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The effect of surface immobilized bisphosphonates on the fixation of hydroxyapatite-coated titanium implants in ovariectomized rats

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ABSTRACT

Immobilized bisphosphonates (BPs) have been introduced to improve implant fixation, however, no information could be found about the efficiency of this approach in osteoporotic bone. This study was designed to evaluate the bone response to surface immobilized BPs on implants inserted in tibiae of ovariectomized (OVX) rats. Three months after bilateral ovariectomy, 40 rats were randomly assigned into four groups for implantation of hydroxyapatite-coated titanium implants with or without immobilized BPs: (1) control group (without BP treatments); (2) pamidronate (PAM) group (1 mg/ml of PAM immersing); (3) ibandronate group (1 mg/ml of ibandronate immersing); and (4) zoledronic acid (ZOL) group (1 mg/ml of ZOL immersing). After implantation periods of 3 months, the peri-implant–bone density, trabecular microstructure, bone–implant interface and mechanical fixation of implants were evaluated by dual energy X-ray absorptiometry, micro-computed tomography, histology and push-out test. We found that three BPs triggered pronounced bone–implant integration and early bone formation around implants in OVX rats, with a rank order of ZOL > ibandronate > PAM. These results provide new evidence that immobilized BPs have positive effects on implant fixation in osteoporotic bone, in addition to their well-documented potency to inhibit implant loosening in normal bone.

1. Introduction

Hydroxyapatite (HA)-coated titanium (Ti) implants have been widely used in orthopaedic and dental applications due to their favorable biocompatibility and osteoconductivity. It has been demonstrated that both Ti and HA are bioactive materials, surface modification of Ti implants with HA may increase the peri-implant bone formation, bone mineralization, and the mechanical stability of the implants [1]. However, implantation of such implants leads to damage of the bone matrix, followed by an inflammatory response which in turn induces implant loosening as a biological consequence of particulate debris [2]. In osteoporotic conditions, estrogen deficiency up-regulates osteoclastic activity, and this osteolysis phenomenon is further intensified [3]. Thereafter, it is desirable to find useful interventions to promote the initial stability and long-term survival of implants, not only for the normal patients but also for the patients suffering from osteoporosis. One of these approaches is the application of bisphosphonates (BPs) as possible candidates for inhibition of peri-prosthetic osteolysis.

BPs have a high affinity for both natural and synthetic HAs, and their powerful anti-resorptive effects in osteoporosis were recognized through directly blocking osteoclastic proliferation and activity, or indirectly acting on osteoclasts via osteoblasts [4]. With a basic P–C–P structure but variable R1 and R2 lateral chains, BPs allow many possible variations: R1 in charge of their binding ability to the bone mineral, while R2 responsible for their anti-osteoclastic properties [5]. Systemic use of BPs has been shown to improve implant fixation by inhibiting particle-induced osteolysis [6,7]. However, considering the undesirable effects such as gastrointestinal ulceration and osteonecrosis of the jaw [8,9], local application of BPs, directly targeting of the location where osteoclast action needs to be controlled, seems more effective [10]. In particular, chemisorption of ibandronate, pamidronate (PAM) and zoledronic acid (ZOL) on the surface of HA from an aqueous solution has been studied [11–14], the method of immobilization of BPs onto HA-coated Ti implants is expected to improve implant fixation through the local action of BPs in vivo. However, similar effects of these BPs in osteoporotic conditions have so far attracted negligible attention, and no information could be found about the comparison among various BPs in their ability to achieve evident increase in implant fixation.

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For a comprehensive investigation of BP adsorptions onto HA, this study was designed to evaluate the bone response to Ti implants coated with HA and followed by immobilization of BPs ibandronate, PAM and ZOL, implanted in tibiae of ovariectomized rats. The bone density distribution around implants and the mechanical fixation of implants were separately assessed by dual energy X-ray absorptiometry (DXA), histology, micro-computed tomography (μCT) and push-out tests.

2. Materials and methods

2.1. Preparation of implants

Eighty custom titanium cylinders, measuring 1 mm in diameter and 10 mm in length, were used as the substrate materials for modification. They were grit blasted with aluminium oxide to produce a rough surface for HA coating adhesion; the average roughness was 5 μm. Then, these titanium cylinders were plasma sprayed with HA using an METCO MN Plasma System and an AR-2000 Thermal Spray Robot (Metco, USA). The average thickness of the HA coating was 50 μm, and crystallinity was 80%. Fig. 1 shows the surface morphology of the HA sample. Finally, the surface-modified implants were divided into four groups for the following procedures:

(1) Control group: immersing implants in distilled water.
(2) PAM group: immersing implants in 1 mg/ml of PAM (Novartis Pharma AG, Switzerland).
(3) Ibandronate group: immersing implants in 1 mg/ml of ibandronate (Roche Diagnostics, Mannheim, Germany).
(4) ZOL group: immersing implants in 1 mg/ml of ZOL (Novartis Pharma AG, Switzerland).

BP immobilizations were conducted at 37 °C for 24 h. Finally, the implants were cleaned with distilled water and sterilized in an autoclave.

2.2. In vitro release testing

The implants ($n = 5$/group) treated with PAM, ibandronate and ZOL were rinsed in 1 ml of eluting agent (adjusted to pH 7.0). After 24 h, the solutions were collected, and the concentrations of BPs were tested by reverse phase liquid chromatography at 220 nm [15], using a Waters 1525 binary HPLC pump (Waters Co., USA), a Rheodyne (Cotati, CA, USA) model 7725i injection valve fitted with a 20 μl loop, and a Waters 2487 dual λ absorbance detector (Waters Co., USA). The three BPs in the samples were separated on a Phenomenex C_{18} column (USA) at 25 °C using a mixture of acetonitrile and *n*-amylamine, and the flow-rate was set as 1 ml/min. Such procedures were performed once a day over 21 days.

