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Short communication

In vitro cytocompatibility of plasma-sprayed dicalcium silicate/zirconia composite coatings

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Abstract

Dicalcium silicate/zirconia composite coatings composed of 70 wt% zirconia and 30 wt% dicalcium silicate were prepared by plasma spraying. Human osteoblasts were used for evaluation of the cytocompatibility of the composite coatings with Ti and hydroxyapatite (HA) coatings used as controls. The cells on the composite coatings possessed abundant extensions, and the cell number was also the highest among these coatings. During the 7 days culture, cell density on the composite coatings maintained higher than that on the Ti coatings, but similar to that on the HA coatings. To evaluate the bioactivity, the composite coatings were immersed in simulated body fluids and cell culture media. Ca–P minerals were deposited on the coating surface after immersion in the cell culture media for 1 day, while only scattered particles were found after soaking in simulated body fluids for 2 days. The enhanced Ca–P mineral formation rate of the composite coatings was explained by the Si–OH functional groups and the adsorbed proteins on the coating surface.

Keywords: Cytocompatibility; Plasma spraying; Composite coatings

1. Introduction

Titanium and its alloys are considered to be one of the best metallic materials for orthopedic and dental implants because of the relatively low modulus and good fatigue strength, corrosion resistance, and biocompatibility (Ref. [1]). Plasma spraying can effectively produce a bioactive surface layer, such as hydroxyapatite (HA), on the titanium substrates and is a very useful way to combine the good mechanical properties of titanium and the bioactivity of HA to meet the requirements of orthopedic and dental implants. However, the low bonding strength and dissolution of HA coatings often cause the failure of HA-coated implants in the long-term usage. Recently, wollastonite and dicalcium silicate have drawn great interests because of their higher bonding strength with titanium alloy substrates as well as their ability to induce Ca–P mineral deposition in simulated body fluids (Refs. [2,3]). In vivo experiments showed that bone tissues could extend and grow into the coatings, and bond directly to the coated implants without fibrous tissue (Ref. [4]). However, dissolution and degradation of the coatings are still problematic with respect to the long-term clinical usage.

In order to mitigate the dissolution and degradation, dicalcium silicate composite coatings with various contents of zirconia were fabricated and durability of the composite coatings in simulated body fluids was evaluated (Ref. [5]). In vitro results showed that bone-like apatite could form on the surface of the coatings with less than 70 wt% zirconia after 7 days of immersion in simulated body fluids. The durability of the composite coatings was enhanced with increase of zirconia contents in the coatings.

In this paper, in vitro cell culture experiments were performed using human osteoblasts to evaluate the cytocompatibility of the composite coatings containing 70 wt% zirconia. Cellular responses to the coating including cell adhesion, spreading and proliferation were investigated.

2. Experimental processes

2.1. Preparation of the composite coatings

Dicalcium silicate powder was synthesized by calcination of stoichiometric mixtures of calcium oxide (CaO) and

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reagent-grade quartz at 1450 °C for 2 h, as reported by Gou et al. (Ref. [6]) and sifted through an 800-mesh sieve. The obtained powder (30 wt%) was mechanically blended with 70 wt% commercially available yttria stabilized zirconia powder (typical size ranging from 50 to 106 μm) as feedstock. Atmospheric plasma spraying (APS) system (Sulzer Metco, Switzerland) was utilized to fabricate the composite coatings (denoted as CZ) with Ti–6Al–4V as substrates (10 mm × 10 mm × 2 mm).

Vacuum plasma spraying system (Sulzer Metco, Switzerland) was used for the preparation of Ti and HA coatings for comparison.

2.2. In vitro cytocompatibility evaluation

Human osteoblasts were obtained by a conventional explanting technique (Ref. [7]). All tested samples were sterilized by an autoclave at 120 °C for 30 min before cell culture. Cells were seeded on samples in 24-well plates at 5×10^4 cells/cm^2 and cultured at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity using the medium composed of Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. The medium was refreshed every other day. After cultivation for 1, 4 and 7 days, the samples were taken out from the culture plates and fixed with 2.5% glutaradehyde in a 0.1 M sodium cacodylate buffer (pH = 7.4) for 1 h. After rinse with phosphate-buffered saline (PBS) (10 min × 3) and sequential dehydration in a series of ethanol (50%, 70%, 95%, 100%) for 10 min × 2, the adhesion and spreading of the cells on the coatings were observed by SEM.

