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Formation of TiO_2 nano-network on titanium surface increases the human cell growth

Chih-Yao Chiang[a,b,1], *Shih-Hwa Chiou*[a,c,d,1], *Wei-En Yang*[d], *Ming-Lun Hsu*[e], *Ming-Chi Yung*[a,b], *Ming-Long Tsai*[c], *Li-Kai Chen*[f], *Her-Hsiung Huang*[d,e,f,*]

[a] *Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan*
[b] *Division of Cardiovascular Surgery, Taipei City Hospital, Taipei, Taiwan*
[c] *Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan*
[d] *Institute of Oral Biology, National Yang-Ming University, Taipei, Taiwan*
[e] *Department of Dentistry, National Yang-Ming University, Taipei, Taiwan*
[f] *Department of Dentistry, Taipei City Hospital, Taipei, Taiwan*

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ABSTRACT

Objectives. This study was to improve human cell growth on titanium (Ti) used for dental implants through formation of a nano-network surface oxide layer created by an electrochemical anodization treatment.

Methods. An electrochemical anodization treatment was used to produce a network oxide layer on Ti surface. Surface characterization of the network layer was carried out using thin film X-ray diffractometer and field emission scanning electron microscopy. Human bone marrow mesenchymal stem cells (hMSCs) were made to express green fluorescent protein (GFP) by retroviral transduction. The GFP signal was measured in situ to assess in vitro and in vivo cell growth on Ti surfaces. In vivo experiments on Ti-supported cell growth were carried out on the back skin of nude mice. Alizarin red staining and immunofluorescent staining were used to observe cell differentiation.

Results. A multilayer TiO_2 nano-network was produced rapidly on Ti surface using a simple electrochemical anodization treatment. The TiO_2 nano-network layer on the anodized Ti surfaces significantly improved in vitro and in vivo hMSC growth, as assessed by measurement of GFP fluorescence, relative to hMSC growth on untreated Ti surface. The TiO_2 nano-network layer on the anodized Ti surfaces can also induce the differentiation of hMSCs after 28-day in vivo test.

Significance. The formation of TiO_2 nano-network on the Ti surfaces can increase the hMSC growth in vitro and in vivo.

* *Corresponding author at*: Department of Dentistry, National Yang-Ming University, No. 155, Sec. 2, Li-Nong Street, Taipei 112, Taiwan. Tel.: +886 2 2826 7068; fax: +886 2 2826 4053.
E-mail addresses: hhhuang@ym.edu.tw, biomaterial@msn.com (H.-H. Huang).
[1] These authors contributed equally to this paper.
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1. Introduction

Titanium (Ti) and its alloys have good mechanical, anti-corrosive, and biocompatibility characteristics, and they are therefore used extensively as dental implants [1–4]. A thin, dense, protective oxide layer (mainly TiO_2) forms rapidly on the Ti surface when exposed to the atmosphere. This produces excellent anti-corrosive and biocompatibility properties. When a Ti implant is introduced into the human body, the surrounding tissue is in direct contact with the Ti-oxide layer on the Ti surface. For this reason, the biocompatibility of the Ti implant depends on the properties of this surface oxide layer such as its microstructure, chemical composition, and morphology. Although Ti is widely used in the clinic, it presents some issues that remain unresolved. For example, the protectiveness of TiO_2 oxide film on Ti surface can be destroyed by fluoride ions [5,6]; patients may have allergic reactions and tissue discoloration when contacting with Ti surface [7]. Furthermore, some of the dental implant failure cases are related to the implant surface characters [8–12]. Therefore, appropriate implant surface treatments may play an important role in obtaining a successful dental implant system, especially when implant is used in poor-quality bone, immediate loading or early loading procedures.

