The effect of super-hydrophilic 8nm TiO_2 Nanotube surfaced implant on the osseointegration in Mongrel dog

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Seong Ho Choi

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This certifies that the Masters
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December 2012

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지도 교수님이신 문 홍석 교수님께 오랫동안 가까이에서 지켜 봐 주시고 많은 지도 편달 주심 감사 드리며 앞으로도 건강한 모습으로 항상 가르침을 주십시오.

오랜 기간 동안 논문에 관해서만 아니라 같이 하는 삶에 대해서도 이야기 해주며 물심 양면으로 지도해 주신 박 영범 교수님께도 감사 드립니다.

저를 이끌어 주시고 지도 해주신 보철학교실 이 호용 학장님, 정 문규 학장님, 한 동후 교수님, 이 근우 학장님, 심 준성 과장님, 이 재훈 교수님, 김 지환 교수님께 감사 드립니다.

많은 변화가 있었고 십년 전 그 때의 저와 지금의 저 모두를 사랑해주시고 항상 든든히 지켜 봐주시는 양가 부모님, 사랑하는 아이들 최 가영, 최 주형 그리고 세상에서 가장 사랑하는 제 처 한서윤에게 감사와 사랑을 전합니다.

2013년 2월

최 성 호 드림

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ABSTRACT

The effect of super-hydrophilic 8 nm TiO_2 nanotube surfaced implant on the osseointegration in mongrel dog

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In modern biomaterial and implant research, the studies on the nanostructure of TiO_2 nanotube are actively being conducted due to its great surface area and good biological applicability compared to the microstructure. Because dental researchs now focus on new generation implant surface with better bioactivity and hydrophilicity, TiO_2 nanotube is one of the influential candidates for next generation.

In 2011, Brammer et al released a research result on the formation of 8 nm TiO_2 nanotube through a new hydrothermal method different from anodization. According to the study, the created 8 nm TiO_2 nanotube exhibited an excellent bioactivity with strong adhesive property and active cell function because of its super-hydrophilic nature. There are few studies about undersize 10 nm nanotube and these 8 nm TiO_2 nanotube study was done in vitro study only, so it is important to carry out in vivo study.

This study aimed to evaluate the impact of the surface of 8 nm TiO_2 nanotube that is created through the new method on osseointegration.

20 unit 8 nm TiO_2 nanotubes formed with a hydrothermal method and RBM (resorbable blasting media) implant which is widely, clinically used, was used as a control group. Under the same environment, animal tests were conducted, using 5 mongrel dogs.

In 8 nm TiO_2 nanotube, the removal torque value was found higher in the 12^{th} weeks than in the 4^{th} weeks. Bone-to-implant contact value in 8 nm TiO_2 nanotube appeared to be higher than that in the control group both in the 4^{th} and 12^{th} week experiments. TiO_2 nanotube showed higher bone volume than the control group both in the 4^{th} and 12^{th} week. In total bone volume, there was statistically significant difference in both groups, p-value was 0.0122. ($p<0.05$)

Therefore, further study is required and super-hydrophilic nature and biomimetric structure should be considered very importantly in 8 nm TiO_2 nanotube research, and 8 nm TiO_2 nanotube is the promising candidate for new generation of implant surface.

Key words: 8 nm titania nanotube, Hydrothermal treatment, Super-hydrophilic, Biomimetric structure

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Seong Ho Choi

Ⅰ. Introduction

Osseointegration refers to a formation of bone to implant interface basically as a foreign body reaction by calcium and phosphorus ion derived from bone matrix in the porous structure in the TiO_2 (titania) layer on the surface of the implant.[1] While in early days, smooth surface titanium required sufficient time for bone osseointegration to happen, many studies have been conducted to reduce the osseointegration time by changing oxide layer on the surface of the implant and secure stable osseointegration between implant and bone.

Schwartz mentioned that osseointegration has connection with layer interface of implant or surface roughness, surface energy, surface composition and topography and it plays an important role in cell adhesion, proliferation and protein adsorption in the early healing process after implant is placed.[1, 2, 3]

In Buser et al's surface roughness study, the rough surface shows faster and stronger osseointegration.[1, 4, 5] This is because it is advantageous for early fixation and affects adhesion and differentiation of osteoblast. Therefore, a commercialized dental implant has been modified in attempt to secure more space for osseointegration by adding micro roughness as well as the macro rough surface.

Currently, most clinically, world-widely commercialized dental implants have physically rough surface compared to the machined smooth surface of early-day implants and there are differences in surface treatment. In addition, there have been many studies on the rough surface of commercialized dental implant and surface treatment. Based on these studies, most of implant products in current markets have 1~2 μm micro-roughness and brought higher success rate and quicker treatment for the patients.

However these implants have critical limitation in difficult cases where additional bone graft, guided bone regeneration (GBR) are required due to poor bone quality around implant. So many dental researchs now focus on new generation implant surface with better bioactivity and hydrophilicity which can induce fast and stable osseointegration.

Recently, new implant surface with 1~100 nm nanometer roughness is developed, finer than micro roughness. Nano roughness has something to do with protein adhesion and osteoblast cohesion, it is reported it plays an important role in the process of osseointegration. In biomaterial research with nano structure, most popular part is TiO_2 nanotube.

TiO_2 nanotube can be easily created on the surface of implant material and its advantage is that millions of nanometer-wide and 10 nm~ 100 μm length nano tubes can be manufactured depending on manufacturing conditions.

There are many studies concerning the nano structure of TiO_2 nanotube because it has a higher surface area and more excellent biological applicability than the microstructure of the commercialized implant.[6-16] Oh et al stated that cells are greatly affected by their surrounding structures and many studies showed that such cells make a positive contribution to cell adhesion, proliferation and function development in the nanostructure.[17] In addition, Brammer et al revealed in their recently published research findings that various kinds of cells cultivated in the nanostructure are different from the existing types.[18, 19]

A study on interaction between cells and nanostructure is one of the most highly recognized biomaterial research areas. It is to study movements and evolution of new cells different from interaction between cells and surface shown in the existing macro- and micro- structures by manufacturing a nano-size structure that has a standardized structure almost the same as or smaller than the cell on the surface of biomaterials. Oh et al stated that when osteoblast was cultivated on the 100 nm diameter and 300 nm long nanotube, osteoblast was easily attached, cultivated, resulting in accelerated cell differentiation, bone formation, and calcification.[20, 21]

Generally, there are many ways to manufacture TiO_2 nanotube such as sol-gel, vacuum evaporation, and anodization. Among them anodization is mostly employed to create TiO_2 nanotube on the surface of titanium and electrolyte solution containing fluoride is also used. [22- 26]

In 2011, Brammer et al released a research result on the formation of 8 nm TiO_2 nanotube through a new hydrothermal method, different from anodization.[27] According to this study, the created 8 nm TiO_2 nanotube exhibited an excellent bioactivity with strong adhesive property and active cell function because of its super-hydrophilic nature.

Interestingly, unlike anodized TiO_2 nanotube, these 8 nm TiO_2 nanotube exhibits mostly multiwalled configuration with 2-6 parallel walls along the length of the elongated nanotubes. Brammer et al stated that these multiwalled nanotubes significantly enhanced osteoblast cell response and nanotube structure provided significantly up-regulated bone forming ability with 2-3 fold alkaline phosphatase level and imitated the extra cellular matrix(ECM) environment of collagen type I fibers having fibrous and tangled nanoscale geometry.[27]

There are few studies about under 10 nm nanotube and these 8 nm TiO_2 nanotube studies were done in vitro study only, this 8 nm TiO_2 nanotube can be a good candidate for next generation implant surface, so it is important to carry out in vivo study.

