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Ultraviolet light-mediated photofunctionalization of titanium to promote human mesenchymal stem cell migration, attachment, proliferation and differentiation

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Abstract

Improving the osteoconductive potential of titanium implants has been of continuing interest in the fields of dentistry and orthopedic surgery. This study determined the bioactivity of ultraviolet (UV) light-treated titanium. Human mesenchymal stem cells (MSCs) were cultured on acid-etched microtopographical titanium surfaces with and without 48 h pretreatment with UVA (peak wavelength of 360 nm) or UVC (peak wavelength of 250 nm). The number of cells that migrated to the UVC-treated surface during the first 3 h of incubation was eight times higher than those that migrated to the untreated surface. After 24 h of incubation, the number of cells attached to the UVC-treated surface was over three times more than those attached to the untreated surface. On the UVC-treated surface, the cellular spread was expedited with an extensive and intensive expression of the focal adhesion protein vinculin. The cells on the UVC-treated surface exhibited a threefold higher bromodeoxyuridine incorporation, a doubling of the alkaline phosphatase-positive area and the up-regulated expression of bone-related genes, indicating the accelerated proliferation and differentiation. The UVC-treated surface did not adversely affect the viability of the cells. These biological effects were not seen after UVA treatment, despite the generation of superhydrophilicity. Thus, we discovered a novel photofunctionalization of titanium dioxide that substantially enhances its bioactivity in human MSCs. Further studies are required to investigate the universal effectiveness of this surface modification for different titanium-containing materials, with varying chemistries and textures, as well as to understand its significance in enhancing in vivo osteoconductivity.

Keywords: UVC; Orthopedic and dental implant; Osseointegration; Superhydrophilicity; Osteoblast

1. Introduction

Titanium implants have become an essential treatment modality in bone and teeth reconstructive surgeries in the orthopedic and dental fields. However, there have been pressing clinical demands for reducing patients' morbidity, improving outcome predictability, avoiding complications and expanding treatment indications. Addressing these demands, considerable efforts have been made to develop new technologies for modifying the surface of titanium to improve its osteoconductivity.

Topographical modification of titanium surfaces has been successfully implemented to improve the bioactivity of titanium [1,2]. The acid-etched surface, which is representative of the microroughened surfaces currently used in the dental implant market, induces distinct osteoblastic phenotypes and functions in vitro, such as up-regulated

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gene expression [2], enhanced production of extracellular matrix [3,4] and mineral deposition [2], and an increased extracellular matrix–titanium interfacial bonding [2–4]. The acid-etched surface affords a higher percentage of bone–implant contact [5], enhances the mechanical quality of the bone [2,6] and increases the biomechanical fixation of implants in vivo [7] compared to a machined surface.

Surface chemistry has been another target in the modification of titanium surfaces. A number of protocols with various subtractive and additive procedures, including the use of different acidic and alkaline liquids to treat titanium surfaces at different processing times and temperatures and the use of chemical deposition techniques under modified electrical environments, have been introduced. Such protocols produce various titanium surface chemistries, particularly in terms of the Ti/O ratio, thickness of the oxidized layer, crystallization properties and surface impurities [8–10], which result in different responses of osteogenic cells [9,10]. For instance, recent studies have shown that hydrofluoric acid treatment of titanium promotes osteoblastic differentiation and enhances protein production [11], resulting in the enhancement of bone–titanium integration [12,13]. Storing the chemically modified titanium in physiological saline solution promotes osteoblastic differentiation and growth factor production [14], thereby enhancing bone–implant integration [15] while maintaining the hydrophilicity and low carbon contamination of the surface [14,15].