MTT assay was applied for determination of the viability and proliferation of the cells. After the original culture medium was removed, 720 μL fresh culture media and 80 μL of MTT solution (5 mg/mL) were added to the well and incubated for another 4 h. Then, the upper medium was removed carefully and the intracellular formazan was solubilized by adding 800 μL dimethyl sulfoxide (DMSO) to each well. The absorbance of the resulting solution was measured using spectrophotometry at a wavelength of 570 nm. Results were reported as absorbance which is directly proportional to the number of the living cells present on the coating surfaces.

3. Results and discussions

3.1. Behaviors of the Cells

Cellular behaviors such as adhesion, morphological change, functional alteration, proliferation, and differentiation are greatly affected by surface properties including composition, roughness, hydrophilicity, surface texture, and morphology. The detailed surface properties of the composite coatings were reported previously (Ref. [5]). In this paper, we further studied their effects on cellular behaviors.

Cell adhesion is a key step before cell growth and proliferation. Fig. 1 showed the cell morphologies after culture for 12 h. All of the cells showed good adhesion on the composite (Fig. 1A), Ti (Fig. 1B) and HA (Fig. 1C)

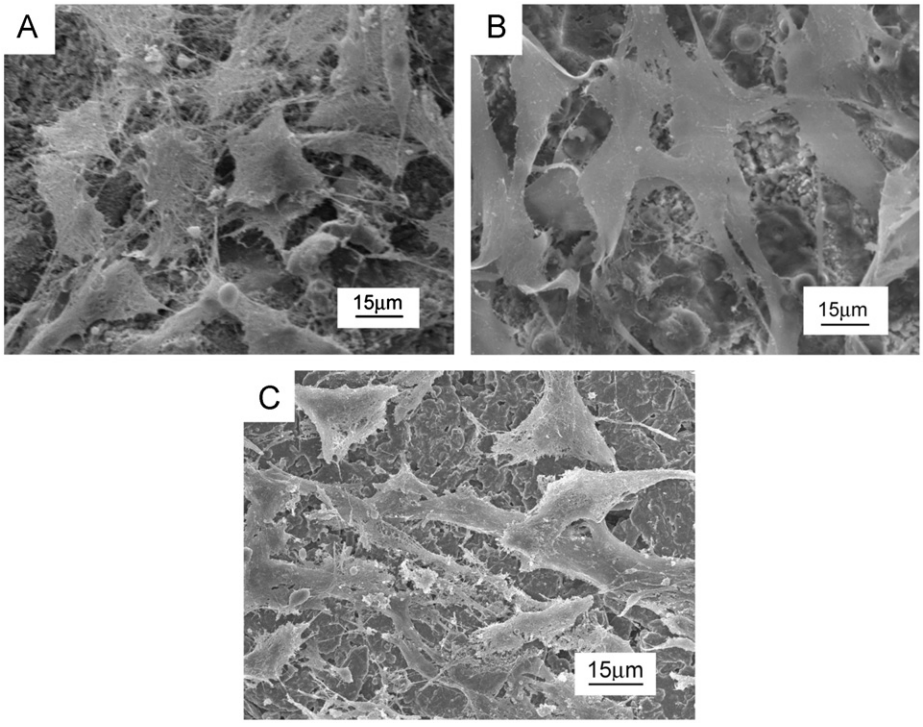

Fig. 1. SEM images of the cells on CZ (A), Ti (B) and HA (C) coating surface.

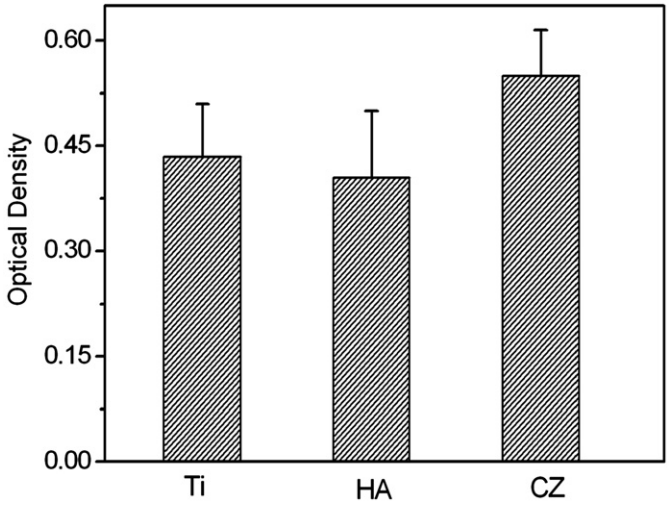

Fig. 2. Comparison of cell attachment on different coatings.

