Title

Influence of bioactive material-applied dental implant surfaces on early healing and osseointegration of bone

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Abstract

The dental implant surface type is one of six key factors that determine the long-term clinical success of implant restoration. Implant surfaces used to consist of bio-inert titanium oxide but are now bio-active surfaces due to coats of materials like calcium phosphate. Bio-active surfaces are known to significantly improve the healing time of the human bone around the inserted dental implant. Here, we characterized various modified implant surfaces by scanning electron microscopy, energy dispersive spectrometry, and surface roughness testing. Their effect on early bone healing was then tested by using the rabbit tibia model to measure removal torque values and bone-to-implant contact ratios. The modified surfaces differed in topography, composition and roughness, but induced similar favorable early bone responses in terms of the early functioning and healing of dental implants. *In vivo* methods that can more sensitively detect clinical differences between various modified surfaces should be developed.

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I. INTRODUCTION

Dental implants are an excellent treatment option for restoring areas that are missing a tooth. Implants were originally made with commercially pure (CP) titanium, which yields a strictly bio-inert titanium oxide (TiO_2) surface. However, it takes a long time (3~6 months) before this implant becomes biologically attached to the bone. As a result, various surface modifications have been introduced to improve the speed with which bone attaches to the implant surface [1-3]. One of those is calcium phosphate ceramic coating, which changes the bio-inert TiO_2 surface into a bio-active surface [4].

Indeed, plasma-sprayed hydroxyapatite (HA) coating, which is another form of calcium phosphate ceramic coating, results in more rapid bone deposition onto the implant surface compared to the TiO_2 surface [5]. However, HA coating is associated with a number of problems, including delamination of the coat, cohesion and adhesion failures, and disintegration with the formation of particulate debris; these problems may be due to the porosity and thickness of the coat, weak interfacial bonding, and the resistance of HA particles to biodegradation [5]. It has also been reported that HA particles may activate osteoclasts, thereby promoting bone resorption [6].

It has been suggested that calcium metaphosphate (CMP) may be a good bone substitute because of its good osteoconductivity and adequate biodegradable properties [7-9]. The dip-and-spin coating technique results in a thin CMP coat of about 1 μm that is associated with a more rapid bone response than the TiO_2 surface [7, 8]. While the CMP coat is considered to overcome the disadvantages of the HA coat [7], few studies have compared the CMP-coated surface to the HA-coated surface directly [10, 11].

The purpose of the present study was to characterize the CMP- and HA-coated surface properties and

to investigate the early bone response to both coated surfaces by an animal experiment.

II. EXPERIMENTS

Screw-shaped dental implants that were 8 mm in length and 3.75 mm in diameter were made from CP titanium and coated with CMP. For this, a CMP sol was prepared by reacting correct amounts of $Ca(NO_3)_2 \cdot 4H_2O$ with $(OC_2H_5)_3P$ in methyl alcohol to obtain a stoichiometric Ca/P ratio of 0.5. The viscosity of the sol was 5-6 cP. After the implant surface was grit-blasted with 300-600 μm HA powder for 10 s, it was coated with the prepared CMP sol by dip-and-spin coating at 8000 rpm for 40 s. The CMP sol-coated implants were then immediately dried at 70°C and heat-treated at 650°C for 5 h in a high vacuum furnace. X-ray diffraction analysis revealed the material phase was δ-CMP (JCPDS No. 09-0363). Control implants were coated with HA by the plasma-spraying coating method, which is most commonly used for clinical applications [5]. The plasma-spray method has been described previously [5, 12-14].

The coated surfaces were characterized as follows. Scanning electron microscopy (SEM, JSM-6480LV, JEOL, Japan) was performed to obtain images of the overall surfaces. Energy dispersive spectrometry (EDS, INCA Energy for JSM-6480LV, Oxford, England) was performed to investigate the compositions of the coated layers. Surface roughness testing (Surface test SV-3000, Mitutoyo, Japan) was performed to measure R_a, which is defined as the arithmetic mean of the departure of the profile from the mean profile.

