TOF-SIMS study of bone mineralization on alkali-treated Ti alloy

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Titanum and titanum alloys have become the most popular metallic materials for orthopedic implants due to their biocompatibility, excellent corrosion resistance, good mechanical properties and low density. The osseointegration of titanum has been a main research focus. Kokubo *et al.* [1–4] invented an alkaline treatment on titanum surfaces to promote bone-like formation and osteoconduction. Although the mechanism of inducing apatite formation on alkali-treated surfaces has been proposed [2], bone mineralization on alkali-treated titanum is still not well understood. The objective of this study is to investigate the chemical composition of new bone formed on the alkali-treated titanum surfaces using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) in order to achieve better understanding of bone mineralization on the alkali-treated titanum surfaces. ToF-SIMS can detect not only chemical composition but also chemical states with high sensitivity and high spatial resolution [5].

The titanum material used in the present study was Ti6Al4V alloy (Baoji Special Iron and Steel Co. Ltd., China). Alkali treatment was performed by immersing the specimens in 100 mL of 5 M NaOH aqueous solution at 60 °C for 24 hrs. After the alkali treatment, the specimens were gently washed with distilled water and dried at 40 °C for 24 hrs in an air atmosphere. Then alkali-treated specimens were then placed on an alumina boat heated to 600 °C at a rate of 5 °C/min for 1 hr and cooled to room temperature in the furnace. Then, the as-prepared specimens were implanted into the distal femur of a dog and fixed on the cortical bone. The detailed surgical operation is described elsewhere [6]. After two months of implantation, the dog was sacrificed retrieve the specimens. After fixing for histological observation, the specimens were embedded in polymerized methyl methacrylate (PMMA) and cut into 250-μm thick sections. The specimens were further ground and polished to 100 μm thickness with a grinding and polishing system (RotoPol-21, Struers A/S, Denmark). The sections were stained with toluidine-blue. Histological evaluations were performed using a light microscope (DM RXA2, Leica Microsystems, Germany).

ToF-SIMS measurements were performed on a PHI 7200 (Physical Electronics, USA) spectrometer equipped with two ion guns (Cs^+ for high mass-resolution spectroscopy and $^{69}Ga^+$ for spatially resolved imaging) and a reflection analyzer. The SIMS mapping was acquired in both positive and negative ion modes using a 25 keV Ga^+ primary source. To obtain high spatial resolution mapping and to control the surface charging, an ion pulse length of 50 ns was used. SIMS images were generated by collecting a mass spectrum at every pixel (400 μm $\times$ 400 μm) as the primary ion beam was rastered across the sample surface. Before starting to collect chemical information, the surface was bombarded using a Ga^+ DC beam (1.5 nA current) for 20 min in order to eliminate surface contamination during the process of specimen preparation.

Histological examinations showed direct bone apposition on the implant surface (Fig. 1). The toluidine-blue staining shows that new bone formed a direct bonding with the alkali-treated titanum surface in the medullary cavity of the dog after the two-month implantation. The results of the ToF-SIMS mapping indicate changes in chemical composition in the region of the bone/Ti interface. Fig. 2 is an image of the integrated secondary ions in an area of the new bone/Ti interface. Spatial distributions of various ions near the interface are revealed in Fig. 3. The Ti and Ca ion distributions clearly indicate the bone boundaries, showing no artifacts in the mapping images, such as ion smearing during sample polishing. The mapping shows a certain degree of P deficiency in the interface while Ca is uniformly distributed

