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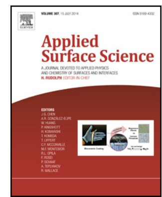

Improvement of biological properties of titanium by anodic oxidation and ultraviolet irradiation

Baoe Li [a,b,1], Ying Li [c,1], Jun Li [a], Xiaolong Fu [c], Changyi Li [c], Hongshui Wang [a], Shimin Liu [d], Litong Guo [e], Shigang Xin [b], Chunyong Liang [a,*], Haipeng Li [a,*]

[a] *School of Materials Science and Engineering, Hebei University of Technology, Tianjin 300130, China*
[b] *Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, China*
[c] *Stomatological Hospital, Tianjin Medical University, Tianjin 300070, China*
[d] *Business School, Tianjin University of Commerce, Tianjin 300134, China*
[e] *China University of Mining and Technology, Xuzhou 221116, China*

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ABSTRACT

Anodic oxidation was applied to produce a homogeneous and uniform array of nanotubes of about 70 nm on the titanium (Ti) surface, and then, the nanotubes were irradiated by ultraviolet. The bioactivity of the Ti surface was evaluated by simulated body fluid soaking test. The biocompatibility was investigated by in vitro cell culture test. The results showed that bone-like apatite was formed on the anodic oxidized and UV irradiated Ti surface, but not on the as-polished Ti surface after immersion in simulated body fluid for two weeks. Cells cultured on the anodic oxidized Ti surface showed enhanced cell adhesion and proliferation, also presented an up-regulated gene expression of osteogenic markers OPG, compared to those cultured on the as-polished Ti surface. After UV irradiation, the cell behaviors were further improved, indicating better biocompatibility of Ti surface. Based on these results, it can be concluded that anodic oxidation improved the biological properties (bioactivity and biocompatibility) of Ti surface, while UV irradiation improved the biocompatibility to a better extent. The improved biological properties were attributed to the nanostructures as well as the enhanced hydrophilicity. Therefore, anodic oxidation combined with UV irradiation can be used to enhance the biological properties of Ti-based implants.
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1. Introduction

Titanium (Ti) and its alloys are widely used as biomedical implant materials due to their favorable properties in mechanics and corrosion resistance. However, the Ti-based implants are typically bioinert, after implantation in body, bone tissues can not grow directly on their surfaces, leading to the poor bonding strength between them [1]. While, an accelerated stable fixation between bone tissues and implants (osseointegration) is of great demand in clinics in view of the early loading of the device, decreased patient morbidity, patient physical agony and health care costs [2]. Therefore, there is necessity to modify the Ti surface to improve its biological properties.

Nanoscience gave us the possibility to improve Ti's biological properties by manufacturing nanostructures on the surface. It has been reported that the bone cells are accustomed to a nanoscale environment rather than to a microscale environment [3]. For instance, osteoblasts were found to proliferate more quickly on nanostructured alumina and hydroxyapatite than on the conventional counterparts [4]. While, in our previous work [5–7], it was proved that the plasma sprayed nano-titania (TiO_2) coating (with nanostructured surface) exhibited excellent bioactivity and biocompatibility after acid/alkali treatment or ultraviolet (UV) irradiation. In contrast, the as-sprayed nano- and micro-TiO_2 coatings as well as the surface modified micro-TiO_2 coatings cannot do so. These results indicated that the biological properties of implants might depend on not only the nanostructured surface but also the acid/alkali treatment or UV irradiation which yielded surface Ti—OH groups during surface modification. Although the biological properties of Ti implants have been greatly improved by employing plasma-sprayed nano-TiO_2 coatings with proper surface modification, this method has the potential to form surface residuals which can be harmful to the osteoblasts [8]. Thus, alternative methods to modify Ti surface to produce well-defined nanostructures as well as Ti—OH groups are desirable.

* Corresponding authors. Tel.: +86 182 02287097; fax: +86 22 60204129.
E-mail addresses: liangchunyong@126.com (C. Liang), lhpcx@163.com (H. Li).
[1] Baoe Li and Ying Li contributed to the work equally.

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One method that may create controllable nanostructures on Ti surface is anodic oxidation. Anodic oxidation is an electrochemical method easily implemented to produce uniform and controllable nanostructured surfaces on a variety of metals and alloys (e.g. alumina, Ti-based alloys), which is an economical, simple, and versatile technique [4]. However, the research works mainly focused on the improvement of the adhesive bonding, corrosion resistance or wear resistance [9–11]. So far, this method has not been widely applied to produce biomedical coatings for orthopedic applications, although in a few studies it was employed to obtain calcium phosphate coatings on titanium surface [12]. As for the introduction of Ti—OH groups on nanostructured Ti surface, UV irradiation is a novel, clean and simple method when compared with acid/alkali treatment. UV irradiation was initially applied on TiO_2 powders to enhance the catalytic performance. In our previous work, this method had been successfully used to convert the plasma sprayed nano-TiO_2 coatings to hydrophilic states, improving the biological properties [7].

