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Effect of surface alkali-based treatment of titanium implants on ability to promote *in vitro* mineralization and *in vivo* bone formation

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ABSTRACT

This study investigated whether a novel alkali-based surface modification enhances *in vitro* mineralization as well as *in vivo* bone formation around titanium (Ti) implants in a femoral condyle model of 36 male Wister rats. All implant surfaces were grit-blasted and then received either acid-etching treatment, alkali-based treatment, or were left untreated (controls). Histological and histomorphometrical analyses were performed on retrieved specimens after 4 and 8 weeks of healing to assess peri-implant bone formation. Results of implants surface characterisation showed notable differences in the topography and composition of alkali-treated surfaces, reflecting the formation of submicron-structured alkali-titanate layer. In the *in vitro* test, alkali-treated Ti surfaces showed the ability to stimulate mineralization upon soaking in simulated body fluid (SBF). *In vivo* histomorphometrical analyses showed similar values for bone area (BA%) and bone-to-implant contact (BIC%) for all experimental groups after both 4- and 8-week implantation periods. In conclusion, the surface topography and composition of the grit-blasted Ti implants was significantly modified using alkali-based treatment. With respect to the present *in vivo* model, the biological performance of alkali-treated Ti implants is comparable to the commercially available, grit-blasted, acid-etched Ti implants.

Statement of Significance

Since success rate of dental implants might be challenged in bone of low density, an optimum implant surface characteristic is demanding. In this work, alkali treatment of Ti implants showed significant advantage of surface mineralization upon soaking in simulated body fluid. Using an *in vivo* rat model, Ti surfaces with either acid-etching treatment or alkali-based treatment evoked robust bone formation around Ti implants. Such information may be utilized for the advancement of biomaterials research for bone implants in future.

1. Introduction

Worldwide, the population is aging rapidly. As a result, many patients are becoming edentulous and need their missing teeth replaced by dental implants in order to restore occlusal function and esthetics [1,2]. Clinically, the use of endosseous titanium implants has been proven to be a reliable alternative to conventional removable prostheses and present high survival/success rates [3]. This concept is based on the process of osseointegration, which involves the establishment of an intimate contact between implant and bone without intervening layers of connective tissue [4]. Once an implant is placed in the bone, a cascade of biological events is initiated at the implant surface [4]. First, new bone formation takes place in the vicinity of implants and subsequently bone deposition onto the implants takes place through osteoconduction [4]. However, dental implant treatment desires rapid healing time for clinical loading and high success rates of osseointegration,

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especially in low density bone commonly seen in elderly patients with completely edentulous jaws [1–3].

Over the years, several modifications of dental implants design and geometry have been developed to enhance bone apposition and biomechanical fixation [5–7]. Specifically, implant modification by changing the physical and chemical surface roughness is appealing from a clinical and commercial perspective. Such surface modifications are expected to promote osteogenicity of titanium (Ti) implants and make the implant surface more attractive for early bone formation [8]. For instance, micro-rough titanium implants have been thoroughly investigated and dominate the market [8]. Further, a plethora of acid-etched implants have been introduced into clinical dental practice [9]. In addition, the acid etched surface was made more hydrophilic by chemical modification (e.g. the SLActive® implant), which was claimed to promote osseointegration [10].

Alternatively, alkali-based treatments have been introduced to modify the surface of Ti implants to achieve optimal surface bioactivity [11–13]. Alkali-treated Ti surfaces were shown to be able to stimulate the deposition of an apatite-like layer from simulated body fluid *in vitro* [13–15]. Comparison of alkali-treated Ti implants with acid-etched Ti implants indicated that the optimized alkali-treatment rendered Ti implants superhydrophilic (contact angle almost 0 degrees), resulting into a much more rapid and extensive surface mineralization compared to acid-etched Ti implants [12]. Additionally, the formation of a micron-sized surface porosity onto alkali-treated Ti surfaces was hypothesized to enhance the bioactivity of Ti implants *in vivo*. Moreover, alkali surface modification has been suggested as a useful method to allow immobilization of drugs and osteogenic biomolecules onto the implant surfaces [16]. Although alkali-treatment was shown to improve the bone-bonding capacity of Ti implants [17], this strategy has attracted less *in vivo* research interest and has rarely been directly compared to acid-etched implants.

