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The enhanced characteristics of osteoblast adhesion to photofunctionalized nanoscale TiO_2 layers on biomaterials surfaces

Tomohiko Miyauchi [a,1], Masahiro Yamada [b,1], Akiko Yamamoto [c,d], Fuminori Iwasa [a,b], Tetsuo Suzawa [e], Ryutaro Kamijo [e], Kazuyoshi Baba [a], Takahiro Ogawa [b,*]

[a] Department of Prosthodontics, School of Dentistry, Showa University, Tokyo, Japan
[b] Laboratory of Bone and Implant Sciences (LBIS), The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, 10833 Le Conte Avenue (B3-087 CHS), Box 951668, UCLA School of Dentistry, Los Angeles, CA 90095-1668, USA
[c] Biomaterials Center, National Institute for Materials Science (NIMS), Tsukuba, Japan
[d] International Center for Materials Nanoarchitectonics, National Institute for Materials Science (NIMS), Tsukuba, Japan
[e] Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan

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ABSTRACT

Recently, UV photofunctionalization of titanium has been shown to be effective in enhancing osteogenic environment around this functional surface, in particular for the use of endosseous implants. However, the underlying mechanism remains unknown and its potential application to other tissue engineering materials has never been explored. We determined whether adhesion of a single osteoblast is enhanced on UV-treated nano-thin TiO_2 layer with virtually no surface roughness or topographical features. Rat bone marrow-derived osteoblasts were cultured on UV-treated or untreated 200-nm thick TiO_2 sputter-coated glass plates. After an incubation of 3 h, the mean critical shear force required to initiate detachment of a single osteoblast was determined to be 1280 ± 430 nN on UV-treated TiO_2 surfaces, which was 2.5-fold greater than the force required on untreated TiO_2 surfaces. The total energy required to complete the detachment was 37.0 ± 23.2 pJ on UV-treated surfaces, 3.5-fold greater than that required on untreated surfaces. Such substantial increases in single cell adhesion were also observed for osteoblasts cultured for 24 h. Osteoblasts on UV-treated TiO_2 surfaces were larger and characterized with increased levels of vinculin expression and focal contact formation. However, the density of vinculin or focal contact was not influenced by UV treatment. In contrast, both total expression and density of actin fibers increased on UV-treated surfaces. Thin layer TiO_2 coating and UV treatment of Co–Cr alloy and PTFE membrane synergistically resulted in a significant increase in the ability of cell attachment and osteoblastic production of alkaline phosphatase. These results indicated that the adhesive nature of a single osteoblast is substantially enhanced on UV-treated TiO_2 surfaces, providing the first evidence showing that each individual cell attached to these surfaces is substantially more resistant to exogenous load potentially from blood and fluid flow and mechanical force in the initial stage of *in vivo* biological environment. This enhanced osteoblast adhesion was supported synergistically but disproportionately by enhancement in focal adhesion and cytoskeletal developments. Also, this study demonstrated that UV treatment is effective on nano-thin TiO_2 depositioned onto non-Ti materials to enhance their bioactivity, providing a basis for TiO_2-mediated photofunctionalization of biomaterials, a new method of developing functional biomaterials.

1. Introduction

Photofunctionalization of titanium surfaces by UV treatment has been discovered recently [1]. The functionalized titanium surfaces facilitate the growth of increased number of cells in osteoblastic cultures, in association with increased cell attachment and proliferation [1,2]. The rate of osteoblastic differentiation is also accelerated but not as substantially as compared to cell attachment and proliferation [1–3]. As a result of these enhanced biological cascades, the *in vitro* osteoblastic phenotypes, such as alkaline phosphatase activity and the formation of mineralized nodules, are considerably increased on UV-treated titanium surfaces compared to untreated titanium surfaces [1]. *In vivo* enhancement in the ability of osseointegration is also remarkable. The biomechanical strength of osseointegration for UV-treated implants is 3-fold more

* Corresponding author. Tel.: +1 310 8250727; fax: +1 310 825 6345.
E-mail address: togawa@dentistry.ucla.edu (T. Ogawa).
[1] These authors equally contributed.

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than for untreated implants at the early healing stage in an animal model [1]. The enhancement remains significant even at the late stage of healing, which was supported by almost 100% bone-implant contact enabled around the UV-treated implants as opposed to less than 55% for the untreated implants [1]. Thus, UV treatment of titanium not only expedites the process of osseointegration but also elevates the level of osseointegration eventually to a near maximum. The effect of UV photofunctionalization distinguishes itself from most of the recently reported surface modification technologies for titanium, e.g., nanotopographical and chemical modifications, which only expedite but do not elevate the process of osseointegration. Photofunctionalization is also expected to be a new approach of surface enhancement to circumvent conventional surface modification technologies due to its technical simplicity, since it does not require additional chemical or mechanical processing of the original titanium surfaces [1,4].

It remains uncertain as to which initial biological processes are particularly enhanced around UV-treated titanium surfaces. Despite the known facts regarding the increased number of osteogenic cells on UV-treated titanium surfaces, it is not clear how this happens at the initial stage of cell–material interaction. It would be particularly interesting to investigate the nature of adhesion of cells to UV-treated titanium surfaces. *In vivo* tissues are subject to considerable forces that can impact cell–extracellular matrix (ECM) interaction and subsequent cell functions. Forces that are applied externally involve compressive and tensile forces from weight, gravity, motion, and muscular function, as well as shear forces from blood and fluid flow [5]. How tissues withstand these forces depends in a large part on the type and density of cells and the strength of cell–ECM interaction [5,6]. In light of biomaterials to be evaluated for their biological affinity, the faster and stronger establishment of adhesion between cells and biomaterial surfaces holds a key.

