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# A histomorphometric study on osteoconduction and osseointegration of titanium alloy with and without plasma-sprayed hydroxyapatite coating using back-scattered electron images

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A quantitative evaluation, at the scanning electron microscopic (SEM) level, was made of the osteoconduction and osseointegration of Ti–6Al–4V implants with and without plasma-sprayed hydroxyapatite coatings (HACs). By employing the Chinese Coin implant model in the lateral femora cortices of canines, different biological properties between HA-coated and uncoated Ti–6Al–4V implants could be compared in one specimen. After 4, 6 and 12 weeks, the implants with surrounding bone were removed and assessed histologically in undecalcified sections under SEM. The osteoconductivity and the ability of osseointegration of implants were histomorphometrically analysed from back-scattered electron images (BEIs) and represented in terms of the new bone healing index (NBHI) and apposition index (AI), respectively. Throughout all implant periods, the HA-coated Ti–6Al–4V implants revealed higher NBHI than the uncoated ones, it appearing that the HA-coated Ti–6Al–4V implant was more osteoconductive than the Ti–6Al–4V was. For HA-coated implant, the evidence of direct bone-to-HAC contact was observed. However, at the bone/Ti–6Al–4V interface, there intervened a fibrous membrane without calcium content, indicating that the Ti–6Al–4V implant was not osseointegrated in the SEM field of view. The maximum value of AI was reached 6 weeks after implantation for HA-coated implant, implying that the HAC had a stimulating influence on bone apposition within 6 weeks of healing. The signs of partial dissolution of HACs within the remodelling canals were evident at the HAC–bone interface 12 weeks after implantation, accounting for the slight decrease in NBHI and the obvious decrease in AI for HAC implant.

## 1. Introduction

The long-term loosening of the cemented total hip prosthesis has led to the research for stable fixation of implant to bone [1]. Currently, hydroxyapatite (HA)-coated titanium implant, presenting itself simultaneously with excellent biocompatibility and mechanical properties, raises much interest as an alternative method of long-term fixation [2–4].

Some studies with regard to plasma-sprayed HA-coated titanium implants have demonstrated that the HA coating (HAC) can promote the formation of normal bone at its surface [5–14]. Therefore, it is generally described as osteoconductive. Furthermore, at the optical microscopic (OM) [5–14] and the scanning electron microscopic (SEM) [15, 16] level, the evidence of osseointegration [17, 18], a direct bone-to-HAC contact, has been reported. However, at the SEM level, there are no quantitative studies on osteoconduction and osseointegration of HA-coated implant reported.

Titanium alloys, primarily Ti–6Al–4V, are used frequently as implant materials because the mechanical properties of these alloys are thought to be superior to those of CP titanium. Some studies clearly demonstrated that Ti–6Al–4V implants can be osseointegrated at the OM level [19, 20]. However, to our knowledge, there is only a 2.5 year post-operative anatomical study where the osseointegration to press-fit Ti–6Al–4V femoral component was reported at the SEM level [3]. As a consequence, the bone/Ti–6Al–4V interface has to be further evaluated. Moreover, the osteoconduction of Ti–6Al–4V implant has not yet been quantitatively investigated.

In this study, at the SEM level, the osteoconductivity and osseointegration of Ti–6Al–4V implant with and without plasma-sprayed HAC were evaluated



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quantitatively using the Chinese Coin implant model in the lateral femora cortices of canines. With the help of this new implant model, the different extent of tissue reactions between the HA-coated and uncoated Ti–6Al–4V implant could be histologically compared from observations of secondary electron images (SEIs) and back-scattered electron images (BEIs) in one specimen. Because the contrast of BEIs is highly atomic-number dependent, this electron image could markedly distinguish the bone from the unmineralized tissue. By histomorphometric analyses from BEIs, the osteoconductivity and the ability of osseointegration of implants were represented in terms of the new bone healing index (NBHI) and apposition index (AI), respectively. The NBHI was defined as the (area of new healing bone/area of bone defect × 100%), and the AI was defined as the (length of direct bone–implant contact/total length of bone–implant interface × 100%).

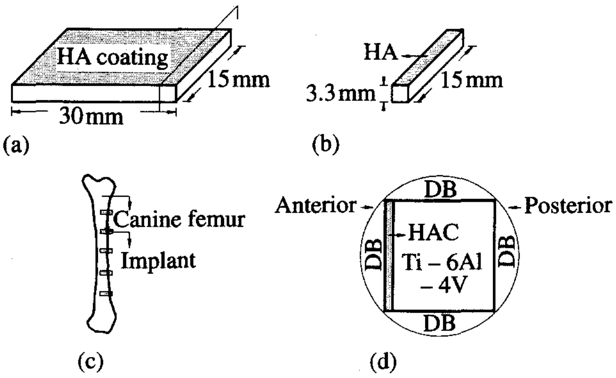



*Figure 1* Schematic configuration of the Chinese Coin implant model. DB = defective bone region and HAC = HA coating. (a) HA coating on a plate of Ti–6Al–4V, (b) specimens for implantation, (c) trans-cortical implantation, (d) Cross-sectional view of the predrilled hole.

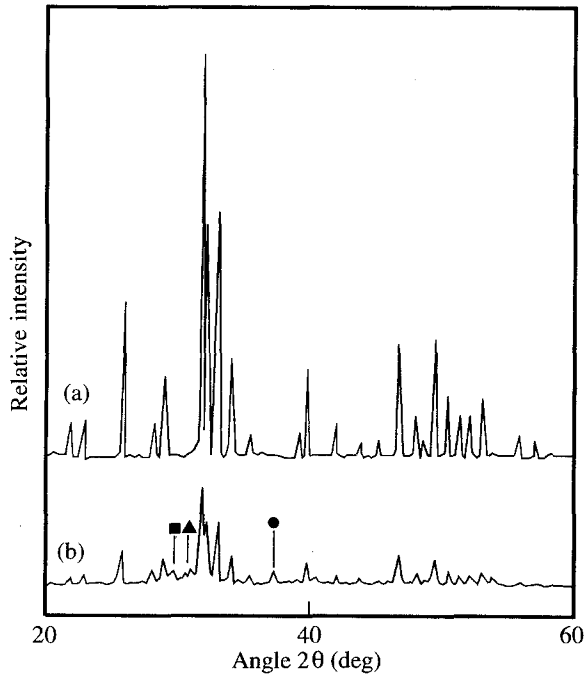



*Figure 2* The X-ray diffraction pattern ($CuK_{\alpha}$) for (a) feedstock HA powders revealing well-crystallized single phase of $Ca_{10}(PO_4)_6(OH)_2$, and (b) HA coating containing impurity phases and less crystallinity. HAP = HA powders and HAC = HA coating. (▲) α, β-$Ca_3(PO_4)_2$, (■) $Ca_4P_2O_9$, (●) CaO.

