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Characterization of Plasma-Sprayed Hydroxyapatite/TiO_2 Composite Coatings

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To enhance the bonding between hydroxyapatite (HA) coating and titanium alloy substrate, HA/TiO_2 composite coatings have been fabricated *via* plasma spraying. Bonding strength evaluation, simulated body fluid tests, and cell culture *in vitro* were carried out to characterize the composite coatings. The results obtained showed that the addition of TiO_2 to HA coating improved the bonding strength of the coating significantly. After being immersed in simulated body fluid (SBF) for a period, the surfaces of HA/TiO_2 composite coatings were completely covered by carbonate-containing apatite, which indicated that the coatings possess good bioactivity. The *in vitro* cell culture indicated good cytocompatibility for HA/TiO_2 composite coatings.

Key words bonding strength, cell culture, HA, plasma spray, SBF test, TiO_2

1. Introduction

One major application of hydroxyapatite (HA) is to serve as a cover material for titanium or other metals used in implants.[1–4] In these cases, the biocompatibility of implants is assured by HA, while the mechanical aspects are provided by the metal. Many methods, such as sol-gel,[5] metal-organic CVD,[6] pulsed laser deposition,[7] and thermal spray,[8] are available for fabrication of HA coatings. Among them, the plasma spray technique has become the most popular method to deposit HA coatings. Plasma-sprayed HA coatings on the surface of titanium alloy implants have found wide application.

However, the long-term stability of the plasma-sprayed HA coating is still questionable. Despite the strong bonding between the HA coating and bone structure, it has been recognized that the mechanical stability of the interface between the coating and metal substrate could be a problem either during surgical operation or after implantation for a period of time.[8,9] To solve this problem, several attempts have been made.[10–12] One of these approaches is to form a composite coating with a mechanically strong, biocompatible but bioinert metal or ceramic and the bioactive, but mechanically fragile HA.[13,14]

For HA-based composite coatings, two problems should be taken into account prior to evaluating the tissue response to the coating *in vivo*. First, fabrication of the composite coating should improve the bonding condition between the coating and substrate significantly. Second, additives in the coating must not significantly reduce the biocompatibility and bioactivity of the material.

In this paper, the preparation of HA/TiO_2 composite coatings by plasma spraying is described. Microstructures of fabricated specimens were examined by scanning electron microscopy (SEM). The bonding strengths of HA/TiO_2 coatings were measured by the ASTM C-633 method. The bioactivity of coatings was evaluated by examining carbonate-containing apatite formation on the coating surfaces in simulated body fluid (SBF). Osteoblast cultures were examined to assess the cytocompatibility of the composite coatings.

