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Investigation of Effective Modification Treatments for Thin Titanium Membranes

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Abstract

Titanium membranes are used for guided bone regeneration in implant therapy. However, as a bioinert material, titanium does not have the ability to accelerate bone formation. Various titanium surface treatments to confer bioactivity have been demonstrated; however, there are concerns about the influence of chemical treatments on the corrosion of thin titanium membranes. This study investigated the influence of surface modifications on the structure of thin titanium membranes. Titanium membranes of 20 μm thickness were treated with acid or alkali solutions, and we evaluated their surface structure, wettability, corrosion depth, and mechanical strength compared to non-treated membranes. Alkali-treated thin titanium membranes displayed the formation of nanoscale pore structures on their surfaces, enhanced hydrophilicity, and less corrosion depth compared with acid-treated membranes. Furthermore, the tensile strength of alkali-treated membranes was comparable to non-treated membranes. These results suggest that alkali treatment is an appropriate surface modification method for thin titanium membranes.

1 Introduction

The existence of sufficient bone volume is a factor for successful treatment with dental implants [1]. In cases with insufficient bone volume at the implant placement site due to bone absorption or trauma, implant threads can be partially exposed when the implant is placed into the bone tissue, and in these cases, guided bone regeneration (GBR) is applied to augment the bone tissue [2, 3]. Barrier membranes play a crucial role in GBR, because epithelial tissues recover more quickly than bone, and can invade into the space required for new bone formation and inhibit the process [4]. Therefore, positioning the barrier membrane at the interface between the epithelium and periosteum retains the space required for bone healing.

Absorbent membranes, such an atelocollagen or polyglycolic acid, display excellent operability and do not require removal, and are used as barrier membranes in periodontal therapy. These absorbent membranes are suitable for small bone defects; however, their application to the grafting of large bone defects is problematic because of their insufficient mechanical strength [5, 6]. Non-absorbent membranes, such as those made from titanium, are superior in mechanical strength to absorbent membranes.

Titanium membranes are used as barrier membrane for GBR because of their superior biocompatibility, mechanical strength, and operability. Therefore various studies have demonstrated that they make and retain space well in grafts of large bone defects [7, 8, 9]. However, titanium does not have the ability to accelerate bone formation because it is bioinert [10, 11].

It is well known that titanium surface topography can be improved by various modification methods [12, 13]. Improved titanium surfaces have bioactive ability, and can promote cell adhesion and osteoinduction [14, 15, 16]. In particular, chemical methods, such as acid etching or alkali treatment, are often used because of their simplicity [17, 18, 19]. A titanium surface modified by a strong acid or alkali solution can form an apatite layer when soaked in body fluid [18, 21, 22]. For the reason, these modified treatments are already being applied as dental implants and titanium plates used in bone reconstruction. Therefore, the creation of bioactive thin titanium membranes would be beneficial for GBR.

Chemical treatments corrode the titanium surface, making it rough, extremely hydrophilic, and suitable for bone formation [23]. The corrosion depth is not a serious problem for solid titanium materials such as implant

fixtures. However, the effects of these treatments on the surfaces of thin titanium membranes are not well established, and they may influence the structure and mechanical strength of these membranes. The purpose of this study was to investigate the influence of surface modification treatments on the mechanical strength and structure of thin titanium membranes.

2 Results

2-1 SEM observations

SEM images of the surface structure of samples from each group are shown in Figure 1. The control group displayed a non-uniform, rough surface (Fig 1a). At high magnification, grooves and indentations were detected, but pore structures were not observed (Fig 1b). The acid group displayed a uniform, rough surface (Fig 1c), which contained microscale pore structures (Fig 1d). The alkali group had a similar structure to the control group, with grooves and indentations (Fig 1e). At high magnification, nanoscale pore structures were observed on the surface, and these structures formed a network (Fig 1f).

