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The effects of functionalized titanium with minTBP-1-IGF-1 for improving osteoblast activity

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ABSTRACT

Titanium possesses mechanical property and excellent biocompatibility but yet suffers from bioinertness and poor osteoblasts adhesion. In this work, minTBP-1 was employed to produce a modified IGF-1 endowed with directionality toward Ti surface. It demonstrated that the modified IGF-1 uniformly covered on titanium surface and the surface hydrophilicity was significantly increased. Osteoblasts exhibited increased cell adhesion and mineralization. Our results illustrate that minTBP-1-IGF-1 could produce significant enhancement in osteoblasts adherence, mineralization and maturation.

1. Introduction

Titanium (Ti) and its alloys are widely used in dental implants due to their good mechanical property, chemical stability, and biocompatibility [1]. Nevertheless, long osseointegration process of traditional dental implants still beset patients. Thus, it is essential for inserted dental implants to form a direct, rapid, strong and long-lasting bond between implant and bone [2]. Among various factors responsible for bone formation, titanium surface properties play a crucial role in osseointegration of implants [3]. In order to promote osseointegration of dental implants, titanium surfaces had been modified and optimized constantly. During the osseointegration process, cell growth factors such as Bone Morphogenetic Proteins (BMPs), Transforming Growth Factors (TGFs) and Insulin-like Growth Factors (IGFs) that are known as competence or progression factors regulate cellular behavior and provide continuity of cell cycle. It has been reported that synthetic surfaces coated with certain peptides such as IGF-1 prompted osteoblast adhesion and proliferation [4]. It is well known that IGF-1 is a mitogenic factor enhancing the growth of osteoblast and bone mesenchymal stem cells [5].

Recently, peptide aptamers (*i.e.* binders) that interact with inorganic materials have been artificially created and used as a "glue" to link the surface of inorganic metals to various bimolecular stuffs [6]. A new peptide aptamer, named TBP-1 (RKLPDAPGMHTW) that explored by a linear 12-per peptide displaying phage library, has been proved to have the characteristic of special interacting with the surface of Ti. Its N-terminal, RKLPDA (minTBP-1), is sufficient for Ti binding [7].

In this study, minTBP-1 was employed to produce a modified IGF-1 endowed with directionality toward Ti surface. We further explored the surface topography and wettability of all samples. The influence of minTBP-1-IGF-1 on the attachment of osteoblast cells to the surface of Ti and the mineralization activity of the osteoblast cells were also tested.

2. Materials and methods

The pure Ti (Cp Ti, TA2) was purchased from Northwest Institute for Nonferrous Metal Research (China). The samples were cut into 2 mm × 32 mm × 32 mm pieces and polished using silicon carbide sand paper with increasing fineness from 200 to 5000. The plates were then cleaned further by sonication in acetone followed by ethanol and Milli-Q water before use. The specimens were divided into two groups: the pure Ti group and the Ti/minTBP-1-IGF-1 group. All specimens were incubated for 30 min in 0.5 ml of 0.5% bovine serum albumin (BSA, Chon fraction V, Iwai chemicals, Tokyo) in TBS and then washed three times in binding buffer (5 M urea, 0.2 M Tris–HCl (pH 9.5), 0.1% Tween 20). For minTBP-1-IGF-1 protein coating, the modified IGF-1 protein were dissolved in binding buffer with a concentration of 1 μg/ml, after which 10 ml

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of the protein solution was placed onto each Ti plate and left for 1 h at ambient temperature. The Ti/minTBP-1-IGF-1 plates were then rinsed twice with binding buffer and three times with TBS.

Surface topography and roughness were observed by atomic force microscopy (AFM, Vecco Instrument Dimension, Icon). Measurements were carried out at scan size of 2 μm and at scan rate of 0.3 Hz (n = 3). In order to obtain further information of the surface wettability of the samples, the contact angles of deionized water droplet (0.5 μl) were measured using an automatic contact angle meter (KRUSS Gmbh, DSA10-Mk2) at room temperature. Three samples from each group were measured and two measurements were performed on each sample to evaluate the average contact angle.

The experimental designs and procedures were approved by the Animal Ethics Committee of School of Stomatology (Xi'an, Air Force Medical University). Osteoblastic cell was isolated from newborn rat calvaria (the Laboratory Animal Center of Air Force Medical University). The third to five generation of cells were used for the experiment. The osteoblasts were cultured in MEM supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 mg/L streptomycin (Invitrogen, USA). Cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C. The culture medium was changed every 3 days.

The osteoblasts were seeded onto the substrates at a density of 2.10×10^4 cells per sample using 6-well plates as the holders for cell adhesion. After the cells were incubated on each sample for 24 h, they were rinsed thrice with PBS, fixed with 4% paraformaldehyde (PFA) solution for 20 min at room temperature, stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma) for 10 min, and rinsed with PBS again. The number of adhered cells attached to the sample was calculated by the cell nuclei numbers. The cell nuclei numbers in 10 × visual field of each sample were counted by CLSM. The cell attachment rate (%) = (the number of adhered cells attached to the sample/the number of inoculated cells) × 100%.