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2. Experimental Procedure

2.1 Specimen Preparation

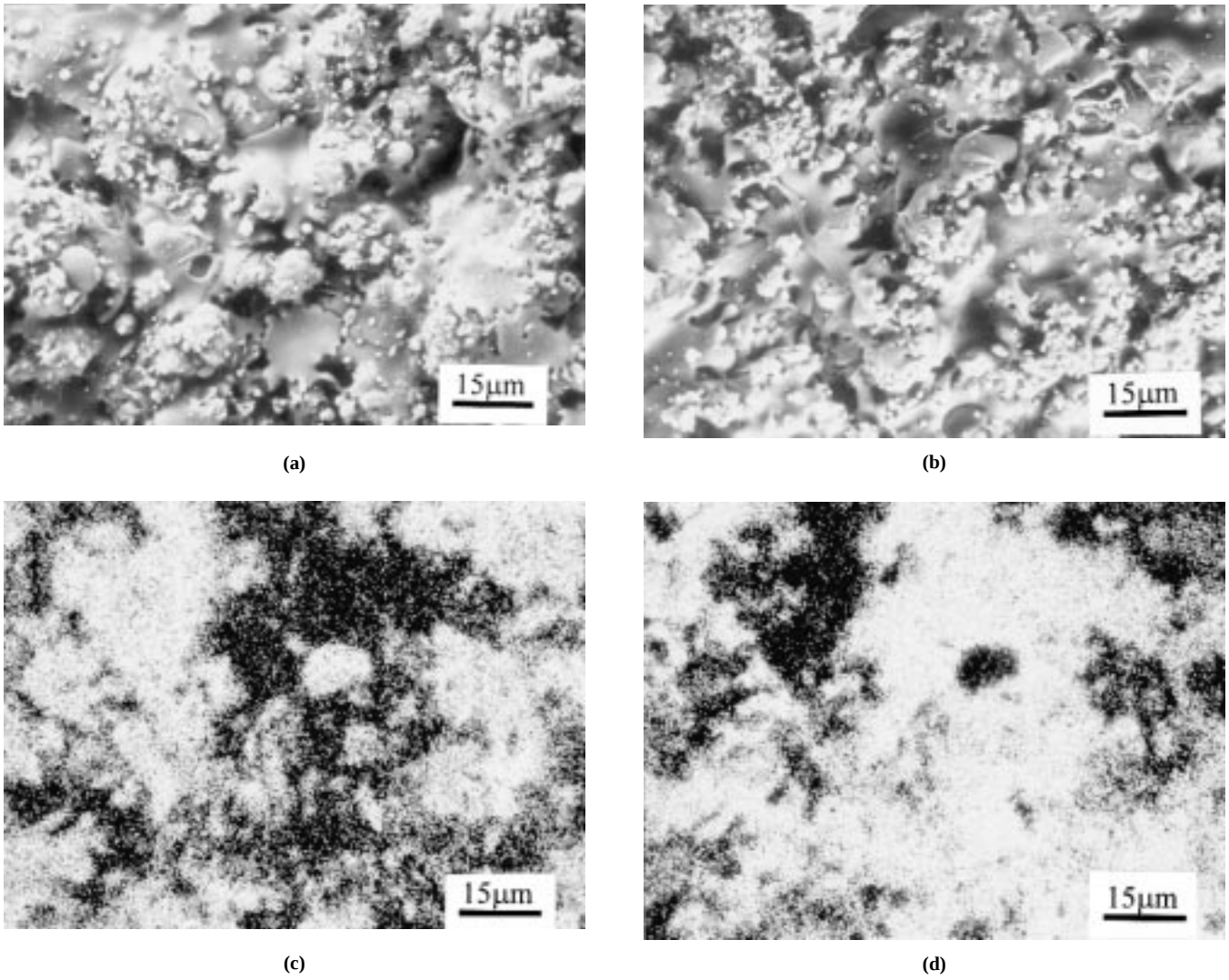
Commercially available HA and TiO_2 powders, with typical sizes of 45 to 160 μm and 15 to 40 μm, respectively, were used. The HA and TiO_2 powders with two compositions, 80 wt.% HA/20 wt.% TiO_2 ("H8TO2") and 40 wt.% HA/60 wt.% TiO_2 ("H4TO6"), were mixed in a ball mill for 5 h. Two geometrics of Ti-6A1-4V substrates were used: one in $10 \times 10 \times 2$ mm plate form for surface characterization, SBF tests, and cell culture tests; and the other one in cylindrical form (Ø25.4 mm) for bonding strength evaluation according to ASTM C-633. An atmospheric plasma spray system (Sulzer Metco, Switzerland) was applied to fabricate HA/TiO_2 composite coatings under suitable spray parameters.

2.2 Bonding Strength and Thermal Expansion Coefficient Evaluation

The bonding strength evaluation of the coatings was performed in accordance with the ASTM C-633 standard. The value obtained represented an average of five test data. For comparison purposes, the bonding strength of HA coatings fabricated *via*

Table 1 Ion concentrations of SBF in comparison with human blood plasma

	Concentration, mM							
	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}	SO_4^{2-}
SBF	142	5	2.5	1.5	4.2	148.5	1	0.5
Blood plasma	142	5	2.5	1.5	27	103	1	0.5



(a) **(b)** **(c)** **(d)**

Fig. 1 Morphologies and element distribution of HA/TiO_2 composite coatings: **(a)** H8TO2 coating, **(b)** H4TO6 coating, **(c)** Ca K_α picture for (b), and **(d)** Ti K_α picture for (b)

