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Original paper

Application of multi-directionally forged high-strength titanium to dental implants in beagle dogs

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ABSTRACT

Pure titanium is widely used as a material in dental implants. However, it possesses inferior mechanical strength. This study aimed to elucidate the efficacy of acid treated multi-directionally forged (MDF) pure titanium in vivo. We verified the temporal changes until osseointegration in beagle dogs. Using two types of experimental materials (conventional pure titanium or MDF pure titanium), new bone formation was assessed using morphological examinations, and the bone-to-implant contact (BIC) value was evaluated at each time point (14, 30, and 90 d after the operation). As such, new bone formation was observed around the acid-etched MDF group, in which the BIC value was highest, followed by that in the acid- etched pure titanium group. MDF pure titanium implants showed early promotion of new boneformation compared to conventional titanium implants. The new acid-treated MDF made of pure titanium could be applied to humans in the future to prove its practicality.

INTRODUCTION

Dental implant fixture has been integrated into a titanium screw type with surface characteristics1). The main materials are divided into pure titanium and titanium alloy. Pure titanium is widely used in implants because of its excellent biocompatibility2). However, it is said to possess inferior mechanical strength, causing fractures3,4). Although the use of titanium alloy can improve the mechanical strength of implants because of the addition of alumina and vanadium, it is limited in its biocompatibility4-6).

In addition to the properties of biocompatibility and mechanical strength, the elastic modulus must be considered. The elastic modulus of cortical bone is approximately 10–30 GPa, which is considerably lower than those of pure titanium and titanium alloy (approximately 110 GPa)7). Studies have reported that this difference in elastic modulus can cause stress shielding, i.e., the nonuniform transmission of stress applied to the implant body8,9), which induces loosening and destruction of an implant body and bone resorption10-13). Therefore, for long-term stability of implants, they must be designed to have excellent biocompatibility, high mechanical strength, and low elastic modulus.

To increase its strength, the conventional grade-2 pure titanium was processed through multi-directionally forging, causing ultra-fine graining of the crystal structure, to achieve multi-directionally forged (MDF) pure titanium14,15). MDF processing applies theoretically infinite processing strain, ensuring a constant shape, by changing the casting direction by 90° for each forged path. MDF pure titanium is about twice as strong as grade-2 pure titanium in terms of mechanical strength and has the same or better physical characteristics as titanium alloys. Moreover, its elastic modulus is as low as about half that of other titanium alloys7). Owing to its improved mechanical properties and high biocompatibility over other materials, it is being studied for application to the framework of removable partial dentures and crowns16,17).

For the acquisition of early osseointegration, the implant surface is an important requirement1). The addition of roughness to an implant surface through mechanical polishing can promote the migration of osteoblasts18). In the past, an implant surface was coated with titanium plasma through spraying and hydroxyapatite coating; nowadays, smooth surfaces, such as those developed through acid etching and blast treatment, have become the standard19-22). Our previous study indicated that compared to the conventional method, the acid-etching method, using 67% sulfuric acid solution, can form nano-level pores with a regular arrangement 23). Furthermore, the use of this newly established acid treatment on pure titanium MDF is effective for early cell proliferation24).

This study aimed to elucidate the efficacy of MDF pure titanium in vivo. In order to apply MDF titanium to humans in the future, we verified the temporal changes until osseointegration through morphological method using beagle dogs. In addition, we adopted a newly established acid treatment method to MDF.

MATERIALS AND METHODS

Animals

Nine female beagle dogs (each weighing 9−10 kg and aged 12 months) were used as experimental animals. The study was designed in accordance with the guidelines of the US National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985), and our protocols have been approved by the Animal Care Committee of Kanagawa Dental University (Yokosuka, Japan, Approval No. 19-015).

Experimental material

Two types of experimental materials were used: conventional pure titanium (Aichi Steel Corp.,Japan) and MDF pure titanium (Kawamoto Heavy Industries., Japan).These materials were produced by cutting and mechanically polishing raw materials with a diameter of 4mm ×1000 mm. Four types of implant fixtures were used in the experiment: conventional pure titanium (machine-surface and acid-etched) as the control group, and MDF titanium (machine-surface and acid-etched) as the experimental group. Each type of implant at each experimental time point (14, 30, and 90 d after the operation) used three fixtures. The manufactured shape was cylinder with a diameter of 3.4 mm and a length of 8.0 mm. The manufacturing method of the implant is described in the following section.

