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Microstructure, bioactivity and osteoblast behavior of monoclinic zirconia coating with nanostructured surface

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ABSTRACT

A monoclinic zirconia coating with a nanostructural surface was prepared on the Ti–6Al–4V substrate by an atmospheric plasma-spraying technique, and its microstructure and composition, as well as mechanical and biological properties, were investigated to explore potential application as a bioactive coating on bone implants. X-ray diffraction, transmission electron microscopy, scanning electron microscopy and Raman spectroscopy revealed that the zirconia coating was composed of monoclinic zirconia which was stable at low temperature, and its surface consists of nano-size grains 30–50 nm in size. The bond strength between the coating and the Ti–6Al–4V substrate was 48.4 ± 6.1 MPa, which is higher than that of plasma-sprayed HA coatings. Hydrothermal experiments indicated that the coating was stable in a water environment and the phase composition and Vickers hardness were independent of the hydrothermal treatment time. Bone-like apatite is observed to precipitate on the surface of the coating after soaking in simulated body fluid for 6 days, indicating excellent bioactivity in vitro. The nanostructured surface composed of monoclinic zirconia is believed to be crucial to its bioactivity. Morphological observation and the cell proliferation test demonstrated that osteoblast-like MG63 cells could attach to, adhere to and proliferate well on the surface of the monoclinic zirconia coating, suggesting possible applications in hard tissue replacements.

1. Introduction

Plasma-sprayed hydroxyapatite (HA) coatings are commonly and clinically used in hard tissue replacement due to their chemical similarity to the mineral components of bones and hard tissues in mammals [1–3]. However, the clinical use of plasma-sprayed hydroxyapatite coatings is plagued by their low crystallinity and poor bonding strength on titanium alloys [4–6]. The low crystallinity gives rise to fast dissolution of the hydroxyapatite coatings during contact with human body fluids, subsequently shortening their lifetime, whereas the poor bonding strength results in delamination, causing safety concerns. It is thus important to explore new bioactive coatings to improve tissue integration and control the friction at the interface of the implants [7].

Chemical and dimensional stability, mechanical strength, toughness, and Young's modulus similar to that of stainless steel alloys make zirconia an excellent ceramic biomaterial for use as a femoral head [8]. In addition, zirconia has also been used as a second phase to improve the bonding strength and fracture toughness of HA coatings [9–11]. Zirconia shows morphological fixation with the surrounding tissues without producing any chemical or biological bonding when implanted [12]. In our previous work, a zirconia film with nanostructured surface prepared by cathodic arc deposition was demonstrated to be bioactive [13]. It was also reported that zirconia gel with tetragonal or monoclinic structure exhibited higher apatite-forming ability in SBF fluids than amorphous gel [14]. A zirconia coating fabricated by micro arc oxidation could also induce apatite precipitation on its surface in modified simulated body fluids [15]. Our previous work showed that plasma-sprayed calcia-stabilized zirconia coating had good bioactivity, which was dependent on the content of the monoclinic phase in the coating [16]. The phase composition and microstructure of the zirconia coating should be related to the fabrication technique and process, which may give rise to a difference in the bioactivity.

Zirconia exists in three crystalline phases: monoclinic, tetragonal and cubic. Tetragonal and cubic zirconia possess superior mechanical properties but undergo low temperature degradation (LTD) in water or water vapor [17,18]. LTD can reduce the mechanical strength and service life of the zirconia-based materials [19,20] and it is thought to be the main reason for the failure of zirconia artificial joint balls [21]. LTD, which depends on the microstructure and fabrication process, is accelerated by micro-cracks, high rough-

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ness and pores [17–19]. Generally, monoclinic zirconia is more stable at room temperature than cubic and tetragonal zirconia. The calculated energy vs. volume data at absolute zero temperature confirmed the higher stability of the monoclinic phase [22]. Therefore, some researchers have made attempts to prepare the dense polycrystalline monoclinic ZrO_2 components. Cutler et al. [23] found that annealing at the cubic stability temperature and rapid cooling were crucial to the fabrication of dense undoped monoclinic zirconia. Samples fabricated by this method exhibited a fracture toughness in the range 3.7–6.0 MPa $m^{1/2}$, a Young's modulus of about 175 GPa, and a thermal expansion ($8.7 \times 10^{-6}\,C^{-1}$) closer to those of Ti alloy than hydroxyapatite [23]. Yttria- and ceria-stabilized zirconia coatings with the tetragonal or cubic phase have been deposited by plasma-spraying and are widely used in thermal protection [24–26]. However, to our knowledge, preparation and characterization of plasma-sprayed monoclinic zirconia coatings without a stabilizer have been seldom reported. Because monoclinic zirconia is a stable phase at low temperature (<1100 °C), its application at high temperature is difficult to realize. However, under low temperature and humidity conditions such as the physiological environment, the advantage of monoclinic zirconia coating can be fully realized.

In this work, plasma-sprayed monoclinic zirconia coatings were deposited onto titanium alloys. The in vitro bioactivity, cytocompatibility, and hydrothermal stability of the plasma-sprayed monoclinic zirconia coating were evaluated to explore their potential applications in hard tissue replacement.