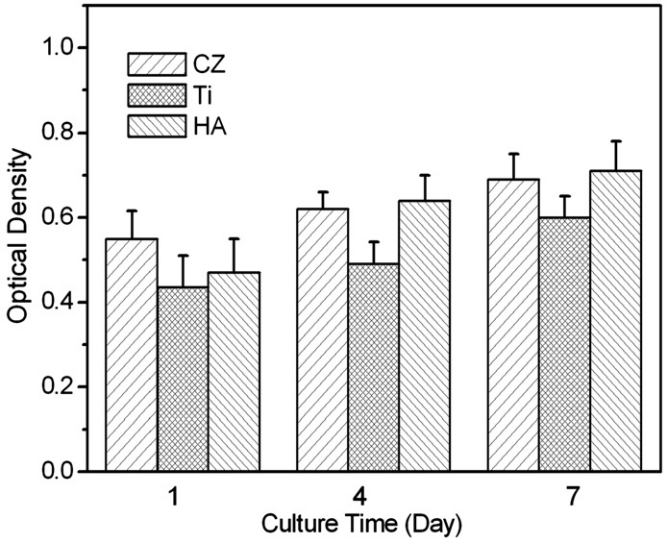

Fig. 3. Cell proliferation on the CZ composite coating surface compared to those on Ti and HA coatings.

coating surfaces. They spread and reached large sizes with compact bodies, appeared polygonal shape with many extending cyto-plasmic processes adhering to the coating surface. The cells on the composite coatings possessed much more cellular extensions than those on the Ti and HA coating surfaces. The number of attached cells after 24 h of incubation was measured by MTT. The highest number of cells was detected on the composite coatings (Fig. 2).

Cell adhesion is primarily mediated by the integrin family of cell-surface receptors (Ref. [8]). Integrins not only anchor cells, support cell spreading and migration, but also trigger signals that regulate survival, proliferation, and differentiation of cells (Refs. [9–15]). Integrins along the cell-substrate interface are critical in controlling the distribution of forces throughout the cells and influence the overall adhesion strength (Ref. [16]). The surface chemistry of underlying substrates is an important factor affecting integrin binding. Keselowsky et al. (Ref. [17]) reported that the integrin binding and cell adhesion is a function of underlying surface chemistry and the effects of surface functional groups are following a trend of $OH > COOH = NH_2 > CH_3$. On the CZ composite coating surface, there are abundant Si–OH functional groups caused by the dissolution of dicalcium silicate and exchange of Ca and H ions in the culture media (Ref. [2]). This kind of OH-rich surface was reported to be favorable for the intergin binding and cell adhesion (Ref. [17]).

Surfaces that favor the binding of integrins and cell adhesion always favor cell proliferation. With the high amount of attached cells, the composite coatings also showed good properties for cells proliferation. During the 7 days of incubation, the cells on the composite coatings maintained a relatively higher proliferation rate (Fig. 3) than those on the Ti coatings, but similar to those on the HA coatings.

3.2. Protein-associated Ca–P deposition

Upon the implantation of biomaterials into human body, many reactions take place at the interface of biomaterials and bone tissue. One is the formation of the so-called biologically equivalent apatite surface (Ref. [18]) which is deposited in an organized fashion in the matrix (either cellular or extracellular) of living organisms. The formation of this apatite layer is one of the multiple sequential steps involved in the bone-bonding process and is very important for the establishment of a direct structural and functional connection between the living bone tissue and biomedical implants. In our experiments, lots of particulate deposits with a honeycomb microstructure were found on the body of cells cultured on the composite coatings after 1 day of incubation in the cell culture media (Fig. 4A). The results of EDS analysis showed that the deposits were mainly composed of calcium, phosphorus and oxygen (Fig. 4B). For comparison, similar samples were immersed in simulated body fluids. By contrast, only scattered globular particles were found on the coating surface after 2 days of immersion (Fig. 4C). These results demonstrated that the rate of Ca–P mineral deposition was greatly improved due to the existence of proteins.

