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Biological responses of neonatal rat calvarial osteoblasts on plasma-sprayed HA/ZrO_2 composite coating

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Plasma-sprayed hydroxyapatite (HA) coating, applied to metal substrates, can induce a direct chemical bond with bone and hence achieve a biological fixation of the implant. However, the poor bonding strength between the HA coating and the substrate has been a concern for the orthopedists. In a previous study, the zirconia-reinforced hydroxyapatite composite coatings (HA/ZrO_2) could significantly improve the mechanical strength before and after soaking in simulated body fluid. This study aims to investigate the biological responses of osteoblasts on plasma-sprayed HA/ZrO_2 coating. The osteoblasts derived from neonatal rat calvarial were cultured in Dulbecco's modified Eagle medium (DMEM) with fetal bovine serum (FBS) on the surface of plasma-sprayed HA coating, HA/ZrO_2 coating, and ZrO_2 coating, respectively. The biological responses were investigated by the cell growth (1, 3, 5, and 10 days) and the cell morphology under scanning electron microscopy (SEM) (3, 6, 12, 24 and 48 h). Examination by SEM revealed that osteoblasts on HA coatings exhibit less spreading during the medium phase (6 and 12 h), while, better morphologies were observed at the latter phases (24 and 48 h). This should be derived by the dissolution of HA coating in the culture medium. On HA/ZrO_2 coating, the cells showed the poor morphologies at the latter phases (24 and 48 h). This could be explained by the no apatite formed at the surface HA/ZrO_2 coating after soaking in simulated body fluid. The lower contents of ZrO_2 coating in HA coating and the addition of other solid solution (ZrO_2–MgO, CaO–ZrO_2, ZrO_2–CeO_2) in HA coating are the two possible methods to improve the cytocompatibility of HA/ZrO_2 coating.

Introduction

Hydroxyapatite (HA) has the same chemical and crystallographic structure as the apatite of living bone, and can bond physicochemically with bone and promote bone growth onto its surface [1–4]. HA is therefore considered as an excellent bone substitute [5, 6]. However, the bulk form of HA was reported to be susceptible to fatigue failure [7, 8]. The problem can be solved by applying HA coatings on metal substrates; this method combines the biocompatibility and required mechanical properties. Recently, the application of plasma-sprayed HA coatings on a bioinert titanium alloy substrate to promote the fixation of orthopedic prostheses has attracted worldwide attention [9].

However, the long-term strength and fracture toughness of HA and HA coatings have been questioned [10, 11]. Zirconia, having the attributes of high strength and stress-induced phase transformation toughening, is a candidate material to strength the ceramics [12–14]. In a previous study, it was proved that the addition of yttria-stabilized zirconia improved the bonding of HA coatings to titanium, and the bonding degradation of zirconia-reinforced HA composite coatings (HA/ZrO_2) was also improved after immersion in simulated body fluid [15]. This could be attributed to the factor that the composite coating, with the addition of ZrO_2 in HA, significantly reduced the dissolution rate in simulated body fluid. During plasma spraying, less OH^- ions were lost in ZrO_2-containing composite coating. These factors, together with the reduced effective surface of the ZrO_2-containing HA coating, were attributed to the reduced dissolution rate of the composite coating.

The interaction occuring at the bone–biomaterial interface largely determines the success or failure of an

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implant [16–20]. The outcome at this site is dependent, in part, on a successful bone formation. The osteoblast is the cell type responsible for deposition of bone within the interface zone between implant and host tissue. It plays a fundamental role in the successful clinical outcome of an implant. Since these responses may subsequently affect the fixation of implants, it is important to understand the reaction between osteoblasts and biomaterials. The osteoblast culture model has been widely adopted to evaluate the biocompatibility of biomaterials because the tissue–implant interface can be partially reproduced. This system allows early evaluation of the degree of cytocompatibility under controlled conditions [21–24].

In this study, the *in vitro* test is to prepare the neonatal rat carlvarial osteoblast on the culture wells which were seated with different plasma-spraying coatings, including HA, HA/ZrO_2, and ZrO_2. The biological responses were assessed as the level of cell growth cultured on the different coatings. The cell morphology on coatings was also observed by scanning electron microscopy (SEM).

Materials and methods

Preparation of specimens

Powders of HA and ZrO_2 manufactured by Merck and Toyo Soda, respectively, were used. Typical sizes of ZrO_2(8 mol % Y_2O_3) and HA (at 50% cumulative mass per cent) were measured as 0.2 and 0.7 μm, respectively. Mixtures of the following compositions were investigated: HA, HA + 10 wt % ZrO_2 (HA/ZrO_2) and ZrO_2. The preparation of plasma-spraying powder have been well described in our previous study [25].

The 12.7 mm∅ × 2.0 mm disk plate used in this study is a surgical grade Ti6A14V alloy (ASTM F136–92). Bioinert Ti6A14V alloy was selected as the substrate. All the specimens were cleaned in acetone ultrasonically and then sand blasted to roughen the surface before plasma spraying with the prepared powders; the spraying parameters are shown in Table I. After plasma spraying, the surface roughness of the specimens is quantified using a surface profilometer (Kosaka Laboratory Ltd., Surfcorder SE-30H).

