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**Effects of thread size in the implant  
neck area on peri-implant hard and soft  
tissues: an animal study**



**Jay-Yong Choi**

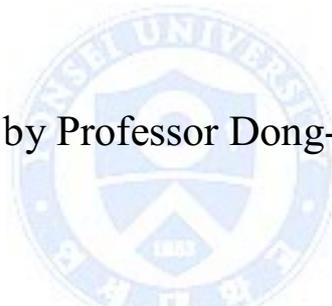
The Graduate School

Yonsei University

Department of Dental Science

**Effects of thread size in the implant  
neck area on peri-implant hard and soft  
tissues: an animal study**

Directed by Professor Dong-Won Lee



A Doctoral Thesis

submitted to the Department of Dentistry  
and the Graduate School of Yonsei University  
in partial fulfillment of the requirements for the degree of  
Doctorate of Dental Science

Jay-Yong Choi

December 2015

This certifies that the Doctoral Thesis  
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2015년 12월

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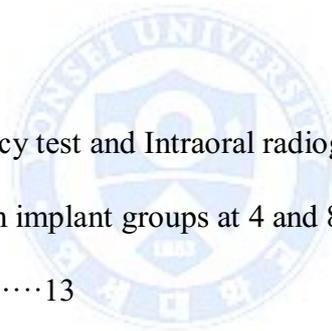


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## **ABSTRACT**

### **Effects of thread size in the implant neck area on peri-implant hard and soft tissues: an animal study**

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The aim of this animal study was to examine the effects of thread size in the implant neck area on peri-implant tissues in terms of BIC and hard- and soft-tissue dimensions. Six Beagle dogs received experimental implants in the mandible 3 month after removal of premolars and first molars (P2, P3, P4 and M1). Two different types of implants were installed in each animal: Anyone microthread<sup>®</sup> as group 1, and Anyone<sup>®</sup> as group 2. Resonance frequency test, intraoral radiography, micro-CT and histomorphometry were used to evaluate peri-implant tissue after implantation periods of 4 and 8 weeks.

No remarkable complication was observed during the healing period in either group. Resonance frequency testing revealed no significant difference between groups. In radiographic evaluation, group 2 showed more bone loss than group 1. However, this

difference was not statistically significant. In the micro-CT analysis, BIC and BIV values, and soft-tissue height was not significant in both groups. Histological analysis revealed no significant difference in BIC ratio, bone density, or bone loss between groups. However, soft-tissue height was significantly greater in group 2 than in group 1 ( $P = 0.0004$ ).

No difference in peri-implant hard or soft tissues was observed according to thread size in the implant neck area.



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**Key words: animal experiments, bone implant interactions, soft tissue-implant interactions, CT imaging, morphometric analysis**

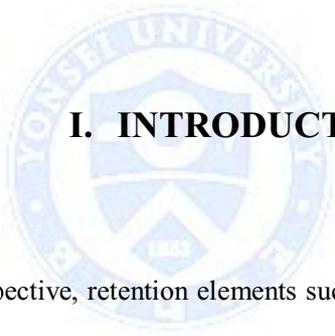
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**I. INTRODUCTION**

From a biomechanical perspective, retention elements such as the threads on the implant neck area provide mechanical stimulation to maintain the marginal bone level (Hansson, 1999). Several studies have found that a certain pitch distance of threads aids the preservation of peri-implant bone (Kong et al., 2009; Motoyoshi et al., 2005). Very small threads with a favorable profile can also effectively aid stress distribution in the bone (Hansson and Werke, 2003).

Some studies have used external type abutment connections. In a clinical study using a machined surface, the use of a non-threaded neck area resulted in the reduction of the marginal bone level to the level of the first fixture thread, and the smooth area seemed to provide an unfavorable condition for bone apposition (Quirynen et al., 1992). The location of

the beginning of the thread in the implant neck area has been found to play an important role in the stabilization of peri-implant marginal bone, and bone loss was related to the lengths of polished necks in various implant systems (Jung et al., 1996).

Clinical studies have been conducted in our department using implants with conical seal designs. In a study of TiOblast<sup>®</sup> surface implants, the presence of microthreads in the implant neck area was satisfactory for the preservation of peri-implant marginal bone (Lee et al., 2007). In another study of sandblasted, large-grit, acid-etched (SLA) surface implants, less bone loss occurred around implants with threads beginning at the top than around those on which the threads began 0.5 mm from the top. This indicates that the microthreads acted to stabilize the peri-implant marginal bone, and their locations played an important role in the stabilization process (Song et al., 2009). A recent clinical study of an implant with a resorbable blast media surface found that macrothreads beginning at the top of the implant neck maintained as much marginal bone as observed with microthreads (Kang et al., 2012). Most clinical studies of marginal bone loss have been based on intraoral radiographs.

