthetized by isofuran inhalation. The skin of the right leg was shaved and disinfected. Then, the soft tissue and periosteum was incised and tibia was exposed aseptically. Osteosynthesis was performed according to the following process. Screw entries on the tibia were determined by each hole in a tentatively fixed plate, to maintain stable fixation of the plate and screws on tibia. After screw entries were marked, the plate was removed. Four holes were drilled using a 1.5-mm-diameter drill. The tibia osteotomy was transacted by bone saw at the proximal portion of the middle one-third. The plate was placed on the tibia again and carefully tightened to the tibia by screws, then stable fixation was confirmed. Tissues were not contaminated by titanium because the tools did not contain titanium. The operated site was washed with 500 mL of saline to remove metal wear debris due to the above manipulation. The wound was closed, and 5000 IU of procaine penicillin G was injected into the muscle on the day of surgery and post-operative day 1. Rabbits were carried back to the above breeding room after implantation. An Elizabeth collar was tied on the neck for four weeks to prevent self-eclipsing. The experiment was continued for 48 weeks.

2.3.2. Muscles group (Muscles, n = 5)

The set of plate and screws was implanted between the gastrocnemius and soleus in the right leg. The head and tail of plate or screws were separately sutured with muscular aponeurosis by nylon yarn to prevent the contact of a part of the set with other parts. The wound was washed with saline, and then closed. The subsequent process was the same as that in osteotomies group.

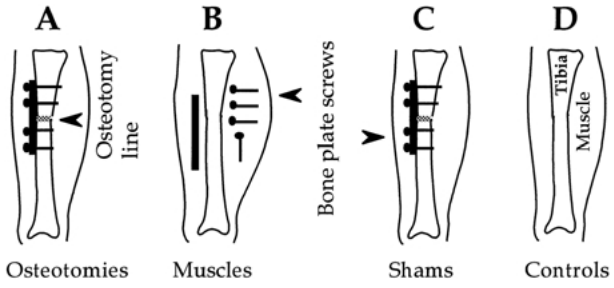

Figure 1 Implantation of plate and screws into rabbit leg. (A) The tibia was cut by saw and fixed with a plate and screws (osteotomies). (B) The plate and screws were implanted into separate muscles (muscles). (C) After surgery (A), the plate and screws were immediately retrieved (shams). (D) No implantation was made (controls).

2.3.3. Osteotomies control group (Shams, n = 6)

Manipulation of implantation in osteotomies was imitated on right tibia. Closing the wound, this implantation was completed. Then, the wound was reopened and implants were retrieved. The right leg was collected for quantitative analysis of titanium.

2.3.4. Negative control group (Controls, n = 6)

Since the left leg of shams was not treated, which was used as a negative control. Tissue for quantitative analysis was collected before shams manipulation.

One osteotomy rabbit kept through 48 weeks was applied to histological analysis. Two rabbits in osteotomies group were excluded during the experimental term, because one of tibia fissured post-operation week (POW) 1. Autophagia occurred repeatedly in the other one within 19 weeks. Therefore, the number of osteotomies for quantification of titanium was four.

2.4. Retrieval of tissues

The analysis of a small part of tissue containing large metallic debris makes a tremendous experimental error. Therefore, all tissues from knee to ankle excluded the plate-screw set and skin. The tissue for histological analysis was collected from another rabbit, which was not used for titanium quantification.

For histological analysis, hard tissue around the screw-hole and soft tissue covering the plate-screw junction were retrieved. The volume of the tissues for transmission electron microscopy (TEM) was approximately $1\,mm^3$.

To prepare the quantitative analysis sample for atomic absorption spectrophotometer (Perkin-Elmer Model 4000) equipped with a graphite furnace atomizer (Perkin-Elmer HGA-500) (GFAAS), the implant set and skin were removed from every sample. The weight of the samples was about 20 g. The samples were carefully handled and stored under $-80\,^{\circ}C$ to hinder the contamination from titanium.

2.5. Observation by the naked eyes and X-ray photography during implantation

In osteotomies and muscles, the implantation site was observed by naked eyes and X-ray photography at POW 1, 2, and 4. Continual observation was executed per four weeks after POW 4.

