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Full Length Article

Electrochemical corrosion characteristics and biocompatibility of nanostructured titanium for implants

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ABSTRACT

In the present study, a nano-grained (NG) surface layer on a commercial pure (Grade-2) titanium sheet was achieved by means of sliding friction treatment. The surface characteristics, *in vitro* corrosion behavior and biocompatibility of NG Ti were investigated, compared with those of the conventional coarse-grained (CG) substrate. The protective passive film on NG Ti surface is thicker than that on CG Ti, leading to its enhanced biological corrosion resistance in simulated body fluid (SBF) solution. In addition, NG Ti shows a much higher hydrophilicity and nano-roughness, which is related to its significantly improved cell attachment, spreading, proliferation and maturation relative to CG Ti. The enhanced biological anti-corrosion properties and biocompatibility render NG Ti a promising biomaterial for implants.

1. Introduction

Titanium and titanium alloys are employed extensively for orthopaedic and dental implant fabrication [1–3]. However, concerns about possible biotoxicity of alloying elements in the long-term performance, such as Al and V in the Ti-6Al–4 V alloy commercially applied, have been driving the development of purity titanium as an alternative to alloys because of its excellent biocompatibility [4,5]. Commercially pure Ti would take a prominent place among its alloys if the loss of strength due to lack of alloying elements are compensated [3,5,6]. Grain refinement is one feasible way. Excitingly, a number of reports revealed that the combination performances (mechanical property, corrosion resistance and biocompatibility) of Ti can be tailored through grain refinement by severe plastic deformation (SPD) techniques such as equal channel angular pressing [5,7], high pressure torsion [8] and groove pressing [9].

The corrosion behavior of Ti alloy is mainly dependent on the passive film properties (such as thickness and layer mode) and the microstructure of the substrate (grain size and dislocation density) [3,10,11]. Numerous results suggest that nanocrystallization does not change the single structure of the passive film but improves its electrochemical stability [6,9,12], which was mainly attributed to a Ti and O ion rich layer at the passive film/metal interface [13]. Moreover, some studies [6,10] have reported the nanocrystalline structure could induce a quickly formed passive layer, which obviously enhanced the corrosion resistance and reduced ion release in corrosion medium. While further work is still needed to deeply understand the formation process of passive film with respect to nanocrystallization effect in Ti alloys.

The biocompatibility of nanostructured metal is dominated by both the surface architecture and microstructure, including grain size distribution, grain boundary misorientation, and so on, which are related to manufacturing processes. The work by Truong [2] and Estrin [14] showed that the enhanced attachment and spreading of human bone marrow-derived mesenchymal stem cells (hMSCs) in the initial stages of culture was caused by increased surface roughness on nanoscale. Huang [15] and Wen's [16] revealed that the significantly improvement of osteoblast adhesion and proliferation on nanocrystalline Ti fabricated by surface mechanical attrition treatment can be attributed to decreased grain size and increased hydrophilicity. Shri et al. [17] demonstrated that the increased protein adsorption and cell growth was remarkably improved by deformation-induced martensites.

Sliding friction treatment (SFT) is a new developed SPD method that can generate nano-grains on metal surface [18,19]. The objective of the present study is to evaluate the suitability of a NG surface layer on Ti prepared by SFT for implant application with respect to *in vitro* corrosion behavior and biocompatibility, and CG Ti is also

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investigated as control. An excellent combination of *in vitro* biological anti-corrosion and biocompatibility properties was achieved by introducing a NG surface layer on pure Ti and the beneficial effect of the NG layer are also discussed.

2. Experimental

2.1. Material preparation and microstructure characterization

A coarse-grained (CG) commercially pure (ASTM Grade-2) Ti sheet with 3 mm thickness was used in this study. The schematic illustration of SFT process can be seen in Ref. [18]. In this work, the processing parameters for SFT were selected as follows: 500 N in load, 0.2 m/s in sliding velocity, 50 mm in amplitude, and 100 in cycle. In order to eliminate the influence of surface pollution during SFT, the top surface layer of 1–3 μm was removed by polishing carefully.

The microstructure of NG Ti and CG Ti was characterized by a transmission electron microscope (TEM, JEOL, JEM-2100) operated at a voltage of 200 kV and an optical microscopy (OM, Leica MPS 30), respectively. For TEM observation, cross-sectional thin foil samples were cut from the samples and thinned by ion thinning at low temperatures. The grain size was calculated and estimated by a combination of linear intercept method and TEM or OM observation.

2.2. Electrochemical corrosion measurement

All the electrochemical experiments were conducted using a IM6 Zahner-electrik Gmbh (Zenniom, Germany) electrochemical workstation in SBF solution at 37 ± 1 °C controlled by thermostatic water bath. A traditional three-electrode system was adopted using a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode, and the measured sample with a certain exposed area (10mm × 10 mm) was used as working electrode. The detailed decomposition of SBF solution can be found in Ref. [20].