This study aimed to evaluate the impact of the surface of 8 nm TiO_2 nanotube that has super-hydrophilic property and biomimetric structure on ossification and osseointegration by measuring removal torque, bone to implant contact and bone volume through animal model studies using mongrel dogs under the same environmental conditions.

Ⅱ. Materials and Methods

2.1 Preparation of 8 nm TiO_2 nanotube via hydrothermal process

For the study, 20 implants with 3.5 mm diameter and 8.5 mm length were manufactured, and 20 same size RBM implant (GS II®, Osstem^{kr}, Korea) which is clinically, widely used are studied as the control group.

Briefly noted to fabricate the TiO_2 nanotube, 20 implants were immersed into 10M aqueous NaOH solution, heated at 120°C for 30 minutes in a PTFE (polytetrafluoroethylene) lined autoclave, and subsequently washed with 0.1M HNO_3 aqueous solution and pure water. Then the samples were annealed in air at 500°C for 1 hour. (Fig. 1)

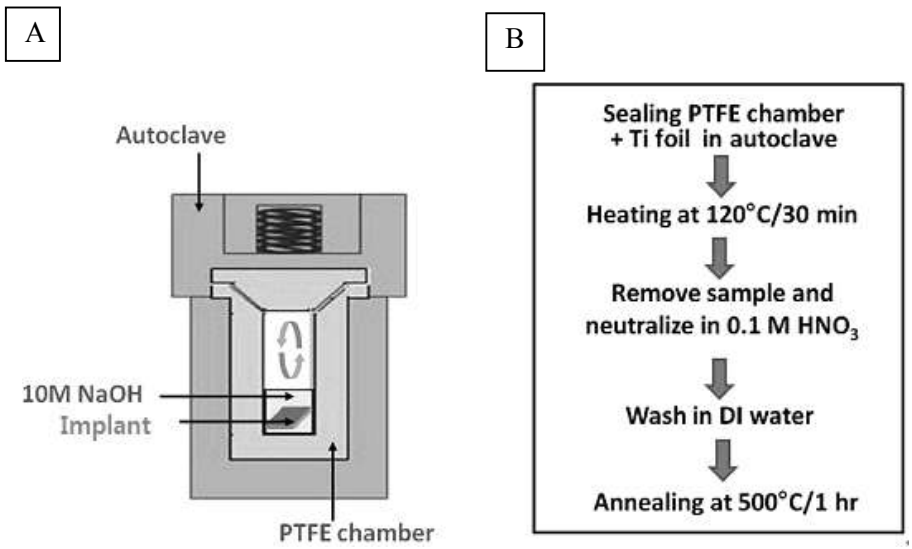

Fig. 1. A; Schematic illustration for hydrothermal processing.
B; Experimental flow chart to form the 8nm diameter TiO_2 nanotube surface.
Reference from Karla S. Brammer Materlals Science & Engineering, University of California, Sandiego, USA.

All the 8 nm TiO_2 nanotube implants formed were validated using scanning electron microscope (SEM XL30, FEI Co, USA) and transmission electron microscope (TEM Tecnai sphere 200 KV). (Fig. 2)

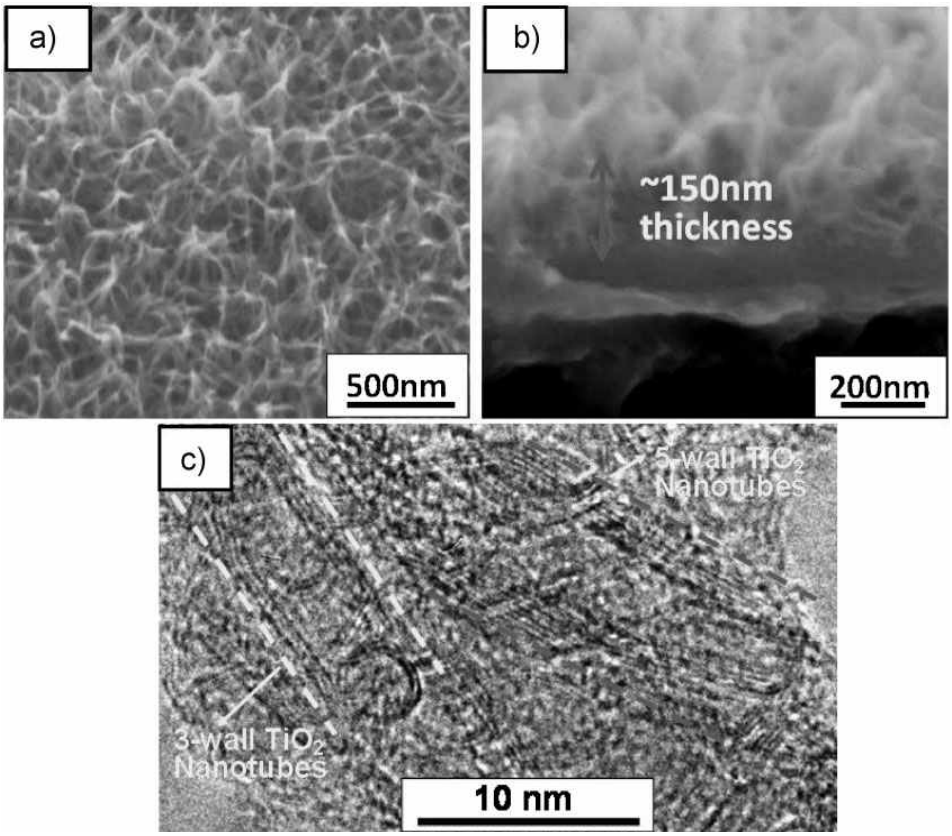

Fig. 2. Scanning electron microscopy (SEM) images showing a) top view and b) cross-sectional view of the TiO_2 nanotube layer having 150 nm layer thickness.
c) Transmission electron microscopy (TEM) image illustrating the multiwall nature of the 8nm diameter TiO_2 nanotubes. Part of the nanotube lengths are indicated by dotted lines.
Reference from Karla S. Brammer Materlals Science & Engineering, University of California, Sandiego, USA.

2.2 Animal test model

5 mongrel dogs aged between 18 and 24 months, weighting more or less than 30kg were used in the animal test.

Each subject was kept in the cage in animal testing lab. Commercial pet food was given to them and they were given freedom to drink water as they wished. The entire experiment was conducted in accordance with the animal test guidelines presented by Department of Laboratory Animal Medicine of Yonsei University College of Medicine.

2.3 Animal testing procedure

Dogs were kept away from eating for 24 hours before the surgery but free to drink water. All surgical operations were conducted under the general anesthesia. Two hours before the surgery, antibiotics, cefazolin sodium(Cefazolin®, Chongkundang pharm. Co., Seoul, Korea) 22 mg/kg was injected intravenously and 15 minutes before the anesthesia, sulfate (Atropine®, Dai Han Pharm, Co., Seoul, Korea) 0.1 mg/kg was injected into the dogs' vein as premedicant. By injecting Xylazine HCI (Rompun®, Bayer Korea, Seoul, Korea) 0.5 mg/kg and ketamine HCI(Ketalar®Yuhan Co., Seoul, Korea) 5 mg/kg intravenously, the dogs were put into anesthesia and during the operation, the inhalation amount of anesthetics, Gerolan (Choongwae Pharmaceutical Co., Seoul, Korea) was adjusted and the anesthesia was maintained by 100% oxygen with 2% Isoflurane.