2. Materials and methods

2.1. Fabrication and characterization of coatings

The pure monoclinic zirconia powders (>99.9%) were purchased from Farmeiya Advanced Materials Co. Ltd. (Jiujiang, China). To improve the flowability, the powders were sintered at 1450 °C for 6 h in air, mechanically ground, and then spheroidized by a plasma jet. Details of the spheroidizing process can be found in our previous paper [27]. After spheroidization, zirconia powders 2–20 μm in size were obtained. Ti–6Al–4V substrates with dimensions of 10 × 10 × 2 mm were ultrasonically cleaned in ethanol and deionized water and then grit-blasted with alumina grit. An atmospheric plasma-spraying system (Sulzer Metco, Switzerland) was utilized to deposit the coatings. Argon (35 slpm) and hydrogen (12 slpm) were used as the primary and auxiliary plasma forming gases, respectively. The powder feeding rate was about 20 g min^{-1} using argon (3.5 slpm) as the carrier gas. The plasma arc current and voltage were 620 A and 68 V. The spraying distance was fixed at 120 mm.

The surface morphology of the coating was examined by field emission scanning electron microscopy (FE-SEM, JSM-6700F, JEOL, Japan). The crystalline phase of the coating was identified using a Siemens D5000 diffractometer with Cu $K_{\alpha 1}$ (λ = 1.54056 Å) irradiation with a scanning step of 0.02. Scans were obtained from 15° to 85° at 2° per minute. Micro Raman spectroscopy (Lamb 1B, Dilor Inc., France) with an excitation wavelength of 632.8 nm was employed to detect the surface phase composition of the coating. The tensile bond strength between coating and substrate was measured in accordance with ASTM C-633-79. For this test, coatings of approximately 380 μm in thickness were sprayed on Ti–6Al–4V rods of 25.4 mm in diameter. A thin layer of E-7 adhesive glue with tensile fracture strength of over 70 MPa was applied. The tensile bonding strength was measured using a universal testing machine (Instron-5592, SATEC, USA) and five samples were tested independently. The results were reported as means ± standard deviation (SD). The nano-hardness and elastic modulus of the coating were determined on three randomly selected polished coating samples using MTS Nano Indenter® XP (MTS Cooperation, Nano Instrument Innovation Center, TN, USA). At least seven indents were made on each coating sample. The final results of nano-hardness and elastic modulus of the coating were the average values of all individual indentations. Vickers indentation (Wilson–Wolpert Tukon2100B, Instron, Norwood, MA) was used to assess crack formation and propagation around the indentation; this test was repeated three times at separate files on the polished coating surface. In order to evaluate its hydrothermal stability, the coating was hydrothermally treated in an autoclave at 134 °C for different time periods. Afterwards, the phase composition was determined by XRD and Vickers hardness was measured by Vickers indentation; at least 10 indents were made.

Before transmission electron microscopy (TEM) observation, the as-sprayed coating samples with a thickness of 1 mm was attached to a tripod polisher with glue, ground, and polished from both sides to a thickness of about 30 μm. Afterwards, the thickness of the coating specimen was further reduced using a low-angle ion-thinning precise ion polishing system (PIPS). TEM examination was carried out using a JEM-2010 TEM at an accelerating voltage of 200 kV.

2.2. Bioactivity evaluation

After ultrasonic washing in ethanol and rinsing with deionized water, the zirconia samples were immersed in a simulated body fluid (SBF) at 36.5 °C without stirring to investigate the bioactivity. The SBF solution was prepared according to the method proposed by Kokubo and Takadama [28]. After immersion for 2, 6 and 12 days, the samples were removed from the SBF solution, washed with deionized water, and then dried at 40 °C. In order to investigate the variation in the Ca and P ion concentrations in the SBF solution, the coating samples were immersed in 25 ml of the SBF solution for 1, 2, 4, 6, 8, 10 and 12 days without stirring. The Ca and P concentrations in the SBF solution after immersion for various time periods were detected by inductively coupled plasma optical emission spectroscopy (ICP-OES, AX, Varian, USA). The surface morphologies of the coating soaked in the SBF solution for 2, 6 and 12 days were evaluated by field emission scanning electron microscopy under secondary electron imaging (FE-SEM, JSM-6700F, JEOL, Japan). The composition of the deposits on the surface was determined by energy-dispersive X-ray spectrometry (EDS) in the electron probe (EPMA, JAX-8100, Japan).

2.3. Cytocompatibility evaluation

2.3.1. Cell culture

The osteoblast-like cell line MG63 (Cells Resource Center of Shanghai Institute for Biological Science, Shanghai, China) was seeded on the surface of zirconia coatings to evaluate the cytocompatibility. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air, in 75 cm^2 flasks (Corning Incorporated, USA) containing 10 ml of α-minimum essential medium (α-MEM) (Minimum Essential Medium alpha-Medium, Gibco, Invitrogen, Inc.), 10% fetal calf serum (FCS) (Excell Biology Inc., South America), 2 mM L-glutamine (Hyclone, USA), 1% antimicrobial of penicillin, and streptomycin (Antibiotic–Antimycotic, Gibco, Invitrogen Corporation). The medium was changed every third day and for the subculture, the cell monolayer was washed twice with phosphate-buffered saline (pH 7.4, Gibco, Invitrogen) and incubated with trypsin–ethylenediaminetetraacetic acid (EDTA) solution (0.25% trypsin, 1 mM EDTA (Chemicon International)) for 5–10 min at 37 °C to detach the cells. The effect of trypsin was then inhibited by adding the full medium at room temperature. The cells were centrifuged (900 rev min^{-1}, 28 °C, 6 min) and resuspended in the complete medium for reseeding onto the coating surface.