MDF titanium

The MDF titanium is a grade-2 pure titanium that has undergone MDF processing (forging temperature: $T=300$ K, cumulative strain: $\Sigma\Delta\varepsilon=0.4-0.6$) and subsequent heat processing, which improves its mechanical properties without changing the chemical composition of the pure titanium[14,15]. The maximum tensile strength is 1004 MPa, the Vickers hardness (Hv) is ≥300, and the elastic modulus is 51 GPa[10].

Acid-etched method

First, 68% sulfuric acid solution was heated to 120°C, and then the implants were soaked in it for 75 s to be etched. After acid etching, the surfaces were washed twice with ultrapure water for 30 s and twice with methanol followed by blow-drying for 30 s. Acid etching was performed 7 days prior to implantation, while gas sterilization was performed 1 day prior[25].

Surgical procedure

Fig. 1 shows the operation procedure conducted in this study.All the animals were fit with the implants under general anesthesia, and were treated to minimize pain and discomfort in accordance with the guidelines of the Institutional Animal Ethics Committee Kanagawa Dental University. Atropine (0.04 mg/kg subcutaneous injection) was administered as a pre-anesthetic agent before general anesthesia. Anesthesia induced by the administration of propofol (6 mg/kg intravenous injection) was maintained using isoflurane. After confirming that the anesthesia was sufficiently effective, the experiment was conducted without causing pain or discomfort to the animals. This study used cylindrical titanium, the implant was placed directly after tooth extraction. The tooth at the planned implantation site in the lower jaw was extracted (P3, P4), the gingiva was detached with a full-layer flap, the bone was reshaped, and the implant was placed in line with the bone margin after drilling (Pilot drill: φ2.8 mm, Twist drill: φ2.8 mm, φ3.5 mm, Straumann, Switzerland) to a depth of 8 mm (left side: conventional titanium, right side: MDF titanium). Each-surface-treated implant was placed on one side, and four types of implants were placed in the same dog. Two implants (different-surface-treated same-titanium) were placed into the mesial root of P3 and P4 to avoid affecting bone formation around other implants. Because this study used cylindrical titanium, the implant was placed directly after tooth extraction. The implantation site was completely closed with absorbable sutures.

Morphological procedures

On the 14th, 30th, and 90th days after the operation, a cannula was inserted into both common carotid arteries and fixed with 0.2% heparin-Ringer's solution by blood removal perfusion (2.5% glutaraldehyde-PBS solution). After collecting the surrounding bone containing the implant fixture, it was fixed by immersion in a 10% formalin solution, dehydrated with an series of alcohol bath of increasing concentration, and embedded with synthetic resin (Technovit, manufactured by Kulzer).

After curing the resin with ultraviolet rays, a 30- µm-thick horizontal cross-ground abrasive-structure specimen was prepared with a microtome for polished specimens (Leica SP1600 Saw Microtome). The bone-to-implant contact (BIC) value for the polished tissue specimen in the central part of the implant was measured using a polarizing microscope (Olympus BX51). The measurement method involved capturing the image of a cross section with a polarizing microscope, analyzing the inverted image with ImageJ, and confirming the ratio of the gap (noncontact part) between the bone and implant interface to the circumference of the implant (Fig. 2). Statistical analysis was performed by one-way ANOVA (GraphPad Prism (v. 6.05); GraphPad Software Inc)22). The surrounding bone was observed using a polarizing microscope (Olympus BX51) after mesio-distal cutting of the jawbone including the implant body and removal of soft tissue with 3% sodium hypochlorite. The implant fixture was then carefully removed. After freeze-drying, bone formation at the implant–bone interface was observed using a scanning electron microscope (SEM; JEOL 600C +).

RESULTS

Morphological observation

Fig. 3 shows the morphological observations (left: sagittal section in stereomicroscope , right: horizontal section in light microscope) of each group at each time point. Fourteendays after surgery, marked bone formation was observed around the implant in the acid-etchedgroup compared with that in the machine-surface group. Thirty days after surgery, more bone formation was observed around the acid-etched MDF group; however, no apparent difference was observed in bone formation between the acid-etched pure titanium group and the machine-surface MDF group. Ninety days after surgery, newly formed bone was observed around all samples.

BIC value

The BIC value 14 days after surgery was the highest in the acid-etched MDF group, followed by the acid-etched pure titanium group. In addition, the machine-surface MDF group and the machine-surface pure titanium group showed significantly lower values than that of the acid-etched group (Fig. 4).

