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ARTICLE

Morphology Improvement of Sandblasted and Acid-Etched Titanium Surface and Osteoblast Attachment Promotion by Hydroxyapatite Coating

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Abstract: The sandblasting-acid-etched (SLA) technique and hydroxyapatite (HA) deposition are the two methods widely used to improve surface characteristics of titanium implants. In the current study, a layer of HA was deposited on SLA-treated titanium (SLA-Ti) by a simple deposition method. The surface topography and surface roughness of SLA-Ti and HA-coated titanium (HA-Ti) were evaluated using scanning electron microscope (SEM) and atomic force microscope (AFM). Protein adsorption, osteoblast chemotaxis as well as osteoblast attachment on both Ti surfaces were additionally analyzed. The results show that SLA-Ti surface is covered with uniform, multiple micropores, whereas HA-Ti surface is covered with a large number of uniform microparticles under multiple microporous background. In comparison to SLA-Ti, the surface roughness of HA-Ti surface is lower. The protein adsorption on SLA-Ti and HA-Ti surfaces is generally even, and the chemotaxis of osteoblast have no differences. After 12 and 24 h of incubation, more osteoblasts are adhered to the HA-Ti surface and more osteoblast-osteoblast connection is observed on HA-Ti surface compared to SLA-Ti surface. These findings demonstrate that HA coating deposited on SLA-Ti surface improves SLA-Ti surface morphology and promotes osteoblast attachment *in vitro*.

Key words: titanium; SLA-Ti surface; hydroxyapatite (HA); protein adsorption; osteoblast attachment

Titanium (Ti) and titanium alloys have been widely used as dental and orthopedic implant materials due to their high strength, excellent anti-corrosive property and good biocompatibility[1]. However, unlike bio-ceramic and bio-glass, Ti cannot be directly bound to the surrounding bone tissues[2,3]. The biocompatibility of Ti is found to be closely associated with surface roughness, surface topography and surface chemical property[4].

Sandblasting-acid-etched (SLA) technique takes the advantages of sand blasting and acid etching to obtain a microporous structure, which greatly improves the bioactivity and osseointegration of Ti implants. However, SLA-treated Ti (SLA-Ti) surface may not be bioactive enough to achieve osseointegration for patients with poor bone quality. Additional surface modification methods are thus needed to improve bone formation ability on SLA-Ti surface. Hydroxyapatite (HA) coating is another important Ti surface modification technology to enhance osseointegration of Ti[5]. In addition to super biocompatibility, HA crystal has an ability to induce bone formation. HA coating on Ti surface could

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yield a bioactive surface, which exhibits both the mechanical property of Ti and the osteoinductive advantage of HA. HA coating techniques include plasma spraying, deposition method, sol-gel technique, and some other methods[5,6]. Plasma spraying is reported to suffer from problems of poor mechanical property that will be caused by a relatively high thickness, a low adhesion of coating to the bottom materials[6], an incomplete HA structure and component. Chemical deposition, which has been extensively studied, is easy to perform and carried out below 100 °C, which allows binding of HA coating to an osseointegration drug such as bone morphogenetic protein (BMP) to be released in the human body. Such a drug-delivery system can decrease the time to heal after implantation, and enhance the survival rate of implants.

In the current study, a uniform HA coating was prepared on SLA-Ti surface with a simple deposition method. Surface topography, biological characteristics including protein adsorption, osteoblast chemotaxis and osteoblast attachment on SLA-Ti and HA-Ti surfaces were assessed.

1 Experiment

The pure Ti plates (5 mm in diameter and 1 mm in thickness) were purchased from Baoji Special Steel Titanium Industry Co., Ltd, Baoji, China. Wet grinding of the titanium surfaces was performed with 240#, 400# and 600# grit silicon carbide abrasive papers, and sandblasting was done with 18#~24# grit white corundum to pre-prepare Ti surfaces. The specimens were rinsed with deionized water, ultrasound-degreased and subsequently rinsed for 10 min each in benzene, acetone and ethanol. For SLA treatment, the specimens were sandblasted with rough corundum of particle sizes of 0.25~0.50 mm for 0.5 min. Then, the specimens were dipped in a bath containing 10 parts of HCl (30%), 80 parts of H_2SO_4 (60%) and 10 parts of water, at boiling temperature for 1 min to produce SLA-treated titanium (SLA-Ti). All procedures were carried out in accordance with the patent description[7].

The thermostatic HA solution was prepared by dissolving 0.25 g HA powder, which was synthesized by our laboratory, in 250 mL hydrochloric acid. The precipitate was removed by filtration sieve and HA solution was stored at 4 °C. Before application, pH of HA solution was adjusted to 7.3 using 0.2 mol/L Tris buffer solution.

For HA deposition treatment, SLA-Ti specimens were vertically suspended in the thermostatic HA solutions at 37 °C for 72 h. After immersion, the specimens were rinsed with deionized water and dried at 50 °C. All specimens were sterilized with ultraviolet light for 24 h prior to use.

The specimens were rinsed with distilled water twice, followed by drying, the surfaces of the specimens were sprayed with platinum and surface topography was observed using scanning electron microscope (SEM; Olympus, Japan). The microstructure was additionally observed by atomic force microscope (AFM) with a scan area of 5 μm×5 μm, and surface roughness was observed with a scan area of 10 μm×10 μm. The surface roughness of three areas in each specimen was randomly measured, and the means were calculated. The difference of the mean surface roughness among different Ti surfaces was tested for statistical significance by analysis of variance (ANOVA), with a $p<0.05$ considered statistically significant.

The simulated body fluid (SBF) was prepared by supplementing F12 medium with 10% fetal bovine serum (FBS), penicillin (5000 unit/mL), streptomycin (5000 μg/mL), and $NaHCO_3$ (2.438 mg/mL). The specimens were incubated in 12-well culture plate containing 500 μL phosphate buffered saline (PBS) for 30 min and then in SBF for 1 h at 37 °C in a humidified environment containing 5% CO_2. Thereafter, the specimens were rinsed with PBS twice on a shaker to remove non-attached protein. After fixed with 4% chilled methanol for 10 min, the specimens were stained with rhodamine, followed by rinsing with PBS and blocking with buffered glycerol. Distribution of adsorbed protein on Ti surfaces was subsequently observed under a fluorescence microscope (Olympus, Japan).

The study protocol was approved by the ethics committee of Sichuan University. Primary osteoblasts were isolated from the fibula of new-born Sprague-Dawley (SD) rats, and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum with 5% CO_2 at 37 °C. The osteoblasts were digested by 0.25% trypsin and 1 mmol/L ethylene diamine tetra acetic acid (EDTA; Sigma). After collected through centrifugation, serum-free DMEM was added to prepare a cell suspension. The specimens were incubated in 12-well plates containing 500 μL SBF/well at 37 °C for 1 h, and thereafter 1 × 103 cells were seeded on Ti surfaces. After 2 h, the specimens were placed on the contra lateral side of the wells. After addition of 2 mL F12 medium, incubation was continued for 12 and 24 h more. Chemotaxis of osteoblast was observed under an inverted phase contrast microscope.

For observation of osteoblast attachment, 1 × 103 cells were seeded on Ti surfaces and incubated at 37 °C in F12 medium. After 12 and 24 h, the specimens were rinsed with PBS twice, fixed with 4% chilled methanol, and rinsed with PBS. The specimens were incubated with anti-actin antibody (SC-1616; Zhongshan Bio-Tech Co., Zhongshan, China) for 1.5 h, rinsed with PBS three times, and then incubated with secondary