Using a veterinary monitoring device (Omicare M1205A, Hewlett Packerd, Palo Alto, CA, USA), ECG, oxygen saturation, and body temperatures were constantly monitored throughout the operation process. For tooth extraction and implant operation, topical anesthesia was additionally conducted using Lidocaine HCI 2% containing epinephrine

(1:80.000 epinephrines, lidocaine HCI, Yuhan Co., Seoul, Korea). For tooth extraction, two premolars around P3 area on the left and right sides of both upper and lower jaws were extracted and 12 weeks of healing period were given to the subjects.

For implant placement, after an incision was conducted on the gingiva, full thickness flap was lifted and the bones were progressively removed under the influence of saline solution. Then, 3.5 mm diameter and 8.5 mm length implants were placed.

After implantion, the flaps were sutured using Monosyn 4/0(Glyconate monofilament absorbable, B-Braun, Aesculap, PA, USA). After the operation, to prevent infection of the surgery wounds, antibiotics (Ampicillin, Jong-geun Dang Pharmaceutical Company, Seoul, Korea: 500 mg/day iv) were intravenously injected and anti-inflammatory drug (Ketopro®, Unibiotech Co., Seoul, Korea) 3 mg/kg was injected for three days. The mouths of the dogs were irrigated every day with 0.2 Chlorhexidine(Chlorhexamed, Bu-Kwang Pharmaceutical Compaceutical Company, Seoul, Korea) for one week and the sutures were removed two weeks later.

In the 4^{th} and 12^{th} weeks, ketamine HCI(Ketalar®Yuhan Co., Seoul, Korea) 5 mg/kg was intravenously injected for stabilization and then, KCI solution was intravenously injected to sacrifice the test animals. The lower jaws were cut, immersed and fixed in 10% neutral formalin.

2.4 Removal torque value measurement (Ncm)

Immediately after sacrificing the subject animals, removal torque value of 20 implants were measured in their upper jaws. Each 5 unit 8 nm TiO_2 nanotube implants in 4^{th} week and 12^{th} week were measured and each 5 unit RBM control implants as well.

2.5 Samples making

After fixing the samples containing implants in the buffered neutral formalin solution for two weeks, dehydration was conducted by increasing the alcohol concentration starting from 70% and moving onto 80%, 90% to 100%.

After embedding using Technovit 7200 (Heraeus KULZER, Dormagen, Germany) and alcohol (1:3, 1:1, and 3:1 ratio), with the hardening completed, tissue slides were made utilizing the cutting system (Exakt 300, Kulzer, Norderstedt, Germany) with buccolingual plane.

For the central section of each sample, the tissue was made 400 ㎛ thick and micro-grinding was conducted with a grinding machine (EXAKT 400CS, EXAKT Apparatebau, Norderstedt, Germany) to make it 15 ㎛ thick. The samples were made according to the method used by Donath and Breuner and dyed with H&E (Hematoxylin & Eosin) for histological observation. (Fig. 3)

2.6 Histologic and histomorphometric analysis of samples

The dyed samples were photographed with magnifications of 12.5, 50 and 100 times, using an optical microscope (Leisa DM 2500, Leisa Microsystems, Germany) to observe osseointegration in the 4^{th} and 12^{th} weeks. (Fig. 3) For evaluation of each sample, the photography was taken around the entire implant with a magnification of 50 times and BIC (bone-to-implant contact ratio) was measured using Image Pro plus 4.5 (Media Cybernetics Inc., Silver Springs, MD, USA). Total bone volume from the lower jaw bone sample, the bone volume around the micro-thread, and the bone volume around the macro-thread were measured.

2.7 Statistical analysis

The mean values and standard deviations of the data were calculated.

One-way analysis of variance (ANOVA) in SPSS 18.0 (SPSS Inc., Chicago, IL, USA) program was used to compare the mean values of the data between the 8nm TiO_2 nanotube group and RBM control group.

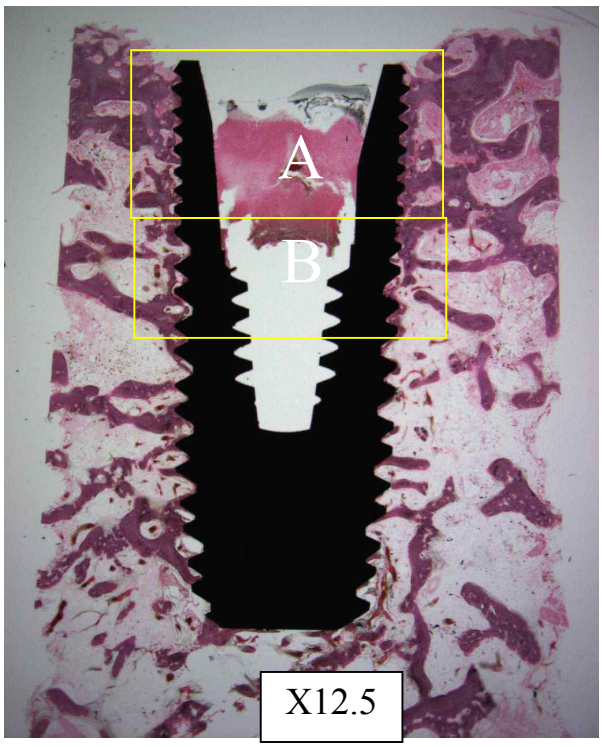

Fig. 3. 8nm TiO_2 nanotube 4week using an optical microscope (Leisa DM 2500, Leisa Microsystems, Germany X12.5)
A; micro-tread contact to cortical bone
B; macro-thread contact to cancellous bone.

Ⅲ. Results

3.1 Removal torque value (RTV) measurement

Immediately after sacrificing the test animals, removal torque values were measured in their upper jaws. In the experiment process, removal torque values failed to be measured with some of the five testing animals. (Table 1.)

Table 1. Removal torque value (Ncm) in the maxilla in 4^{th} and 12^{th} week.

Maxilla	RTV 4weeks	RTV 12weeks
8 nm TiO_2 nanotube	15.7	21.5
	22.7	26.2
		28.9
		39.8
	20.0	35.5
RBM control	15.2	13.8
	12.9	18.9
	22.7	31.7

Removal torque value of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the control RBM group, but there is no statistically significant difference.

In the 8 nm TiO_2 nanotube experimental group, the removal torque value increased more in the 12^{th} week than in the 4^{th} week. But there is no statistically significant difference.

In the control RBM group, the removal torque value increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.(Fig. 4)

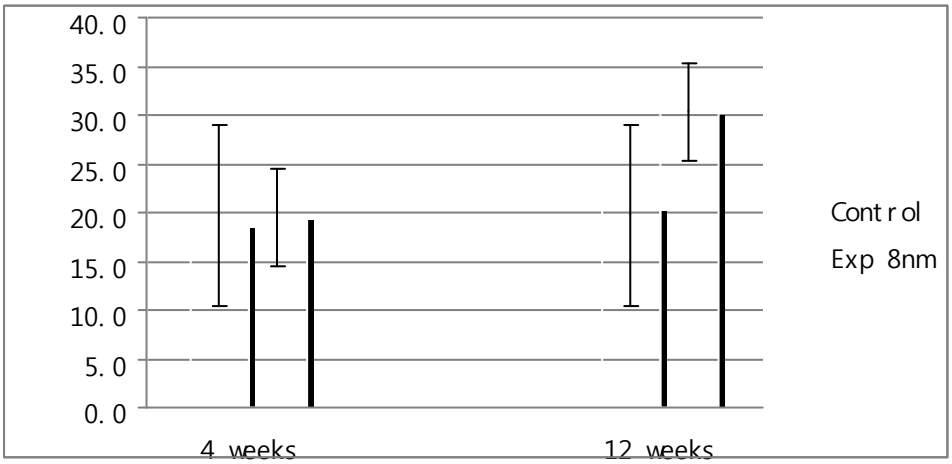

Fig. 4. The mean of removal torque value in the 4th and 12th week.