## 2. Materials and methods

### 2.1. Fabrication of HA-coated Ti–6Al–4V implants

High-purity feedstock HA powder was used in the coating process. Plates of bioinert Ti–6Al–4V alloy (ASTM F-136) were selected as substrates. Prior to spraying, the surfaces were degreased for removing organic contaminants and blasted with $Al_2O_3$ grits to effect the surface roughness. Well-prepared surfaces were coated with HA (Fig. 1a) by means of an atmospheric plasma-spraying technique (APS, Plasma-Technik, M 1100–C). The coating thickness was in the range of 100 ± 50 μm. The phase composition of plasma-sprayed HACs was identified by X-ray diffraction. Comparing with feedstock HA powders, the HACs contained a small amount of impurity phases such as α-, β-tricalcium phosphate, calcium oxide, and tetracalcium phosphate (Fig. 2). The degree of crystallinity was decreased to about 35% with respect to the starting materials. The surface roughness, ($R_a$) was 12.55 ± 1.76 μm for HAC and 3.56 ± 0.31 μm for grit-blasted Ti–6Al–4V.

### 2.2 The Chinese Coin implant model

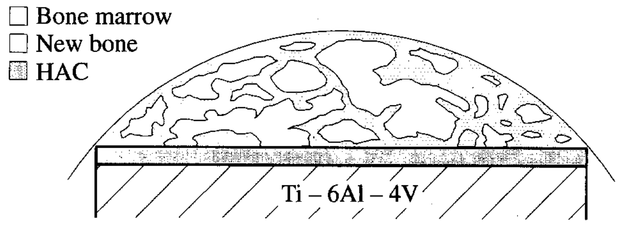
Rectangular HA-coated Ti–6Al–4V samples with dimensions 3.3 mm × 3.3 mm × 15 mm, were used for implantation (Fig. 1b). All implants were cleaned by ultrasonically washing in reagent-grade acetone followed by ultrasonically rinsing in distilled water. Steam sterilization was used prior to implantation.

The lateral cortices of dogs were drilled trans-cortically by three-stage hand reaming to the final defect diameter, using sterile surgical techniques. During all drilling procedures, copious saline was used to minimize any bone thermal trauma and to move the bone debris. The drill sequence employed was 1/16 in (approximately 1.587 mm) and 1/8 in (approximately 3.175 mm) drill bits. Final sizing was done using a 3/16 in (approximately 4.763 mm) drill to produce a defect approximately 0.097 mm oversized compared to the diagonal (4.666 mm) of the square face of the rectangle. Then, the implants were inserted into pre-drilled holes by finger pressure with the HAC facing anteriorly (Fig. 1c). The cross-sectional view of the hole with implant containing simultaneously one HAC face, three Ti–6Al–4V faces, and four defined bone defect with approximately equal area, was quite like a Chinese Coin (Fig. 1d). The defective bone regions (DB) were designed for bone healing. A total of 50 implants were inserted into the femora of five dogs. Each femur of the animals contained five implants. Roentgenographic screening of all animals used in this study ensured that the femora would accept the implants, that the animals were skeletally mature, and no osseous abnormalities existed.

## 2.3. Specimens for histological evaluation

After periods of 4, 6 and 12 weeks, the dogs were sacrificed (two dogs were killed at 4 weeks). The intact femora were retrieved and each implant was isolated transversely using a band saw. The implants were fixed in 10% buffered formalin solution and dehydrated in graduated ethyl alcohol solutions from 70%–100%. Then the implants were embedded in polymethylmethacrylate (PMMA). Each undecalcified implant block was sectioned perpendicularly to the long axis of the implant into three to five thin slices (120–150 μm)in the cortical bone region with a low-speed diamond saw (Isomet). Well-polished slices were carbon coated and examined in the anterior (HAC face) and posterior (Ti–6Al–4V face) area of bone defect under SEI–SEM and BEI–SEM.

The quantity of defective bone and new bone area of the implants was measured using a computer-assisted image analysis system for histomorphometry (MD-30 Plus, Ver. 3.0, Leading Edge Pty Ltd.). To accomplish this, the BEI–SEM photographs were projected on to the monitor through a CCD video camera (Sony, Model XC-77). After grey-level treatment by the image analysis system, the area of new healing bone could be sharply distinguished within the defective bone region and could be calculated. Then, the osteoconductivity of the implant was represented in terms of the new bone healing index (NBHI), defined as the (area of new healing bone/area of defective bone × 100%) (Fig. 3). The ability of osseointegration of implants was addressed as the apposition index (AI), defined as the (length of direct bone–implant contact/total length of bone–implant interface × 100%) (Fig. 3).




$$\mathrm{NBHR} = \frac{\text{the area of new bone}}{\text{the area of bone defect}} \times 100\%$$

$$\mathrm{AI} = \frac{\text{the length of direct bone-implant contact}}{\text{the length of bone-implant interface}} \times 100\%$$

*Figure 3* Schematic representation of new bone healing index (NBHI) and apposition index (AI). HAC = HA coating.

## 3. Results

The surgical operations were tolerated well by the dogs, with no complications noted. At harvesting, all implants were clinically stable and no signs of inflammation or adverse tissue reactions could be seen around the implant sites.

### 3.1. Histological evaluation

#### 3.1.1. *Four weeks*

At 4 weeks the defective bone regions were prominently repaired with new bone. The SEIs and BEIs revealed that the HA-coated implants had better osteoconduction (more new bone repaired) compared with the uncoated ones (Fig. 4). Along the HAC surface, a direct bone-to-HAC contact (osseointegration)