2.6. Visual observation

In osteotomies, the color of tissues around the implant was observed by naked eye before being stored.

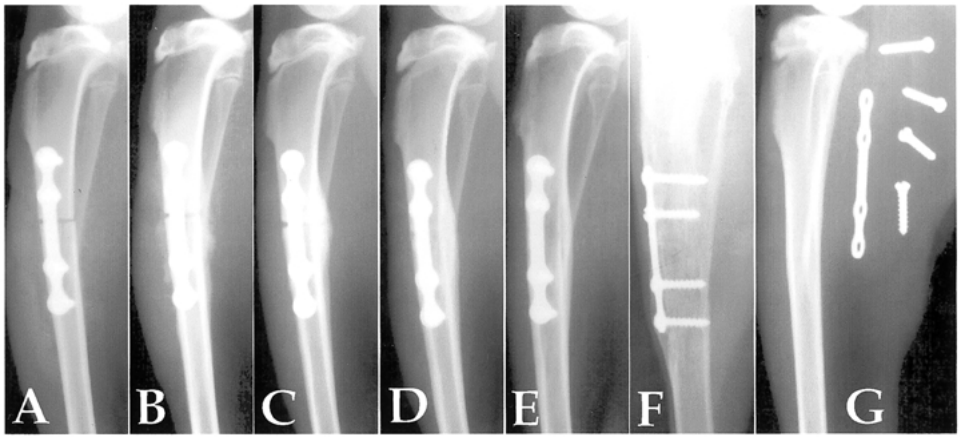

Figure 2 X-ray photographs at POW 1-48 in osteotomies (A–F) and POW 2 in muscles (G). (A) POW 1. (B) POW 2. (C) POW 4. (D) POW 8. (E) POW 24. (F) POW 48. (G) POW 2 in muscles.

2.7. Microscopic observation

To prepare light microscopy (LM) and TEM samples for histological analysis, tissues were fixed by perfusion with fixation solution from the aorta to leg. Soft tissue and decalcified or undecalcified tibias were sliced, and stained by hematoxylin eosin. The samples were histologically observed using LM and TEM. Wear debris were identified using energy-dispersive X-ray analysis (EDX). The retrieved implant sets were ultrasonically cleaned for 15 min three times, and the implant sets were stored in desiccator at room temperature. The screw holes of the plate were observed using scanning electron microscopy (SEM).

2.8. Quantification of titanium in tissues

Tissues were ashed at 600 °C in covered Vycor crucibles in a muffle furnace for 6 h. 2 mL of concentrated hydrofluoric acid was added to the ash, then 1 mL of concentrated nitric acid and 3 mL of double-distilled water were added. The mixture was heated at 60 °C for 10–20 min to dissolve the ash. The resultant sample was diluted to a final volume of 10 mL.

Titanium in the samples was quantified by GFAAS. A hollow cathode titanium lamp was used as a light source. The detection limit of titanium was 100 p.p.b. in soft tissue and 200 p.p.b. in bone. Quantification was performed in triplicate for each sample. Data were statistically analyzed with Fisher's PLSD ANOVA.

3. Results

3.1. Observation by X-ray photography

In osteotomies, an osteotomy line was observed clearly on the tibia at POW 1 (Fig. 2). Stable fixation and alignment of the plate and screws with tibia were observed during 48 weeks. External callus formed at POW 2 and maintained until POW 8. The osteotomy site was repaired as a primary healing in POW 8. The osteotomy line disappeared at POW 24. Slight bone resorption was observed under the middle plate at POW 48. In muscles, the suture of plate and screws with muscular aponeurosis proceeded well to POW 48. No abnormality in soft tissues or bone was observed during POW 48.