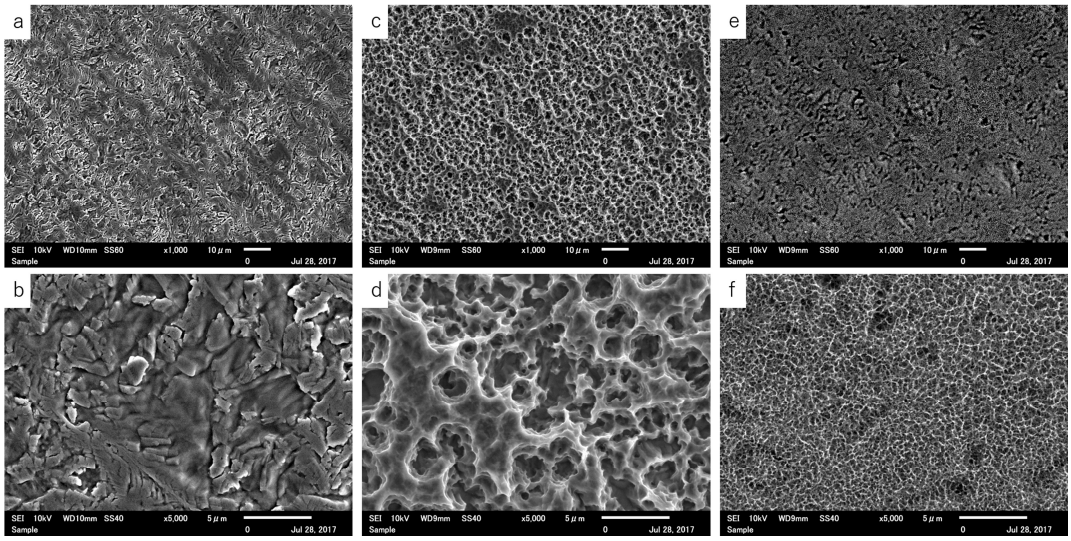

Fig.1. SEM images of each sample.

Control group: (a) ×1000 displayed a non-uniform, rough surface, (b)× 5000 had grooves and indentations, but no pore structures. Acid group: (c) ×1000 displayed a uniform, rough surface, (d) ×5000 contained microscale pore structures. Alkali group: (e) ×1000 had a similar structure to the control group, with grooves and indentations, (f) ×5000 had nanoscale pore structures.

2-2 Corrosion depth

Table 1 shows the thickness of each sample. The acid group displayed a significantly large corrosion depth compared to the other groups, while the degree of corrosion in the alkali group was slight and comparable to the control.

Table 1. Thickness

group	μm(SD)
control	20.0 ± 0.75
acid	15.1 ± 0.64*
alkali	19.7 ± 0.46

SD: standard deviation

P<0.001

2-3 Tensile strength

Table 2 shows the tensile strength of each sample. The tensile strength was significantly decreased by acid treatment. Conversely, there was no significant difference in strength between the alkali and control groups, indicating that alkali treatment did not weaken the membranes.

Table 2. Maximum tensile strength

group	N(SD)
control	309.5 ± 29.3
acid	158.4 ± 20.2*
alkali	295.0 ± 48.3

SD: standard deviation

P<0.001

2-4 Evaluation of wettability

Figure 2 shows the shapes of the water drops applied to each sample. The water drop on the control membrane had a semicircular shape and was slightly extended (Fig 2a). The drop on the acid-treated membrane had a round shape and was not extended (Fig 2b), while the drop of water on the alkaline membrane was extended dramatically, and is not visible in the

image in Fig 2c. Table 3 shows the contact angle of each sample. The angle in the alkali group was almost 0°, significantly lower than other groups, suggesting that the alkali membranes had much higher hydrophilicity.

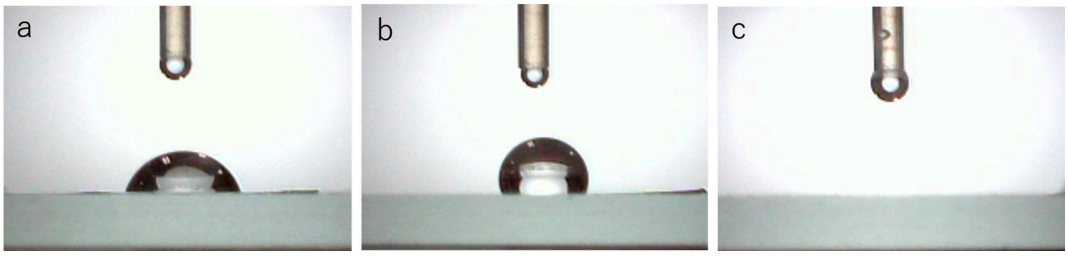

Figure 2 The shapes of the water drops applied to each sample.

(a) control (b) acid (c) alkali group.

Table 3. Contact angle

group	
control	71.1±1.63*
acid	103.1±3.81*
alkali	almost 0*

SD: standard deviation

*significant differences in each group respectively

P<0.001

3 Discussion

To regenerate large sections of bone, the GBR membrane needs to be malleable enough to easily conform to bone morphology, and have adequate mechanical strength to maintain its form until the new bone has formed. There is a correlation between mechanical strength and thickness; thicker

membranes have higher strength [24]. However, thick membranes are less flexible and formable, creating sharp edges when cutting, trimming, and bending them along the defect site. Thick membranes show less tissue adhesion, which permits penetration of soft tissue from the gap, preventing new bone formation. Thin membranes follow the bone morphology and do not create air pockets, which is advantageous for bone formation. However, with decreased mechanical strength, thin membranes can collapse into the defect cavity, decreasing the bone formation space and consequently, the volume of new bone formed. There are reports that membranes of 100 to 200 µm thickness are suitable for healing large-scale bone defects [25]. However, the lack of flexibility of membranes of this thickness gives them poor operability. An advantage of titanium membranes is that they maintain their mechanical strength even when thin. A thickness of 20 µm is most suitable for GBR treatment and accordingly, commercially available and clinically applied titanium membranes are 20 µm thick. Our experiments were conducted with membranes of this thickness as well. Thin titanium membranes are manufactured through the extension of a titanium metal mass by applying pressure through the gap between two rollers, until the