Prior to all electrochemical measurements, the specimens were initially reduced potentiostatically at −1.0 V for 300 s to remove air-formed oxides on the surface and then kept in solution until a stable corrosion potential was reached. The open circuit potential (OCP) measurement was carried out for 30 min starting from the electrode immersing into the electrolyte. The electrochemical impedance spectroscopy (EIS) measurements were carried out under potentiostatic condition at OCP with 10 mV amplitude AC voltage signal, and the applied frequency range was from 10^5 Hz down to 10^{-2} Hz. The impedance date was analyzed using ZSimpWin 3.0 software. The potentiodynamic polarization (PDP) curves were obtained after 30 min immersion in SBF solution, in the range from −1 V to 2 V using a scan rate of 0.5 mV/s. The corrosion potential (E_{corr}) and corrosion current density (j_{corr}) were determined by Tafel slope extrapolation, and the passive current density (j_{pp}) was obtained from the passive zone where the corrosion current remained approximately constant. Mott-Schottky experiments were performed in the potential range of −0.5–1 V (vs. SCE) with the applied frequency of 1 kHz. For electrochemical experiments, the SBF solution of 500 ml was changed for each tested sample, and all electrochemical tests were repeated three times as per the proposed ASTM standard to ensure reproducibility and statistically analyzed to gain the standard deviations.

After electrochemical measurement, the surface morphologies were observed by a JSM-6460 scanning electron microscope (SEM), and the chemical composition of the surface oxide film on NG and CG Ti was analyzed by X-ray photoelectron spectrometer (XPS, ESCAKAB-250Xi, USA).

2.3. Surface characterization

Before biocompatibility experiments, surface topography and roughness were observed by atomic force microscopy (AFM, Dimension, Icon) with the contact mode at a rate of 1 Hz. In order to obtain further information of the surface wettability of the NG and CG Ti, the contact angles of deionized water droplet (0.5 μl) were measured using an automatic contact angle meter (KRUSS Gmbh, DSA10-Mk2) at room temperature. Three samples from each group were measured and two measurements were performed on each sample to evaluate the average contact angle. The surface energies of the NG and CG Ti were also calculated based on contact angle measurements, and the method for surface energy calculation can be seen in Ref. [21].

2.4. Investigations of biocompatibility

2.4.1. Protein adsorption assay

For the protein adsorption assay, bovine serum albumin (FBS, Thermo Science, USA) was used as a standardized model protein, and 1 ml droplet of Dulbecco's modified Eagle/Ham's F121:1 (DMEM) medium (Thermo Scientific, USA) containing 10% fetal bovine serum albumin was pipeted onto the samples surface placed in 24-well plate. After incubation for 1, 4, and 24 h at 37 °C, the samples were transferred to new 24-well plates and washed three times with phosphate buffer saline (PBS, Sigma, USA). Afterwards, 500 μl of 1% sodium dodecyl sulfate (SDS) solution was added to these wells and shaken for 15 min to detach proteins from the samples surface. The total protein concentrations in the collected SDS solutions were determined using NanoDrop 2000C device (Thermo Scientific, USA) at wavelength of 280 nm.

2.4.2. Cell culture

Cell culture experiments were performed using human fetal osteoblast cell line (hFOB1.19) provided by Institute of Biochemistry and Cell Biology of Chinese Academy of Sciences (Shanghai, China). The sheet samples were placed in 24-well culture plates and incubated into DMEM medium supplemented with 10% FBS, 0.3 mg/ml Geneticine418 (Sigma, USA), 0.5 mM sodium-pyruvate (Sigma, USA) and 1.2 g/l Na_2CO_3, and incubated in a humidified atmosphere incubator with 5% CO_2 at 37 °C, and the complete medium was refreshed every 2 days.

2.4.3. Cell cytotoxicity

Cytotoxicity was evaluated on cells cultured in medium conditioned by the presence of implants. The lactate dehydrogenase (LDH) activity in the culture media was used as an index of cytotoxicity. The LDH activity was determined spectrophotometrically according to the manufacturer's instructions.

2.4.4. Cell adhesion and proliferation assessment

The cells (hFOB1.19) were seeded on the sample substrates with a density of 8×10^4 cells/ml in 24-well tissue culture plate. The adhesion of cells was assessed after 1, 4 and 24 h of incubation in 24-well plates. At the end of each time period, the complete medium was removed from each well, and the samples were washed three times with PBS then transferred to new 24-well plates. The cells adhered on the samples were subsequently digested out of the samples with 0.3 ml 0.25% trypsin (Sigma, USA) for 5 min, then 0.7 ml complete medium was added to stop digestion. The released cells were counted with a hemocytometer on a Nikon Eclipse inverted fluorescence microscope. In order to study the proliferation of the hFOB1.19 on the samples, an initial density of 10^4 cells/ml was seeded on the surface of each substrate and incubated for 3, 7 and 14 days.