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In vivo behavior of surface-modified titanium implants after chemical processing at room temperature

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We evaluated new bone formation on the nano-modified surfaces of titanium plates implanted in the rat femur by comparing the ratio of bone-to-implant contact (%BIC). To create the nanostructure, titanium discs were incubated in a 10 M NaOH solution at 30°C for 24 h, and then washed and dried. Plates of titanium with nanostructures (the experimental group) and unprocessed titanium 1 mm thick and 4 mm in diameter (control group) were implanted into the femurs of the rats. After 14, 21 and 28 weeks, the %BIC for the control and experimental groups was 0.2% and 3.6% at 2 weeks, 18.2% and 24.7% at 3 weeks, 37.5% and 53.0% at 4 weeks respectively. These data suggest that modifying the nanostructure of the titanium surface, which is one of the main materials used in implants, allows induction of bone formation. (J Osaka Dent Univ 2014 ; 48(1) : 29–35)

Key words : Nano structure ; Implant ; Osseointegration

INTRODUCTION

There has been a concerted effort among materials scientists and clinicians worldwide to improve the performance of dental implants with the aim of accelerating and maintaining their integration into hard and soft tissues and/or extending their range of application.[1] The surface characteristics of implant materials affect the rate and extent of osseointegration.[2] Vandrovcova *et al.* have recently reviewed the growing evidence demonstrating that surface-modified materials are highly effective for adhesion, growth, and osteogenic differentiation of cells.[3] A recent advance in dental implant research is the modification of the surface of implant materials at the nanometer level.[3, 4] Techniques that provide an increased surface area and finer surface roughness may yield better tissue-titanium mechanical interlocking.[5] However, more importantly, such nanoscopic features are also believed to directly affect osteogenic cell behavior around implant fixtures possessing non-conventional surfaces, creating a biomimetic relationship between alloplastic surfaces and host tissues by mimicking the natural cellular environment at the nanometer level.[3, 4, 6]

The nanostructure used in this study is known as a titanium nanosheet (TNS), which is a kind of low-dimensional nanostructured oxide material and is similar to that of TiO_2 nanotubes (TNT) created by processing in a chemical solution of TiO_2 powders,[7] titanium deposition using the process of TiO_2 sputtering, and/or electrochemical oxidization processing of the titanium metal. Recently, it was shown that nanotube and TNS structures can be created by treatment of titanium surfaces with an aqueous solution of 10 M NaOH at 30°C. The surface properties and structures of the materials play important roles in the adsorption of proteins, which might influence cell behavior. A previous study reported that TNS produced via chemical processing promotes the osteogenic differentiation of rat bone marrow cells.[8]

In vitro experiments have shown that cell activity may be modulated by nanoscale structures.[9–12] Reproducing the nanotopography present in bone may improve early and long-term interactions between host bone and osseointegrated implants. Moreover, the biomolecules and cells involved in the early healing

phase after implant installation interact at the nanometer level. Recently, an in vivo study demonstrated enhanced early bone formation on nanostructures. The aim of the present study was to evaluate whether TNS structures enhance osseointegration compared with that on an unprocessed titanium surface in a rat model.

MATERIALS AND METHODS

TNS production

Table 1 shows the process of TNS production. Titanium disks 4 mm in diameter were punched from sheets of 1-mm-thick grade 2 unalloyed titanium (Daido Steel, Osaka, Japan). The disks were immersed in an aqueous solution of 10 M NaOH and then incubated in an oil bath at 30°C for 24 h. Unprocessed titanium disks were used as the control. The disks were then washed with distilled water until the wash solution reached a conductivity of 5 μS/cm. The disks were then dried at room temperature and the surface topography was qualitatively evaluated using a scanning electron microscope (S-4000 ; Shimadzu, Kyoto, Japan).