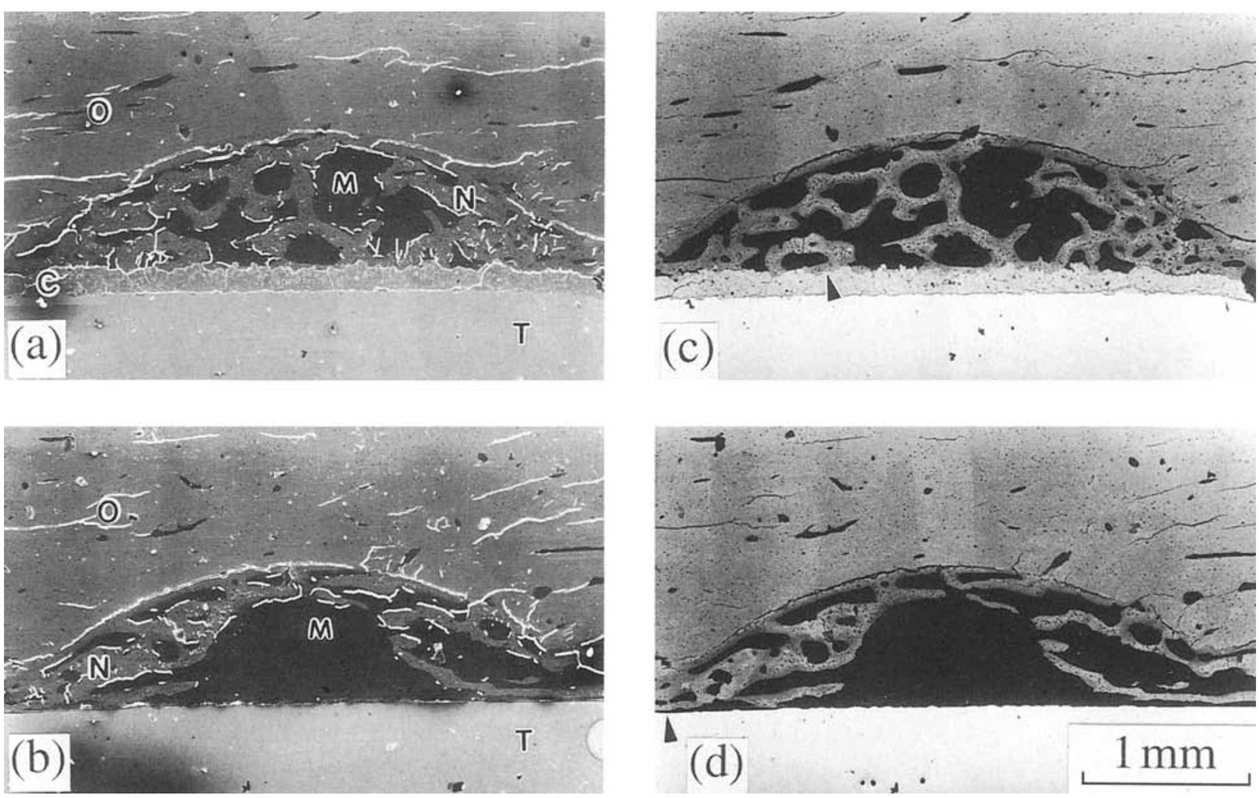



*Figure 4* Images of histological section at defective bone region 4 weeks post-implantation: (a) SEI–SEM at HAC face, (b) SEI–SEM at Ti–6Al–4V face, (c) BEI–SEM at HAC face, and (d) BEI–SEM at Ti–6Al–4V face. O, original cortical bone; N, new bone; M, bone marrow; C, HA coating; T, Ti alloy.

was observed within some regions near the corner of the implant (Fig. 5), while in other regions bone marrow was apposed to the HAC. With respect to the Ti–6Al–4V surface, bone marrow was apposed to the Ti–6Al–4V implant in most regions. At limited regions near the corner of the implant, a thin layer marked "F", together with an artificial gap intervening at the bone/Ti–6Al–4V interface, was noted from SEI (Fig. 6a). However, after the BEI investigation (Fig. 6b), this layer was not shown, suggesting that it was a fibrous membrane. By further wavelength dispersive spectrometer (WDS) X-ray mapping analysis, it was confirmed that this layer was fibrous membrane without calcium content (Fig. 6c). These findings indicated that the Ti–6Al–4V implant did not conform to the concept of osseointegration under SEM examination.

### 3.1.2. Six weeks

In comparison with 4 weeks, more new bone was repaired within the defective regions 6 weeks after insertion. From the observations of SEIs and BEIs, the amount of new bone at the HA-coated implant face remained greater than that at the uncoated implant face (Fig. 7). The regions of direct bone-to-HAC contact increased and extended to the centre of the edge face of the implant (arrow, Fig. 7c), whereas only in a few regions was bone marrow directly apposed to the HAC still observed. This finding suggested that the HAC was osseointegrated even when there existed an initial gap larger than 1 mm. The evidence of fibrous membrane interposing at the bone/Ti–6Al–4V interface near the centre of the edge face of the implant (Fig. 8) again demonstrated that the Ti–6Al–4V implant did not exhibit any ability of osseointegration.

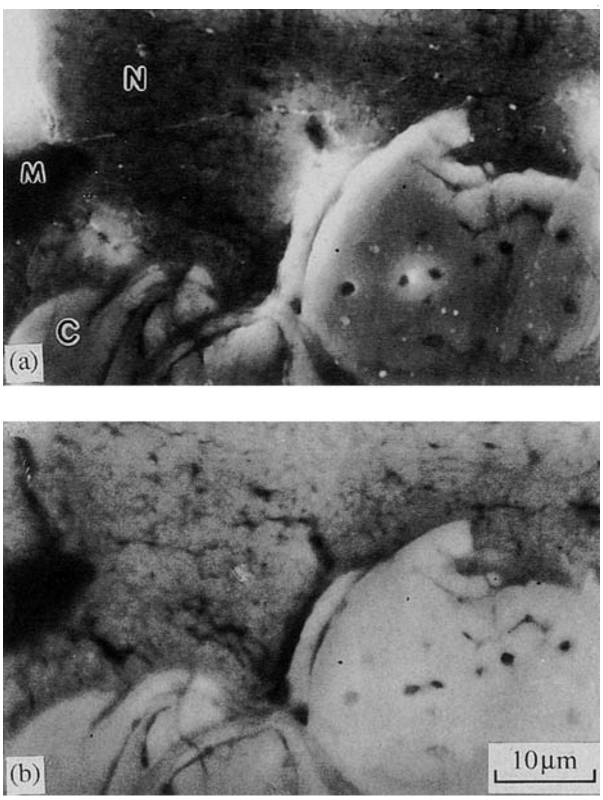



*Figure 5* Images of HAC–new bone interface near the corner of the implant (arrow, Fig. 4c) showing direct bone-to-HAC contact 4 weeks after implantation: (a) SEI–SEM, and (b) BEI–SEM. N, new bone; M, bone marrow; C, HA coating.