An animal experiment was performed to evaluate the early bone response to the coated surfaces. This experiment was approved by the Animal Research Committee of Seoul National University Bundang Hospital

(approval number: BA0906-045-026-01). Eighteen mature New Zealand white rabbits weighing 2.5-3.5 kg were implanted with a CMP-coated implant in one tibia and an HA-coated in the other as described previously [7, 8]. To assess bone attachment to the implant surfaces, eight rabbits were sacrificed by an intravenous administration of KCl after 2 (n = 4) and 6 (n = 4) weeks of healing, the implants were removed, and the bone-to-implant contact (BIC) ratios were measured. For this, each implant was surgically removed *en bloc* with an adjacent bone collar, immediately fixed in 4% neutral formaldehyde, and embedded in light-curing resin. Un-decalcified, cut, and ground sections were prepared by using the Exakt® system (Exakt Apparatebau, Norderstedt, Germany) based on a method described by Donath *et al* [15]. The sections were ground to an approximate thickness of 30 μm and stained with hematoxylin and eosin. The specimens were examined by using a light microscope (Olympus BX microscope, Olympus, Tokyo, Japan) at 100× magnification. BIC ratios were measured by image analysis software (Kappa PS30C Imagebase, Kappa Opto-electronics GmbH, Gleichen, Germany). The remaining ten rabbits were sacrificed after 2 (n = 5) and 6 (n = 5) weeks of healing to measure the removal torque values (RTVs). For this, the installed implants were exposed and an implant mount was securely engaged to the implant. The mount was firmly grabbed by the jaws of a removal torque (RT) tester (Model MGT50, Mark-10 Corporation, Hicksville, NY) and reverse torque was applied. The peak torque that initiated reverse rotation was measured. Nonparametric Wilcoxon's signed rank test was used to test the statistical significance of differences between the CMP- and HA-coated surfaces in terms of BIC ratios and RTVs. P values less than 0.05 were considered to be statistically significant.

III. RESULTS AND DISCUSSION

Figure 1 shows the SEM images of the CMP- and HA-coated surfaces. Both surfaces displayed many irregularities like depressions and small indentations. Fine and homogeneous grains of coating were found on the CMP-coated surface (Figure 1(a)) while HA particles that were not homogeneous in size and distribution were observed on the HA-coated surface (Figure 1(b)). Such lack of homogeneity may explain why HA surfaces release particulate debris that activates bone resorption.

Table 1 shows the composition of both coated surfaces, as determined by EDS. Titanium was not detected on the HA-coated surface because of the thick HA-coating layer. The Ca/P ratio of the CMP-coated surface (0.58 ± 0.01) was lower than that of the HA-coated one (1.76 ± 0.09). Although it is generally accepted that, to ensure predictable implant performance, the chemical purity of HA should be as high as possible with a Ca/P ratio of 1.67, low Ca/P ratios such as that of the CMP coating in this study have been reported to have a high initial affinity for positive ions like Ca^{2+} and Mg^{2+}, which promotes bone deposition [5, 15].

The means and standard deviations (SDs) of R_a for the CMP- and HA-coated surfaces were 1.16 μm ± 0.04 μm and 3.33 μm ± 0.80 μm, respectively. Since the optimum roughness value for bone attachment is about 1.5 μm [16-19], the roughness of the CMP coating was estimated to induce a bone response better than that of the HA coating in this investigation.

The BIC ratio means and SDs 2 weeks after implantation were 25.2% ± 1.9% for the CMP-coated implants and 25.3% ± 2.4% for the HA-coated implants. After 6 weeks of healing, the BIC ratios for the CMP- and HA-coated implants were 53.8% ± 4.4% and 54.2% ± 5.0 %, respectively. No significant differences were

found between the groups after either 2 or 6 weeks of healing ($p>0.05$). Microscopically, a discrete coating layer was not observed on the slides of the CMP-coated implants, whereas an HA-coating layer that was about 50-100 μm thick and had multiple porosities was found on the HA-coated implant sections (Figure 2). This may relate to the fact that the dip-and-spin technique produces a thin CMP coating of about 1 μm [7], whereas plasma spraying yields a thicker and more porous HA coating layer that could be prone to coating delamination and the release of coat segments [5]. Such cohesion failure could generate isolated HA particles that could promote osteolysis and implant failure if the particles are not properly resorbed [5]. Making a thin coating layer is likely to overcome those disadvantages.

The mean resistance to RT for the CMP-coated implants at 2 and 6 weeks was 11.9 Ncm ± 1.6 Ncm and 32.4 Ncm ± 4.4 Ncm, respectively. The mean RTVs for the HA-coated ones were 12.7 Ncm ± 2.4 Ncm at 2 weeks and 33.4 Ncm ± 4.1 Ncm at 6 weeks. There were no significant differences between the groups in terms of the RTVs ($p>0.05$).

