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Research on cell behavior related to anodized and hydrothermally treated titanium surface

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ABSTRACT

In vitro cell response is believed related to the physical and chemical properties of substrate. In this study, the cell adhesion affected by mechanical stimulation from substrate was evaluated by culturing the MG-63 osteoblast-like cells on Ti plates with different chemical composition and surface topography. Three types of surface, surface with machined grooves, with pores, and with pillars, was fabricated by mechanically abraded (control), anodized (AO) and anodized following with hydrothermally treated (HYT) Ti plates, individually. Cells exhibited earlier spreading on the AO and HYT surface after 5 h culturing, resulted from chemical factor, i.e., calcium and phosphate containing on the surface. After 24 h cells completely flattened on the HYT surface but not on the AO surface; this improved cell adhesion behavior was primarily attributed to physical factor that is specific surface topography provides cell relatively large mechanical stimulation. The finite element method was used to evaluate the stress distributions which cells were suffered. For the HYT group, analyzed data indicated that cell received larger stress stimulation than control group ($P > 0.01$); therefore it can explain the fact that the superior cell adhesion resulted from the specific geometry of HYT coated-surface.

1. Introduction

Titanium and its alloys are regarded to have good biocompatibility, however the bonding between Ti implants and bone tissues is not sufficient. This bonding is attributed to a mechanical interlocking of titanium surface defects and pores in the bones. Accordingly, this bioinert surface needs an extra surface modification by coating a bioactive material such as hydroxyapatite (HA) on it [1,2]. A method comprising anodization and hydrothermal treatment to form a crystalline HA layer on a Ti substrate was proposed [3–8]. The process consisted of two steps: (1) forming an oxide film that contains Ca and P by anodic oxidation, and then (2) hydrothermally treating this oxide film to precipitate HA crystals on the surface.

This HA layer has been observed to remain stable during the process of bone formation in vivo [9,10] and bone matrix mineralization in vitro [11] since its high crystallinity, which is different from plasma-sprayed HA. Moreover the hydrothermal treatment formed HA promotes could promote in vitro cell responses have been reported [12–14], which was explained by means of the changes of surface composition and morphology but the mechanism is not clear.

Cell adhesion was following attachment, spreading and flattening, which depended on the surface chemical and physical properties of the substrate, such as composition, wettability, roughness, stiffness and topography. The surface composition and wettability were considered to directly affect conditioning molecules adsorption which may be helpful to attract cell attachment. After cell attachment, cell began to move on the substrate by spreading while changing its shape. It has been found that the stiffness of substrate could guild cell movement [15], which is attributed to cell produce different degree of strain by suffered mechanical stress originated from substrate. However, there is still not a method been proposed to efficiently evaluate the stress and strain that cell suffering [4]. This study fabricate three types of

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surface, i.e., the mechanical abraded, the anodized, and the anodized following with hydrothermally treated surface to evaluated the how surface topography affect cell adhesion.

Finite element analysis (FEA) is an effective tool that can be applied to quantify the stress distribution in biomechanical field, including spine, hip, knee and dental implant [16–19]. Mechanical behavior of cell–surface interface is one of the important osseointegration factors, but there is lack of investigation in experiment and theoretical analysis until now. To examine the biomechanical behavior of cell on different coated surfaces, the magnitude of the maximum stresses must be considered.

2. Materials and methods

2.1. Sample preparation and treated processes

$10 \times 10 \times 1$ mm coupons of commercial pure Ti were abraded with silicon carbon papers up to grade 800, and were then cleansed with acetone for 5 min followed by ethanol for 3 min in an ultrasonic cleaner, and finally dried at room temperature.

The electrolyte for anodizing treatment was composed of 0.04 mol/l β-glycerophosphate disodium pentahydrate ($C_3H_7Na_2O_6P{\cdot}5H_2O$, β-GP) and 0.2 mol/l calcium acetate monohydrate [$Ca(CH_3COO)2{\cdot}H_2O$, CA] in distilled water. The solution was constantly stirred using a magnetic stirrer at 25 °C. The pH of the solution was around 7.8. A high density graphite plate was used as the cathode, and Ti plate was galvanostatically anodized at a constant current density of 50 mA/cm^2 up to 300 voltage using a direct current power supply (GWinstek GPR-30H10D). After anodizing, the sample was cleansed with distilled water, and then dried at room temperature.