A total of three groups (HA coating, HA/ZrO_2 coating, ZrO_2 coating) was investigated, and all the specimens are subjected to ultrasonic wash five times in de-ionized water and one time in absolute alcohol. This is then packed in double-sealed autoclaving bags, steam sterilized at 121 °C for 30 min, dried at 121 °C for 15 min.

TABLE I Plasma spraying parameter employed for the preparing the HA coatings, HA/ZrO_2 coating, and ZrO_2 coating

Parameter	
Primary gas, flow rate (1 min^{-1})	Ar, 41
Secondary gas, flow rate (1 min^{-1})	H_2, 8
Powder carrier gas, flow rate (1 min^{-1})	Ar, 3.2
Powder feed rate (g min^{-1})	20
Power (kW)	40.2
Stand-off distance (cm)	7.5
Surface speed (cm min^{-1})	2400
Transverse speed (cm min^{-1})	60

Plasma spraying was performed with a Plasma-Technik system (M-1100 C).

Cell culture

Osteoblasts were isolated by sequential trypsin-collagenase digestion on calvaria of neonatal (< four days old) Sprague–Dawley rats using the procedure of Cohn and Wong [21]. Cells were collected from the third and fourth digestions. Phenotype and function of the cells were characterized by the presence of alkaline phosphatase and deposition of calcium phosphate mineral *in vitro*. Osteoblasts were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and maintained in a humidified, 5% CO_2/balance air incubator at 37 °C. Subcultured cells were used for experiments after two or three passages.

The sterilized disks were placed in 24-well culture plates. These tissue culture plates coated with 12% poly-HEMA (hydroxyethylmethacrylate) held the biomaterial samples to ensure that the osteoblasts would grow on the biomaterials only and not the tissue culture wells [20]. For experiments, the osteoblasts cells were seeded on the discs at a density of 75 000 cells/ml and flooded with growth medium supplemented with 4% FBS, 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate. Culture medium was replaced every two days during culture.

Cell morphology

Specimens were prepared for SEM after 3-, 6-, 12-, 24- and 48-h cultures. The medium was pipetted out from the dishes, and the plates were rinsed several times with cacodylate buffer (pH 7.2), and fixed for 2 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffered at pH 7.2; with post-fixation in 1% OsO_4 in buffer for 1 h; treatment with 1% tannic acid in buffer for 1 h; dehydrated in an ascending alcohol series; and immersed in HMDS (hemethyldisilazane) for 10 min in lieu of critical point drying. Finally, after sputter coating with gold, the specimens were examined using a Hitachi S-2500 scanning electron microscope at an accelerating voltage of 25 kV.

Cell number

The cell growth on each biomaterial sample was determined after 1-, 3-, 5-, and 10-day cultures. At the designated times, the osteoblasts were released from the biomaterial surface by addition of 0.05% trypsin containing 1 mM EDTA in phosphate-buffered saline (PBS), the cell suspension was then counted with a hemacytometer.

Statistical analysis

Each data point represents the mean ± standard deviation of five individual cultures. The analysis of one-way variance (ANOVA) was used to evaluate the significance differences between cell growth on different kinds of materials. Differences were considered significant at $p < 0.05$.

TABLE II Average surface roughness (μm) of HA coating, HA/ZrO_2 coating, and ZrO_2 coating, respectively

Materials	Roughness
HA coating	10.37 ± 1.35
HA/ZrO_2 coating	11.34 ± 1.38
ZrO_2 coating	11.67 ± 1.31

$n = 5$ specimens per group. The value is presented by mean $\pm$ standard deviation.

Results

Surface roughness

After plasma spraying, the surface roughness of HA coating, HA/ZrO_2 coating, and ZrO_2 coating is shown in Table II. The smoothest surface of $10.37 \pm 1.35\,\mu m$ is measured at the surface of HA coating, and the surface roughness of HA coating is significantly smoother than ZrO_2 coating (refer to Table II). Although the surface roughness of ZrO_2 coating is rougher than HA/ZrO_2 coating, there is no statistical difference between HA/ZrO_2 coating and ZrO_2 coating by one-way ANOVA.

Cell morphology

After 3-h culture in medium containing 4% FBS, the osteoblast morphologies on the surface of specimens are observed by SEM (Fig. 1). Some of cells grown on HA coating exhibit spherical shapes, and some are polygonal and contact the surface of HA coating by focal adhesions. Cells cultured on the surface of HA/ZrO_2 and ZrO_2 coatings have a morphology very similar to those cultured on HA coating.