Therefore, in this paper, to improve the biological properties (bioactivity and biocompatibility) of Ti implant, anodic oxidation was used to fabricate controllable nanostructures on Ti surface, and then the nanostructured Ti surface was irradiated by UV light to induce Ti—OH groups. The influence of the nanostructures and Ti—OH groups on biological properties was investigated. The bioactivity of the Ti implant was evaluated by simulated body fluid (SBF) soaking test to examine bone-like apatite formation, and the biocompatibility was investigated by in vitro cell culture test to examine the cell adhesion, proliferation and gene expression.

2. Materials and methods

2.1. Preparation of samples

Commercially pure Ti plates in dimensions of 10 mm × 10 mm × 1 mm were polished with no. 1000 SiC abrasive paper, and then ultrasonically cleaned in acetone, ethanol and deionized water for 5 min, respectively. Anodic oxidation experiments were carried out using a direct current (dc) voltage source (WYK-150, Yangzhou, China). The Ti plates were used as anodic electrode while graphite (40 mm × 40 mm × 5 mm) was used as cathodic electrode. The distance between anodic and cathodic electrodes was 40 mm. 1 M NaF solution was used as electrolyte. The anodization process was carried out under a constant voltage of 10 V at room temperature for 1 h. During the experiment, the solution was stirred using a magnetic stirrer. After the anodic oxidation treatment, the samples were rinsed with deionized water, and then irradiated by UV light in deionized water for 24 h. A 125 W high-pressure mercury lamp was used as the UV light source. The primary wave-length of the UV light produced by the mercury lamp was 365 nm. For comparison, the as-polished Ti plates without anodic oxidation as well as the anodic oxidized Ti without UV irradiation were used as two control groups.

2.2. Surface characterization

Surface morphology of the Ti samples was observed using scanning electron microscopy (SEM, HITACHI S-4800). The crystal structure was examined by X-ray diffraction (XRD) analysis on a RIGAKUD/MAX2500 diffractometer with Cu *K*a radiation. The surface roughness was examined by atomic force microscopy (AFM, Agilent 5500), and the hydrophilicity was assessed from the measurements of the contact angle between the deionized water and sample surface at room temperature. Drop volumes of 2 μL were chosen to avoid gravitation-induced shape alteration and to diminish the evaporation effects. The drop image was acquired using a digital camera (1280 × 960 pixel) attached to a microscope and processed by an image analysis software.

2.3. Bioactivity evaluation

The samples were soaked in 30 mL of SBF at 37 °C without stirring to evaluate their bioactivity. The ionic concentrations in SBF are nearly equal to those in human blood plasma. It was prepared by dissolving analytical-reagent grade chemicals of NaCl, $NaHCO_3$, KCl, $KH_2PO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, and Na_2SO_4 into distilled water and buffered at pH 7.4 at 36.5 °C with 45 mM trimethanol aminomethane ($(CH_2OH)_3C(NH_2)$) and 1 M HCl [13]. After soaking for 2 weeks without stirring, the growth of bone-like apatite on sample surfaces was investigated by SEM and XRD.

2.4. Biocompatibility evaluation

2.4.1. Cell morphology and proliferation

The samples were sterilized by an autoclave sterilizer at 121 °C for 30 min before cell culture test. MC3T3-E1 murine preosteoblasts which were supplied by Tianjin Medical University (China) were seeded on the sample surfaces at a density of 1×10^4 cells/cm^2. The cells were cultured in the Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Grand Island, NY, USA) with 10% fetal bovine serum (Hyclone, Logan, UT, USA) and 3% penicillin/streptomycin. Cultures were maintained at 37 °C in an incubator with a fully humidified atmosphere of 5% CO_2. After culturing for 1, 4 and 7 days, the cellular proliferation on the sample surfaces was evaluated by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO) colorimetric assay according to the manufacturers' instructions [14]. To observe the cellular attachment on the sample surfaces, the cells adhered on the sample surfaces were fixed with 2.5% glutaraldehyde in phosphate-buffered saline (PBS, pH = 7.4) for 4 h, and then rinsed with PBS twice, followed by dehydration in a grade ethanol series and critical point drying. After gold sputtering, the cell morphologies were observed by SEM.

2.4.2. Osteogenic gene expression analysis

After the cells were cultured on the Ti plates for 7 days, total RNA was isolated from cells using a monophasic solution of phenol and guanidine isothiocyanate (Trizol, Invitrogen Life Technologies, Carlsbad, CA, USA), following the instructions of the manufacturer. RNA was quantified at 260 nm using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, USA) and then reverse transcribed to cDNA using NI-RT Master Mix cDNA Synthesis kit (NEWBIO industry, Tianjin, China) that contains oligo (dT) in a final volume of 20 μL to evaluate the gene expression [15]. Each cDNA was diluted 1:10 with RNase-free water, and then frozen (−20 °C) until the PCR reactions were carried out.

Reverse transcription-polymerase chain reaction (RT-PCR) was carried out using the SYBR Green PCR reagent (Qiagen, USA) with the primer sets listed in Table 1. The length of the resulting amplicons and the GeneBank accession number are also shown in Table 1. Highly purified gene specific primers for osteoprotegrin (OPG) and the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were synthesized commercially (Shengong, Shanghai, China). Levels of each mRNA were normalized to that of GAPDH mRNA. The PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide to determine their sizes.

To determine the exact gene expression amount between different groups, real-time PCR was also performed using the SYBR green detection by Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). For quantitative PCR, 10 μL

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