Ultraviolet (UV) light treatment of titanium induces temporal chemical alteration within the superficial TiO_2 molecule, resulting into a photocatalytic chemical reaction. The photochemical reaction of semiconductor oxides has attracted considerable interest in the environmental and clean-energy sciences. It has been applied to develop pollutant-degrading, anti-fogging, anti-bacterial and stain-proofing materials [16,17]. An example of the UV light-mediated physical changes of TiO_2 is seen in the generation of a superhydrophilic surface. In this phenomenon, UV treatment is assumed to alter the molecular structure of surface TiO_2 [18,19] by creating surface oxygen vacancies at bridging sites, resulting in the conversion of relevant Ti^{4+} sites to Ti^{3+} sites, which are favorable for dissociative water adsorption. Moreover, other mechanisms have also been proposed: for example, enhanced wetting is caused by surface chemistry alteration by TiO_2 photocatalytic activity, such as decontamination of hydrocarbon from TiO_2 surfaces [20,21]. UV light energy greater than 3.2 eV is needed to induce TiO_2 photocatalytic activity to excite an electron from the valence band to the conduction band, which corresponds to approximately 370 nm wavelength of UV light, referred to as UVA. Meanwhile, the direct decomposition of hydrocarbon by UVC light in the lower range around 250 nm wavelength is also of interest.

We tested a hypothesis that UV light treatment of titanium enhances its bioactivity relevant to osseointegration. This study examined the effects of UV light pretreatment of titanium disks on the behaviors and functions of human mesenchymal stem cells (MSCs) cultured on its surface. An acid-etched titanium surface, which is one of the most commonly used surface textures in dental implants, was used with the aim of further improving its bioactivity.

2. Materials and methods

2.1. Titanium samples and surface characterization

Commercially pure grade-2 titanium disks (20 mm diameter) were machine-prepared and acid-etched with 67 wt.% sulfuric acid (H_2SO_4) at 120 °C for 75 s. Some disks were treated with UV light for 48 h under ambient conditions. We used two different light sources, UVA and UVC. UVA was generated by using a 6-W mercury lap (UVP, Cambridge, UK) and had intensities of ca. 2 mW cm^{-2} ($\lambda = 360 \pm 20$ nm) and 0.0 mW cm^{-2} ($\lambda = 250 \pm 20$ nm), while UVC was from a 15-W bactericidal lamp (Toshiba, Tokyo, Japan) with intensities of ca. 0.1 mW cm^{-2} ($\lambda = 360 \pm 20$ nm) and 2 mW cm^{-2} ($\lambda = 250 \pm 20$ nm). The surface morphology was evaluated using scanning electron microscopy (SEM; XL30, Philips, Eindhoven, Netherlands). The hydrophilicity of the titanium surfaces was measured using an automatic contact angle measuring device (DCA-VZ, Kyowa Interface Science, Saitama, Japan) at the contact angle of 1 μl H_2O. Additional image analyses were undertaken to evaluate the spread area of 10 μl H_2O on titanium disks.

2.2. Human mesenchymal stem cell culture

MSCs (Poietics, Cambrex Bio Science Walkersville, East Rutherford, NJ) were cultured in MSC growth medium that consisted of MSC basal medium and growth supplements (SingleQuots). The growth supplements contained fetal bovine serum (FBS), L-glutamine and penicillin/streptomycin. Cells were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After 2–4 passages, the cells were cultured in MSC osteogenic induction medium, consisting of MSC basal medium and an osteogenic induction supplement (SingleQuots) that contained FBS, L-glutamine, penicillin/streptomycin, dexamethasone, ascorbate and β-glycerophosphate. At 80% confluency of the last passage, cells with and without osteogenic induction were detached using 0.25% trypsin–1 mM EDTA–4Na and seeded onto titanium disks at a density of 3×10^4 cells cm^{-2}. The culture medium was renewed every 3 days. Cells with osteogenic inductive medium were used in alkaline phosphatase activity and gene expression assays, and those without were used in migration, attachment, cytomorphology, viability and proliferation assays.

2.3. Migration assay

Migration of human MSCs to titanium surfaces was examined using a cell invasion assay (345-024K, Trevigen, Gaithersburg, MD). The assay was established to investigate chemotaxis, migration and/or invasion of various cells [22–24]. The assay uses a 96-well transwell containing 8 μm

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