An experimental study of an implant with a TiOblast<sup>®</sup> surface verified the advantages of microthreading over a smooth fixture neck with no threading in terms of the establishment of bone-implant contact (BIC) and maintenance of marginal bone level (Abrahamsson and Berglundh, 2006).

The level of supporting bone and soft-tissue dimensions around implants are important in implant maintenance and are affected by surgical and prosthetic parameters and related variables (Belser et al., 2004). Resonance frequency analysis has been used to measure the mechanical stability of implants for osseointegration; this noninvasive technique is easy to use in the clinical setting (Lachmann et al., 2006a; Lachmann et al., 2006b; Oh et al., 2009). BIC is an important measure of implant stability and osseointegration (Klokkevold et al., 1997).

Micro-computed tomography (CT) has become a standard tool for the assessment of the three-dimensional (3D) architecture of trabecular bone (Muller, 2002). Most studies of tooth structure have been based on the examination of two-dimensional (2D; histological) images, and serial histological sectioning is time consuming and destructive. 2D methods can result in malorientation errors in the section plane due to anisotropic structures.

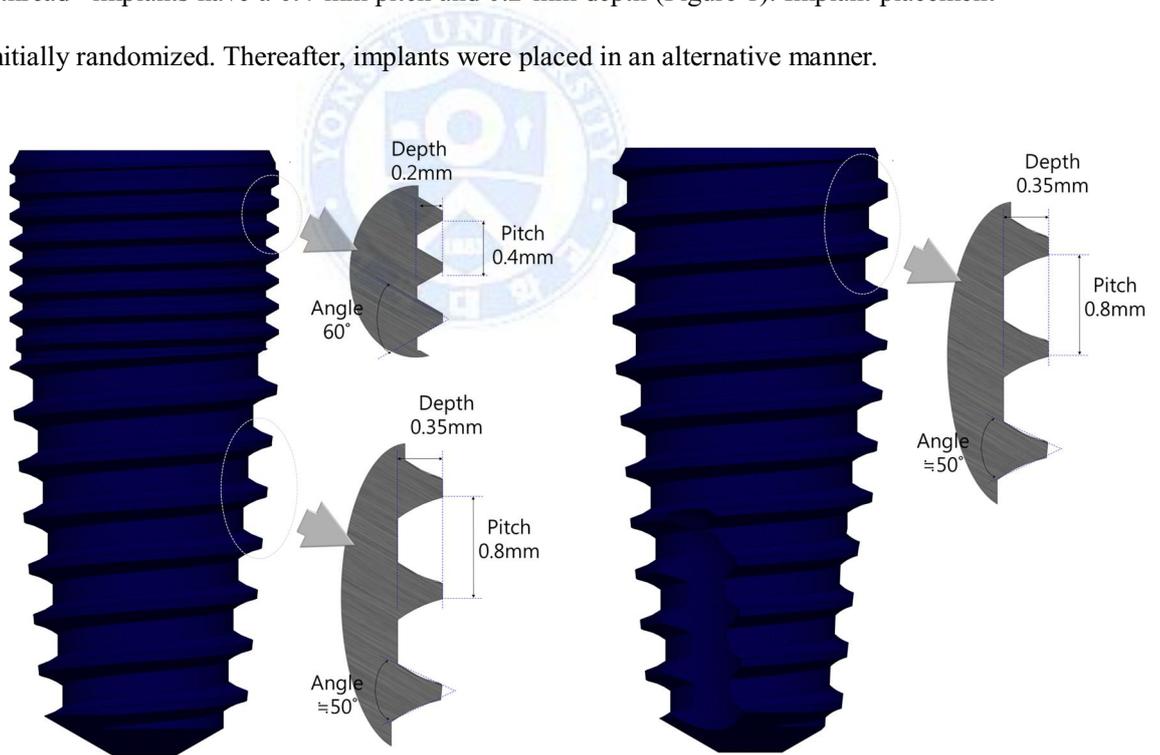
Although clinical studies have compared the effects on marginal bone level with microthreaded and macrothreaded implants, no animal study examining this issue has been conducted. Thus, the aim of this animal study was to examine the effects of thread size in the implant neck area on peri-implant tissues in terms of BIC and hard- and soft-tissue dimensions.