The cells (2×10^5 cells/well) were seeded in 6-well plates onto the substrates. Mineralization of the cells was measured by staining the cells with 0.2% Alizarin red s (ARS) after 21 days of incubation. After microscopic observation, Alizarin red dye (Sigma) was extracted from cells using 10% acetylpyridinum chloride, and absorbance was measured at 570 nm.

The data were expressed as mean ± standard deviation. The statistical significance of differences was determined by one-way analysis of variance (ANOVA) using SPSS 16.0 statistical software. A P value less than 0.05 was considered to be statistically significant.

3. Results and discussion

AFM images displayed the morphological properties of both surfaces on a lower scale. The pure Ti surface showed a Ra value of 8.23 ± 0.85 nm, while the minTBP-1-IGF-1 coating involved a reduction of the roughness minTBP-1-IGF-1 displaying a Ra value of 3.18 ± 0.41 nm (Fig. 1a and b). The modified IGF-1 uniformly covered on titanium surface (Fig. 1d).

Surface wettability is a surface characteristic known to affect the biological response to the implant. Most studies have found that hydrophilic surfaces tend to enhance the early stages of osteoblast adhesion, proliferation, differentiation and bone mineralization compared to hydrophobic surfaces [8]. It has been proved that integrins expression can be regulated by surface wettability. Integrins are heterodimeric transmembrane receptors with noncovalently bound α and β subunits, and α5β1 mediates cell attachment and proliferation. It has been confirmed that the expression of α5β1 increases as the microtextured surfaces became more wettable [9]. As shown in Fig. 2a, the pure Ti surface was hydrophobic with a contact angle of 83.5°. However, the contact angle of Ti/minTBP-1-IGF-1 surface was 19.3° (Fig. 2b), implying that the surface hydrophilicity was significantly increased. The minTBP-1-IGF-1 might be conducive to promote the biological response of cells.

The initial cell adhesion behavior was visualized by DAPI staining (Fig. 3). As shown in Fig. 3a and b, after incubation for 24 h, the cells are distributed uniformly on the Ti surfaces and compared with the pure Ti group the Ti/minTBP-1-IGF-1 group had a larger cell density. The quantitative data in Fig. 3c showed that the cell attachment rate of both group had significant differences. The cell attachment rate of the Ti/minTBP-1-IGF-1 group was well above the pure Ti group, indicating minTBP-1-IGF-1 effectively promoted the adhesion of osteoblast cells. IGF-1 is known to stimulate the processes of osteoblast growth and differentiation [4]. Based on the above experimental result, minTBP-1-IGF-1 could play the role of local delivery of IGF-1.

ARS staining was performed after the osteoblast cells were treated with or without minTBP-1-IGF-1 for 21 days (Fig. 4). Under minTBP-1-IGF-1 conditions, there was extensive calcium nodule formation in the Ti/minTBP-1-IGF-1 group (Fig. 4b), indicating that the cells the Ti/minTBP-1-IGF-1 group displayed a higher mineralization level than those of the pure Ti group and osteoblasts were

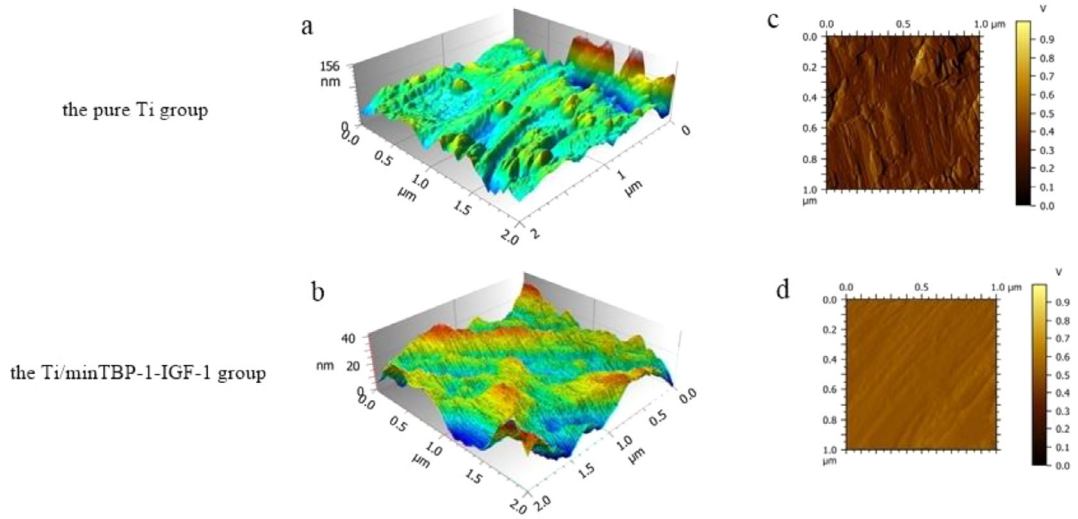

Fig. 1. AFM images of Ti surface: (a and c) the pure Ti group; (b and d) the Ti/minTBP-1-IGF-1 group.