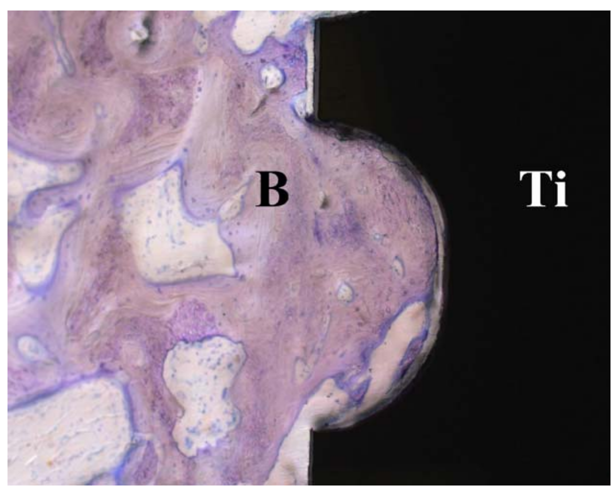

Figure 1 Optical microscopy photograph after toluidine-blue surface staining (original magnification = ×100). Newly-formed trabecular bone was observed on the surface of alkali-treated surface. B, bone; Ti, Ti6Al4V.

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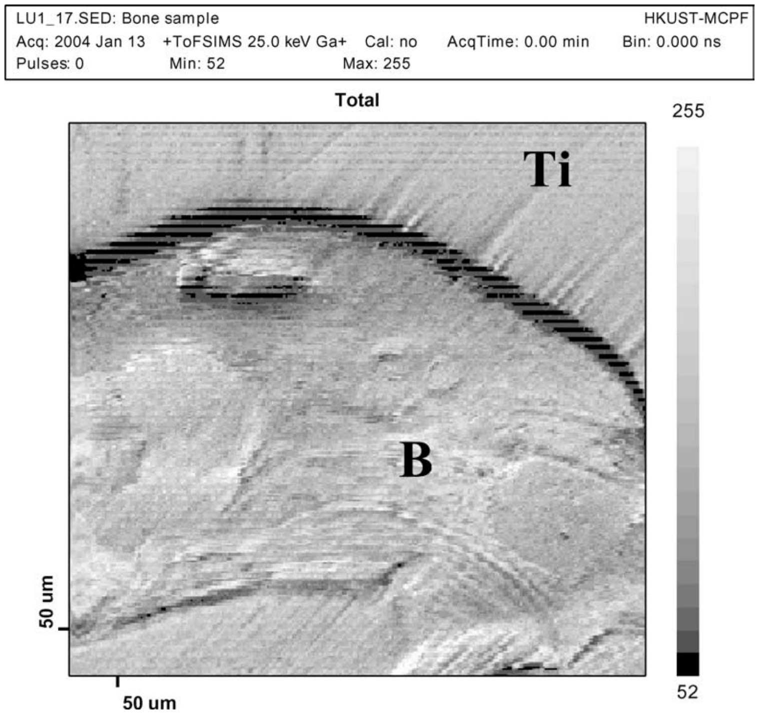

Figure 2 The integrated secondary ion image. B, bone; Ti, Ti6Al4V.