The aim of the current study was to subject grit-blasted Ti implants to an additional alkali- or acid-based treatment to stimulate their *in vitro* mineralization capacity upon soaking in simulated body fluid (SBF). Subsequently, these implants were evaluated *in vivo* upon installation in the femoral condyles of healthy rats to test their capability to stimulate the formation of a new bone tissue onto the implant surfaces. It was hypothesized that besides surface bioactivity, alkali surface modification would generate more bone formation along the surface of Ti implants compared to conventional grit-blasted, acid-etched Ti implants.

2. Materials and methods

2.1. Implants material

Commercially pure titanium (cpTi-grade 2) implants were manufactured by Machinefabriek G Jansen (Valkenswaard, the Netherlands). For *in vitro* testing, 40 Ti disks with a diameter of 12 mm and a thickness of 1 mm thick were used. For the *in vivo* experiments, 72 root-shaped, threaded Ti implants were provided. The conical 'root' shape of implant body had the following dimensions: length of implant 6 mm, diameter at crestal side 2.8 mm, diameter at apex 2.5 mm, thread depth 0.5 mm, distance between threads 0.5 mm.

2.2. Surface treatments of Ti implants

First, all Ti implants (disks and root-shaped) were grit-blasted with alumina powder (250–500 μm, Korox®, BEGO) at a pressure of 0.5 MPa for 10 s (distance is ~25 mm). Following grit-blasting, Ti implants were ultrasonically washed in ultrapure water, acetone, and 2-propanol for 10 min, and dried in an oven at 40 °C. Then, Ti implant surfaces either received an acid-etching treatment, an alkali treatment, or were left grit-blasted (serving as control).

2.2.1. In vitro testing

For acid etching, Ti disks were incubated in a mixture of 7 ml sulfonic acid (95–97%, Merck, Darmstadt, Germany), 7 ml hydrochloric acid (37–38%, Schariau Chemise S.A., Barcelona, Spain), and 7 ml ultrapure water (MilliQ®; 18 MΩ.cm, Millipore) at 85 °C for 90 s. After acid-etching, Ti implants were ultrasonically washed in ultrapure water for 5 min twice, and dried in an oven at 40 °C. For alkali-treatment of grit-blasted titanium, Ti disks were treated with a 5 mol/L sodium hydroxide (NaOH) solution at 60 °C. The volume of NaOH solution was poured to 5 mL per implant disk. Treatment times in alkali solution were 1, 2, 3, 5 and 7 days. Occasionally, machined Ti disks without further surface treatment were used for comparison purposes in the *in vitro* test.

2.2.2. In vivo testing

For acid etching, root-shaped Ti implants were immersed in acid etching solution for 90 s (as described above), and then ultrasonically washed in ultrapure water for 5 min. For alkali-treatment, Ti implants were fixed to a tetrafluoroethylene holding device, and put in 65 mL of 5 mol/L NaOH solution (5 mL of NaOH solution per implant) for 24 h at 60 °C. Following surface treatment, all implants were gently washed in ultrapure water, and dried in a furnace at 40 °C for 24 h. Untreated root-shaped implants were left as only grit-blasted controls. Finally, implants were sterilized by gamma radiation (SynergyHealth, Ede, The Netherlands).

2.3. Implants surface characterization

2.3.1. Surface roughness

Average surface roughness values (Sa and Sdr) were determined for all Ti implant groups using a Universal Surface Tester (UST, Innowep, Würzburg, Germany). The (Sa) parameter represented the arithmetic mean of the roughness area from the mean plane. The (Sdr) parameter indicated the ratio between the developed surface area and a flat reference area.

To obtain a reliable surface characterization on screw-type implants, the thread tops, valleys, and flanks were evaluated as described previously [18]. Therefore, a mean value of roughness was obtained based on 9 measurements (3 thread tops, 3 thread valleys, and 3 thread flanks) onto surfaces of 3 implants per group. Ti implants were sputter-coated with a thin film of gold and observed under a field emission scanning electron microscope equipped with electron beam 3D surface roughness analyzer (FE-SEM: ERA-8900FE, Hachioji, Tokyo, Japan) at a voltage of 15 kV to assess the surface topography.

2.3.2. Surface composition

Surface composition was examined by an electron probe micro-analyzer (EPMA: JXA-8220, JEOL, Tokyo, Japan) and X-ray photoelectron spectroscope (XPS; Axis-Ultra, Kratos-Shimadzu, Kyoto, Japan). The EPMA measurement was performed at an accelerating voltage of 15 kV. All XPS spectra were obtained with a monochronized Al Kα line (1486.6 eV, voltage 15 kV, and current 10 mA). The binding energy was normalized to the hydrocarbon (C–H) in C 1s peak (285.0 eV) on each implant.