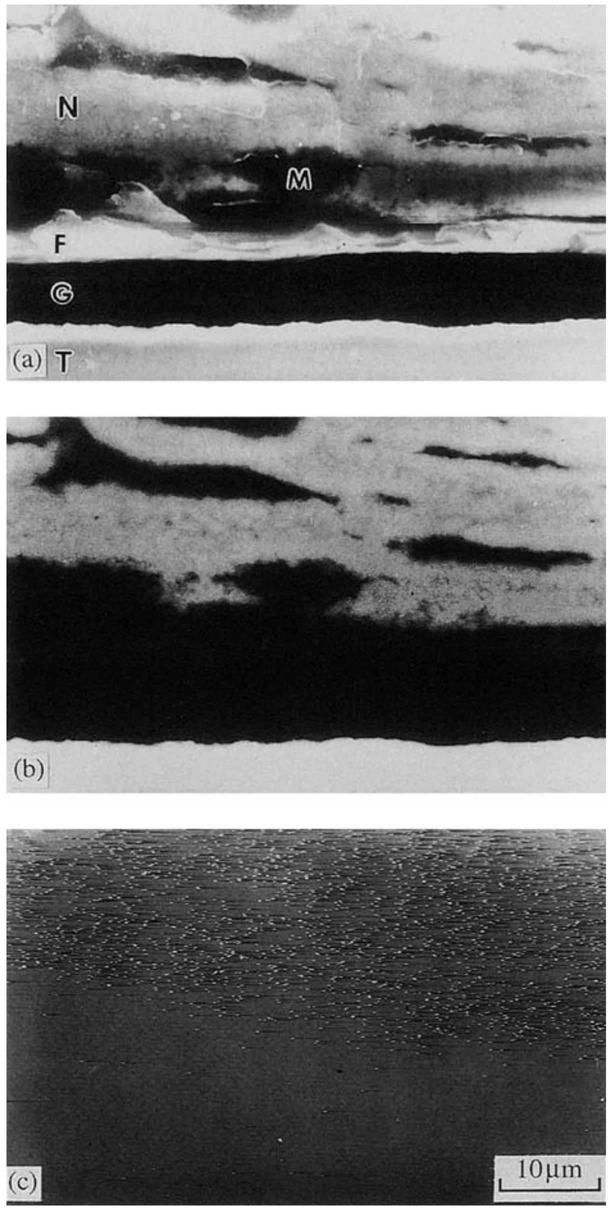



*Figure 6* The new bone–Ti–6Al–4V interface near the corner of the implant (arrow, Fig. 4d) showing a thin layer of fibrous membrane together with an artificial gap interposed 4 weeks post-implantation. (a) Image from SEI–SEM, (b) image from BEI–SEM, and (c) WDS X-ray mapping of Ca trace. N, new bone; M, bone marrow; F, fibrous membrane; T, Ti alloy; G, artificial gap.

### 3.1.3. Twelve weeks

With increasing survival time to 12 weeks, the SEIs and BEIs showed that the amount of new bone seemed unchangeable at both HA-coated and uncoated implant sites compared with the observations 6 weeks after insertion. The HA-coated implants still revealed a greater amount of new bone than the uncoated ones (Fig. 9). However, it is noted that the regions of direct bone-to-HAC contact decreased obviously, e.g. by comparing Fig. 9c with Fig. 7c. Some

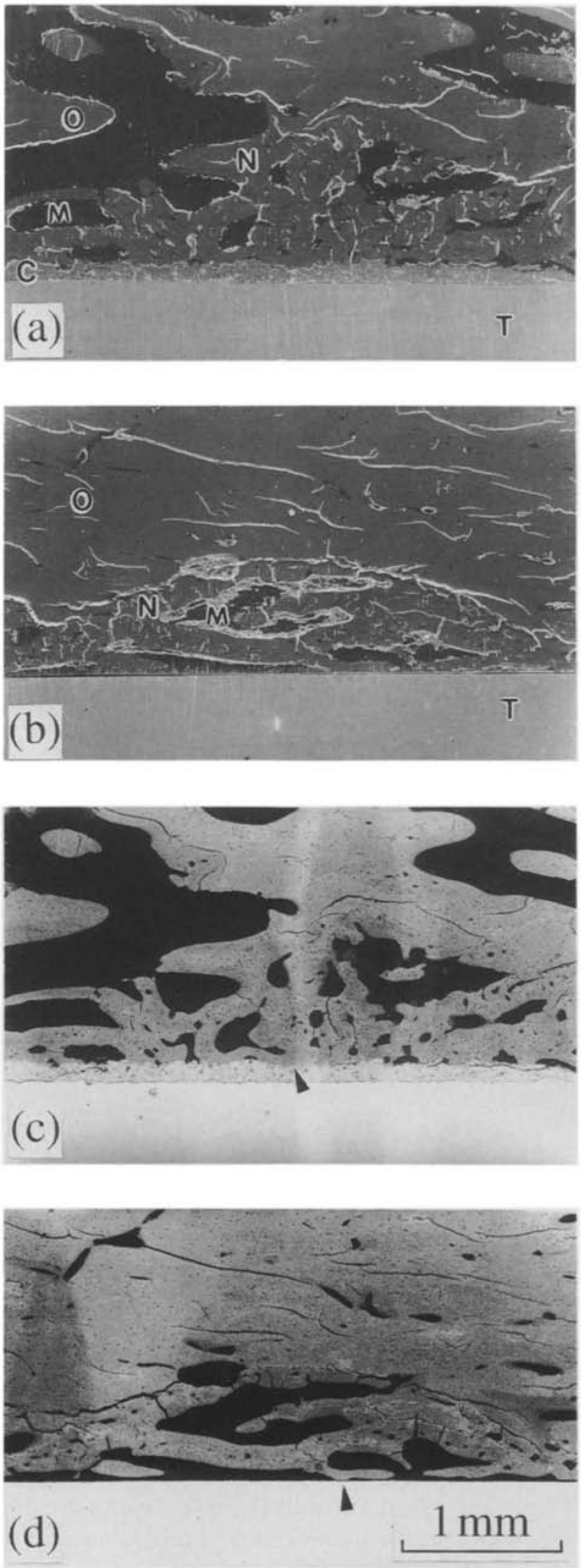



*Figure 7* Images of histological section at defective bone region 6 weeks post-implantation: (a) SEI–SEM at HAC face, (b) SEI–SEM at Ti–6Al–4V face, (c) BEI–SEM at HAC face, and (d) BEI–SEM at Ti–6Al–4V face. O, original cortical bone; N, new bone; M, bone marrow; C, HA coating; T, Ti alloy.