3.2 Histologic finding

The experimental group of 8 nm TiO_2 nanotube showed much more bone between the thread. (Fig. 5-12)

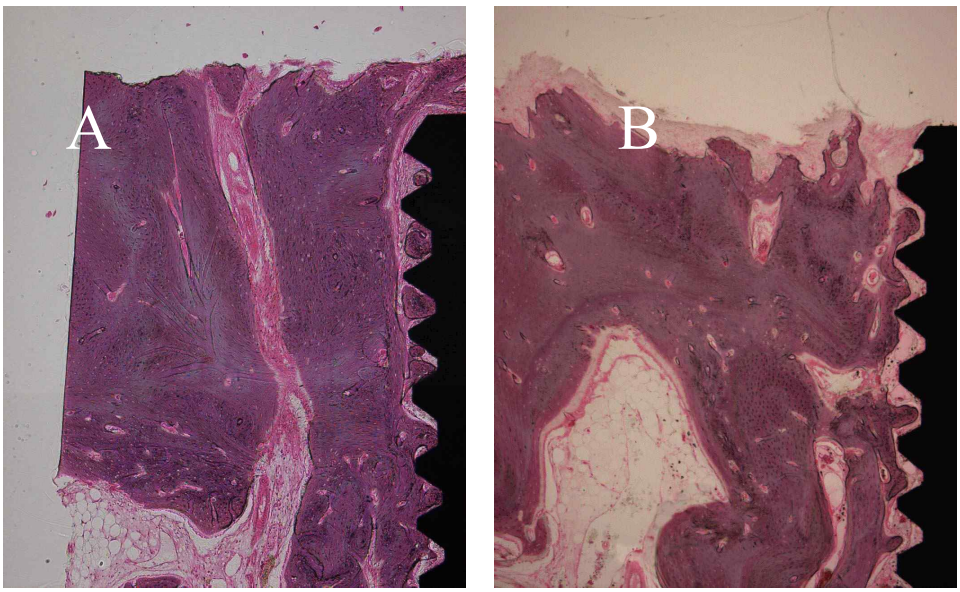

Fig. 5. A; 8 nm TiO_2 nanotube 4^{th} week micro-thread(X50)
B; RBM control 4^{th} week micro-thread(X50)

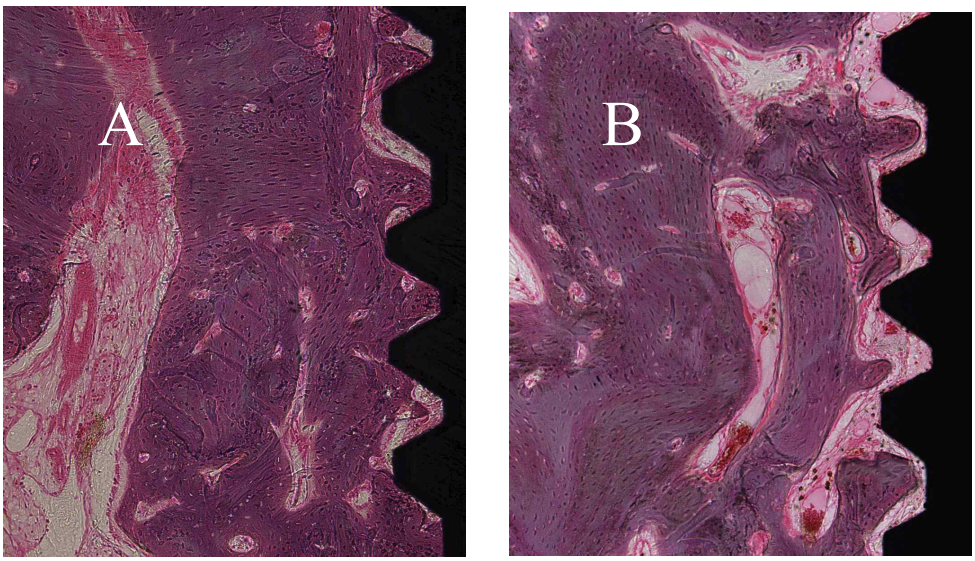

Fig. 6. A; 8 nm TiO_2 nanotube 4^{th} week micro-thread(X100)
B; RBM control 4^{th} week micro-thread(X100)

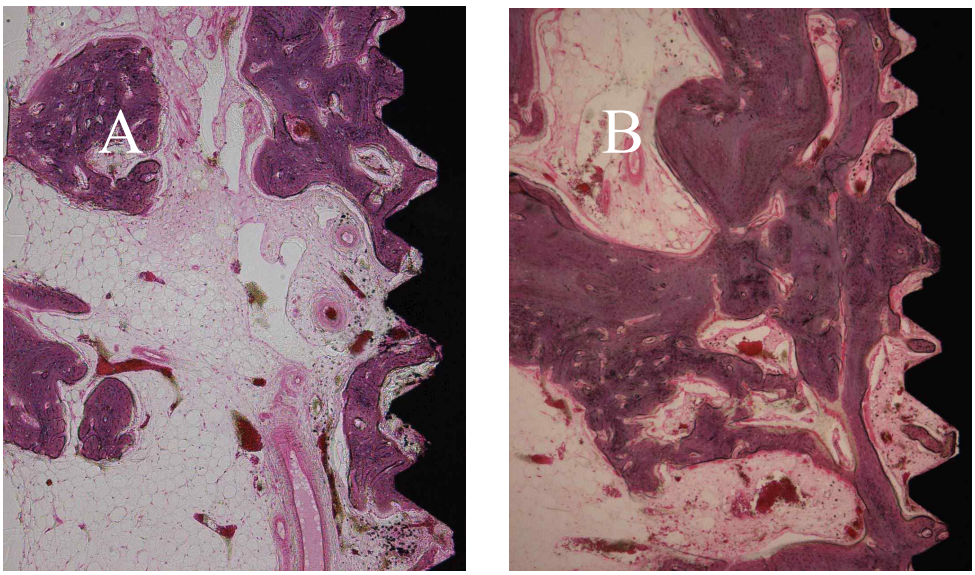

Fig. 7. A; 8 nm TiO_2 nanotube 4^{th} week macro-thread(X50)
B; RBM control 4^{th} week macro-thread(X50)