Figs 2(a)–2(c) represent the micrographs from SEM of osteoblasts cultured for 6 h on all three specimens (HA coating, HA/ZrO_2 coating, ZrO_2 coating). As shown in Fig. 2, osteoblasts cultured on HA coating have a less spreading morphology than HA/ZrO_2 and ZrO_2 coating, even though there were a lot of cells attached to the surface of HA coating. On the surface of HA/ZrO_2 and ZrO_2 coating, osteoblasts appear to spread, elongate and take on the morphology of the underlying respective specimens. Furthermore, the cells on HA/ZrO_2 and ZrO_2 coating extend filopodia to across the valley of topography and form multiple points of attachment of the cell to the surface.

After 12-h culture, cells cultured on HA coatings were also spreading less than HA/ZrO_2 and ZrO_2 coating by the observation of SEM. With increasing culture period from 6 to 12 h, cells on surface of HA/ZrO_2 coating further spread and almost completely took on the morphology of the underlying respective specimens (Fig. 3(b)). However, osteoblasts on ZrO_2 coating were not significantly changed in morphology with increasing culture period from 6 to 12 h (Fig. 3(c)).

Figs 4(a–c) represent randomly taken SEM of

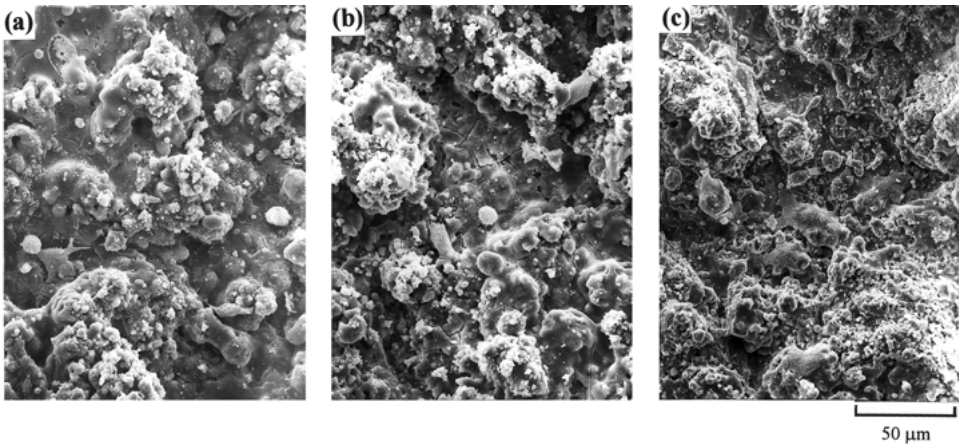

Figure 1 Scanning electron micrographs of osteoblasts cultured for 3 h (a) HA, (b) HA/ZrO_2, and (c) ZrO_2 coatings. On three different coatings, cells have a very similar morphology.

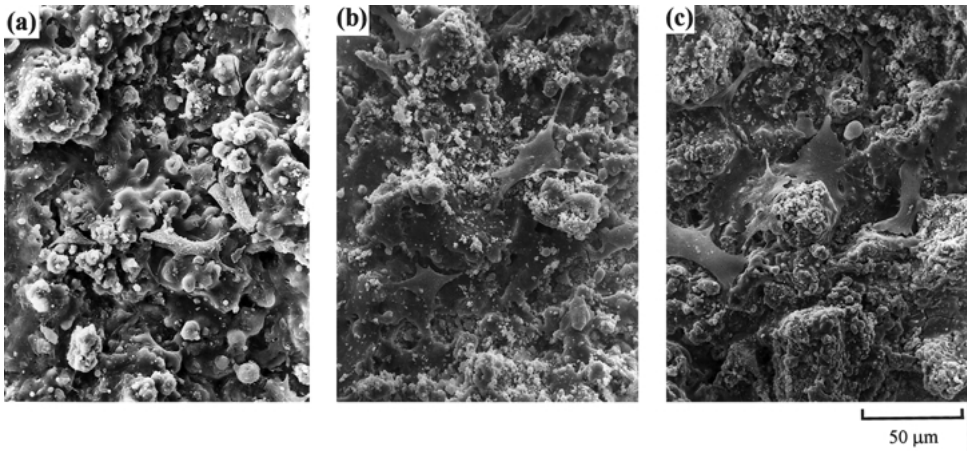

Figure 2 Scanning electron micrographs of osteoblasts cultured for 6 h on (a) HA, (b) HA/ZrO_2, and (c) ZrO_2 coatings. The osteoblasts cultured on HA coating have a less spreading morphology than HA/ZrO_2 and ZrO_2 coatings.