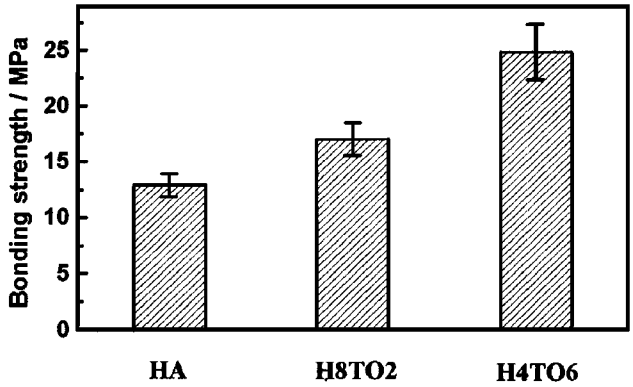


Fig. 2 Bonding strength of HA coating and HA/TiO_2 composite coatings

atmospheric plasma spraying under appropriate spray parameters was also examined. The linear thermal expansion coefficient (CTE) of the H4TO6 coating was measured in a thermal analyzer (Netzsch 402ES-3, Germany).

2.3 SBF Test

After being ultrasonically washed in acetone and rinsed in deionized water, specimens were soaked in the SBF solution. The SBF solution, of ionic concentration shown in Table 1, was buffered at pH of 7.4 using trimethanol aminomethane-HCl. Duplicate samples were immersed in SBF for l, 7, and 14 days at 36.5°C without stirring.

2.4 Cell Culture

Osteoblasts were isolated *via* sequential collagenase digestions of neonatal rat calvaria and cultured in Dulbecco's Modified Eagle Medium, supplemented with 10% fetal bovine serum in a 37 °C, humidified, 5%CO_2/95% air environment. Prior to cell culture experiments, all samples were degreased, ultrasonically cleaned, and sterilized in a steam autoclave at 120°C for 30 min. Each of these samples was placed inside one well of a 24 well culture plate. The osteoblasts were seeded onto the samples

at a density of 5×10^4 cells/well. One milliliter of growth medium was also cautiously added to each well. The growth medium was changed every 2 days. The cells were then incubated for 1, 4, and 7 days at 37 °C in air with 5%CO_2 added.

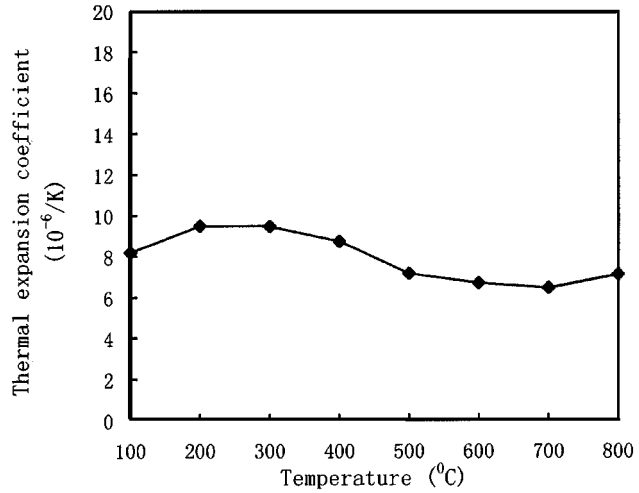


Fig. 3 Linear CTEs of H4TO6 coating

At harvest, the culture media were removed and the samples rinsed three times with 0.1M phosphate-buffered saline (PBS) and fixed with 2% glutaric dialdehyde for 45 min. After fixation, the samples were rinsed with PBS sequentially dehydrated in an ethanol-water series of 30, 50, 70, and 95% ethanol, each step taking 10 to 20 min, followed by 1 h in 100% ethanol, and were finally critical point dried with CO_2. The dried specimens were then submitted to surface characterization.