The capability of cell retention should be emphasized more for endosseous implant materials as a tissue-generating, as well as load-bearing and motion-resisting device, than for other biomaterials and other purposes. When implant surfaces are exposed to the same number of mesenchymal stem cells in the bone marrow, the initial step required for faster generation of bone depends on how many cells are retained against the blood and fluid flow and micromotion at the cell–implant interface. Assay systems utilized to evaluate the adhesive properties of cells include counting the cells or quantifying the volume of cells remaining on the material surfaces after physical and chemical detachment protocols. The detachment protocols applied include use of enzymes [7], fluid flow [8,9], and spinning device [10–12]. These assays provide a comparative measurement of the probability of adherent cells after a certain level of detachments, but do not provide a quantitative/absolute value of the strength of cell adhesion or the information on the quality of cell adherence, such as the critical force and energy required for complete cell detachment. A recent study developed a laser-spallation technique to detach cells or cultured ECM to quantify the interfacial tensile strength between the cells/ECM and material surfaces [13]. This technique requires the cell culture to be dried and exposed to the laser in a larger area of the culture and is therefore a cell population-based evaluation. We previously developed a shear test-based single cell detachment technique [14,15]. This technique allows for obtaining a direct and absolute value of the shear force required to detach a single cell from a material surface. The measurement can be performed in a culture medium without any treatment of the cells, such as enzyme treatment, drying, or fixation.

In perspective of applications of photofunctionalization of titanium to implantable or tissue engineering materials other than titanium, it could be beneficial to coat these materials with TiO_2, if successful photofunctionalization can take place. A series of previous studies related to titanium photofunctionalization only dealt with bulk Ti that possess oxidized and crystallized superficial layer distinct from TiO_2 formed using coating/depositioning techniques [16–18]. The degree and type of oxidation and crystallization of titanium surfaces affects their biological capability [18,19]. Differently prepared titanium surfaces show different absorbance curve of UV light, resulting in the difference in UV-responsiveness [1]. Whether UV treatment is effective to depositioned TiO_2 needs to be answered.

Another crucial question is whether UV-enhanced bioactivity is obtainable on TiO_2 surfaces without roughness or topographical features. Ti bulks used in the previous studies were prepared by either machining, sandblasting, or acid-etching, all of which show the roughness at the micro and supra-micron levels. The average roughness values are 100–600 nm and such roughness features *per se* stimulate osteogenic cells and promote osteoblastic differentiation [20–22]. The reported biological effects of photofunctionalization involve the expedited and enhanced cell spread on UV-treated microroughened titanium surfaces, which otherwise manifests a delayed and limited spread on such surfaces [1,3]. Cells inherently spread more quickly and largely on smoother material surfaces. Therefore, a hypothesis that needs to be addressed here is that UV treatment is effective on very smooth TiO_2 surfaces with virtually no topographical feature, to significantly improve its bioactivity. More importantly, there is a fact that possibly denies this hypothesis. Cell sheet technology, which has attracted attention as a new method of tissue engineering, is based on the principle and fact that hydrophilic and morphologically smooth surface is not capable of retaining cells and releases them [23–25]. Polymer poly(*N*-isopropylacrylamide) (PIPAAm) is temperature-responsive and converts its surface property from hydrophobic to hydrophilic. The cell culture dish made of PIPAAm allows spontaneous release of cultured cells as a piece of sheet during this conversion. UV treatment converts titanium surfaces from hydrophobic to hydrophilic [1,2]. We are interested in elucidating if this adverse effect, in light of bone-implant integration, takes place on UV-treated, very smooth, titanium surfaces.

This study examined the critical shear force and the total energy required to detach a single osteoblast adhered to a UV-treated TiO_2 surface, a 200 nm-thin sputter-coated layer of TiO_2 on very smooth and flat glass plates with virtually no roughness or topographical features. A previous study demonstrated that such chemical deposition-based coating up to a thickness of 250 nm does not alter the original surface morphology [18]. The degree of establishment in focal adhesion and cytoskeletal development was also examined to seek the underlying mechanisms for the possibly modulated cell adhesion to UV-treated TiO_2 surfaces. We further determined the effectiveness of UV treatment of TiO_2 that was coated onto other biomaterial surfaces, cobalt–chromium (Co–Cr) alloy and polytetrafluoroethylene (PTFE)-based wound healing membrane, to enhance their bioactivity. Co–Cr alloy is a biotolerant material and used as an endosseous implant for orthopedic reconstruction. PTFE, a synthetic fluoropolymer of tetrafluoroethylene, is well known as Teflon and chemically stable. Although PTFE is biologically inert and does not show osteoconductive potential, it is widely used as a space-making barrier membrane in bone regeneration therapies, such as periodontal surgery and bone augmentation before the placement of dental implants.

2. Materials and methods

2.1. TiO_2 nanoscale coating and surface characterization

Glass plates (17 × 17 mm square, 1.0 mm in thickness; Superfrost/Plus Microscope Slides, Fisher Scientific, Pittsburgh, PA) were coated with titanium dioxide (TiO_2; 99.99%, Kurt J. Lesker Co, Pittsburgh, PA) using a sputter-deposition system (Denton Discovery 550, Moores town, NJ) with a deposition rate of 18.5 Å/min,