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Surface nanocrystallization of hydroxyapatite coating

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Abstract

Nanocharactered biomaterials, such as nanopowders, nanocrystalline compacts and nanostructured films, as well as materials with nanoscale roughness, have attracted much attention recently, due to their clear effects on cell response. Surface nanocrystallization of plasma-sprayed hydroxyapatite (HA) coating can be realized by conventional post-heat treatment. This study reveals that 20–30 nm nanocrystals formed on HA coatings post-heat treated at 650 °C, and the increase in holding time increased the number of surface nanocrystals and intensified their aggregation. Hard aggregation occurred when HA coatings were repetitively post-heat treated. This indicates that the surface nanocrystallization is controllable. Cell experiments were carried out with rat calvarial osteoblasts. The post-heat treated HA coatings exhibit an obviously better osteoblast response than the as-sprayed coatings. Well-flattened cells attached themselves to the coating surfaces, with a good interaction between their filopodia and the nanocrystallized region. It is proposed that the surface nanocrystallization should be taken into account when the post-heat treatment process is introduced for the fabrication of HA coatings.

Keywords: Surface nanocrystallization; Hydroxyapatite; Coating; Post-heat treatment; Osteoblast compatibility

1. Introduction

Plasma spraying is one of the most widely used methods for the fabrication of biomedical hydroxyapatite (HA) coatings, and it is generally agreed that HA decomposes partially during air plasma spraying [1–5]. The coating process results in structural alterations of HA including: (i) the formation of amorphous calcium phosphate; (ii) a loss of OH^- groups; and (iii) the formation of secondary calcium phosphate phases [2]. Plasma-sprayed HA coatings have been proved to contain quite a large amount of amorphous phase, being an oxyapatite, and hydroxyapatite was proved to be an oxyhydroxyapatite with a small quantity of HA [3]. Meanwhile, as the plasma power level is increased, the crystallinity and OH^- ion content of the coatings decrease, while the amount of non-HA calcium phosphate compounds increases [4]. Thermal decomposition of apatite in coatings is catalyzed by underlying titanium during plasma spraying and post-heat treatment [6].

The amorphous phase, which is a product of the structural destruction of HA during plasma spraying, is more commonly found at the coating–substrate interface [5], and is deemed to have a detrimental effect on the long-term stability of the coating–substrate interface after implantation, because the amorphous phase could be rapidly dissolved when exposed to body fluid. Extra phases, such as tricalcium phosphate (TCP), tetracalcium phosphate (TTCP) and CaO, might have a similar effect. At the same time, thermal decomposition of HA will intensify its structural difference from bone apatite, which may reduce the bonding efficiency between them.

Post-heat treatment has been reported to be effective in improvement not only in the structural integrity [7–9] but also in the mechanical properties of HA [10,11]. A stable HA coating with high crystallinity not only promotes cell

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proliferation and bio-integration [12,13], but also is beneficial to the long-term effectiveness of the coating, especially when used to shield the release of metallic ions. Many factors affect the restoration of structural integrity of the coating during post-heat treatment. In our previous work, factors influencing phase composition and structure of plasma-sprayed hydroxyapatite coatings during post-heat treatment were systematically investigated [14].

At the same time, ultrafine nanosized HA particles have been found on traditionally post-heat treated HA coatings [15]. Compared to conventionally crystalline HA, in addition to improving the bone bonding to HA, nanostructured HA is speculated to enhance the proliferation of osteoblasts, synthesis of intracellar proteins, alkaline phosphatase activity and deposition of calcium-containing mineral, thus enhancing the bonding of orthopedic/dental implants to juxtaposed bone and improving the overall implant efficacy [16,17]. Cytotoxicity results with human osteoblast cells showed excellent cell attachment and cell spreading on compacts made from HA nanopowders [18]. Nanocrystalline SiHA coating on titanium (Ti) caused a significant increase in human osteoblast-like cell growth density with culture time as compared with the uncoated Ti. Also, rapid dissolution of the coating is not favorable to very early cells attachment [19]. Meanwhile, high crystallinity of the HA coating [20] and stoichiometric elements content in dense HA ceramic [21] are more beneficial to cell growth.