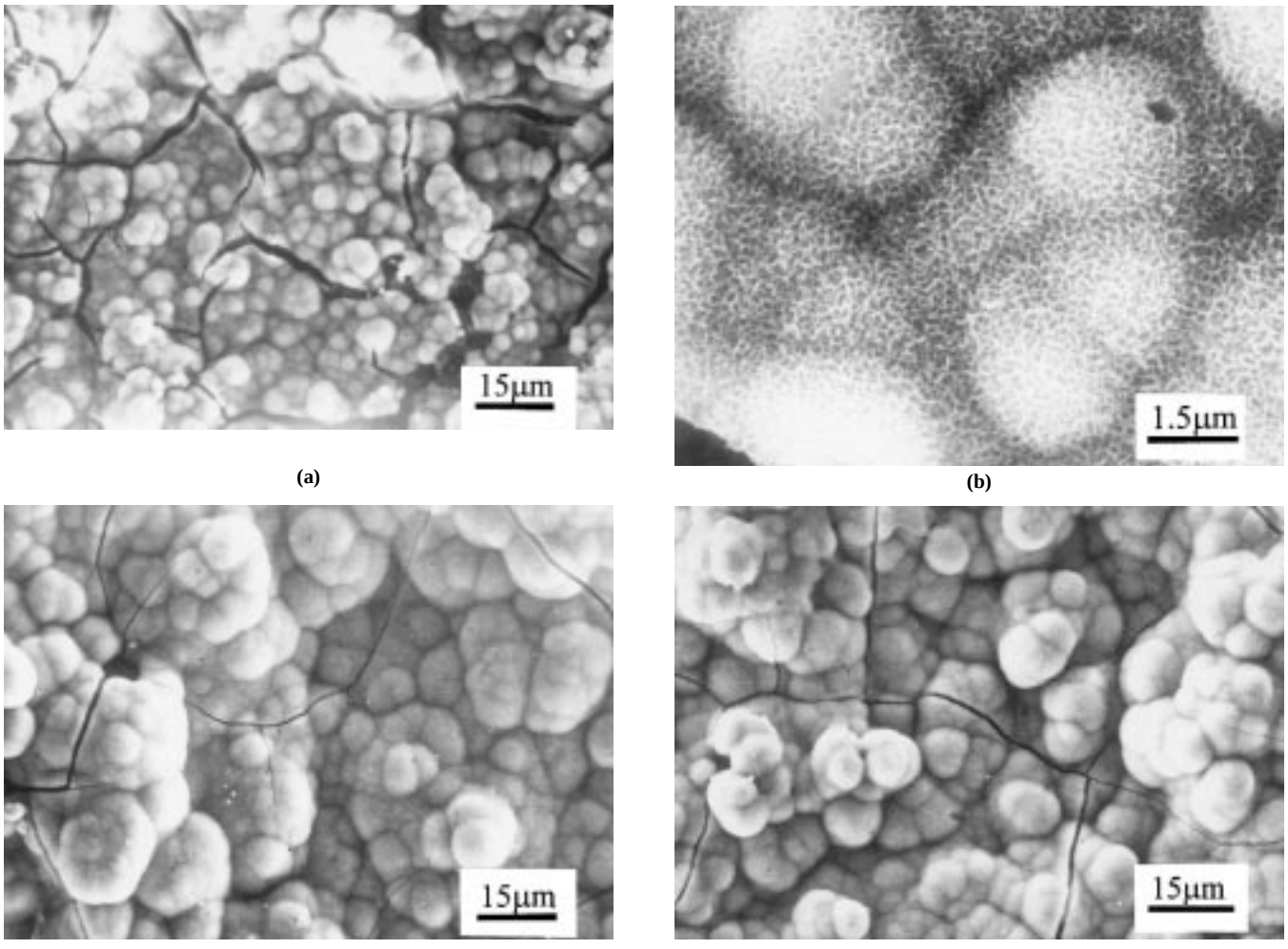
2.5 Surface Characterization

The microstructures of the coatings before and after the SBF test were observed by SEM. The resultants generated on the coating surfaces in SBF were characterized by x-ray photoelectron spectroscopy (XPS). Osteoblasts cultured on the coating surface were also observed by SEM.