3.2. Visual observation

Visual observation in osteotomies at POW 48 indicated that neogenetic bone covered the plate and strongly bonded to the surface of the plate. Neogenetic bone also fused the host bone cortex. A large number of fine and

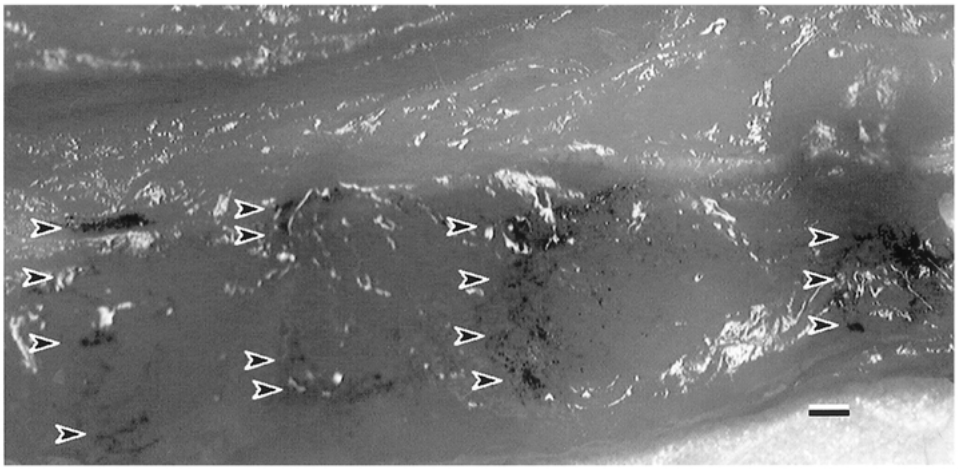

Figure 3 Soft tissue covered implants retrieved at POW 48 in osteotomies. Arrowheads show that fine debris concentrated in the soft tissue side near the plate-screw junctions. Scale bar: 1 mm.

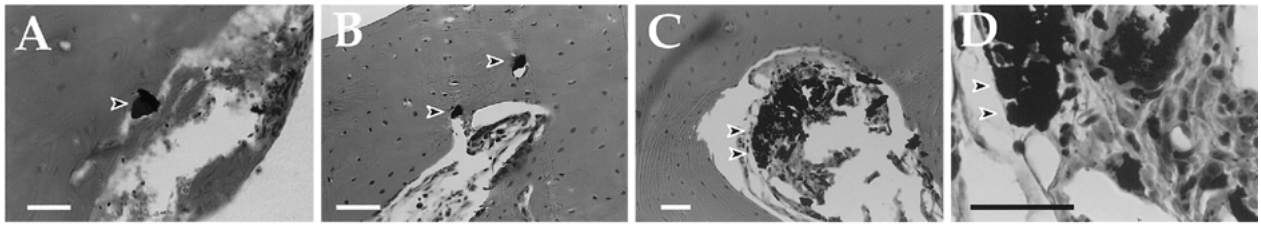

Figure 4 Pictures of tissues using light microscopy at POW 48 in osteotomies. (A) Undecalcified slice of new bone contacting the titanium plate sample. (B) and (C) Decalcified slice of new bone contacting the plate. (D) Higher magnification picture of picture (C). Arrows show wear debris. Scale bar: 60 μm.