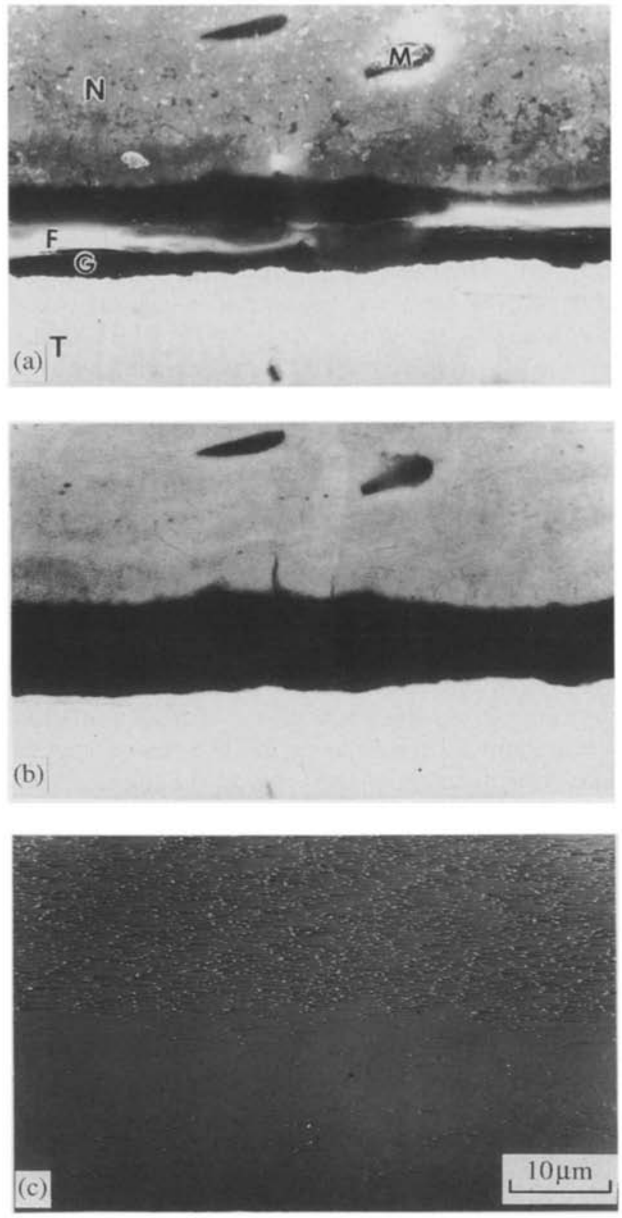



*Figure 8* The new bone–Ti–6Al–4V interface near the centre of the edge face of the implant (arrow, Fig. 7d) showing a thin layer of fibrous membrane together with an artificial gap interposed 6 weeks post-implantation. (a) Image from SEI–SEM, (b) image from BEI–SEM, and (c) WDS X-ray mapping of Ca trace. N, new bone; M, bone marrow; F, fibrous membrane; T, Ti alloy; G, artificial gap.

remodelling canals (R) were observed at the HAC–bone interface (Fig. 9c). Moreover, granular particles of about 1–3 μm (Figs 10b and 11), dissociated from the HAC, were obviously observed within the remodelling canal. The granular particles were further proved to be HA by means of energy dispersive spectrometry (EDS) (Fig. 12). On the other hand, at the bone/Ti–6Al–4V interface, the phenomenon of osseointegration was still not found.

### 3.1.4. New bone healing index (NBHI)

To evaluate the osteoconduction quantitatively, the NBHI of each implant was calculated from BEIs (Table I), and the values for the HA-coated and uncoated Ti–6Al–4V implants were compared statistically by Student's *t*-test (Table II). As listed in Table I, at each of the time periods, the mean NBHI of HA-coated implants was greater than that of uncoated ones. After 4 weeks healing, the mean NBHI was