3. Results and Discussion

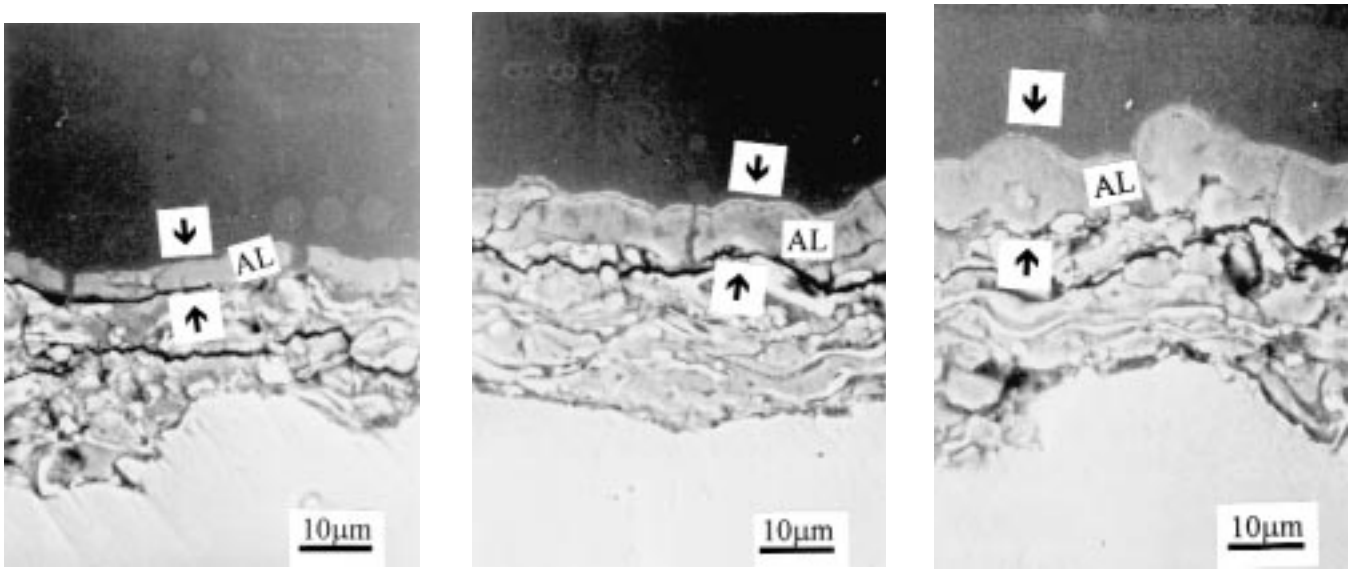
3.1 Surface Morphologies

Morphologies and element distribution of HA/TiO_2 composite coatings are shown in Fig. 1. The H8TO2 and H4TO6 coatings are characterized by a rough surface and some pores, which



(a) **(b)** **(c)** **(d)**

Fig. 4 Morphologies of H4TO6 coating after immersion in SBF: **(a)** 1 day, **(b)** higher magnification for (a), **(c)** 7 days, and **(d)** 14 days



(a) (b) (c)

Fig. 5 Cross section of H4TO6 coating after immersion in SBF: **(a)** 1 day, **(b)** 7 days, and **(c)** 14 days. "AL" indicates the apatite layer

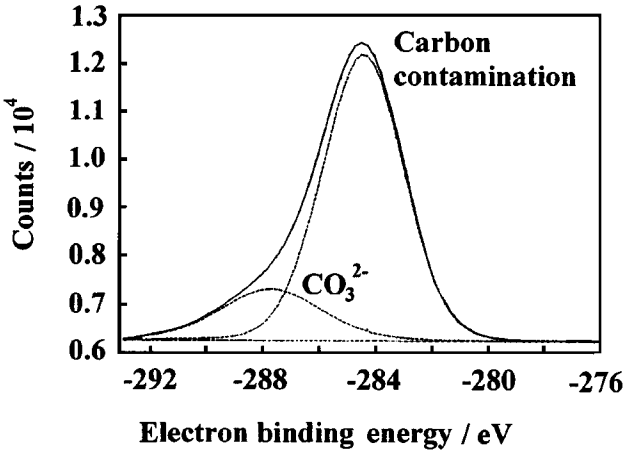


Fig. 6 XPS C1s spectrum of H4TO6 coating after immersion in SBF for 14 days

are considered to be beneficial to bonding with surrounding tissues *in vivo.*

Calcium and Ti K_α x-ray maps of Fig. 1(b) shown in Fig. 1(c) and (d), respectively, indicate that these elements are well distributed in the coating. Thus, the HA and TiO_2 phases are distributed evenly in the coating.