Thirty days after surgery, the BIC value was the highest in the acid-etched MDF group, and almost no difference was observed between the machine-surface MDF group and the acid-etched pure titanium group. The machine-surface pure titanium group showed significantly lower values than all other samples.

Ninety days after surgery, the BIC values of the acid-etched MDF, machine-surface MDF, acid-etched pure titanium, and machine-surface pure titanium were all higher than 90, with less difference between the two (acid-etched and machine-surface) groups.

Fluctuation of BIC value during the experimental period

Figure 5 shows the change in the BIC value over time. The MDF acid-etched group showed the highest value in all periods, whereas the machine-surface pure titanium group showed the lowest value. MDF titanium in the acid-etched state induced new bone formation at an early experimental stage.

SEM observation

The SEM image of the peri-implant bone is shown in Figure 6. On the 14th day, bone formation was observed densely around the acid-etched MDF group, whereas in the control group, immature new bone was observed to be developing around the implant. On day 30, new bone formation was confirmed around all implants. Acid-etched MDF titanium was confirmed to have denser bone formation than other samples. On the 90th day, dense bone formation was confirmed in all samples. Among them, the acid-etched MDF group was confirmed to have densely bone formation than that in other samples. In addition, the machine-surface MDF group was observed to have smoother and denser bone formation than that of the machine-surface pure titanium group.

DISCUSSION

In this study, we morphologically verified the surrounding bone formation when an MDF pure titanium implant was applied in vivo. Our findings indicate that MDF pure titanium implants exhibited relatively early promotion of new bone formation, compared to that in conventional pure titanium implants. Early acquisition of osseointegration is a very important factor in implant treatment as it allows for shorter unloading periods26). Shortening the unloading period has been reported to enable early loading and improve the QOL of patients27). Our findings showed in vivo data that confirms MDF pure titanium in the acid-etched state induces new bone formation earlier than pure titanium because the crystal structure of MDF pure titanium becomes ultrafine via giant strain processing.

Grain refinement is a method that can reinforce polycrystalline metal materials without the addition of alloying elements28). In recent years, research on zirconia implants has progressed owing to their superior biological, aesthetic, and mechanical properties29,30). Since a material exhibiting high strength has a high elastic modulus, stress is preferentially applied to the implant fixtures, and the disadvantage is that the resorption of the marginal bone occurs owing to stress shielding, which hinders normal stress transmission to the surrounding bone11-14). MDF pure titanium has both high tensile strength and low elastic modulus[15,28]. Our findings suggest that the MDF implant exhibits high stabilization at an early stage in vivo.Thus, MDF titanium is most ikely to be a suitable implant material as it is highly biocompatible and has a low elastic modulus, with mechanical properties comparable to those of titanium alloys.

Brånemark began the clinical application of implants, which involved processing with machine polishing, and it took a long time to obtain osseointegration. Next, titanium plasma-spray coating—coating plasma on the titanium surface—was realized, and research on rough surfaces became active[31]. It has been reported that a moderately rough surface (1.5 μm) has a higher BIC value than those of other rough implant surfaces[32]. In addition, the sand-blasted, large-grit, acid-etched surface, which was acid-etched after sand-blasting the titanium surface, could obtain earlier osseointegration than the titanium plasma-spray coated surface and machine surface[33]. The acid treatment method used in this study involved roughening the surface via sulfuric acid etching, and the effectiveness of surface treatment using concentrated acid etching and sulfuric acid etching, combined with UV irradiation, was verified[34,35]. Some studies have also demonstrated the effectiveness of surface treatment for new materials[36,37]. A previous study confirmed that the MDF pure titanium and acid treatment method can be used to induce smooth and dense osteoblasts earlier than in the case of pure titanium under the same conditions[23]. In this study, the MDF pure titanium surface had a fractal structure with fine and uniform pores after using the acid treatment method, suggesting that the proliferation of osteoblasts could be induced at an early stage. The three-dimensional fractal structure of MDF pure titanium exhibits a more complex microstructure in one pore, which is considered to have influenced hydrophilicity and osteoblast proliferation[23,24]. Cleaning with methanol during this acid treatment method is a common technique in the metal industry, and it is possible to clean the surface faster by utilizing its volatility[38]. This experiment also considered that the ultrafine surface structure was implanted in the bone without being contaminated, which effectively worked for cell proliferation. Thus, our study indicated using experimental animals, that both the MDF and newly established acid-etched methods are required for induction of bone formation at an early stage.