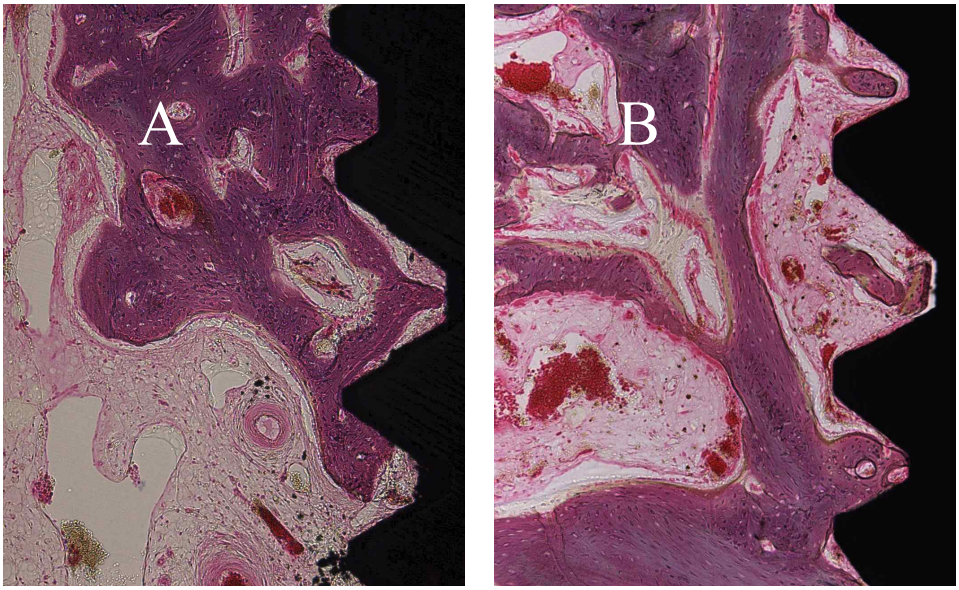

Fig. 8. A; 8 nm TiO_2 nanotube 4^{th} week macro-thread(X100)
B; RBM control 4^{th} week macro-thread(X100)

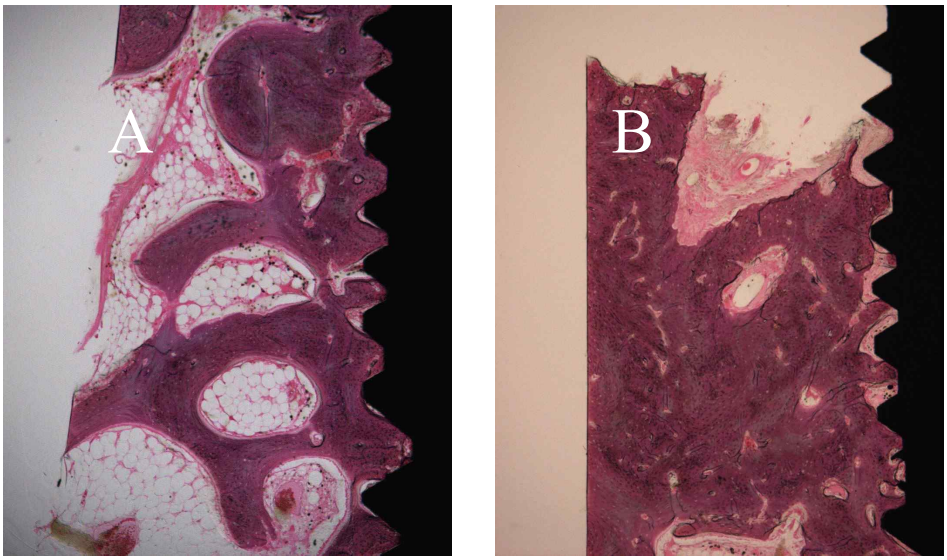

Fig. 9. A; 8 nm TiO_2 nanotube 12^{th} week micro-thread(X50)
B; RBM control 12^{th} week micro-thread(X50)

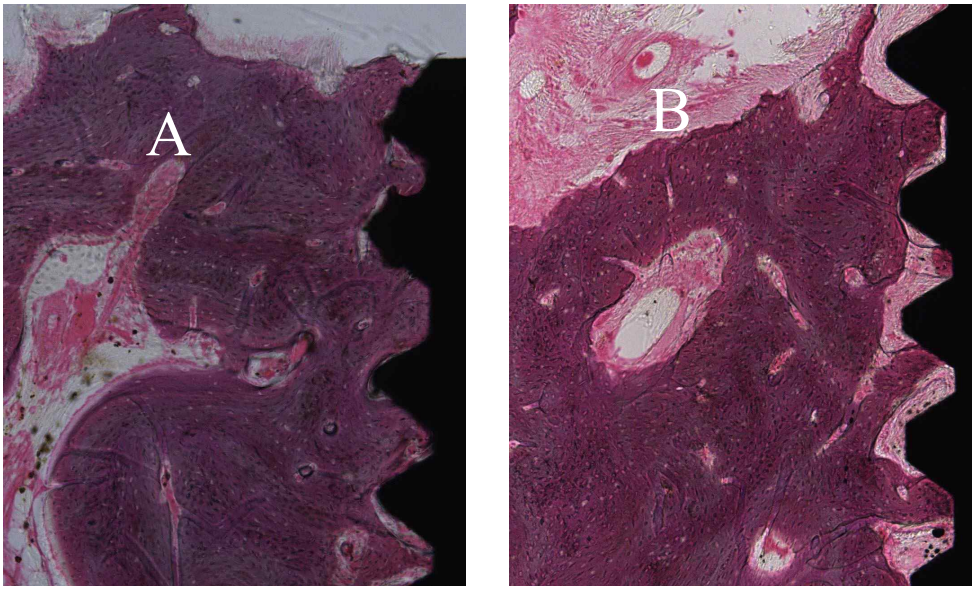

Fig. 10. A; 8 nm TiO_2 nanotube 12th week micro-thread(X100)
B; RBM control 12th week micro-thread(X100)

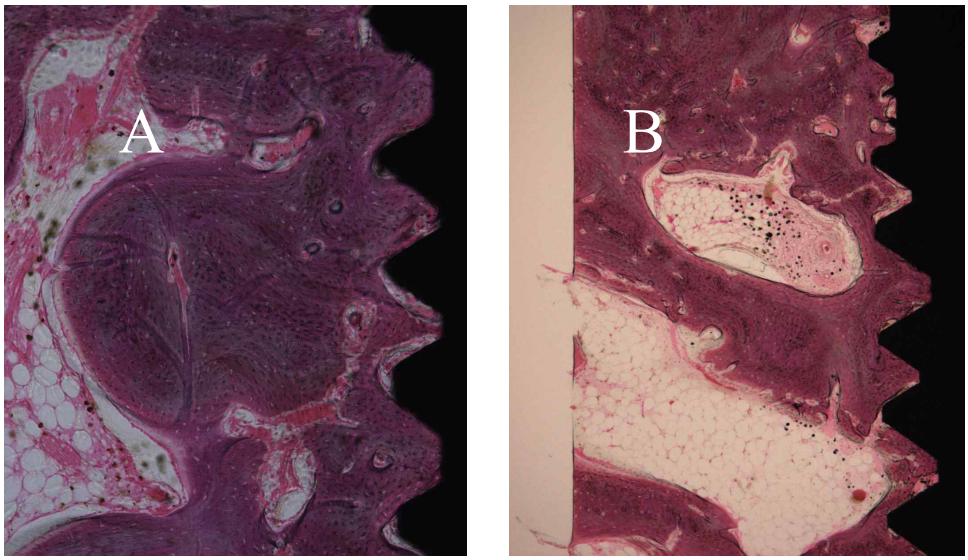

Fig. 11. A; 8 nm TiO_2 nanotube 12th week macro-thread(X50)
B; RBM control 12th week macro-thread(X50)