3.2 Bonding Strength

The bonding strengths of the HA coating and HA/TiO_2 composite coatings are shown in Fig. 2 and indicate the lowest bonding strength of 12.9 MPa for the HA coating. However, when TiO_2 was added, the bonding strength of the coating was improved significantly. The bonding strength increased with an increase in TiO_2 content to 24.8 MPa for the H4TO6 coating.

The relatively poor adhesion of the HA coating mainly arises from the mismatch of CTEs between the Ti alloy substrate and HA coating.[15] The linear CTE of HA is about 15×10^{-6} K^{-1}, while that of the Ti-6Al-4V alloy substrate is only 8.8×10^{-6} K^{-1}. In the process of plasma spraying, the residual stress is manifested at the coating/substrate interface, which weakened the bonding strength between the coating and the substrate. As shown in Fig. 3, the CTE of the H4TO6 coating decreases significantly due to the addition of TiO_2, which is about 7 to 9×10^{-6} K^{-1}, very close to the CTE of the substrate. Therefore, the residual stress was reduced during plasma spraying and, thus, the bonding strength of the coating was improved.

3.3 SBF Test

The SEM examination results of the H4TO6 composite coating after immersion in SBF for 1, 7, and 14 days are presented in Fig.4. Figure 4(a) shows that the coating surface is covered by a loose and thin, newly formed layer consisting of small granules after 1 day's immersion in SBF. This dunelike layer is characterized by microcracks, similar to cracks forming naturally on a dry mud deposit. At higher magnification of the layer in Fig. 4(b), very small crystallites can be observed. With the continued immersion in SBF, the layer became denser and the granules in the layer gradually grew, as shown in Fig. 4(c) and (d). Figure 5 shows the cross sections of the H4TO6 coating after immersion in SBF. This layer grows in thickness with the increase of immersion time. After 14 days immersion, the thickness of the layer reached 10 μm (Fig. 5c).

The XPS analysis of the elements present on the coating surface after 14 days' immersion in SBF shows that the formed layer is comprised of O, Ca, P, and C. The XPS C1s spectrum shown in Fig. 6 indicates that the C1s spectrum is comprised of two peaks: one at 284.6 eV and another at 287.8 eV, corresponding to carbon contamination and the carbonate group CO_3^{2-}.[16] The SEM pictures and XPS results reveal that the layer