CONCLUSION

In summary, the effectiveness of the proposed method was demonstrated in vivo. MDF pure titanium, with an ultrafine grain structure, excellent mechanical properties, and a new acid treatment method that enables further titanium surface modification may benefit implant fixtures from both material and biological perspectives. Future work can now concentrate on the application of the proposed method to humans to examine the practical implementation and performance of the approach.

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Figure captions

Fig. 1 Surgical procedure

(A) Experimental animals were under general anesthesia before all experiments. (B) The tooth at the implant site was divided by a turbine. (C) The tooth was extracted, and gingival detachment with a full thickness-flap bone surgery was performed. (D) After drilling according to the conventional method, the implant was placed along the bone margin. (E) Completely closed layer with absorbent suture. (F) The wound closed without dehiscence.

Fig. 2 Polished section specimens and sagittal bone specimens

(A), (B) Horizontal polished section specimens with the central part of implant fixture and ImageJ inverted image. (C) Sagittal lyophilized specimen of jawbone and implant fixture. Scale bar : 500 μm.

Fig. 3 Morphological observation in each group

Morphological observation of each group 14 d (A-D), 30 d (E-H), and 90 d (I-L) after surgery. Machine surface (B, D, F, H, J, L), newly established acid treatment (A, C, E, G, I, K), pure titanium (C, D, G, H, K, L), and MDF titanium (A, B, E, F, I, J). On the 14th postoperative day, marked bone formation was observed around the implant in the acid-etched group, in contrast to the machine-surface group. Left side: sagittal section in stereomicroscope scope, right side: horizontal section in light microscope. Scale bar : 500 μm.

Fig. 4 BIC value in each group

(A) Comparison of BIC values of all samples 14 days after the implant. The acid-etched group show significantly higher values than that of the machine-surface group. (B) Comparison of BIC values of all samples 30 days after the implant. The acid-etched MDF shows the highest value. (C) Comparison of BIC values of all samples 90 days after the implant. All the samples show high values. *<0.05.

Fig. 5 Fluctuation in BIC value during the experimental period

Acid-etched MDF shows a higher value than those of other groups at each time point.

Fig. 6 SEM images in each group

Bone interface image of each group 14 days (A-D), 30 days (E-H), and 90 days (I-L) after surgery. Machine surface (B, D, F, H, J, L), newly established acid treatment (A, C, E, G, I, K), pure titanium (C, D, G, H, K, L), and MDF titanium (A, B, E, F, I, J). BI (bone interface), NFB (newly formed bone). Scale bar : 500 μm.

