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Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2017) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Nanotubular topography enhances the bioactivity of titanium implants☆

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Received 10 January 2017; accepted 26 March 2017

Abstract

Surface modification on titanium implants plays an important role in promoting mesenchymal stem cell (MSC) response to enhance osseointegration persistently. In this study, nano-scale TiO_2 nanotube topography (TNT), micro-scale sand blasted-acid etched topography (SLA), and hybrid sand blasted-acid etched/nanotube topography (SLA/TNT) were fabricated on the surfaces of titanium implants. Although the initial cell adherence at 60 min among TNT, SLA and TNT/SLA was not different, SLA and SLA/TNT presented to be rougher and suppressed the proliferation of MSC. TNT showed hydrophilic surface and balanced promotion of cellular functions. After being implanted in rabbit femur models, TNT displayed the best osteogenesis inducing ability as well as strong bonding strength to the substrate. These results indicate that nano-scale TNT provides favorable surface topography for improving the clinical performance of endosseous implants compared with micro and hybrid micro/nano surfaces, suggesting a promising and reliable surface modification strategy of titanium implants for clinical application.

Key words: Nanotubular; Topography; Bioactivity; Titanium implant

Currently titanium and titanium-based alloys are most widely used in orthopedic and oral implants. However, a long osseointegration period of 3-4 months is normally required due to the bio-inert nature of titanium, which makes the whole process of implant treatment even longer. Therefore, researchers are searching for efficient surface modification strategies to enhance the bioactivity of titanium implant. Efficient osseointegration of implants relies on the recruitment of osteoblast precursors to the tissues around the implants, followed by osteogenic differentiation, osteoblast maturation, matrix deposition and mineralization. Mesenchymal stem cells (MSCs) are multipotent stem cells which can be induced to differentiate into osteoblasts, adipocytes and chondrocytes, depending on stem cell niches composed of various microenvironment signals. The signals can be roughly divided into two categories: biochemical and biophysical signals including the topography, geometry, elastic modulus[1] and dimension of the substrate. Among the diverse signals, physical factors, especially the topography cues, have been proved to be more lasting, controllable and much safer.

Conflict of interest statement: The authors have no conflict of interest to declare.
Funding: This work was supported by the National Nature Science Foundation of China (NCFC 81550013, NSFC81672659, NSFC81472523, NSFC81272953) and the Guangdong Natural Science Foundation (2015A030313017).
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http://dx.doi.org/10.1016/j.nano.2017.03.017
1549-9634/

Until now, the majority of commercially available implants are moderately textured with micro-roughened topography through sand blasting[2,3] acid etching[4] and micro-arc oxidation,[5] which present micro-scale pits or holes on the surface of implants. The conventional sandblast-acid etching (SLA) technique enhances osseointegration and initial stability of implants owing to the mechanical interlock with surrounding bone. However, compromised proliferation of osteoblasts was observed on microscale surfaces.[6,7]

Inspired by the nanoscale fundamental elements of bone matrix, biomimetic nanostructures are of particular interests for potentially better osseoinduction, by which osteogenesis is induced.[8] Among the diverse nanostructures, titanium dioxide nanotube has grabbed much attention due to its simple fabrication process and controllable size.[9] It has been demonstrated that the surface chemistry and topography of the TiO_2 nanotube influence the cell behavior such as adhesion, spread, proliferation, migration and differentiation as well.[10,11] In addition, surface structures composed of micro- and nanoscale components have been demonstrated to promote osteogenesis for mimicking the hierarchical structure of the bone matrix.[12,13] Zhao et al[13] fabricated hierarchical micropore/nanorod-patterned strontium (Sr) doped hydroxyapatite (HA) coatings and observed that the coating with an interrod spacing of 67 nm displayed advanced biological effect. Zhou et al[14] showed that SLA-treated surfaces with 80-nm nanotubes enhanced osteogenesis in comparison with SLA. However, whether nano-scale nanotube coating or micro-scale SLA topography performs better bioactivity has not been reported yet. Systematic research is also lacking on whether the hybrid hierarchical structure would induce synthetic biological effect compared to solo treatment.