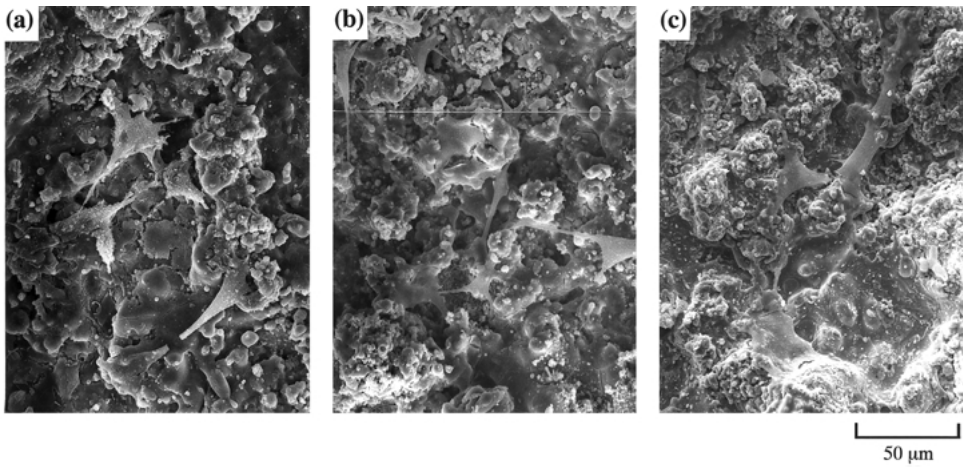

Figure 3 Scanning electron micrographs of osteoblasts cultured for 12 h on (a) HA, (b) HA/ZrO_2, and (c) ZrO_2 coatings. The cells cultured on HA coating exhibit less spreading than HA/ZrO_2 and ZrO_2 coatings.

osteoblasts cultured for 24 h on HA coating, HA/ZrO_2 coating, and ZrO_2 coating. Cells on HA coating have a more flattened morphology with increasing culture period from 12 to 24 h (Fig. 4(a)). Filopodia were also seen to connect distant cells. In this period, cells on surface of HA/ZrO_2 and ZrO_2 coating spread very well. However, some fractured osteoblasts have been found by the observation of SEM, and this phenomenon indicates the cells to be in poor condition.

After 48 h, cells grown on HA coating spread well, and some cells were more closely in contact with substrate (Fig. 5(a)). Osteoblasts cultured on surface HA/ZrO_2 and ZrO_2 coating have a poor cell morphology, sometimes even with the destruction of the cell membrane.

Cell number

As shown in Fig. 6, the cell growths of osteoblasts on the surface of each specimen were evaluated with cell number after culturing for 1, 3, 5, and 10 days, respectively. The mean cell number per area increases significantly $(p < 0.01)$ over time for all specimens. After culturing for 1 day, cell number per area on HA coating surface is significantly higher $(p < 0.01)$ than HA/ZrO_2 coating and ZrO_2 coating. Although the cell number on HA/ZrO_2 coating is higher than ZrO_2 coating, the significant difference is not found by one-way ANOVA.

After 3-day culture, the number per area of cells on HA coating is higher than HA/ZrO_2 coating and ZrO_2 coating, and the differences in cell number per area were significant $(p < 0.001)$ for HA/ZrO_2 coating and ZrO_2 coating. The cell numbers per area on HA/ZrO_2 coating was 1.6 times that of ZrO_2 coating, and the difference in cell number per area was evident $(p < 0.001)$ between HA/ZrO_2 coating and ZrO_2 coating.

After 5-day culture, the cell number per area on HA coating was still significantly higher $(p < 0.01)$ than HA/ZrO_2 coating and ZrO_2 coating by one-way ANOVA, and the cell number per area on HA/ZrO_2 coating was also statistically higher $(p < 0.01)$ than ZrO_2 coating.

After 10-day culture, the number per area of cells on HA coating was higher than HA/ZrO_2 coating and ZrO_2 coating, and the differences in cell number per area was significant $(p < 0.0005)$ for HA/ZrO_2 coating and ZrO_2 coating. Although the cell number on HA/ZrO_2 coating was higher than ZrO_2 coating, the significant difference was not found in the one-way ANOVA statistical test.

Discussion

In the previous study [26, 27], both intramedullary and transcortical implant models in the canine femora were developed to evaluate the shear strength and the failure

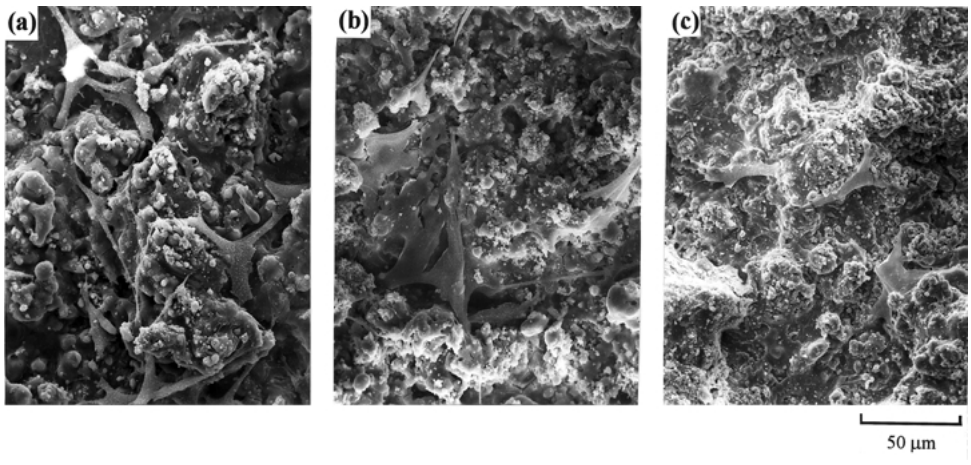

Figure 4 Scanning electron micrographs of osteoblasts cultured for 24 h on (a) HA, (b) HA/ZrO_2, and (c) ZrO_2 coatings. On surface of HA/ZrO_2 and ZrO_2 coatings, some fractured osteoblasts are observed by SEM, indicating the cells to be in poor condition.