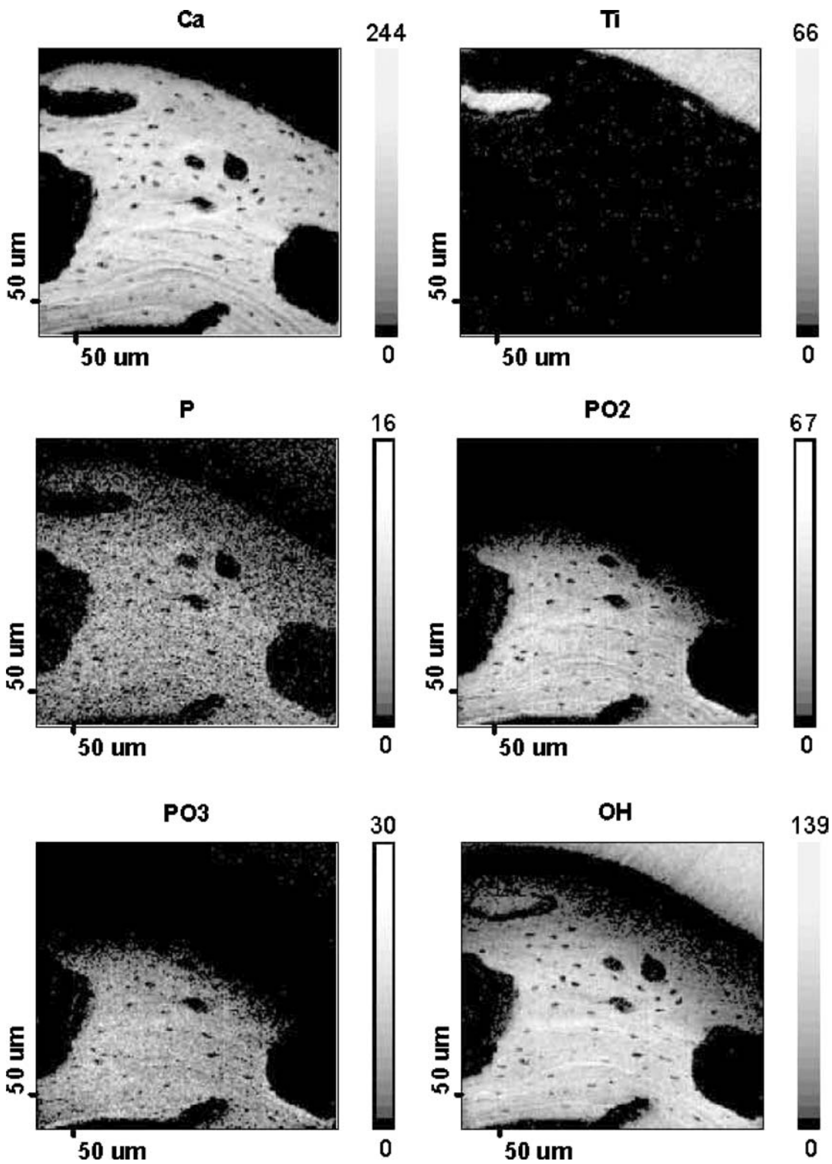

Figure 3 The ion distribution at the bone/implant interface.

in the new bone. Lodding *et al.* [7] used SIMS to examine the interface between non-alkali-treated Ti and rabbit bone after one-year of implantation. They found that calcified bone did not extend all the way to the metal implant, and that both Ca and P were deficient in the interface zone. Apparently, our results indicate that the Ca deposition rate was faster than that of P on the alkali-treated titanum surface. This finding is consistent with the report by Yang *et al.* [8] in which they describe that calcium ions deposited initially on the alkali-treated Ti surface in a calcium phosphate solution.

SIMS mapping of ionic groups provides additional information on bone mineralization. As shown in Fig. 3, the ion intensity of OH, PO_2 and PO_3 also decreases with distance from the interface and reaches its minimum at the interface of the bone/implant. Note that PO_2 and PO_3 are fragments of PO_4 in SIMS of calcium phosphates, and thus their distribution represents that of PO_4 [9, 10]. The mapping images shown in Fig. 3 indicate there are lower concentrations of PO_4 and OH near the interface. Note that these ion groups are the constituents of hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), the main mineral component of bone. PO_4 and OH deficiency near the interface area implies either existence of an intermediate mineral phase other than HA, or existence of the HA with a high degree of crystal imperfections. This phenomenon in the new bone formed on the alkali-treated titanum has not been reported before, and its effects on bone mineralization process need to be further explored.

Acknowledgments
The project was financially supported by the Research Grant Council of Hong Kong (HKUST 6037/02E). The authors express their gratitude to Prof Xingdong Zhang of the Engineering Research Center in Biomaterials, Sichuan University, Chengdu, China, for animal experiments and Dr. Qin L, at Department of Orthopaedics and Traumatology, the Chinese University of Hong Kong, China, for histological examinations.

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Received 16 March
and accepted 6 May 2004