Different methodologies are being used in an effort to improve the interfacial reactions between the biological tissue and the implant, such as increasing the attachment of tissues on implant surface. Among the surface treatment methods for Ti-based implants, surface topography modification has been used to improve the response of cells to the implant. Porous equi-atomic Ti–Ni alloy (Actipore™), whose outermost surface main oxide TiO_2 is similar to that on Ti surface, can be regarded as fully cytocompatible and genocompatible, making it a good candidate for long-term implantation [13]. Osteoblast-like cells attach and spread well on surface-modified Ti metal. These cells have been observed to grow into the pores of the metal surface and form an extracellular matrix [14]. Obviously, producing a suitable porous structure on a Ti-based implant surface is crucial when biocompatibility is a principal concern. Therefore, many researchers have investigated the approaches used to produce porous Ti-based implant surface in order to attain an ideal biological response [15–17]. One approach has been to immerse the Ti metal in an alkaline solution at 60 °C for 24 h, followed by thermal treatment at 600 °C for 1 h. This procedure creates a sodium titanate layer on the surface of porous Ti. The sodium titanate induces bone-like apatite formation in simulated body fluid. The method also produces a bioactive macroporous surface layer on the Ti substrate [15]. In addition, studies have found that after undergoing specific chemical and thermal treatments, a similar porous, bioactive Ti metal can be developed [16,17]. However, most immersion, chemical or thermal treatments to produce a bioactive porous Ti surface have been relatively complex or time-consuming. Lately, electrochemical techniques have been explored as a potential alternative for producing porous Ti-based metal for medical implants [18–20]. Furthermore, electrochemical techniques have been used to produce self-organized and highly ordered TiO_2 nanotubes on a Ti surface in a fluoride solution [21,22]. The in vitro cytocompatibility report revealed that the nano-structured Ti surface has much higher cells (fibroblast mice cells L929) colonization than the conventional Ti surface [23]. However, comprehensive information on the in vitro and in vivo responses of cells as they interact with the porous or nano-scale Ti surfaces produced by electrochemistry is limited.

Our hypothesis is that a nano-scale oxide structure on a Ti surface can improve cell growth. In this study, a faster and simpler electrochemical anodization treatment was used to create a structured nano-network layer of Ti-oxide on a Ti surface for dental implant applications. The growth of green fluorescent protein (GFP)-labled human bone marrow mesenchymal stem cells (hMSCs) on the anodized Ti surfaces was evaluated in vitro and in vivo.

2. Materials and methods

2.1. Preparation of materials

Commercially available pure Ti discs (Goodfellow Cambridge Ltd., Cambridge, UK) were used as the test specimens (ϕ 15 mm; thickness, 1 mm). The disc surfaces were polished with silicon carbide paper (#1500). An electrochemical workstation (Jiehan 5000, Jiehan Technology Co., Taiwan) was used to apply anodic currents of I_1 and I_2 A ($I_1 < I_2$) to the polished Ti specimens in a 5 M NaOH electrolyte solution at 25 °C. The corresponding specimens were designated as I_1 and I_2, respectively. The applied currents were under 0.2 A. The duration for the anodization treatments was controlled to within 30 min. The untreated, polished Ti specimen was designated as I_0, and this corresponded to an applied current of 0 A. The total number for every I_0, I_1 and I_2 specimen group was 24, respectively. The test Ti specimens were sterilized with UV light for 1 h before cells were cultured on the specimen surface.

2.2. Surface characterization

The crystalline structure of the anodized surface layer on the Ti specimens was characterized using a thin film X-ray diffractometer (TF-XRD) (D-max/IIB, Rigaku, Tokyo, Japan). The morphology of the anodized layer on the Ti specimens was analyzed using field emission scanning electron microscopy (FE-SEM) (JSM-6700F, JEOL, Tokyo, Japan). The morphological dimensions of the anodized structure on the Ti specimens were determined from the FE-SEM micrographs using Image-Pro® Plus image analysis software (Version 4.5.1, Media Cybernetics, Inc., Silver Spring, MD, USA). Each test group had three samples for every surface characterization analysis mentioned above.

2.3. In vitro cell growth of GFP-labeled hMSCs

For cell growth on Ti specimen surfaces, we isolated and cultured adult human bone marrow mesenchymal stem cells (hMSCs) according to previously published protocols [24,25]. These possess multi-lineage potential and the ability to differentiate into various cell types from all three germ layers,