Thus, the animal experiment did not reveal any significant differences in the bone attachment parameters (BIC and RTV) despite the fact that the two surfaces differed from each other in terms of surface properties. A previous study comparing three types of modified implant surface (CMP-coated, anodized, and blasted surfaces) also did not find that they differed significantly in terms of the animal test result, although the modified surfaces induced superior initial bone responses relative to the effect of an unmodified implant surface [8]. The animal experiment used in this study may not be sensitive enough to determine the clinical influence of various implant surfaces that differ in their physical or chemical properties. A more sensitive method should be

developed.

IV. CONCLUSIONS

In this study, the CMP- and HA-coated implants induced similar early bone responses even though they differed in their surface characteristics, namely their topography, composition, and roughness. However, the thin CMP coating layer may be superior to the thick HA coating layer, which is associated with several disadvantages. A more sensitive method that reveals the different clinical effects of various modified implant surfaces should be developed.

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Table captions

Table 1. Composition of the CMP- and HA-coated surfaces as measured by EDS.

Tables

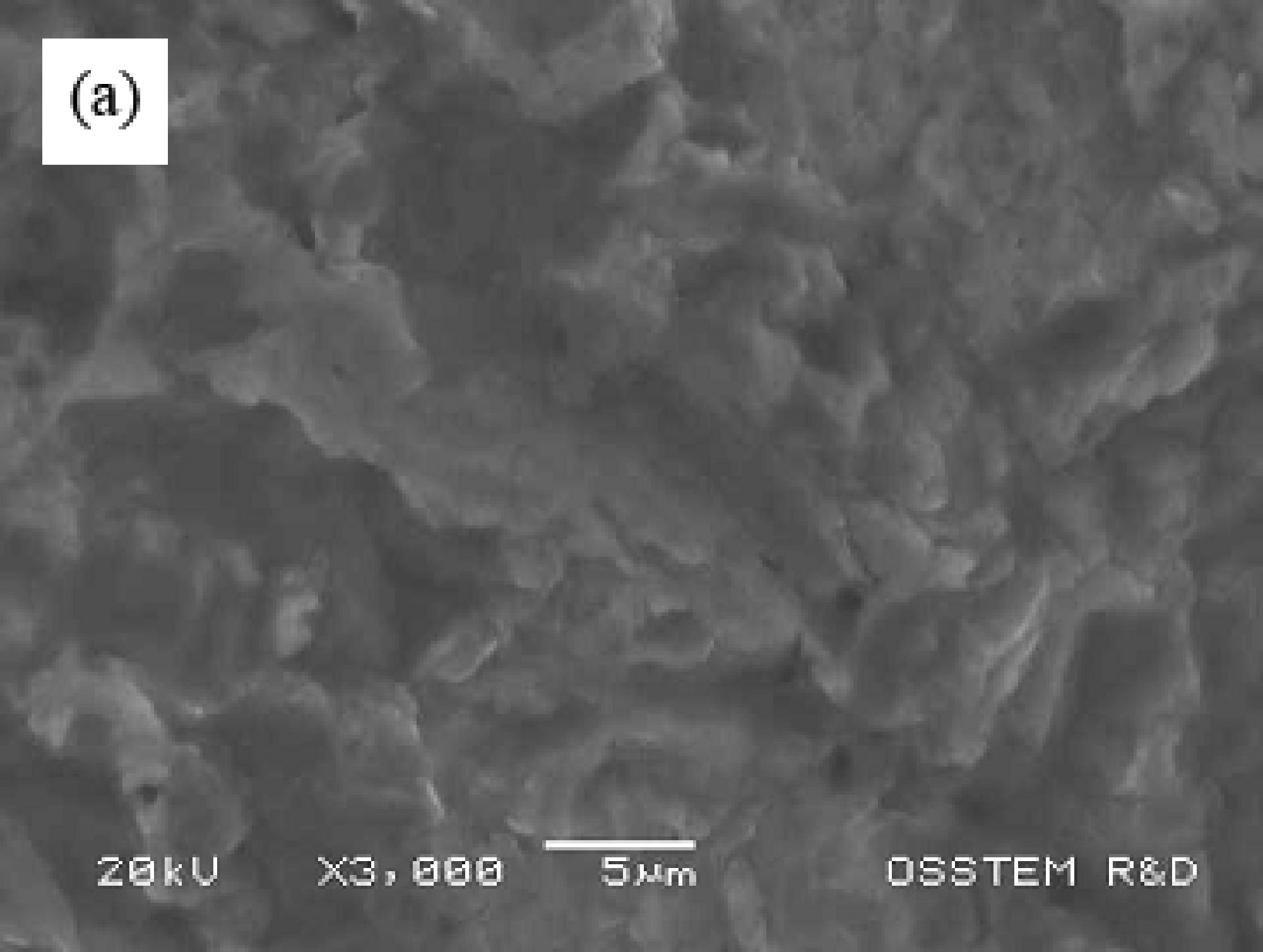
Table 1.