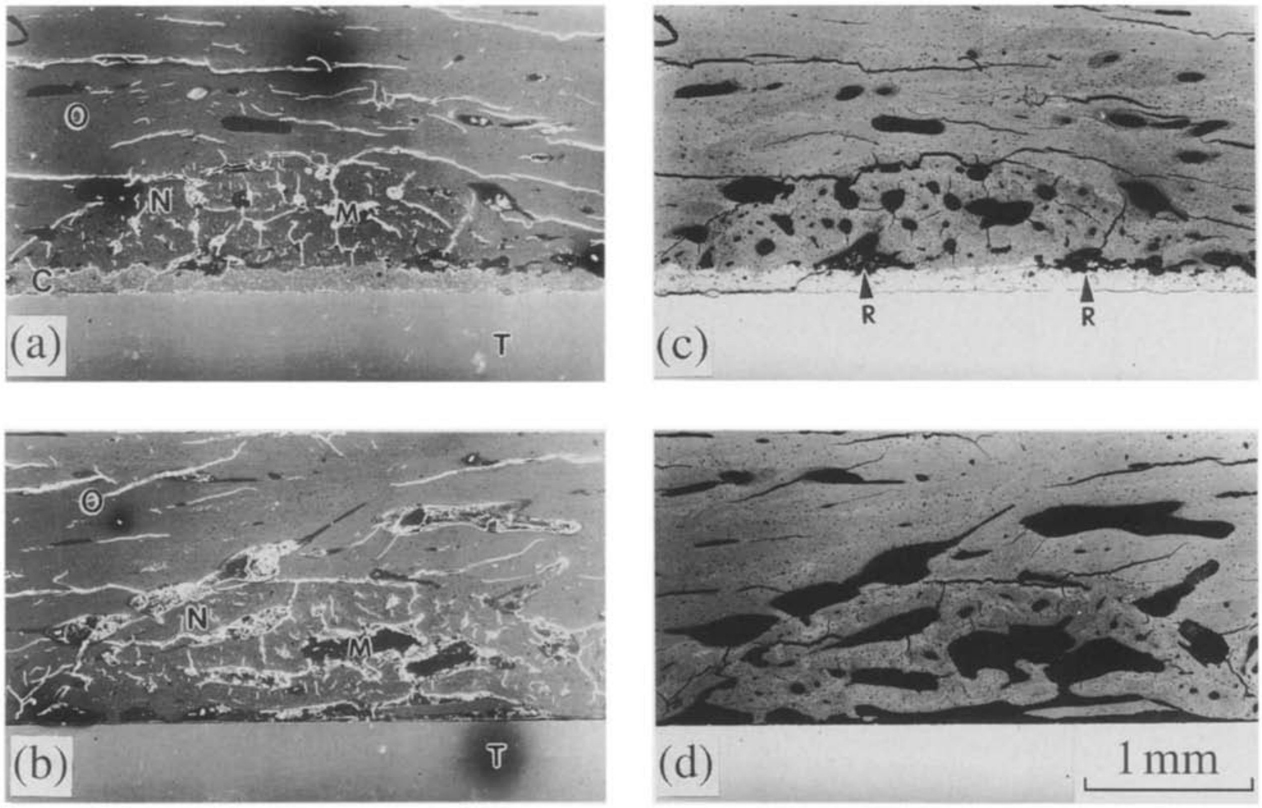



*Figure 9* Images of histological section at defective bone region 12 weeks post-implantation. (a) SEI–SEM at HAC face, (b) SEI–SEM at Ti–6Al–4V face, (c) BEI–SEM at HAC face, and (d) BEI–SEM at Ti–6Al–4V face. O, original cortical bone; N, new bone; M, bone marrow; C, HA coating; T, Ti alloy; R, remodelling canal.

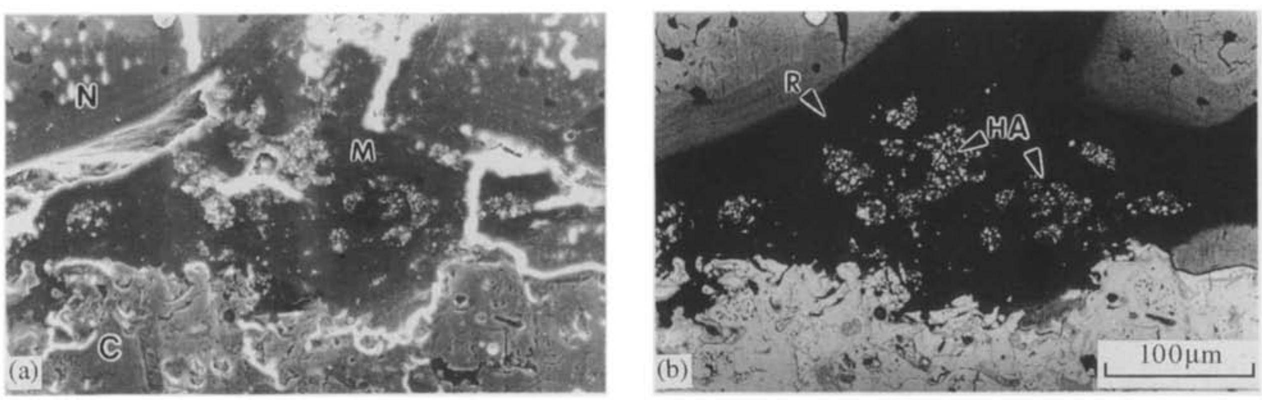



*Figure 10* Partial dissolution of HAC observed in the remodelling canal at the HAC–bone interface (arrow, Fig. 9c). (a) Image from SEI–SEM and (b) image from BEI–SEM. N, new bone; R, remodelling canal; C, HA coating.

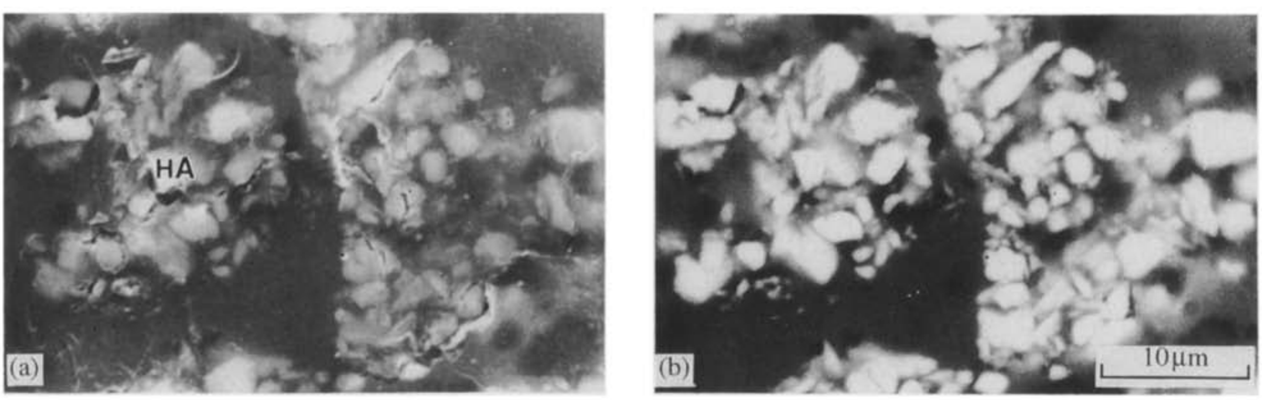
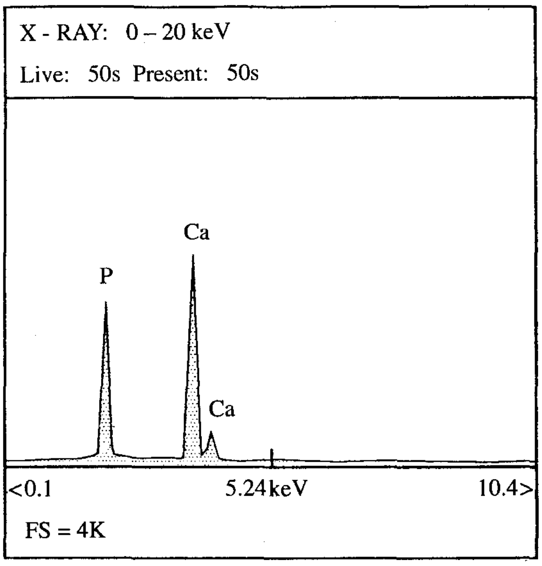



*Figure 11* Granular HA particles, of about 1–3 μm, dissociated from the partially dissolving less-dense outer HAC layer (arrow, Fig. 10b).




All elements analysed, normalised

| Element | ZAF | Element (%) | Atomic (%) |
|---|---|---|---|
| Ca$K$ : 2 | 1.007 | 68.845 | 63.084 |
| P $K$ : 2 | 1.491 | 31.086 | 36.863 |
| Ti$K$ : 2 | 0.714 | 00.070 | 00.053 |
| Ai$K$ : 2 | 0.904 | 00.000 | 00.000 |
| Total | | 100.00 | 100.00 |

*Figure 12* EDS analysis of granular particles confirming that these particles were HA.