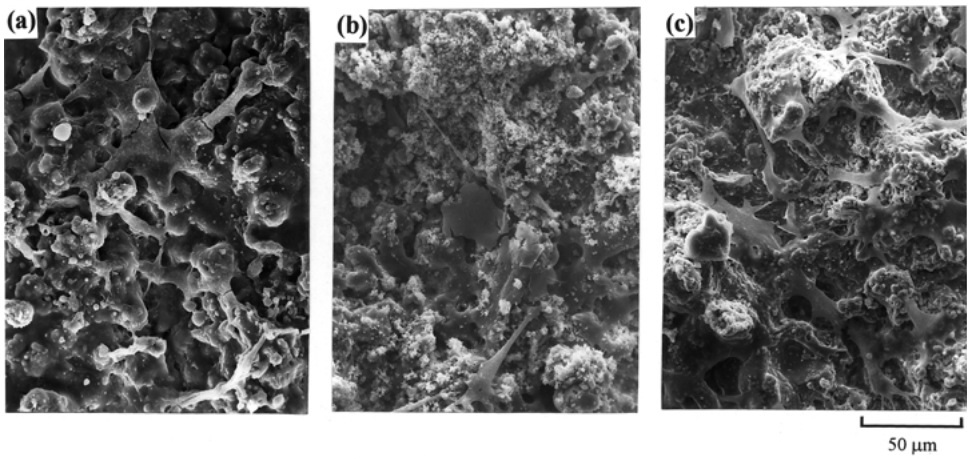

Figure 5 Scanning electron micrographs of osteoblasts cultured for 48 h on (a) HA, (b) HA/ZrO_2, and (c) ZrO_2 coatings. On HA coatings, osteoblasts showed a healthy cell morphology in comparison with HA/ZrO_2 and ZrO_2 coatings.

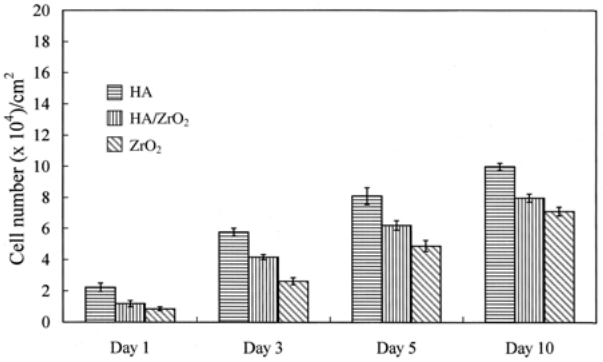

Figure 6 The growth of osteoblasts on different specimens. When compared to HA/ZrO_2 coating and ZrO_2 coating, the higher number of cells grown on HA coating is observed with statistical difference during all culture periods. Values are the mean $\pm$ standard deviation ($n = 5$).

model of plasma-sprayed HA coatings to cancellous and cortical bone, respectively. After the push-out test, the failure model of HA coatings to bone were observed in three regions, (I) the failure site at or near the HA coating-bone interface, (II) failure sites at the HA coating–titanium interface, and (III) failure sites inside the lamellar structure of the coating. In order to improve the bonding strength of HA coating–titanium interface, the concept of a bond coat to provide a mechanical or chemical bond between HA coating and bond coat can be exercised [28]. In the previous study [15, 25], the mechanical strength of HA coating can be increased by the addition of ZrO_2. However, the biocompatibility of plasma-sprayed HA/ZrO_2 coating needs further investigation before clinical use. An indication of biocompatibility on bone apposition and bone formation is in association with the bone cell response induced by biomaterials. Recently, cell cultures were applied to investigate the biological response of bone–implant interface during early phase [29, 30]. The purpose of the present study is to compare HA/ZrO_2 coating with HA coating, and ZrO_2 coating. The cell morphology and number obtained from osteoblasts cultured on these experimental coatings was evaluated.