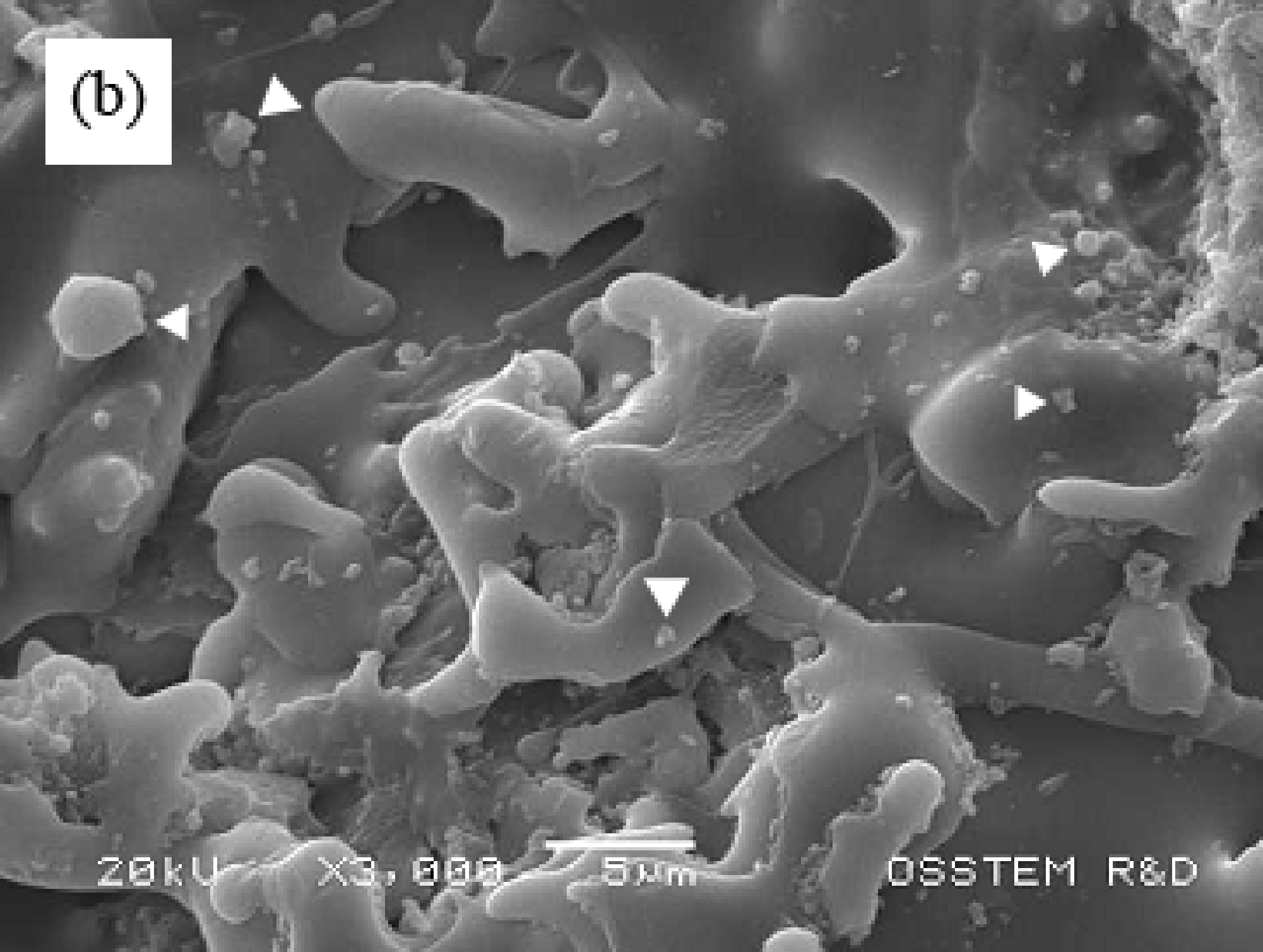
	C (atomic %)	O (atomic %)	Ti (atomic %)	P (atomic %)	Ca (atomic %)	Ca/P ratio
CMP-coated	1.34 ± 0.08	36.95 ± 0.70	52.03 ± 1.11	6.13 ± 0.29	3.56 ± 0.19	0.58 ± 0.01
HA-coated	10.38 ± 2.52	57.77 ± 1.86	Not detected	11.51 ± 0.66	20.34 ± 2.01	1.76 ± 0.09