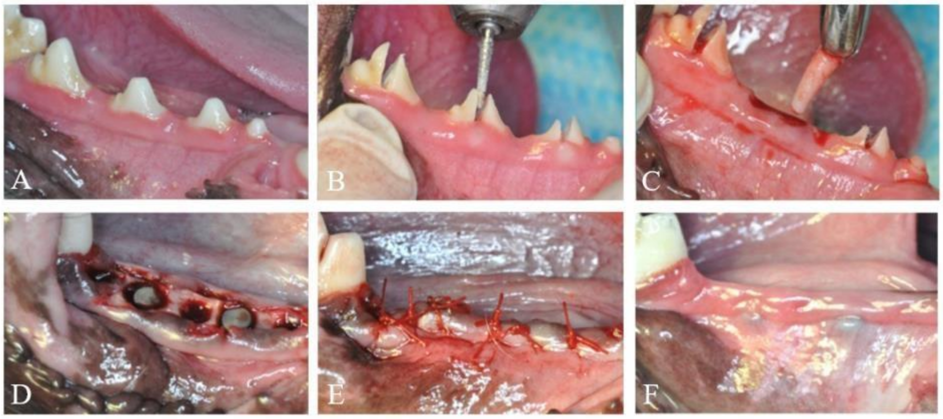

Fig. 1 Surgical procedure

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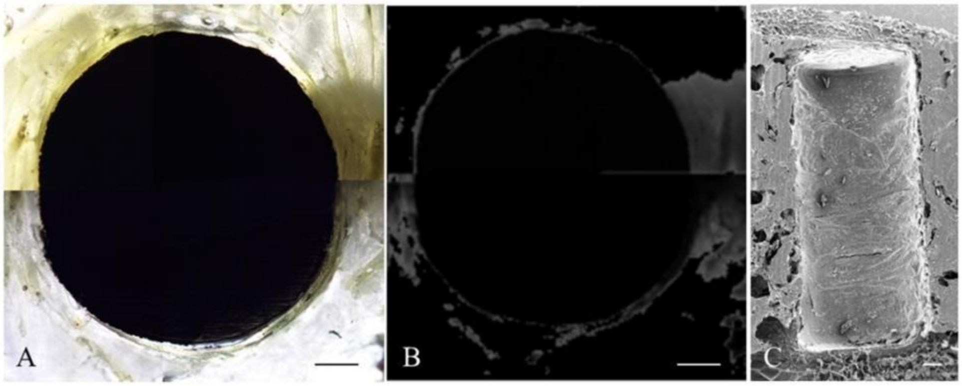

Fig. 2 Polished section specimens and sagittal bone specimens

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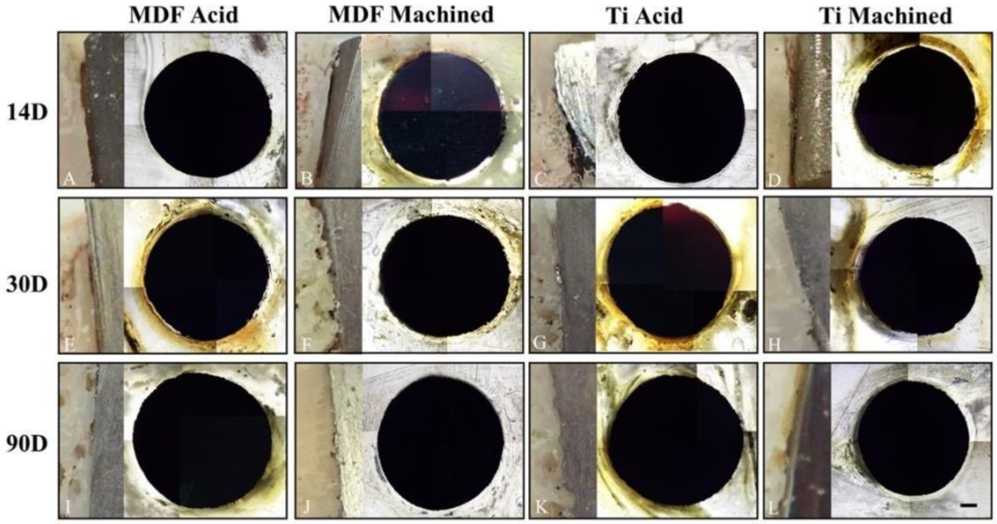

Fig. 3 Morphological observation in each group

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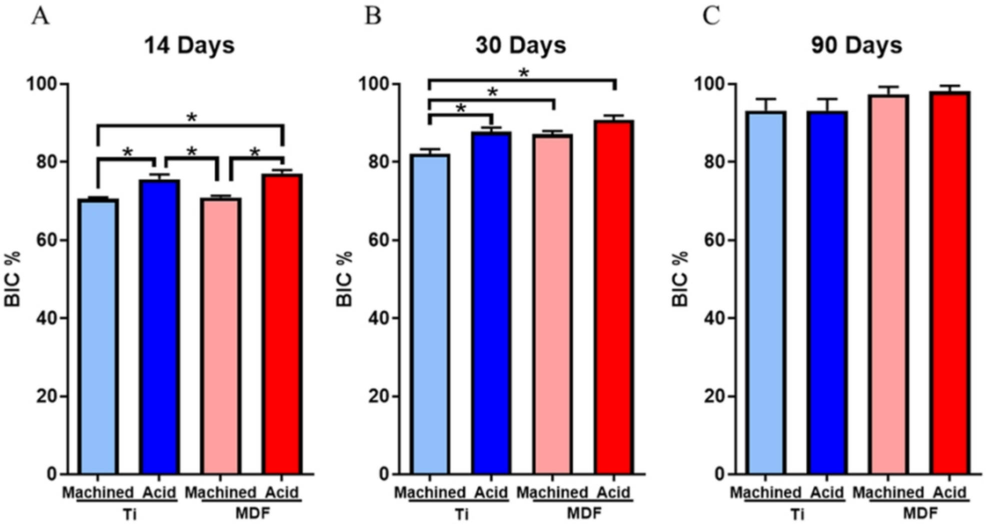