Experimental animals

Seven male Sprague-Dawley rats of approximately 9 week of age were used in this study (Shimizu Laboratory Supplies, Kyoto, Japan). During the experimental period, the animals were kept in an animal room maintained at a constant temperature of 24 ± 0.5°C and allowed free access to food (MF ; Oriental Yeast, Osaka, Japan) and tap water. The experiment was approved by the Osaka Dental University Animal Research Committee (approval number : 12-04006) and complied with the guidelines for animal experiments.

Table 1 The TNS deposition process

Titanium
↓
Treatment with 10 M NaOH at 30°C
For 24 hr
↓
Treatment with distilled water and measure of electrical conductivity of the solution (>5 μS/cm^3)
↓
Drying at room temperature
↓
TNS

Surgery

The rats were anesthetized by isoflurane inhalation, and then subjected to continuous intraperitoneal injection of pentobarbital sodium (Nembutal ; Dainippon Sumitomo Pharma, Osaka, Japan). The animals were fixed to the operating table, their skin was opened and the muscle exposed the left femur bone. A hole was drilled in the exposed bone using a dental turbine handpiece. Two titanium plates were placed vertically in the cavity and the plates were covered with the adjoining muscle tissue, which was sutured using absorbable suture and a 3–0 needle. The skin on the top was sutured with silk thread. The rats were euthanized 2, 3 and 4 weeks after implantation by an overdose of pentobarbital anesthetic.

Staining and histology

Formalin-fixed, paraffin-embedded sections were cut serially at 4 μm and stained with hematoxylin and eosin (HE). Titanium surfaces in the test and control groups were examined under an optical microscope equipped with a digital camera (BX 50 ; Olympus Optical, Tokyo, Japan). First, we observed the perimeter of the titanium plates. Direct contact was assumed when observation by optical microscope (Osteo Measure, Atlanta, GA, USA) revealed contact between the new bone without interposition of other tissue. We then calculated the ratio of bone-to-implant contact (%BIC).

RESULTS

Sample analysis by SEM

SEM analysis of the titanium surfaces after modification in NaOH at 30°C showed a network structure at the nanometer scale. Figure 1 shows SEM images of the relatively smooth surface features of the untreated titanium surface, whereas the chemically treated titanium surface of the test group displayed a nanometer-level fine network structure, as was observed previously by Pattanayak *et al.*[13]

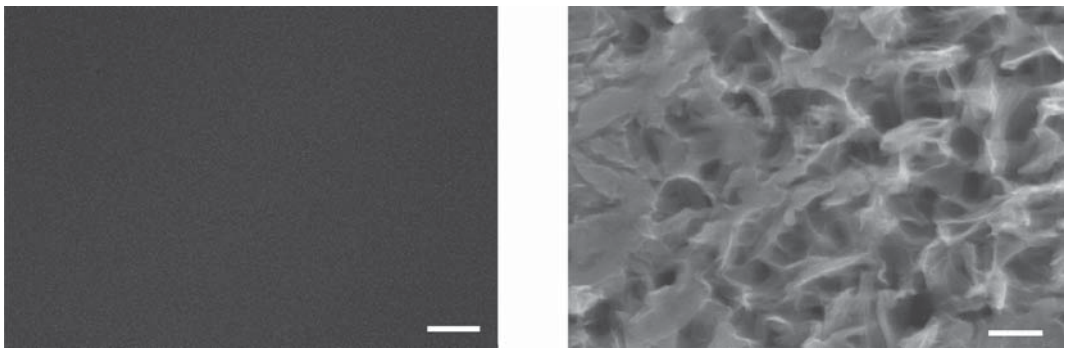

Fig. 1 SEM images of the control and TNS implant surfaces (Bar : 10 nm)