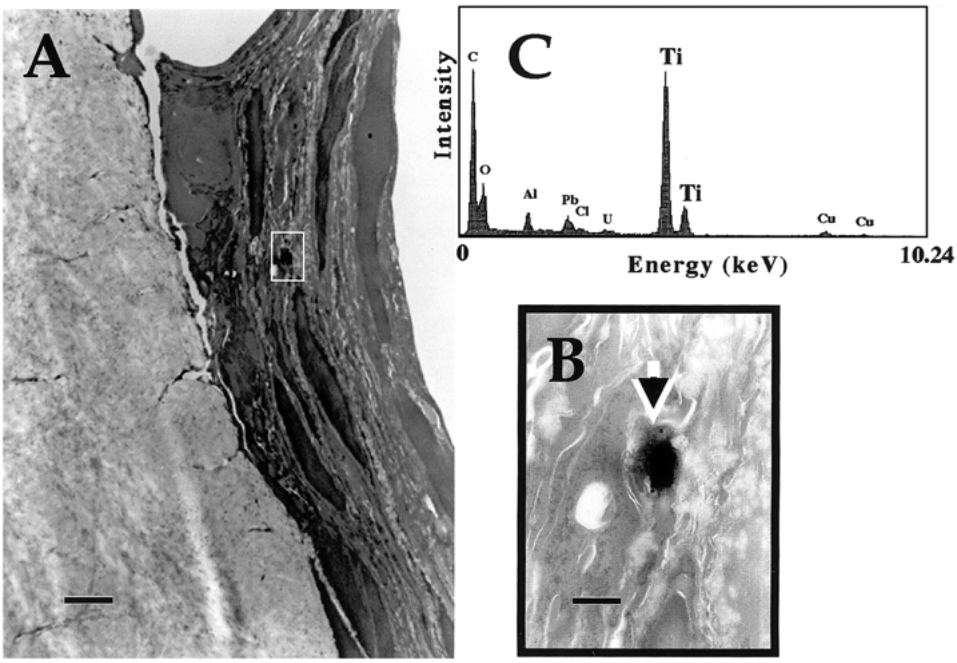

Figure 5 Transmission electron micrographs (A and B) and EDX data (C) from osteotomies. B is a higher magnification of A. The arrow in B is the site analyzed by EDX. Scale bar: 2 μm in A and 0.2 μm in B.

silver-colored debris were observed in soft tissues covering the plate-screw junction. The debris on/in soft tissues contacting the plate-screw junctions are shown in Fig. 3. The tissue contacting debris had not discolored. Implants did not loose and did not show any tarnish.

3.3. Microscopic observation

Opaque debris of 0.1–50 μm were observed in tissues near the plate-screw junction using LM (Fig. 4). No osteocyte necrosis, superfluous osteoclast, and immunocomperent cell was observed by LM in tissues near the plate-screw junction. Although many debris were locally observed in bone and soft tissue, a normal bone remodeling was confirmed. No inflammation was observed during the healing process or superfluous granulation tissue or foreign nodules.

The interface between the debris and bone matrix was unclear with TEM (Fig. 5). The debris were identified as titanium by EDX (Fig. 5). The fretting traces showed polished silver color by naked eye at screw hole of plate and inside of screw head in osteotomies at POW 48. Fig. 6 shows scanning electron micrographs of hole of the plate where is the plate-screw junction. Manufactured scratches were observed in muscles and controls (Fig. 6(A)). Rubbing and wear scratches were observed in shams (Fig. 6(B)). On the other hand, fretting trances were observed in osteotomies (Fig. 6(C)).

3.4. Quantification of titanium

Amounts of titanium in the wet tissue from knee to ankle excluded the implant set and skin in each group were shown in Fig. 7. In osteotomies, the amount of titanium was detected as 9.45 ± 1.50 p.p.m. In muscles, titanium was detected as 0.98 ± 0.47 p.p.m., 10.3% that in osteotomies. In shams, titanium was detected as 4.05 ± 1.04 p.p.m., 42.9% that in osteotomies. In controls, no titanium was detected.

4. Discussion

Good biocompatibility of titanium depends mainly on its corrosion resistance *in vivo*, induced by an insoluble oxide film as a passive film [8]. The film that consists of amorphous or low-crystalline and non-stoichiometric TiO_2 readily regenerates in aqueous solutions even if destroyed [9]. When the film is destroyed, metal ions are actively released from titanium. Titanium is detected in tissue around titanium implant [1–7], despite its high corrosion resistance, mostly due to repetitive destruction of the film by wear and fretting [1, 2].