Histological observation with SEM demonstrated that osteoblasts cultured on HA/ZrO_2 coating exhibited a very similar morphology to those on HA and ZrO_2 coating after 3-h culture. However, with increasing culture period from 3 to 6 and 12 h, the osteoblasts cultured on HA coating has a less spreading morphology than HA/ZrO_2 and ZrO_2 coatings. After 24 h, some fractured cells on HA/ZrO_2 and ZrO_2 coatings were observed, while, osteoblasts on HA coating have more flattened and spreading morphologies. Increasing the culture period to 48 h, some cells grown on HA coatings were more in close contact with substrate by SEM. On HA/ZrO_2 coating, osteoblasts exhibited a damaged and broken cell membrane, indicating poor cell morphology. The relation between cell morphology on HA, HA/ZrO_2, ZrO_2 coatings and culture period could be divided into three stages. During the early phase (3 h), cells on HA/ZrO_2 and ZrO_2 coating exhibited a similar morphology to HA coating. In the medium phases (6 and 12 h), osteoblasts grown on HA/ZrO_2 and ZrO_2 coatings show a better morphology than HA coating by SEM, while, in the latter phase (24 and 48 h), cells cultured on HA/ZrO_2 and ZrO_2 coating exhibit a poorer morphology than HA coating.

Schwartz *et al*. [29] have mentioned that materials properties such as surface energy, composition, roughness, and topography are believed to be of critical importance at the implant–tissue interaction. As shown in Table II, there was no significant difference of surface roughness found between HA coating and HA/ZrO_2 coating. The osteoblasts behavior affected by surface roughness for HA coating and HA/ZrO_2 coating could not be considered. In this study, the result of cell morphologies on coating is possibly related to both the phase composition and dissolution behavior of coating in the culture medium. In the previous studies [25], the phase composition of HA and HA/ZrO_2 coating was identified by XRD analysis, and the contents of phase composition are shown in Table III. After plasma-sprayed process, apart from the HA phase, several impurity phases including α-$Ca_3(PO_4)_2$, β-$Ca_3(PO_4)_2$, $Ca_4P_2O_9$, and

TABLE III Relative phase contents (%) in HA and HA/ZrO_2 coating [25]

Phase	α-TCP	β-TCP	$Ca_4P_2O_9$	CaO
HA	15.09	7.87	7.05	12.85
HA/ZrO_2	40.39	8.16	9.52	7.08

CaO have been identified in the HA coating [25]. Although the phase composition, except for the existence of ZrO_2 and $CaZrO_3$, of HA/ZrO_2 coating is similar to HA coating, the increase of α-$Ca_3(PO_4)_2$ phase and decrease of CaO phase were observed in the HA/ZrO_2 coating [25]. After plasma-sprayed process, the cubic phase of ZrO_2 still maintained in the HA/ZrO_2 coatings, and the monoclinic and tetragonal phases were not found in the X-ray diffractometry (XRD) pattern [31]. After autoclaving treatment, the monoclinic and tetragonal phases were still not found in the HA/ZrO_2 coatings by XRD analysis.

During early phase (3 h), the initial dissolution of HA coating did not significantly influence the osteoblasts behavior on its surface compared to more stable HA/ZrO_2 coating. After culture of 6 and 12 h, the osteoblasts morphologies could be mainly affected by the factor of dissolution behavior of coatings. The dissolution of HA coating could not provide a stable structure for spreading and flattened expression of cells. During the latter phase (24 and 48 h), the expression of cells could be significantly influenced by the phase composition of coating rather than dissolution behavior because the dissolution rate of HA coating became slower than those of early and medium phase. The dissolution of HA coating became slower than those of early and medium phase. The dissolution of HA coating in simulated body fluid have been well investigated by many researchers [32, 33], and their results indicate that the dissolution rate of HA coating significantly decreases after longer immersion periods. In order to acquire more stable HA coating, Anselme *et al*. immersed plasma-sprayed HA coating various days in complete culture medium [34]. Their results show that adult human bone-derived cells were apparently sensitive to the changes in the coating surface induced by liquid phase, and cell numbers significantly increase after longer immersion periods. As shown in Table III, the addition of ZrO_2 increased the impurity phases (α-TCP, β-TCP and $Ca_4P_2O_9$) in the HA/ZrO_2 coating, and the more significant dissolution of HA/ZrO_2 coating would be found after soaking in medium. However, Edward *et al*. [15] have investigated the properties of HA/ZrO_2 coating, the same sample preparation as this study, immersed in simulated body fluid. The researchers have found that the significant dissolution were found in the HA coatings compared to HA/ZrO_2 coatings. After soaking in simulated body fluid, the disappeared impurity phase have been found in HA coatings. Besides, the dissolution/precipitation phenomena have been observed on the HA coating, and the transformation of the HA coating into carbonate HA has been identified by XRD and Fourier transformed infrared spectroscopy (FTIR). In comparison with HA coatings, the HA/ZrO_2 coating also preserved the original phases and no formation of new phase in simulated body fluid. These results indicated that there were no dissolution/precipitation phenomena formed on the HA/ZrO_2 coatings in simulated body fluid. By WDS mapping, Zr element has uniform distribution in HA/ZrO_2 coating. Although the addition of ZrO_2 increased the α-TCP phases in the HA/ZrO_2 coating, factors such as less loss OH^- ions during plasma spraying and the decreased effective surface of HA were attributed to the reduced dissolution rate of the composite coating.