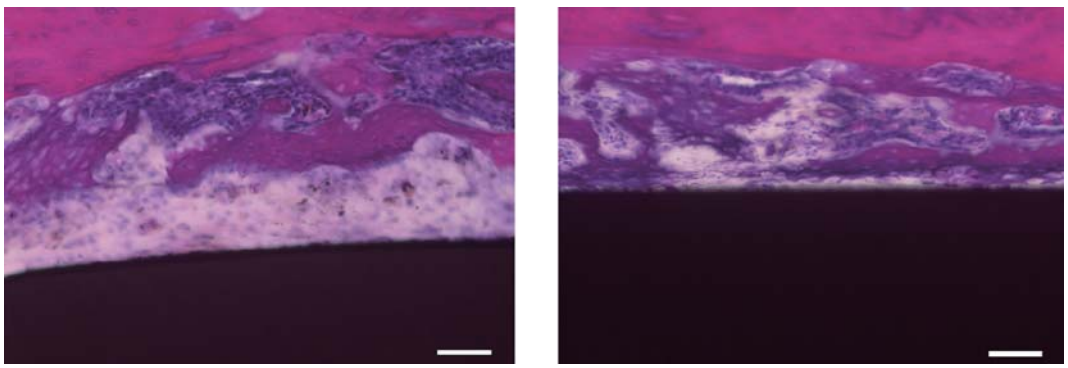

Fig. 2 Light microscopy of hematoxylin and eosin stained tissue sections of the control and TNS groups 2 weeks after surgery (Bar : 100 nm)

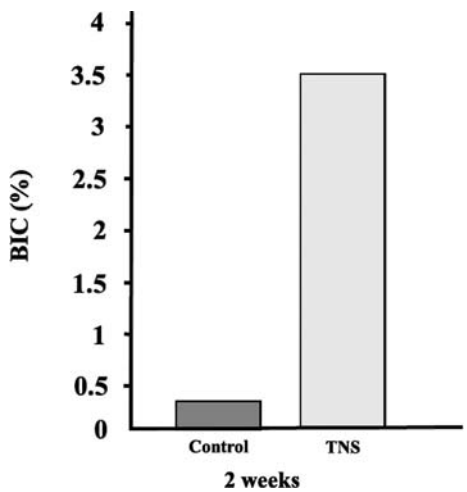

Fig. 3 The BIC ratio was hardly observed (0.2%) in the control group and 3.6% in the experimental group.

Bone-implant integration 2 weeks after surgery

Figure 2 shows the non-decalcified tissue specimens of HE staining after implantation in the rat femurs for 2 weeks. Granulation tissue between the bone and titanium plate was observed in both the experimental group and the controls. The ratio of bone contact with the titanium plate was extremely low (0.22%) in the controls, but 3.6% in the experimental group (Fig. 3). No immune response was observed to the titanium plates.

Bone-implant integration 3 weeks after surgery

Figure 4 shows non-decalcified tissue specimens stained with HE 3 weeks after surgery. In the test group, we observed new bone around the trabeculae and titanium plate. At the site in close contact with the titanium plate, we observed osteoblast formation and osteoid tissue. In the controls, fibrous connective tissue between the new bone and titanium plate showed almost no flat expansion of trabecular bone. The BIC

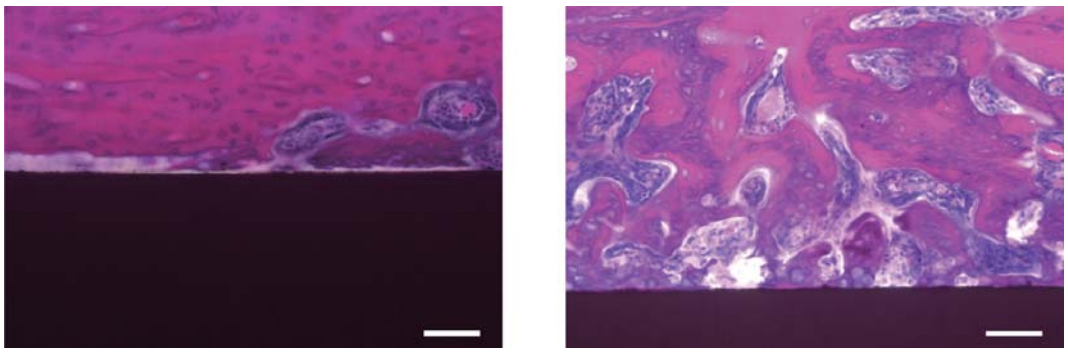

Fig. 4 Light microscopy of hematoxylin and eosin stained tissue of the control and TNS groups 3 Weeks after surgery (Bar : 100 nm)