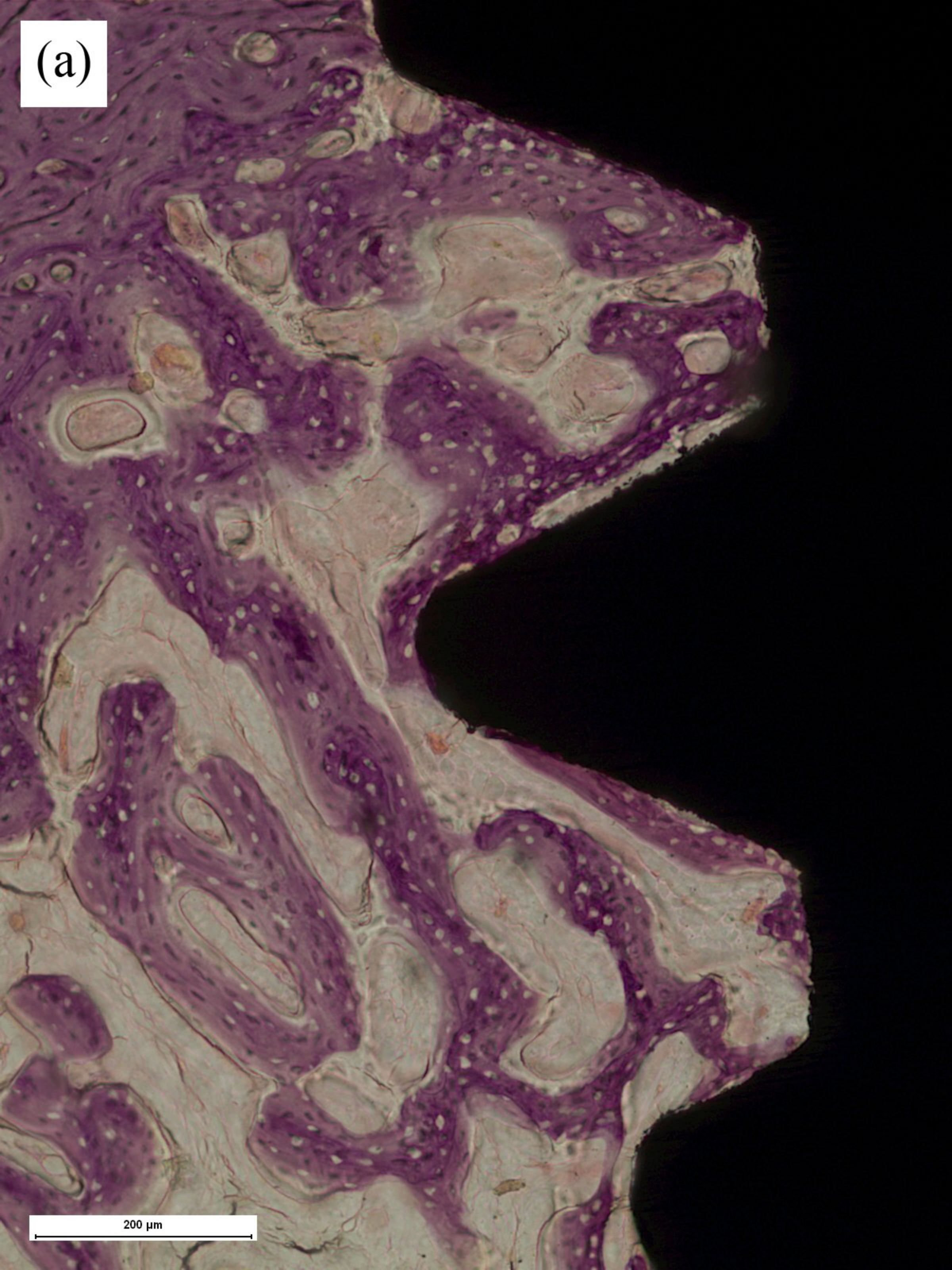
Figure captions

Fig. 1. SEM images of (a) the CMP coat, which was generated by the dip-and-spin technique, and (b) the HA coat, which was produced by plasma spraying. Large and small HA particles were irregularly distributed on the coat surface (white arrows).