Kokubo *et al*. [35–37] soaked glass ceramics, A-W, in SBF, and found that the surface was covered with a layer of apatite phase in seven days. After implanting the A–W into the tibias of rabbits, the bone came in direct contact with the surface of A–W after eight weeks [35]. The alumina containing A–W(Al) has a similar phase composition to A–W, but with different glass matrices. In *in vitro* test, the A–W(Al) did not form the apatite phase on its surfaces in SBF [35]. In the *in vivo* test, the A–W(Al) did not bond with the bone even after 25 weeks past implantation in the same implant model with the A–W. Kokubo *et al*. [38] soaked the Ceravital type glass ceramics in SBF, and the apatite phase was observed on their surfaces. Using the same implant model of A–W, the Ceravital type glass ceramics bonded to living bone in the metaphyses of the tibias of rabbits. The above results indicated that the biocompatibility of bioactive glass/glass ceramics mainly depend on the formation of apatite phase on the surface than the phase composition of materials. In this study, the higher contents of α-TCP, β-TCP and $Ca_4P_2O_9$ in HA/ZrO_2 coating would provide the more unstable surface for cell attachment compared to HA coating during early phase, because the α-TCP, β-TCP and $Ca_4P_2O_9$ are easy to dissolve compared to HA phase after soaking in medium. However, significant dissolution was found in the HA coatings compared to HA/ZrO_2 coatings in the previous study, and the result indicated that the increase of impurity phases has not significantly influenced the biological properties of HA/ZrO_2 coatings than the presence of ZrO_2 in coatings. Besides, the formation of apatite on HA/ZrO_2 coating have not been observed after soaking in simulated body fluid, and this phenomenon could possibly explain the results of osteoblast morphologies and numbers on HA/ZrO_2 coating. Although the addition of ZrO_2 is possible to reduce the original biocompatibility of HA coating, the HA/ZrO_2 coating still provided better biocompatible property than ZrO_2 coating. The assumption could be confirmed by the results of cell numbers per area on HA/ZrO_2 and ZrO_2 coating, respectively.

Although the dissolution behavior of HA coating is more significant than HA/ZrO_2 coating, the dissolution of Ca and P ions from HA coating could provide better environment for cell growth. Lugscheider *et al*. [39] observed that HA coating dissolved saline solution and that HA coatings stimulate L929 cell growth. They did conclude that the positive effect on cell growth of Ca and P content increases in medium. In this study, the dissolution of the HA coating could not provide more stable substrate but an optimum medium condition for cell growth. This could also explain better cell morphologies during later phase and higher cell numbers per area on HA coating than HA/ZrO_2 coating.

In order to improve the biocompatibility of HA/ZrO_2 coating, two methods for further investigation are suggested: (1) The contents of ZrO_2 in HA coating could be decreased to acquire similar phase composition and contents to HA coating, and (2) Adopt the Y_2O_3-stabilized ZrO_2 as reinforced materials, and the addition of stabilizing oxide, Y_2O_3, to pure ZrO_2 (this allows us to acquire a modest phase with higher mechanical proper-

ties at room temperature). The other solid solutions (ZrO_2–MgO, CaO–ZrO_2, ZrO_2–CeO_2) could also be used to reinforce HA coating.

Conclusion

This work was carried out to compare the osteoblast morphologies and numbers on plasma-sprayed HA coatings, HA/ZrO_2 coating, and ZrO_2 coating, respectively. In the *in vitro* experiments, the divergence of osteoblast morphologies on three different coatings could be divided into three phases of culture periods. The cell morphologies on coatings were affected by the factors of phase composition and dissolution behavior of coating after immersion in culture medium. During the medium phase (6 and 12 h), cells grown on HA/ZrO_2 coating exhibited better morphologies than HA coating. While, cells grown on HA/ZrO_2 coating showed inferior morphologies than HA coating at later phase (24 and 48 h). The authors suggest that the lower contents of ZrO_2 in HA coating and the addition of another stabilizing oxide (CaO, MgO, and CeO_2) to ZrO_2 are the two possible methods to improve the cytocompatibility of HA/ZrO_2 coating *in vitro*.