The previous research of our group has shown that nanotube coating on titanium surface greatly improved platelet adhesion and inhibited adhesion of microcosm compared with SLA-treated topography.[15,16] In order to further figure out the optimal implant surface topography scale to induce osteogenesis, serial scales of topographies (nano-scale, micro-scale, and hybrid micro/nano scale) were constructed on the surfaces of titanium implants *via* SLA and anodic oxidation. The surface properties of the different surfaces were investigated. Bioactivity evaluations were carried out by observing the *in vitro* biological response of rat bone marrow-derived MSCs and *in vivo* osteogenesis in rabbit femur models.

Methods

Specimen preparation of different titanium surfaces

Pure Ti foils of 10 mm in diameter and 0.6 mm in thickness (Baoji Titanium Industry, China) were used as the substrate. Four surface treatments were applied to the Ti substrate, namely machined smooth Ti (control group), sandblasted and acid etched Ti (SLA group), TiO_2 nanotubes (TNT group), and sandblasted/acid etched TiO_2 nanotubes (SLA/TNT group). Both the top and bottom surfaces were ground with #400 to #1500 *SiC* sandpaper successively; ultrasonically cleaned with acetone, anhydrous alcohol, and deionized water in sequence; and finally air dried at room temperature. Specimens grouped in control were left untreated. SLA surfaces were prepared by blasting silicon dioxide particles onto machined Ti surfaces, and then acid-etching using 10%HCl:10%H_2SO_4 (1:1, v/v) at 60 °C for 30 min. TNT surfaces were prepared in an electrochemical cell by anodizing using a DC power supply. Ti substrate was used as anode and a copper sheet was used as cathode. The anodic oxidation procedure was performed in aqueous electrolyte containing 0.15 M NH_4F and 0.5 M $(NH_4)_2SO_4$ at 20 V for 30 min. SLA/TNT surfaces were first prepared by sandblasting and acid-etching as described for SLA group, and then anodized by the same protocol used for TNT group. After the treatment mentioned above, the specimens were rinsed with distilled water and sterilized by autoclave sterilization. Tissue culture polystyrene (TCPS) was used as positive control in *in vitro* study.

Surface characterization of different titanium surfaces

The topography of Ti discs sputter-coated with gold was observed by scanning electron microscope (SEM: LEO1530VP FESEM, Zeiss, Germany). Phase identification was carried out by an X-ray diffractometer in θ-2θ geometry using Cu Kα radiation. The surface roughness of samples was determined using a profilometer of laser scanning confocal microscope (LSM700, Zeiss, Germany). The following parameters were calculated: Sa (the arithmetic average of the 3D roughness), and Sq (the quadratic average of the 3D roughness).[17] Hydrophilicity of the titanium surfaces was evaluated using contact angle analyzer (OCAl5, Dataphysics, Germany).

Protein adsorption assay

After incubation in 5 mg/mL bovine serum albumin (BSA) (MP Biomedicals) at 37 °C for 4 h, the protein adsorbed onto the discs was detached in 1% sodium dodecyl sulfate (SDS) at 50 °C, 100 rpm/min for 1 h. The protein concentration was determined using MicroBCA protein assay kit (Pierce).

The bonding strength of coating on TNT and SLA/TNT

To measure the bonding strength of nanotube coating to titanium substrate, micro-scratch test was performed using a Micro Scratch Tester[18] (WS-92). The parameters were set as follows: indenter load: 0.01 to 50 N; pressure rate: 10 $N \cdot min^{-1}$; operating speed: 2 $mm \cdot min^{-1}$. The critical load, the load when the delamination of the coating first occurred, was recorded. Images of the scratches were obtained by the SEM apparatus used above.

Cell culture of BMSCs

BMSCs were harvested from 4-week-old male Sprague–Dawley rats, according to protocols as we described.[19] Briefly, bone marrow was flushed from the hind limbs and spun down at 800 rpm for 5 min. The pellet was resuspended in α-MEM (Gibco) containing and 10% fetal calf serum (FCS, Gibco), and cultured in a humidified atmosphere with 5% CO_2 at 37 °C. When the culture grew to about 80% confluence, the MSCs were trypsinized using 0.25% trypsin (Sigma) and subcultured. The cells at passage 2-4 were used in the following *in vitro* experiments.