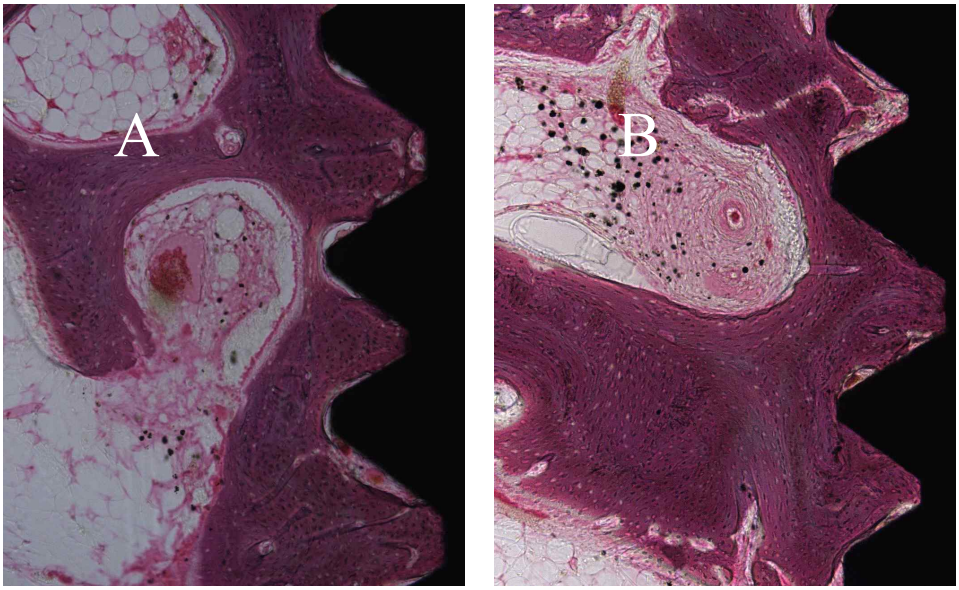

Fig. 12. A; 8 nm TiO_2 nanotube 12^{th} week macro-thread(X100)
B; RBM control 12^{th} week macro-thread(X100)

3.3 Histomorphometric analysis

3.3.1 Bone-to-implant contact value (BIC)

BIC values of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the RBM control group, but there is no statistically significant difference.

In the 8 nm TiO_2 nanotube experimental group, the BIC value increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.

In the RBM control group, the BIC value increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.

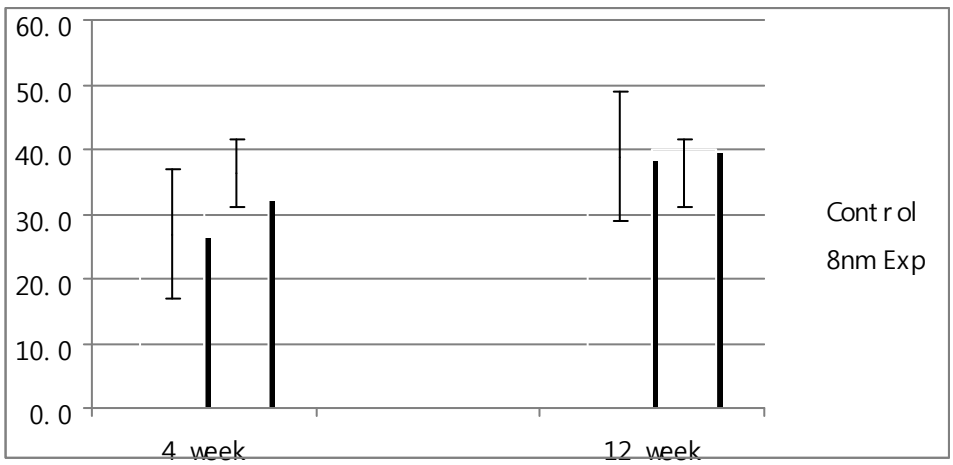

Fig. 13. The mean of BIC value in the 4^{th} and 12^{th} week.

3.3.2 Bone volume (BV)

3.3.2.1 micro-thread bone volume

Micro-thread bone volume (BV) values of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the RBM control group, but there is no statistically significant difference.

In the 8 nm TiO_2 nanotube experimental group, the micro-thread bone volume values increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.

In the RBM control group, the micro-thread bone volume (BV) values increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.

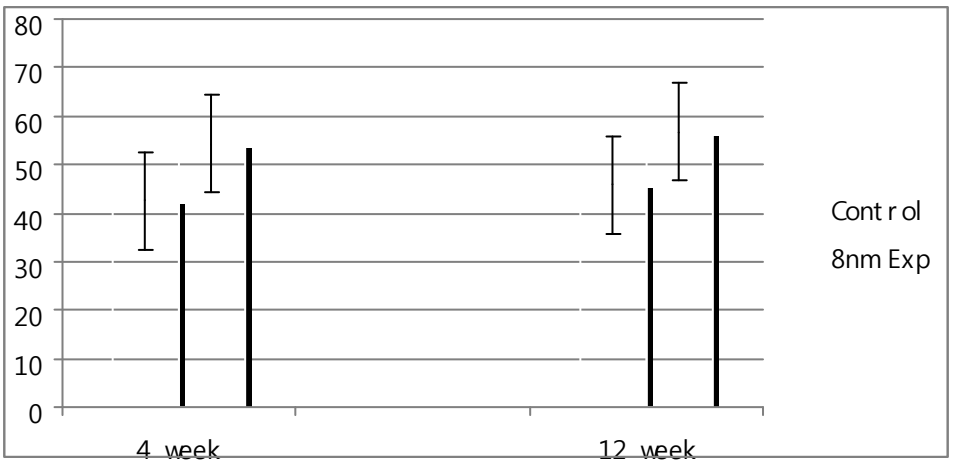

Fig. 14. The mean of Micro-thread bone volume in the 4^{th} and 12^{th} week.

3.3.2.2 The macro-thread bone volume

The macro-thread bone volume (BV) values of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the RBM control group, but there is no statistically significant difference.

In the 8 nm TiO_2 nanotube experimental group, the macro-thread bone volume (BV) values increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.

In the RBM control group, the macro-thread bone volume (BV) values increased more in the 12^{th} week than in the 4^{th} week, but there is no statistically significant difference.

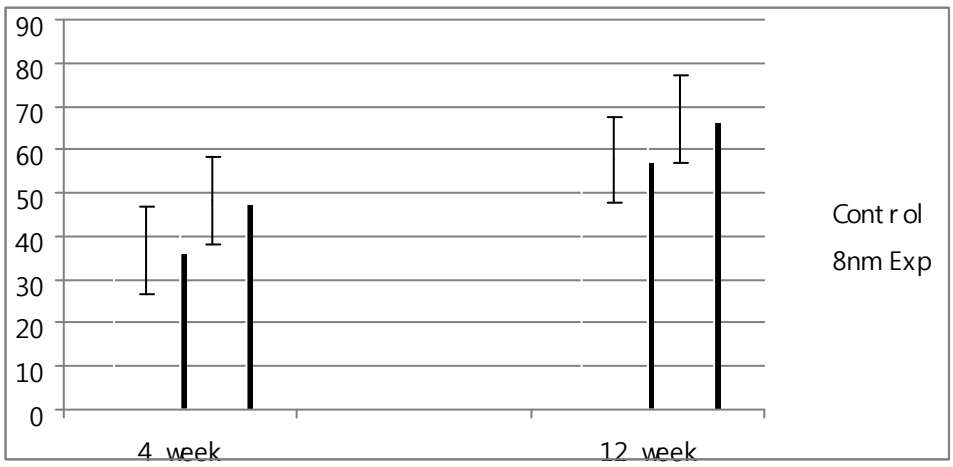

Fig. 15. The mean of macro-thread bone volume in the 4^{th} and 12^{th} week.