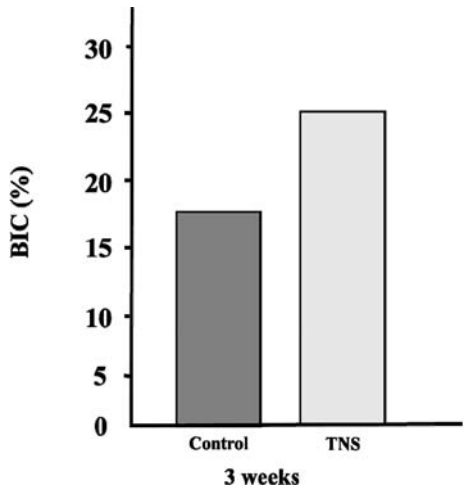

Fig. 5 The BIC ratios of the titanium plate and the new bone 2 weeks after implantation were 18.2% and 24.7% in the control and experimental groups, respectively, indicating more contact in the latter

ratios of the titanium plate and new bone 3 weeks after implantation were 18.2% and 24.7% in the controls and experimental group, respectively, indicating more bone contact in the latter (Fig. 5).

Bone-implant integration 4 weeks after surgery

The non-decalcified tissue specimens with HE staining at 4 weeks in Figs. 6 and 7 show the BIC. In the experimental group, woven new bone and small capillaries were apparent near the titanium plate, and a large number of osteoblasts appeared close to the contact surface. The calcification of new bone and formation of bone marrow were greater than in the specimens at 4 weeks. By contrast, there still existed visible fibrous tissues between the implant and new bone in the controls. Layer plate structures were seen on the outside around the new bone of the titanium plates.

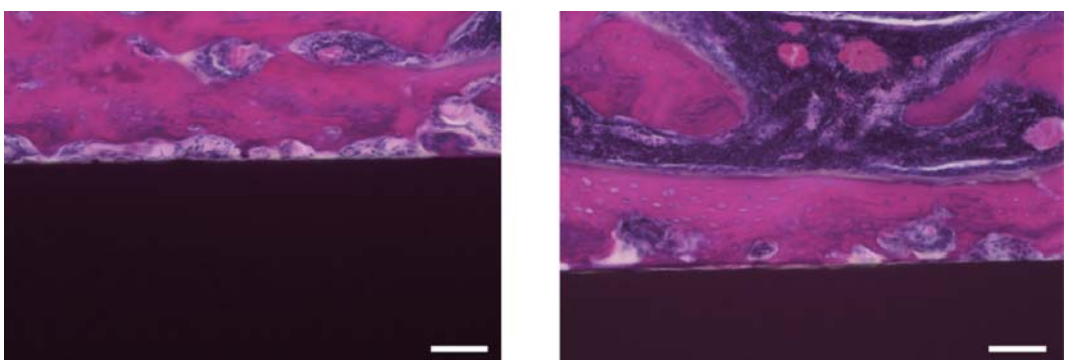

Fig. 6 Light microscopy of hematoxylin and eosin stained tissue sections of the control and TNS groups 4 weeks after surgery (Bar : 100 nm)

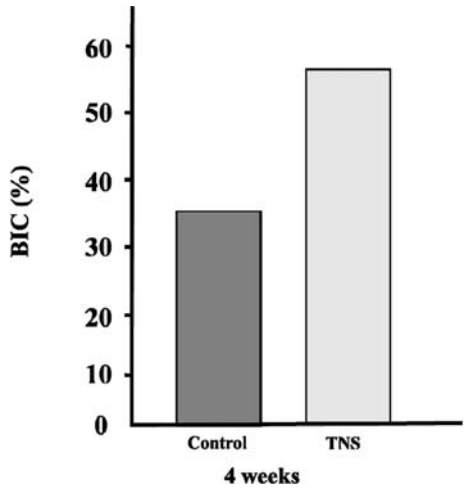

Fig. 7 The BIC tatio showed that bone contact was greaterin the experimental group (53.0%) than in the controls (37.5%)

The ratio of bone contact with the titanium plate (% BIC) was greater in the experimental group (53.0%) than in the controls (37.5%).