2.3. Experimenal design and implantation procedure

The animal study was approved by the Animal Care Committee of Sichuan University. Forty female Sprague–Dawley rats, at the weight of 190–210 g, were bred individually in cages for this experiment. The rats were kept staying in identical environments and receiving standard rodent diets. After 10 days of acclimatization period, all rats received bilateral ovariectomization (OVX). Bred for 3 months after OVX, they were randomly assigned into four groups to undergo the following implantation. The implants were inserted into the position approximately beneath the growth plate of both tibiae as shown in Fig. 1.

The rats were anesthetized by intraperitoneal injections of 10% chloral hydrate (3.3 ml/kg), and the implantation was conducted under aseptic conditions via a longitudinal medial incision above the knee. Both limbs of the same animal received two implants immobilized by the same BP. First, a channel was made through the intercondylar eminence into the medullary canal. Next, the implants were inserted into the channels. Finally, the skin was sutured.

The rats were kept on rearing for another 3 months and sacrificed. Tibiae were harvested, and surface soft tissues were cleaned off prior to a series of structural and functional measurements. For each rat, one tibia was used for histological examination, and the other tibia was used for DXA, micro-CT and biomechanical testing (all in a blinded manner).

2.4. Bone mineral density (BMD) measurement

DXA (Lunar PIXImus, GE Medical Systems, WI, USA) was used for BMD measurement, with a small animal computer software. The tibiae ($n = 10$/group) were placed in identical positions, immersed in the water of 2 cm deep for scan. The region of interest was defined as the peri-prosthetic zone. Following scanning, the values of bone mineral content (BMC) and area of tibiae and implants were separately obtained, and then the peri-implant BMD, BMC and area were calculated.

2.5. Histomorphometry

The cleaned proximal tibia specimens ($n = 10$/group) were trimmed, dehydrated progressively in increasing concentrations of alcohol solutions (70%, 80%, 90%, 95%, 100%), then embedded in methylmethacrylate. Hard tissue sections of 100 μm thickness were cut using an Leica SP1600 microtome (Leica, Germany), and the ones at approximate 3.0 mm below the growth plate were stained in 1% toluidine blue. Histomorphometry was performed to quantify the direct bone contact of bone–implant interface and peri-prosthetic new bone area using an Leica DMI 6000B micro-systems.

2.6. Micro-CT analysis

Bone–implant interface and trabecular microarchitecture at the proximal tibiae were monitored by a micro-CT 80 scanner (Scanco Medical, Bassersdorf, Switzerland). The tibiae with implants ($n = 10$/group) were positioned into in a custom jig with water to be scanned crania-caudally, at 70 kV and 114 mA [16]. The obtained 1000 binary images with a resolution of 2048×2048 pixels and an isotropic voxel size of 10 μm were reconstructed to 3D image for qualitative and quantitative evaluations ($\sigma = 1.2$, support = 1, threshold for bone = 205, and threshold for implant = 700). The thresholds in this analysis system were set as values adequate to separate bone and implant by using discrimination analysis. The volume of interest (VOI) was defined as the 100 slices from 3.0 mm below the growth plate and limited in a ring from implant axis with a diameter of 2.0 mm. The following morphometric indices were directly analyzed from the binarized VOI: bone volume ratio (BV/TV), connectivity density (Conn.D), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and trabecular number (Tb.N). In addition, the %OI was calculated to assess the bone–implant interface details, defined as the ratio of bone voxels to total voxels in direct contact with the implant.

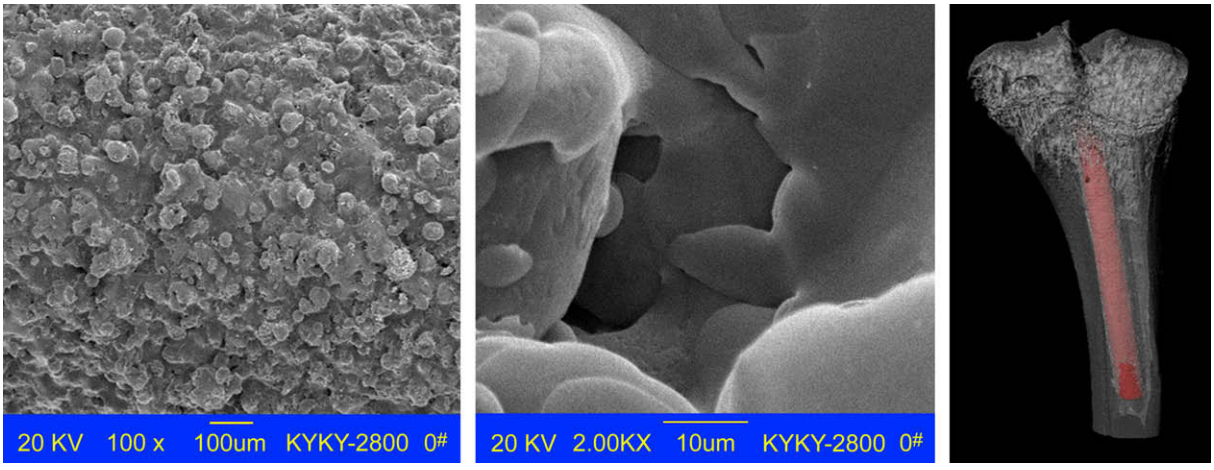

Fig. 1. HA-coated Ti implant: scanning electron micrographs of plasma sprayed HA, and micro-CT image of an implant in tibia.