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References

1. C. A. VAN BLITTERSWIJK, S. C. HESSELING, J. J. GROTE, H. K. KOERTEN and K. DE GROOT, *J. Biomed. Mater. Res.* **24** (1990) 433.
2. C. A. VAN BLITTERSWIJK, J. J. GROTE, W. KUIJPERS, C. J. G. BLOK-VAN HOEK and W. TH. DAEMS, *Biomaterials* **6** (1985) 243.
3. H. A. HOOGENDOORNM W. RENOOIJ, L. M. A. AKKERMANS, W. VISSER and P. WITTEBOL, *Clin. Orthop.* **187** (1983) 281.
4. T. KITSUGI, T. YAMAMURO and T. KOKUBO, *J. Biomed. Mater. Res.* **24** (1990) 259.
5. R. E. HOLMES, R. W. BUCHOLZ and V. MOONEY, *J. Bone Joint Surg.* **68A** (1986) 904.
6. R. W. BUCHOLZ, A. CARLTON and R. HOLMES, *Clin. Orthop.* **240** (1989) 53.
7. M. JARCHO, C. H. BOLEN, M. B. THOMAS, J. BOBICK, J. F. KAY and R. H. DOREMUS, *J. Mater. Sci.* **11** (1976) 2027.
8. G. DE WITH, H. J. A. VANDIJK, N. HATTU and K. PRIJS, *ibid.* **16** (1981) 1592.
9. J. E. LEMONS, *Clin. Orthop.* **235** (1988) 220.
10. K. DE GROOT, R. GEESINK, C. P. A. T. KLEIN and P. SEREKIAN, *J. Biomed. Mater. Res.* **21** (1987) 1375.
11. C. C. BERNDT and J. H. YI, *Mater. Sci. Forum* **34** (1988) 469.
12. Y. MURASE, E. KATO and K. DAIMON, *J. Am. Ceram. Soc.* **69** (1986) 83.
13. S. R. WITEK and D. BUTLER, *J. Mater. Sci. Lett.* **4** (1985) 1412.
14. Q. L. GE, T. C. LEI and Y. ZHOU, *Mater. Sci. Technol.* **7** (1991) 490.
15. E. CHANG, W. J. CHANG, B. C. YANG and C. Y. YANG, *J. Mater. Sci.: Mater. Med.* **8** (1997) 201.
16. T. MATSUDA and J. E. DAVIES, *Biomaterials* **8** (1987) 275.
17. S. F. TARRANT and J. E. DAVIES, in ''Biomaterials and Clinical Application'', edited by A. Pizzoferrato, P. G. Marchetti, A. Ravaglioli and A. J. C. Lee (Elsevier, Amsterdam, 1987) pp. 585–590.
18. H. S. CHEUNG and M. H. HAAK, *Biomaterials* **10** (1989) 63.
19. F. B. BAGAMBISA and U. JOOS, *ibid.* **11** (1990) 50.
20. D. A. PULEO, L. A. HOLLERAN, R. H. DOREMUS and R. BIZIOS, *J. Biomed. Mater. Res.* **25** (1991) 711.
21. D. V. COHN and G. L. WONG, in ''Skeletal Research'', edited by D. J. Simmons and A. S. Kunin (Academic Press, New York, 1979) pp. 3–20.
22. P. BOONEKAMP, Thesis, University of Leiden, The Netherlands (1982).
23. W. A. PECK, S. J. BIRGE and S. A. FEDAK, *Science* **146** (1964) 1476.
24. D. MASQUELIER, B. HERBERT, N. HAUSER, P. MERMILLOD, E. SCHONNE and C. REMACLE, *Calcif. Tiss. Int.* **47** (1990) 92.
25. E. CHANG, W. J. CHANG, B. C. YANG and C. Y. YANG, *J. Mater. Sci.: Mater. in Med.* **8** (1997) 193.
26. B. C. WANG, T. M. LEE, E. CHANG and C. Y. YANG, *J. Biomed. Mater. Res.* **27** (1993) 1315.
27. C. Y. YANG, B. C. WANG, T. M. LEE, E. CHANG and G. L. CHANG, *ibid.* **36** (1997) 39.
28. H. KURZWEG, R. B. HEIMANN, T. TROCZYNSKI and M. L. WAYMAN, *Biomaterials* **19**, 1507.
29. Z. SCHWARTZ and B. D. BOYAN, *J. Cell. Biochem.* **56** (1994) 340.
30. J. FOLKMAN and A. MOSCONA, *Nature* **273** (1978) 345.
31. B. Y. CHOU and E. CHANG, *Biomaterials* **20** (1999) 1823.
32. P. FRAYSSINET, F. TOURENNE, N. ROUQUET, P. CONTE, C. DELGA and G. BONEL., *J. Mater. Sci. Mater. Med.* **5** (1994) 11.
33. C. Y. YANG, B. C. WANG, E. CHANG and B. C. WU, *ibid.* **6** (1995) 258.
34. K. ANSELME, P. SHARROCK, P. HARDOUIN and M. DARD, *J. Biomed. Mater. Res.* **34** (1997) 247.
35. T. KOKUBO, in ''CRC Handbook of Bioactive Ceramics'', edited by T. Yamamuro, L. L. Hench and J. Wilson (CRC Press, Florida, 1990) pp. 41–49.
36. T. KOKUBO, *Biomaterials* **12** (1991) 155.
37. K. OHURA, T. NAKAMURA, T. YAMAMURO, T. KOKUBO, Y. EBISAWA, Y. KOTOURA and M. OKA, *J. Biomed. Mater. Res.* **25** (1991) 357.
38. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA, T. NAKAMURA and T. YAMAMURO, *J. Mater. Sci.: Mater. Med.* **4** (1993) 127.
39. E. LUGSCHEIDER, M. KNEPPER, B. HEIMBERG, A. DEKKER and C. J. KIRKPATRICK, *ibid.* **5** (1994) 371.

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