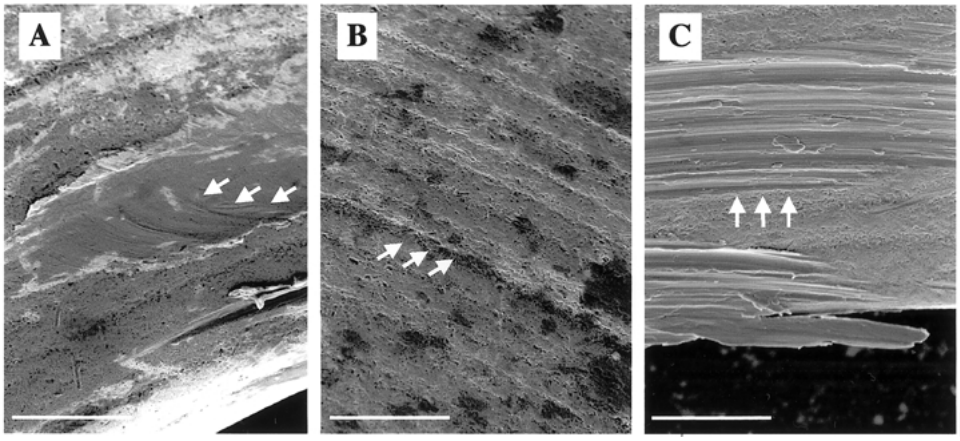

Figure 6 Scanning electron micrographs of hole of the plate where is the plate-screw junction at POW 48. Fretting trances in osteotomies (A), manufactured scratches in muscles (B), and rubbing and wear scratches in shams (C). Scale bar: 100 μm.

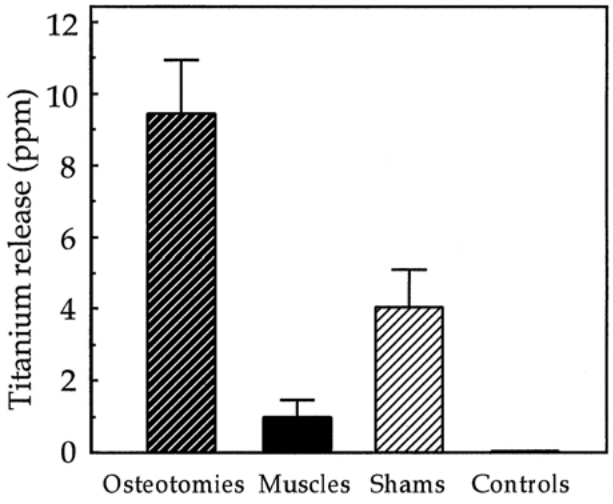

Figure 7 Amounts of titanium detected in tissues in each group.

In this study, titanium detected in tissues was apparently released from the implants because no titanium was detected in control. Even in shams, titanium was detected in soft tissue and tibia, 42.6% that in osteotomies, in spite of the careful wash of the wound with saline before closing it, clearly indicating that wear debris was formed from surgical manipulation for implantation. It is thus not possible to remove wear debris completely. This indicates that shams experiments are important as a positive control for studies on the quantification of metal release from an implant in living body.

The largest amount of titanium was detected in osteotomies. This amount includes those in shams and muscles, indicating that the main cause of metal release from implants was wear and fretting during the experimental acquisition and wear due to implantation surgery *per se*. A repetitive load due to the weight and action of rabbit was concentrated in the plate and screws during implantation. Stress generated by load was concentrated to the plate-screw junction and fretting occurred at the junction. Fretting between the plate and screws generated wear debris. The surface oxide films on plate, screw, and wear debris were thus repeatedly destroyed at each time wear debris generated, and titanium ions were released into tissues before the regeneration of the film. The contact of plates with screws may cause crevice corrosion. Titanium element released as ions and wear debris from the plate-screw-set accumulated in tissues near the set.

Titanium was detected in soft tissues and tibia near implants in muscles, 9.6% that of osteotomies, indicating that titanium is released from implants in the absence of wear and fretting due to certain biochemical factors. A recent study showed that metal ions are released from titanium with active oxygen species generated by macrophages [10]. Active oxygen species are generated by macrophage [11–13]. Active oxygen species are one of the causes of titanium release in the absence of wear and fretting.

On histological evaluation in osteotomies, a primary wound healing and bone remodeling were observed. No superfluous immunocyte invasion, foreign-body inflammation, and osteolysis were recognized despite large amounts of titanium ions and wear debris in the tissues. The maximum immunological response might occur before observation at POW 48. In other words, the damage in the healing process of soft tissue and bone due to titanium ions and wear debris were little and no sign of abnormal morphological damage was observed. Tissue reconstruction proceeded normally in defiance of titanium release in this study.

5. Conclusions

Major causes of titanium release from bone plate-screw sets implanted into the legs of rabbits for 48 weeks were surgical handling in implantation and wear and/or fretting. Wear debris are formed by surgical manipulation for implantation. The shams experiments are important as a positive control for studies on the quantification of metal release from an implant in living body. Titanium is also released from implants in the absence of wear and fretting due to certain biochemical factors.

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