The Ti plate after anodizing was following hydrothermally treated at 250 °C for 6 h in an autoclave (volume: 53.6 ml) containing 26.8 ml distilled water. After hydrothermal treatment, the sample was cleansed with distilled water and then dried in a stream of room temperature air. The abraded, anodized, and anodized following hydrothermally treated specimen was denoted as control, AO and HYT, respectively.

The surface morphology was observed using a scanning electron microscope (SEM; Model: PHILIPS XL30). The crystallinity and phases were identified via glancing angle X-ray diffraction (XRD; Model: PHILIPS X'Pert) with an incidence angle of 1° and at a scanning speed of 2°/min. The composition was quantitatively analyzed by an electron probe X-Ray microanalyzer (EPMA; JEOL, JXA-8600SX).

2.2. Cell culture

The test specimens ($10 \times 10 \times 1$ mm) were placed into a 24-well polystyrene plate. Before cell culture, all the specimens were shined by ultraviolet ray (UV) for 24 h. The test specimens were sterilized and washed several times with Dulbecco's modified Eagle's medium (DMEM, Gibco) and phosphate-buffered saline (PBS, 0.1 M, pH 7.2). The culture medium consisted of DMEM containing 10% fetal bovine serum (FBS), 100 μg/ml of streptomycin, and 100 units/ml of penicillin. The MG-63 cell suspension with a density of 1×10^4 cells/ml was added into the culture well. 500 ml culture solution and 50 μl 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolim bromide (MTT) label solution were added into every culture well before placing the plate inside a culture chamber at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The test specimens were cultured for various periods of time, i.e., 8 h and 24 h. The extent of adhesion of MG-63 cells on the surfaces of specimens was evaluated through the observations of cell morphologies using SEM.

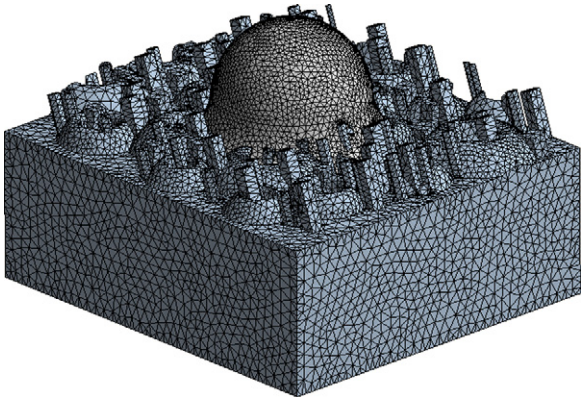

Fig. 1. The 3D solid mesh model of cell on HYT-coated surface.

2.3. Finite element analysis

The 3D model of the coated structures constructed based on SEM images. The different coated surfaces and cells were combined and meshed using the ANSYS software program (Workbench 12.1, ANSYS Inc., Canonsburg, PA) with the 10-node tetrahedral finite elements (Fig. 1). Based on the results of the convergence testing, 0.2 μm of element size was utilized and the final model included approximately 19,000 nodes and 11,500 elements.

The stress distributions of cell were evaluated during initial stage of cell adhesion, and ten cells were placed on the random position of different coated surfaces, then the maximum average stresses were compared among groups. The bottom nodes of the bone tissue in FE models were fixed in all direction as the boundary condition. The coated surface-cell connection was assumed "no separated" contact. For simulating varying coated surfaces, the maximum stresses were investigated. The Cell-substrate adhesion force was adopted 150 nN for 37 °C [20], and the elastic properties of cell was 10 kPa, which were determined from the previous literature [21].

2.4. Statistical analysis

Nonparametric Kruskal–Wallis analyses were performed using SPSS software. The data obtained from ten replications were expressed as mean ± standard deviation (SD) and were analyzed statistically, with the level of significance set at 5%.

3. Results and discussion

3.1. Surface morphology

Fig. 2(a) shows the surface morphology of the control specimen consisted of scratches resulting from mechanical grinding. In contrast, the surface of AO specimen was covered with craters of 1–2 μm in diameter as shown in Fig. 2(b). For HYT specimen, as shown in Fig. 2(c), numerous fine columnar HA crystals precipitated on the surface.

3.2. Microstructure

The AO surface containing 7.9 at% Ca, 5.1 at% P, 67.8 at% O and 19.2 at% Ti and was analyzed by EPMA. Also the distribution of amount of Ca and P was identified, which decreased from outer surface to the inner part of oxide layer.

Fig. 3 shows the XRD patterns of the control, AO and HYT specimen. The control specimen comprised α-Ti however the native oxide film on the Ti surface is too thin to be detected. For AO