Fig. 4 BIC value in each group

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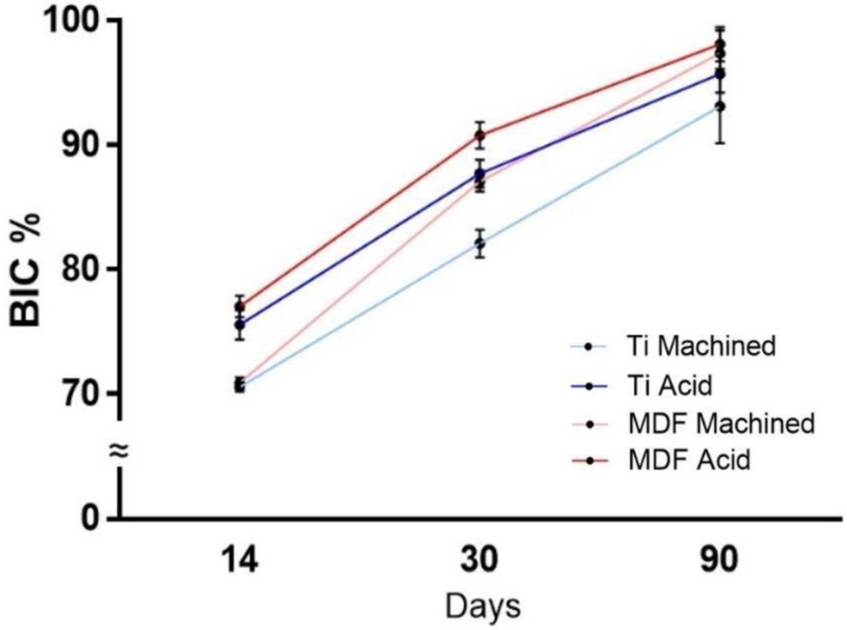

Fig. 5 Fluctuation in BIC value during the experimental period

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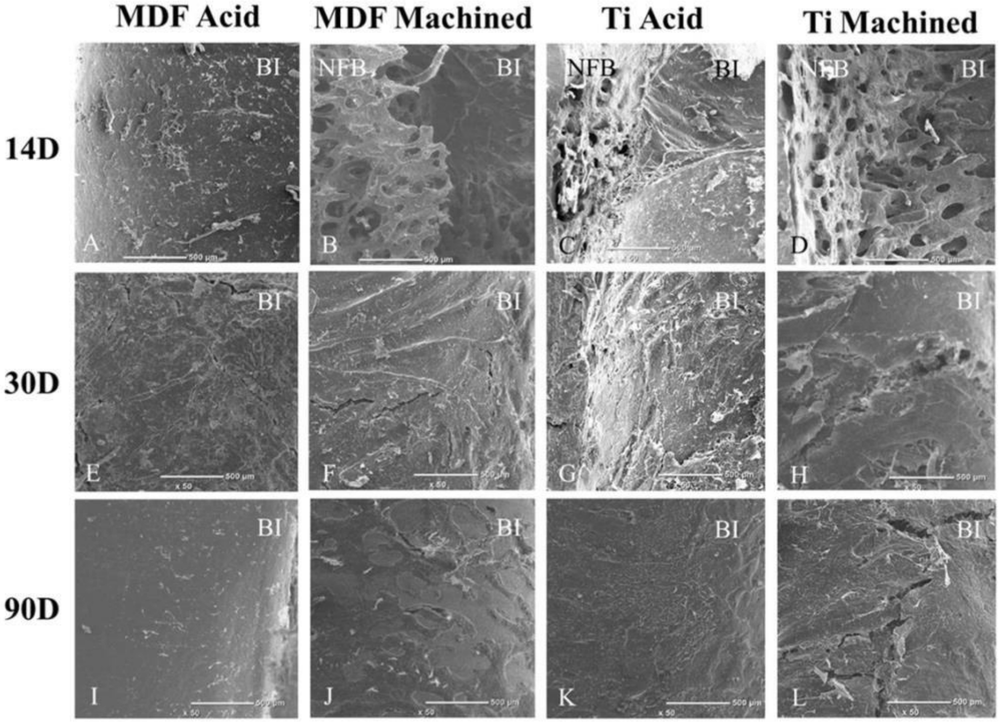

Fig. 6 SEM images in each group

220x159mm (300 x 300 DPI)