targeted thickness is achieved. Consequently, the surface topography of non-treated thin membranes had a roughened structure. In the study, we used acid and alkali treatments for surface modification. Titanium is corroded by strongly acid solutions such as H_2SO_4 and HCl [26]. The surface of acid treatment shown regularly rough surface with the micro scale pore structures. Although rough surface was created on titanium surface, irregular roughed surface and groves which observed in non-treatment were not detected. The aspect consider that corrosion by strong acid changed slightly smooth topography rather than irregularly roughed non-treatment membrane. Similarly aspect indicate the result of corrosion depth. Thickness and mechanical tensile strength of acid treatment was significantly decreased compare with non-treated membrane and alkali membrane. The SEM image was significant, according to the results of corrosion depth and tensile strength measurements.

Several studies have reported that acid treatment modifies the wettability of titanium surfaces [13, 23]. In wettability test, the contact angle of acid treatment was significantly higher than non-treated and alkali treatment. Currently, we have no clear explanation for this discrepancy. However, these

past studies were performed using finely polished, smoothly surfaced titanium disks as control samples. In our study, the thin membrane control had an irregular rough surface caused by the manufacturing process. Thus, acid treatment seems to be altered the thin titanium membrane from a rough surface to a smooth surface, acid membrane showed high contact angle. These results indicated that acid treatment changed slightly rough surface and reduced the mechanical strength for titanium thin membrane. Conversely, the surface of alkali-treated membranes displayed a regular, rough surface and uniformly dense nanoscale pore structures, consistent with previous reports [22, 27]. The alkali-treated membranes displayed enhanced hydrophilicity, which may be attributed to the nanoscale pore structure. Increased hydrophilicity promotes cell adhesion and nutrient supply, and is advantageous for bone regeneration [28]. Previous studies have compared alkali-and acid-treated implants, and found that implants treated with alkali displayed enhanced mineralization of the implant surface [29]. These results indicate that alkali treatment produces a hydrophilic topography with nanoscale pore network. Moreover, the influence of alkali treatment on the strength and thickness of thin titanium

membranes was only slight. Therefore, titanium shows corrosion resistance against alkali treatment, but not acid treatment.

Taken together, our results suggest that alkali treatment is an appropriate thin titanium membrane surface modification method for the development of bioactive titanium membranes.

4 Methods

4-1 Sample preparation

Thin titanium membranes were washed in an ultrasonic cleaner with acetone and distilled water for 60 min each, and dried in a 37°C oven. Then membranes were divided three groups; acid, alkali, and control. For the acid group, membranes were soaked in a 1:1 (w/w) solution of 66.3% H_2SO_4 (w/w) (NACALAI TESQUE, INC., Kyoto, Japan) and 10.6% HCl (w/w) (NACALAI TESQUE, INC., Kyoto, Japan) at 60°C for 60 min, with gentle shaking. The volume of the solution was 20 mL/membrane. After incubation, the membranes were washed with distilled water and dried in a 37°C oven. For the alkali group, membranes were soaked in 5N NaOH solution (NACALAI TESQUE, INC., Kyoto, Japan) and incubated as above for 24 h, then washed and dried. For the control group, membranes were washed with distilled

water and dried in a 37°C oven.

4-2 Scanning electron microscopy (SEM)

For surface structure assessment, a square sample from each group was attached to a sample stage with carbonate adhesive tape, and imaged by scanning electron microscope (SEM, JSM-6010PLUS/LA, Nihon Denshi Oyo Co. Ltd., Tokyo, Japan). Samples were evaluated by measuring the central thickness of the samples.

4-3 Corrosion depth

Each membrane thickness was measured by digital micro meter instrument (MDH-25M, Mitutoyo co. Ltd., Kanagawa, Japan). The degree of corrosion depth was compared with before and after treatment.

4-4 Tensile strength

Mechanical strength evaluation used a rectangular membrane from each group. Both the top and bottom sides of each membrane were fixed to the testing machine (AUTO GRAPH AGS-X, Shimadzu), and the samples were pulled at a constant speed (5 mm/min) until their breaking points were reached. The maximum tensile stress value was used to represent the

mechanical strength of the membrane.

4-5 Evaluation of wettability

First, each square membrane sample was divided into four 10 μm squares, which were fixed to the stage. Then, a 10-μL drop of pure water was gently applied to each sample. Ten seconds after the water and the membrane touched, an image was taken with an S-image device. Then, the contact angles of the dropped water were measured using ImageJ (National Institutes of Health, USA). These were obtained using a half-angle method, by measuring the angle of the straight line connecting the end point and the vertex of the droplet, and then doubling this value.

4-6 Statistical analyses

All data were analyzed at the 5% significance level using one-way analysis of variance followed by Tukey's test, and are expressed as the mean ± standard deviation (SD).

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Contributed reagents/materials/analysis tools: Kazuya Doi, Reiko Kobatake, Takayasu Kubo. Wrote the paper: Kazuya Doi, Reiko Kobatake, Kazuhiro Tsuga.

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