In this work, surface nanocrystallization of plasma-sprayed HA coatings was achieved by use of conventional post-heat treatment; attention is focused on the controllability of the nanocrystalline surface of the HA and the cell response on nanocrystallized coating surfaces.

2. Materials and method

2.1. Specimen fabrication

Fully crystallized HA powder with particle sizes of 38–75 μm were produced in our laboratory and were used as the starting materials. The HA powder was synthesized by a wet chemical method, sintered at 900 °C for 0.5 h, milled and sieved to the required sizes. Commercially pure titanium buttons, with a diameter of 10 or 13 mm and a thickness of 2 mm, were used as the substrate materials. Here we chose pure Ti instead of Ti–6Al–4V as the substrate because (i) potential adverse biological effects of Al and V have been widely reported, such that many new Ti alloys, e.g. Ti–Zr and Ti–Nb, have been developed; and (ii) for the experiment in this work, we obtained conveniently pure Ti samples in our laboratory. Prior to plasma spraying, the substrate surfaces were sandblasted with Al_2O_3 grit of size 500 μm. Coatings were produced by plasma spraying using a Sulzer Metco 9 M instrument. Nitrogen and hydrogen were used as the plasma arc gases and nitrogen as the powder carrier gas. The arc current and voltage were 500–600 A and 70–75 V, respectively. The powder feed rate was 30–40 g min^{-1} and the spray distance was 110 mm. The post-heat treatments of the coatings were carried out in air at 650 °C for 30, 60, 90 and 120 min, respectively. Some of the coatings underwent a 650 °C × 60 min → 650 °C × 90 min → 650 °C × 120 min multi-step post-heat treatment.

2.2. Microstructural characterization

The phases of the various coatings were analyzed by X-ray diffractometry (XRD) using a D/max-γB X-ray diffractometer operated at 40 kV and 100 mA, with a scan speed of 4° min^{-1}. Grazing angle diffraction measurements were performed on the coatings by employing an X'Pert PRO X-ray diffractometer using Cu Kα radiation and a grazing angle of 0.5°. The 2θ angles ranged from 10° to 60°, with a 0.005° s^{-1} scanning speed.

An S-S70 scanning electron microscope, operated at 20 kV, was employed to examine the surface of the coatings. Nanoscale morphologies of coating surfaces were observed by employing a JEOL JSM6700 F field emission scanning electron microscope operated at 5 kV and 10 μA. The surfaces of the samples were sputter-coated with gold. The coating microstructure was analyzed by transmission electron microscopy (TEM) using an H-800 transmission electron microscope operating at 200 kV. For the preparation of TEM samples, coatings were cut from specimens, ground from the substrate side and finally ion beam thinned to electron transparency.

2.3. Cell culture

Osteoblast cells were extracted from the calvariae of infant Wistar rats, 24 h old and primarily cultured at a density of 5.0×10^4 cells ml^{-1} in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. All cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere under standard sterile conditions. Culture media were changed after 24 h for the first time and then every other day. This animal use was approved by the Laboratory Animal Committee, Shandong Province, China.

Coating samples were placed in a 24-well tissue culture plate and the third subculture of cells was seeded at a density of 3.0×10^4 cells ml^{-1} onto the surfaces of the samples. The proliferation of cells cultured on surface nanocrystallized coatings was evaluated by counting cell numbers. Cells were plated at 3.0×10^4 cells ml^{-1} and cultured for 36, 96 and 144 h. They were then detached by trypsinization and counted using a hematocytometer. The results were the averages of each triplicate set from each of four tests.

Cell attachment studies were performed on the coatings via scanning electron microscopy (SEM). Prior to SEM investigations, cells were fixed by using 2.5% glutaraldehyde. Osteoblasts were dehydrated through sequential washings in 50%, 75% and 95% ethanol solutions, followed