Fig. 2. Histological views at 100× magnification of the (a, c) CMP- and (b, d) HA-coated implants after 2 (a, b) and 6 (c, d) weeks of healing. The CMP coating layer is too thin to be detected by the light microscope, whereas the HA coating layer, which is about 50~100 μm thick, is readily observed (white arrows).

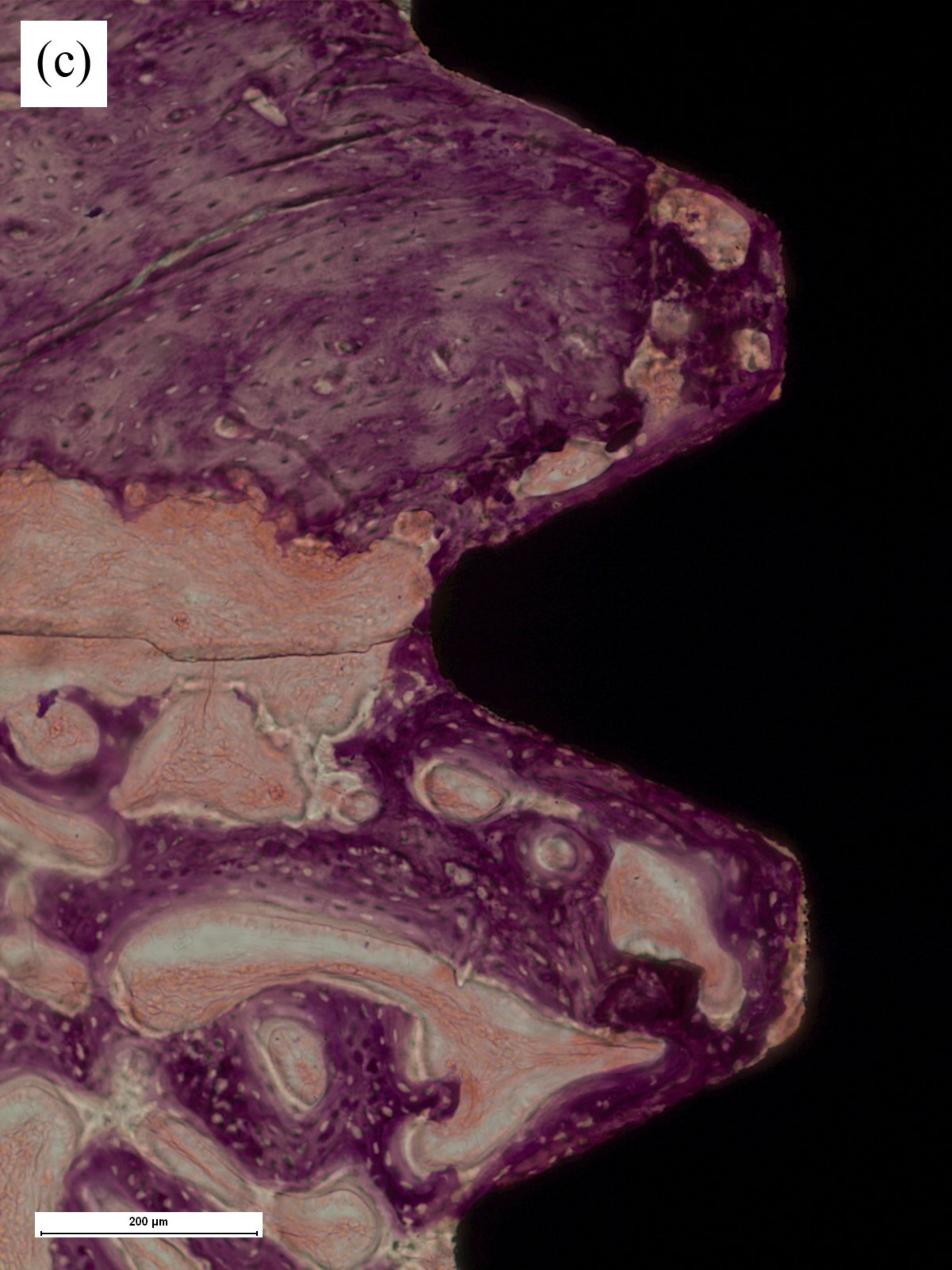

(a)
20kV X3,000 5㎛ OSSTEM R&D


(b)
20kV X3,000 5μm OSSTEM R&D


(a)
200 µm

(b)

200 μm


(c)
200 µm