3.3.2.3 Total bone volume

Total bone volume (BV) values of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4th and 12th week, compared against those of the RBM control group.

There is no statistically significant difference in 4th week, but in 12th week there is statistically significant difference in both groups, p-value was 0.0122. ($p<0.05$)

In the 8 nm TiO_2 nanotube experimental group, the total bone volume (BV) values increased more in the 12th week than in the 4th week, but there is no statistically significant difference.

In the RBM control group, the total bone volume (BV) values increased more in the 12th week than in the 4th week, but there is no statistically significant difference.

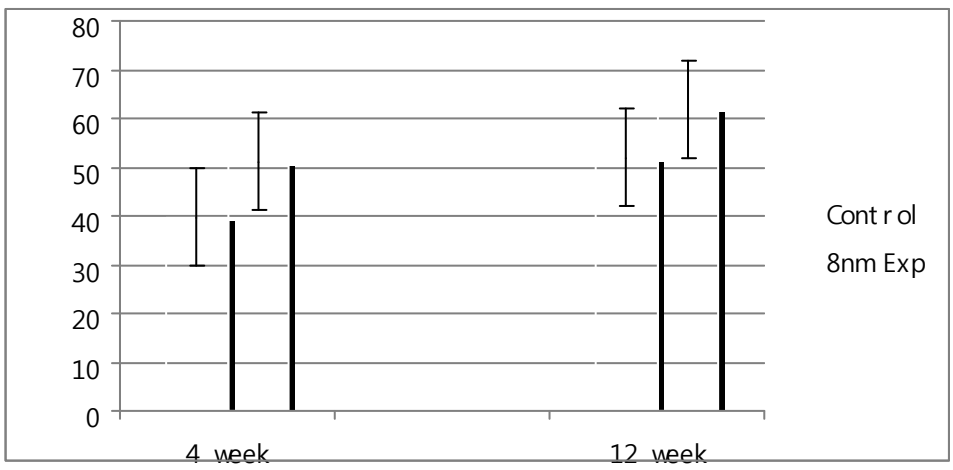

Fig. 16. The mean of total bone volume values in the 4th and 12th week.

Ⅳ. Discussion

Many dental researchs focus on development of new generation implant surface, with better bioactivity and hydrophilicity which can induce fast and stable osseointegration in attempt to overcome the limitation of present clinially used implant. TiO_2 nanotube formed on implant surface is popular portion in biomaterial researchs, there are many studies being conducted concerning the nanostructure of TiO_2 nanotube.[6-16] These studies reported that TiO_2 nanotube has higher surface area and more excellent biological applicability than the microstructure of the commercialized implant.

As research results showing the reaction of cells become activated on the nanostructure of nano-level TiO_2 nanotube are released, there are many studies aiming to observe the behavior of cells towards the nanostructure less than 100 nm.[28-32] According to cell experiments on TiO_2 nanotube implemented by Oh and Brammer, an increase in protein adsorption, cell adhesion and cellular motility was observed when TiO_2 nanotube was formed on the surface of implant.[33] The differentiation of stem cell into osteoblasts was able to be induced upon the diameter of nanotube, and the adhesion and shape of osteoblasts, and ability of ossification also induced upon the diameter of nanotube. Even a slight change to the diameter of TiO_2 nanotube at the level of cells around the nanostructure brings drastic difference to forms of cells such as adhesion, elongation, behavior, and differentiation.[34-38]

However most research studies about TiO_2 nanotube have been focused on the nanometer level of precision for cell-material interactions for enhanced bioactivity in vivo study and there are just a few studies about TiO_2 nanotube in vitro study.

Anodization is generally used to create TiO_2 nanotube, utilizing electrolyte solution containing fluoride. The formation of nanotube structure based on the nanotube formation mechanism is affected by anodization voltage and fluoride concentration in electrolyte solution. The greater the anodization voltage gets, the wider the diameter of the nanotube can be made.[22-26]

In this study, 8 nm TiO_2 nanotubes were formed by a hydrothermal treatment of titanium implant substrates rather than anodization method. In hydrothermal process, temperature and time are very important parameters, it would be interesting to investigate potential time-temperature combinations to vary geometric length, diameter, and configuration of the nanotubes. By new technique produced 8 nm diameter TiO_2 nanotube revealed multiwalled configuration, uniquely the overall morphology showing densely packed and looked like collagen type I fibers having fibrous and tangled nanoscale geometry in nature. (Fig. 2)

About the nanotube surface, the surface is super-hydrophilic with a 0° contact angle compared to a moderately hydrophilic surface on control RBM surface. The surface energy is therefore much higher on the nanotube surface. This may have implications in the increased cell spreading during the initial stages of adhesion. Hallab et al reported that the surface energy might be a more important determinant during cell adhesion and more useful than surface roughness for generating cell adhesion on engineering scaffolds.[39] In addition, Suh et al reported that a higher surface energy induced a stronger osteoblast cell attachment on hydrothermally processed nanotube surface.[40-42] A stronger adhesion mechanism, with cells anchoring into the surface nanotubes, would ensure mechanical interlocking between implant and growing bone, as indicated by Oh's study. [43] However, the Brammer's study was carried out in vitro study, it is necessary to

study the property of 8 nm TiO_2 nanotube which super-hydrophilic and biomimetric structure in vivo study.

For this study, 8 nm TiO_2 nanotube implant was manufactured with a new method of hydrothermal which was employed by Brammer and in-vivo experiments using 5 mongrel dogs were conducted.

What we see the results in this study, RTV of the experimental 8 nm TiO_2 nanotube group turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the RBM control group, but there is no statistically significant difference. Also BIC value of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the control group, as Johansson and Albreksson stated that removal torque value and BIC value are in direct proportion.[45]

In RTV and BIC value, there were differ greatly in 4^{th} week and in 12^{th} week the difference between the groups decreased. This indicates that 8 nm TiO_2 nanotube implant has a good early fixation and appears to be suitable for early loading better than RBM control group as well. The high RTV and BIC value of 8 nm TiO_2 nanotube results from the attributes of the super-hydrophilic surface of 8 nm TiO_2 nanotube, especially in 4^{th} week.

A number of research studies have reported that an implant with a rougher surface has a greater removal torque value, displaying a higher bone-to-implant contact. [39, 40-42, 44]

8 nm TiO_2 nanotube via hydrothermal method is super-hydrophilic showing zero contact angles, which helps bone-to-implant contact working for initial stage of implant plantation. Furthermore, unlike the RBM control group, high value of BIC from 8 nm TiO_2 nanotube turns it into excellent bone adhesive, as indicated by Hallab.[39]

The higher BIC value of 8 nm TiO_2 nanotube compared to the control group indicates that 8 nm TiO_2 nanotube, which has super-hydrophilic property, can achieve excellent osseointegration.

And 8 nm TiO_2 nanotube shows higher bone volume for 4^{th} week and 12^{th} week test than compare group. Especially, 12^{th} week test proves total bone volume is statistically different from RBM compare group.