TABLE I Results of new bone healing index (NBHI) measured from BEIs

| Weeks | HAC Ti–6Al–4V | Uncoated Ti–6Al–4V |
|---|---|---|
| 2 | <br>($n = 11$) | –<br>($n = 11$) |
| 4 | 61.52 ± 10.52<br>($n = 21$) | 48.47 ± 11.50<br>($n = 21$) |
| 6 | 85.09 ± 3.41<br>($n = 11$) | 76.27 ± 5.47<br>($n = 11$) |
| 12 | 79.90 ± 5.66<br>($n = 11$) | 70.54 ± 6.20<br>($n = 11$) |

Note: Values are given as means ± S.D. $n$ = number of slices for histomorphometry.

61.52% for HA-coated implants and 48.47% for uncoated implants. Six weeks after implantation, the mean NBHI for both types of implants apparently increased. The HA-coated implants had the highest NBHI (85.09%) at this period and remained at this level of NBHI up to 12 weeks, although a slight decrease of NBHI from 85.09% to 79.90% was measured 12 weeks after insertion. However, with uncoated implants, a lower NBHI (76.27%) was measured compared to HAC implants 6 weeks after implantation, and this value also decayed slightly 12 weeks after insertion.

Statistical analysis of the NBHI data was read as follows. At each of the time periods, the HAC implants had significantly greater NBHI than the Ti–6Al–4V implants, implying that the HA-coated implants were always more osteoconductive than were the uncoated ones. The increase in NBHI for both implants from 4 weeks to 6 weeks was statistically significant. However, the slight decrease in NBHI for both implants from 6 weeks to 12 weeks was not significant. It is noted that there was no significant difference between NBHI for the $HAC_4$ and the $TI_{12}$ (labelled "a" in Table II), suggesting that the HA-coated implant had a prominent response in osteoconductivity at early time, and that the Ti–6Al–4V implant could not achieve the equivalent osteoconductivity until 12 weeks post-implantation. The fact that there was no significant difference between NBHI for the $HAC_{12}$ and the $TI_6$ (labelled "b" in Table II) was attributed to the decrease in NBHI for HA-coated implant 12 weeks after implantation.

### 3.1.5. *Apposition index (AI)*

From the observations of BEIs, the evidence of osseointegration was found only for HAC implants. Results of apposition index measurements are listed in Table III. At 4 weeks, the mean value of AI was 61.52%. The maximum value of AI (83.45%) was reached 6 weeks after implantation. With increasing survival time to 12 weeks, an obvious decrease in AI from 83.45% to 66.90% was found. The Student's $t$-test was performed to compare the corresponding AI

TABLE II Results of Student's $t$-test applied to the data of NBHI

| | $HAC_4$ | $HAC_6$ | $HAC_{12}$ | $TI_4$ | $TI_6$ | $TI_{12}$ |
|---|---|---|---|---|---|---|
| $HAC_4$ | – | 0.0001 | 0.0001 | 0.0004 | 0.0002 | NS[a] |
| $HAC_6$ | | – | NS | 0.0001 | 0.0002 | 0.0001 |
| $HAC_{12}$ | | | – | 0.0001 | NS[b] | 0.0014 |
| $TI_4$ | | | | – | 0.0001 | 0.0001 |
| $TI_6$ | | | | | – | NS |
| $TI_{12}$ | | | | | | – |

Notes: Numbers shown are the $p$ values for the level of significance of the $t$-test indicated using a two-tailed alternative hypothesis and not assuming equal variances. Statistical significance was defined as $p \leqslant 0.01$.
NS, the test is not statistically significant; $HAC_4$, HA-coated Ti–6Al–4V 4 weeks after insertion; $TI_4$, uncoated Ti–6Al–4V 4 weeks after insertion; etc.
[a] The HA-coated implant had a prominent response in osteoconductivity at an early time (4 weeks).
[b] The NBHI of $HAC_{12}$ was reduced.

TABLE III Results of apposition index (AI) measured from BEIs

| Weeks | HAC Ti–6Al–4V |
|---|---|
| 2 | –<br>($n = 11$) |
| 4 | 61.52 ± 19.24<br>($n = 21$) |
| 6 | 83.45 ± 7.99<br>($n = 11$) |
| 12 | 66.90 ± 14.04<br>($n = 11$) |

Notes: Values are given as mean ± S.D. $n$ = number of slices for histomorphometry.

TABLE IV Results of Student's $t$-test applied to the data of AI

| | $HAC_4$ | $HAC_6$ | $HAC_{12}$ |
|---|---|---|---|
| $HAC_4$ | – | 0.0011 | NS |
| $HAC_6$ | | – | 0.0029 |
| $HAC_{12}$ | | | – |

Notes: Numbers shown are the $p$ values for the level of significance of the $t$-test indicated using a two-tailed alternative hypothesis and not assuming equal variances. Statistical significance was defined as $p \leqslant 0.01$.
NS, the test is not statistically significant; $HAC_4$, HA-coated Ti–6Al–4V 4 weeks after insertion; etc.

data (Table IV). It is clear that both the increase of AI from 4 weeks to 6 weeks and the decrease in AI from 6 weeks to 12 weeks are statistically significant. The appearance of remodelling canals was suggested to account for the decrease in AI 12 weeks after implantation.

## 4. Discussion

In this study, the different biological properties between HA-coated and uncoated Ti–6Al–4V implants were evaluated quantitatively using the Chinese Coin implant model (rectangle in hole), being different from the traditional implant model (cylinder in hole). From the viewpoint of histological assessment, this new implant model offered many advantages. Firstly, the healing of the new bone could be clearly visible within defective bone regions under microscopes. As a result, the osteoconductivity of implants could be histomorphometrically assessed and represented in terms of NBHI. Secondly, as the new bone attached to the implant surface, the phenomenon of osseointegration could be investigated. Thus, the AI value was measured. Thirdly, from the measured NBHI and AI data, the different extent of tissue reactions between HA-coated and uncoated implant could be easily compared and distinguished in one specimen. Finally, the different biological properties between various coating materials and their substrates could also be evaluated quantitatively according to this implant model.

The histological observations demonstrate that, using SEI–SEM and BEI–SEM, the HA-coated implants induce more bone healing than the Ti–6Al–4V implants in the cortical regions throughout all implant periods, implying that a stimulating effect does exist on osteoconduction of HACs after short-term follow-up. However, this finding shows a little difference from the study of Jansen *et al.* [21], who investigated cylindrical HA-coated CP-titanium plugs (with CP-titanium screws serving as controls) implanted in the tibia of rabbits, with up to 3 months follow-up. Their histological results demonstrated that, at the OM level, there were no marked differences in bony reaction between two types of implants in the cortical regions, although the HAC appeared to induce more bone formation in the medullary cavity. Different implant materials used as controls are thought to be reasons why our results do not agree with theirs.