It is well known that a thin layer of proteins is immediately formed on the biomaterial surface after implantation. The conformation of the adsorbed proteins changes upon contact with biomaterials and these proteins provide a higher capacity to bind calcium ions (Refs. [19,20]). Resulting from the limited lifetime of proteins (for example, half-lifetime of albumin is 7 days (Ref. [21])), the thin layer of calcium–protein complexes degrades and then releases calcium ions into media, which leads to a local supersaturation of Ca and P ions facilitating the deposition of Ca–P minerals. In this study, the dissolution of dicalcium silicate in the composite coatings not only led to the increase in calcium ion concentration in the media but also resulted in the formation of Si–OH rich layer due to the

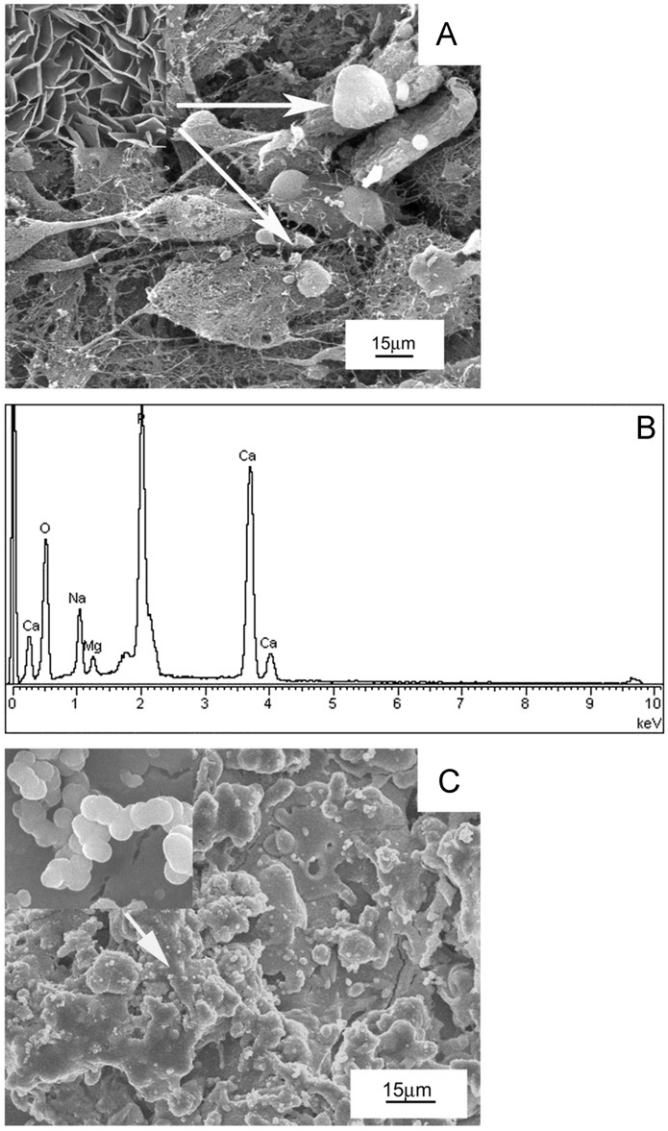

Fig. 4. SEM images of the deposited Ca–P minerals on the composite coatings surface: (A) after 1 day of incubation in culture media, (B) EDS spectrum, and (C) after immersion in simulated body fluids for 2 days.

preferential release of Ca ions, which in turn enhanced the protein adsorption (Ref. [17]). The calcium ions might be re-adsorbed to the coating surface by reaction with Si–OH groups or active sites of the adsorbed proteins, and thereafter induced the formation of Ca–P minerals. The active sites in the coating surface with adsorbed proteins were much more than those immersed in simulated body fluids, which contributed to the acceleration of Ca–P mineral deposition. The inductive effects of protein on Ca–P deposition were also observed by Vasin et al. (Ref. [22]). They reported that more intense calcification was observed on the samples with pre-adsorption of proteins than the original or control samples.

4. Conclusions

Composite coatings with 70 wt% zirconia and 30 wt% dicalcium silicate were prepared by plasma spraying. Human osteoblasts could well adhere, spread, and proliferate on the coating surface. Honeycomb-like Ca–P particles were found on the composite coating surface after 1 day of incubation in cell culture media, while only scattered globular Ca–P minerals were found after immersion in simulated body fluids. The good cytocompatibility and ability to induce Ca–P deposition of the composite coatings were attributed to the dissolution of dicalcium silicate and the resultant formation of Si–OH functional groups on the coatings.

Acknowledgments

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