Micro-thread bone volume has observed the reaction of crestal bone and 8 nm TiO_2 nanotube, while macro-thread bone volume has done one of cancellous bone. 8 nm TiO_2 nanotube group has showed higher bone volume than RBM compare group in both micro-thread and macro-thread volumes. For cancellous bone has higher blood flood rate and is more hydrophilic than cortical bone, super-hydrophilic 8 nm TiO_2 nanotube is considered to help bone development.

Moreover, there were different frequently in bone volume in 4^{th} week between the experimental 8 nm TiO_2 nanotube group and RBM control group, but totally bone filled in between screw threads was observed in both groups in 12^{th} week. This means bony remodeling has been completed to stabilized condition and all clinical appliance of RBM implant, the control group, works for 8 nm TiO_2 nanotube implant.

The commercialized implant in the control group adopts RBM (resorbable blasting media) as its surface is roughened by a blasting method using HA, ß-tricalcium phosphate (ß-Tcp) or the mixture of the two, the remnants hardly causes any problem in biocompatibility after washing and etching employing weak acid is possible. RBM implant (GSII, Osstem^{kr}, Korea), used in the control group, is widely used in clinics and clinically recognized, showing good results in animal study but super-hydrophilic 8 nm TiO_2 nanotube displayed even better results in

biomechanical, histologic, and histomorphometrics in comparison to the control group.

Based on these results it can be assume that in 4^{th} week there were different greatly between the groups, but 12^{th} week the difference between the groups decreased in RTV, BIC value and bone volume value. These indicated that even most of implant products in current markets have brought high success rate and quicker treatment for the patients with sound bones, 8 nm TiO_2 nanotube will bring high success rate and faster treatment for the patient with sound bone and poor quality bone, abnormal case, early loading case.

8 nm TiO_2 nanotube has an attribute of showing more clear reproducibility and compatibility compared to commercialized macro and micro structures but the formation of nanotube which consistently reproduces and the most appropriate size of nanotube are still under vigorous study.

Also, as the study of nanotube formation has been mostly focused on securing faster osseointegration, the increase of the survival rate of implant using nanotube, the development of permanent implant and the reactivation of nanotube remain to be research challenges that need to be explored in the future.

Now there could be conclusion as 8 nm TiO_2 nanotube is biomimetric structure and has super-hydrophilic property, predictably makes bone formation faster and could support custom implant production for imperfect bones or abnormal cases, critical situation as bone graft, GBR, immediate or early loading. Many follow-on research studies are needed and 8 nm TiO_2 nanotube will be considered very importantly in TiO_2 nanotube research and 8 nm TiO_2 nanotube is the promising candidate for new generation of implant surface.

Ⅴ. Conclusion

In this study, implants were manufactured with super-hydrophilic 8 nm TiO_2 nanotube formed through a new, hydrothermal method which was recently adopted and announced by Brammer et al. And they were compared with the control group which clinically, widely used implants. The study was conducted using 5 mongrel dogs under the same environments and the super-hydrophilic 8 nm TiO_2 nanotube showed better results in biomechanical, histologic, and histomorphometric analysis.

1. Removal torque value and BIC values of the experimental group of 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the RBM control group, but there is no statistically significant difference.

2. Total bone volume (BV) values of the 8 nm TiO_2 nanotube turned out to be higher both in the 4^{th} and 12^{th} week, compared against those of the RBM control group, there is no statistically significant difference in 4^{th} week, but in 12^{th} week there is statistically significant difference in both groups, p-value was 0.0122. ($p<0.05$)

3. There were assume an aspect that in 4^{th} week there were different conspicuously between the groups, but 12^{th} week the difference between the groups decreased in RTV, BIC value and bone volume value.

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국문요약

초친수성 8 nm TiO_2 나노튜브 형성 임플란트의 성견에서 골 유착도 연구

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최근 생체재료 및 임플란트 개발과 연구 중에 티타니아 나노튜브 (TiO_2 Nanotube)의 나노 구조는 마이크로 구조에 비하여 높은 표면적과 우수한 생물학적 응용력 때문에 연구들이 활발히 진행되고 있으며, 차세대 임플란트 표면 개발에 TiO_2 nanotube는 유력한 후보중에 하나이다.

일반적으로 TiO_2 nanotube를 제조하는 방법은 양극산화법이 주로 사용되며, 양극산화법으로 형성된 나노튜브를 이용한 생체 내 및 생체 외 골 유착 실험 연구가 발표되고 있는 반면 10 nm보다 작은 크기의 TiO_2 nanotube 연구는 극소수이며, 생체 외 세포 실험이 연구되고 있지만 생체 내 실험은 매우 소수이다.

2011년 Brammer등은 기존 양극산화법과 다른 hydrothermal한 새로운 방법으로 8 nm TiO_2 nanotube를 형성하는 논문을 발표하였는데, 형성된 8 nm TiO_2 nanotube는 super-hydrophilic한 성질 때문에 강한 세포 부착성(adherent)과 활발한 세포 활성도 (cell function)로 우수한 bioactivity를 보인다는 결과를 발표하였다.

본 연구는 hydrothermal 방법으로 8 nm TiO_2 nanotube 임플란트를 형성하고, 임상적으로 널리 쓰이고 있는 RBM 임플란트를 대조군으로 하여, 동일한 환경에서 생체 내 성견 동물 실험하여, 새로운 방법으로 형성된 8 nm TiO_2 nanotube 표면이 골 형성 및 골 유착에 미치는 영향을 평가하고자 하였다.

8 nm TiO_2 nanotube를 TEM level에서 관찰하면 이전의 양극산화법으로 형성된 나노튜브에 비해 multi-walled한 형상을 관찰할 수 있었다. 이는 자연상태의 collagen fiber type I 과 유사하며, 골조직과 가장 유사한 판상형의 나노 구조와 hydroxyl apatite와 유사한 크기 및 인산 칼슘을 많이 함유하는 화학적 조성을 갖는 생체모방형(biomimetric) 구조를 갖는다는 것이다.

8 nm TiO_2 nanotube는 RBM 대조군보다 높은 removal torque value를 보였다. 8 nm TiO_2 nanotube는 4주군보다 12주군에서 removal torque value가 증가하였다. 8 nm TiO_2 nanotube는 RBM 대조군에 비해 4주군, 12주군 모두 더 높은 BIC 값을 보였다. 8 nm TiO_2 nanotube는 4주군, 12주군 모두 더 높은 Bone volume 값을 보였다. 또한 4주군에서 RTV, BIC value, Bone volume value이 RBM 대조군보다 차이를 보이다가 12주군에서 차이가 줄어드는 동일한 양상을 보였다. 이는 임상적으로 인정받은 RBM 임플란트 보다 8 nm TiO_2 nanotube가 우수한 osseointegration을 보이며, 식립 초기에도 빠르며 안정된 뼈 형성능을 보인다는 것이다.

본 연구에서 형성된 8 nm TiO_2 nanotube는 초친수성과 생체모방성 세포보다 작은 크기의 정형화된 구조를 구현함으로써 우수한 osseointegration을 보이므로, 이에 대해 많은 후속 연구가 필요하다. 8 nm TiO_2 나노튜브의 초친수성과 생체모방형 구조는 앞으로 임플란트 연구에서 중요할 것이며, 차세대 임플란트 표면 개발에 유력한 후보라고 사료된다.

핵심 단어: 8 nm titania nanotube, Hydrothermal method, Super-hydrophilic, Biomimetric structure