The evidence of direct bone-to-HAC contact can be seen under SEI–SEM and BEI–SEM, coinciding with other reports [15, 16] where osseointegration of HACs was observed at the SEM level. However, as evidently indicated by the results, a thin layer of fibrous membrane interposing at the bone/Ti–6Al–4V interface is observed, revealing that the Ti–6Al–4V implant is not osseointegrated at the SEM level with WDS X-ray chemical analysis. This finding is markedly inconsistent with studies [19, 20] where the osseointegration was found for Ti–6Al–4V implant at the OM level. Furthermore, this finding has a partial difference from the study of McCutchen *et al.* [3], who investigated a 2.5 year post-operative anatomical specimen of a press-fit titanium alloy femoral component. Their results indicated that the Ti–6Al–4V alloy was osseointegrated within some bone/Ti–6Al–4V interfaces at the SEM level with low magnification (X 1100 for SEI and X 900 for BEI). The design of implant model, the resolution of the microscope, and the surgical techniques are considered to be the probable reasons why our results do not match with the results of the above mentioned studies.

In the present study, from both SEI–SEM and BEI–SEM 12 weeks post-implantation (Figs 9 and 10), the granular HA particles dissociated from the partially dissolving less-dense outer HAC layer are evidently seen within the remodelling canals. The granular HA particles were confirmed as unmelted particles in HAC [22]. This finding is similar to the study of Gottlander and Albrektsson [14] and Denissen *et al.* [15], in whose studies, the pieces of HA loosened from the HAC were observed within the remodelling canals at the OM level 1 year 9 months after implantation. In the present investigation, because there were no osteoclasts found near the HACs at the SEM level, the dissolution of HAC cannot be further deduced to be resorption, as suggested by other investigators [4, 13, 21, 23, 24], who had discussed the signs of resorption of plasma-sprayed HACs at the OM level. In the majority of their studies, the occasional complete dissolution of HACs [21, 23] or the osteoclastic resorption of HACs [4, 13, 24] were reported. Nevertheless, some studies failed to find the resorption of HAC after 32 weeks implantation [9]. The different results with respect to the dissolution or the resorption of HAC might be attributed to different microscopes applied or to different characteristics, especially the extent of crystallinity, of HACs manufactured by different groups. As shown in Fig. 2, the X-ray diffraction pattern of the HAC gave a predominantly amorphous pattern with a crystallinity below 35%. This state of the calcium phosphate was suggested to be less stable and prone to biological degradation, accounting for the partial dissolution of HAC, 12 weeks post-implantation.

In this study, the histomorphometric measurements are analyses from BEI–SEM. The reason for this is that the BEIs may provide excellent images for quantitative analysis and, consequently, in comparison to other images (microradiographic and stained images), more correct data are reached [25]. The NBHI for HA-coated implant is 61.52% at 4 weeks and 85.09%

at 6 weeks, suggesting that the HAC has early osteoconductivity. The fact that HA-coated Ti–6Al–4V implants reveal higher NBHI than uncoated ones throughout all implant periods demonstrates the HA-coated Ti–6Al–4V implants are more suitable than Ti–6Al–4V implants for implant applications.

After histomorphometric analysis from BEIs, the AI for HA-coated Ti–6Al–4V implants is 61.52% at 4 weeks, 83.45% at 6 weeks, and 66.90% at 12 weeks, it appearing that the HAC has a stimulating influence on bone apposition within 6 weeks of healing. Studies regarding the percentage of direct bone–implant contact have been reported at the OM level. Hayashi *et al.* [12] performed a quantitative histological evaluation of HA- and $TiO_2$-coated Ti–6Al–4V and uncoated Ti–6Al–4V by determining the affinity index in femora of dogs. Their results showed that the HA-coated implant had the highest AI (81.1%) 4 weeks after insertion, and this superiority was maintained up to 96 weeks. Soballe *et al.* [10] investigated the effect of HAC on skeletal attachment in non-interference-fit (1 mm gap) and press-fit 4 weeks after implantation in dogs. After histomorphometric analysis, the results showed that the percentage of direct bone–implant contact for non-interference-fit and press-fit was 40% and 60%, respectively. A study by Buser *et al.* [13] demonstrated that the percentage of direct bone–implant contact for HA-coated implant was 60.6% 3 weeks after implantation and 69.5% 6 weeks after implantation. Gottlander [14], gave the percentage of direct bone–implant contact for HA-coated CP titanium screws as 65.1% 6 weeks after insertion and 59.2% 1 year after insertion. By comparing our results of AI measurements with theirs, no marked deviations are seen among AI values measured. However, it is noted that a significant decrease of AI is observed 12 weeks after implantation in this present study. This unexpected finding is due to the partial dissolution of HAC, which is suggested to form the remodelling canals at the HAC–bone interface, resulting in a decrease of AI.

## 5. Conclusions

The osteoconduction and osseointegration of Ti–6Al–4V implants with and without plasma-sprayed HACs were assessed quantitatively at the SEM level. The following concluding remarks may be made.

1. The differences in osteoconduction and osseointegration between HA-coated and uncoated Ti–6Al–4V implants could be compared from the Chinese Coin implant model in one specimen.

2. At the SEM level with high resolution, evidence of osseointegration, direct bone-to-HAC contact, was observed for HA-coated Ti–6Al–4V implant, even when there existed an initial gap larger than 1 mm. However, at the bone/Ti–6Al–4V interface, there intervened a fibrous membrane without calcium content confirmed by WDS X-ray chemical analysis, indicating that the Ti–6Al–4V implant was not osseointegrated at the SEM level.

3. The signs of partial dissolution of HACs could evidently be observed 12 weeks after implantation. Granular particles of about 1–3 μm, within the remodelling canals, were proved to be HA by means of EDS.

4. The histomorphometric analysis from BEIs demonstrated that the NBHI of HA-coated Ti–6Al–4V implant was higher than that of uncoated one throughout all implant periods, implying that the HAC was more osteoconductive than was the Ti–6Al–4V.

5. The maximum value of AI for HA-coated Ti–6Al–4V implant was reached 6 weeks after implantation, it appearing that the HAC had a stimulating influence on bone apposition within 6 weeks of healing.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