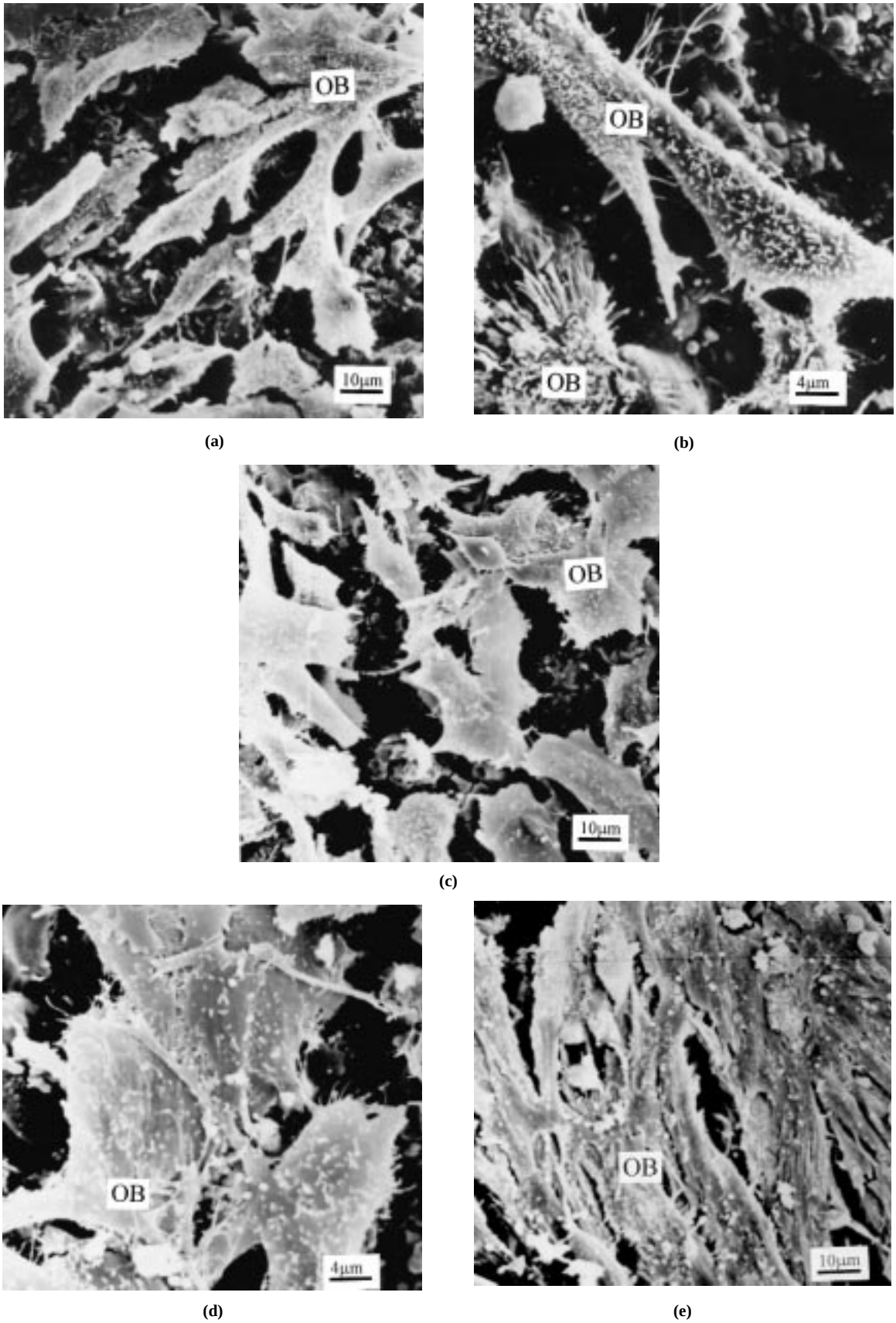


Fig. 7 Morphologies of osteoblasts cultured on the H4TO6 coating: **(a)** 1 day, **(b)** 1 day, **(c)** 4 days, **(d)** 4 days, and (**e**) 7days.

formed on the composite coating is carbonate-containing apatite.[16,17] The formation of the carbonate-containing apatite is considered to be one of the characteristics of the bioactivity for biomaterials.[17,18]

Although the addition of TiO_2 reduced the HA content in the coating, it did not affect the formation of carbonate-containing apatite layer in the SBF. The good bioactivity for the HA/TiO_2 composite coating has been confirmed by the SBF test in this study.

3.4 Cell Culture

The appearance of the osteoblasts cultured on the coating were examined by SEM. Osteoblasts grew and attached to the H4TO6 coating surface after 1 day's culture, although they were small and sparse (Fig. 7a). At higher magnification (Fig. 7b), osteoblast attached to the coating firmly and the pseudopodia of cells spread out across the coating, indicating good compatibility between the osteoblasts and the coating. The osteoblast morphology after 4 days' culture shown in Fig. 7(c) indicates that the cells became larger and denser as compared with Fig. 7(a). The higher magnification (Fig. 7d) shows that the osteoblasts have grown as multilayers in some regions. In Fig. 7(e), after 7 days' culture, osteoblasts completely spread on the coating surface and form a continuous layer in which individual cells cannot be distinguishable.

Therefore, the osteoblast cultures carried out on the H4TO6 coating indicate good cytocompatibility for the coating.

4. Conclusions

The HA/TiO_2 composite coatings were deposited successfully *via* plasma spraying. The bonding strengths of coatings were improved significantly by the fabrication of composite coatings. The characteristics of composite coatings in SBF showed that the composite coatings possess good bioctivity. Excellent cytocompatibility for HA/TiO_2 composite coatings was confirmed by the osteoblast cultures carried out on the H4TO6 coating.

Acknowledgment

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