DISCUSSION

Osseointegration of dental implants is dependent on their surface characteristics, including both the surface topographical properties and chemical composition.[4, 13] Modification of the surface characteristics of an implant results in altered rat bone marrow cell responses, and can change the interaction of the implant with the surrounding hard tissue. Topographical properties of nanostructures on titanium surfaces play an important role in modulating cell responses at the implant-tissue interface, which substantially affects tissue integration of the implant.[14] Komasa *et al.* suggested that a TNS structure on the titanium surface can regulate osteogenic differentiation of bone marrow cells and enhance mineralization.[8] In this study, based on histological analyses and BIC ratios, the TNS-modified titanium surface increased the capability of in vivo bone implant integration compared with that of the unprocessed titanium surface.

A recent study showed that treatment with an aqueous solution of NaOH produces a rough nanoscopic surface.[15] SEM images of our test disks demonstrated that the TNS-modified surface had appropriate surface roughness without cracks. Svanborg *et al.* showed that although a surface may appear smooth at the micrometer scale, it could have considerable roughness at the nanometer scale.[16] Other studies have also shown that nanostructure surface modification causes significant differences in the surface appearance in SEM images,[5, 17, 18] including titanium discs modified with an alkaline solution.[19] These differences in surface nanostructures are known to modulate osteogenic differentiation and mineralization of titanium implant materials.[3, 4] The process of bone formation and remodeling around titanium implants occurs at different rates and intensities at different places around the implant. At a given point in time, the BIC ratio can be measured along the implant surface.

In the present study, some interesting differences were observed in the bone response between TNS-modified and unprocessed titanium surfaces. A low degree of bone-to-metal contact was observed in both groups after 2 weeks. After 3 and 4 weeks, the TNS-modified titanium implants showed higher BIC ratios than those of the unprocessed titanium implants. The different bone responses after 3 and 4 weeks were caused by differences in the surface properties of the implants. The TNS structure has a deep oxide layer, surface topography, and surface roughness. Most studies of the surface properties in the bone response to titanium have been concerned with the surface topography.

The surface roughness of titanium for osteoblast attachment in vitro and bone responses in vivo have recently been studied by other groups.[20–23] Previous studies also have shown that TNS processing increases the attachment of rat bone marrow cells and endothelial cells.[8] These findings have been corroborated by in vivo studies showing a clear tendency for increased bone formation around implant surfaces that have increased roughness.[24, 25] Differences in the surface roughness at the nanoscale level may influence the bone around titanium implants. Our results also strongly indicate that the nanometer-level surface structure is important. Moreover, the oxide titanate layer of the TNS structure might play an important role in the tissue response to titanium implants. The influence of the monolayer on surface contamination

and biological responses has not been studied systematically. However, because the submonolayer absorbs hydrocarbons that are known to dramatically influence the chemical properties of surfaces, such as their wetting behavior, it is also likely that the bone interaction with nanomolecules will be influenced. The effect of surface contamination on implant surfaces has been discussed in more detail elsewhere.[26]

The results in the present study suggest that ingrowth of bone is the dominant factor affecting shear strength between the implant and bone. Direct bonding between the bone and implant is useful for implant fixation. The TNS structure created by chemical processing at room temperature has been shown to enhance early implant fixation by ingrowth of the bone into pores, which is considered important in the early stage of osseointegration between the implant surface and bone. Thus, the TNS structure improves the implant success rate. This can be attributed to changes in the surface nanotopography and the chemical composition following the alkaline modification. The modification method used here is convenient because the incubation in NaOH is at room temperature and requires no template.[27]

CONCLUSION

These data suggest that the surface modification of TNS induces bone formation in vivo. We concluded that further development of advanced implant materials using nanotechnology will improve osseointegration.

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