## II. MATERIALS AND METHODS

### 1. Implants

The implants used in this study were Anyone Microthread<sup>®</sup> (group 1; Megagen, Seoul, Korea) and Anyone<sup>®</sup> (group 2; Megagen). Both implant types have nanostructured calcium-coated (XPEED<sup>®</sup>) surfaces with 3.5-mm diameters and 7-mm lengths. For the threads in the fixture neck area, Anyone<sup>®</sup> implants have a 0.8-mm pitch and 0.35-mm depth and Anyone Microthread<sup>®</sup> implants have a 0.4-mm pitch and 0.2-mm depth (Figure 1). Implant placement was initially randomized. Thereafter, implants were placed in an alternative manner.



**Figure 1.** Schematic figure of implants used. Anyone Microthread (left) and Anyone (right).

## **2. Animal model**

Six beagle dogs aged about 1.5 years and weighing approximately 13–15 kg were used for this study. Animal selection, care, management, the surgical protocol, and animal preparation for surgery were conducted in accord with the guidelines issued by the Ethics Committee on Animal Experimentation of Chonnam National University. The dogs were given 2 weeks to acclimatize. They were fed a soft dog-food diet and had free access to water.

## **3. Experimental procedure**

A total of 24 implants were inserted in the mandibles of the six beagle dogs. The dogs underwent extraction of the left mandibular premolars and first molar (P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub> and M<sub>1</sub>), followed by extraction of the right mandibular premolars and first molar 1 month later. Implants were inserted in the left and right mandibular sockets 2 and 3 months later, respectively, allowing a 3-month healing period for each side of the mandible (Figure 2).



The final drill diameter was 3.2 mm. Thereafter, implants were inserted at the bone crest level. A healing abutment was connected to each fixture. Finally, the flaps were closed using 4-0 polyglycolic acid sutures. The dogs were kept on a soft diet for 2 weeks after the surgical procedure.

Postoperatively, amoxicillin (20 mg/kg p.o., twice a day; Dong Wha Pharm, Seoul, Korea) and meloxicam (0.1 mg/kg p.o., once a day; Boehringer Ingelheim Vetmedica GmbH,

Ingelheim, Germany) were administered for 7 days. Plaque control was maintained by daily flushing of the oral cavity with 0.12% chlorhexidine gluconate for 2 weeks. The animals were anesthetized and euthanized at 8 weeks post-surgery by intravenous injection of concentrated potassium chloride (Daejung, Sigeung, Korea). Following euthanasia, block sections including implants, alveolar bone, and surrounding mucosa were collected.

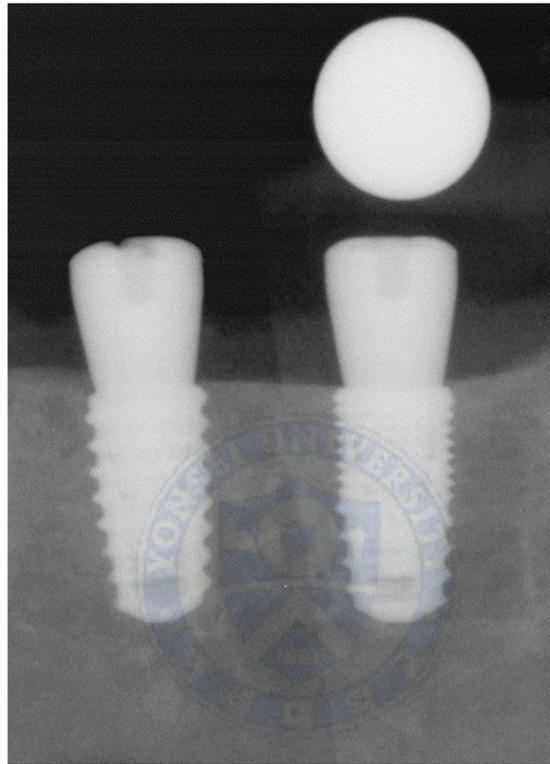
#### **4. Resonance frequency test**

To analyze the mechanical stability, resonance frequency test was performed. One examiner performed the testing using an Osstell meter (Osstell Mentor, Integration Diagnostics, Göteborg, Sweden) and Smart peg (Osstell, Straumann AG, Basel, Switzerland) at the time of each surgery and at sacrifice. Implant stability quotients (ISQs) were measured on the buccal and lingual sides of implants.

#### **5. Intraoral radiographs**

Intraoral radiographs were taken 3 months after each extractions (at the time of implant

placements) and at sacrifice to measure marginal bone loss. Radiographs were taken with a portable device (Elytis, Trophy, France) using the parallel cone technique (70 kV, 8 mA, 0.250 s). A 5.5-mm spherical metal bearing was placed to aid length measurement (Figure 3).



**Figure 3.** Intraoral radiograph with 5.5-mm spherical metal bearing

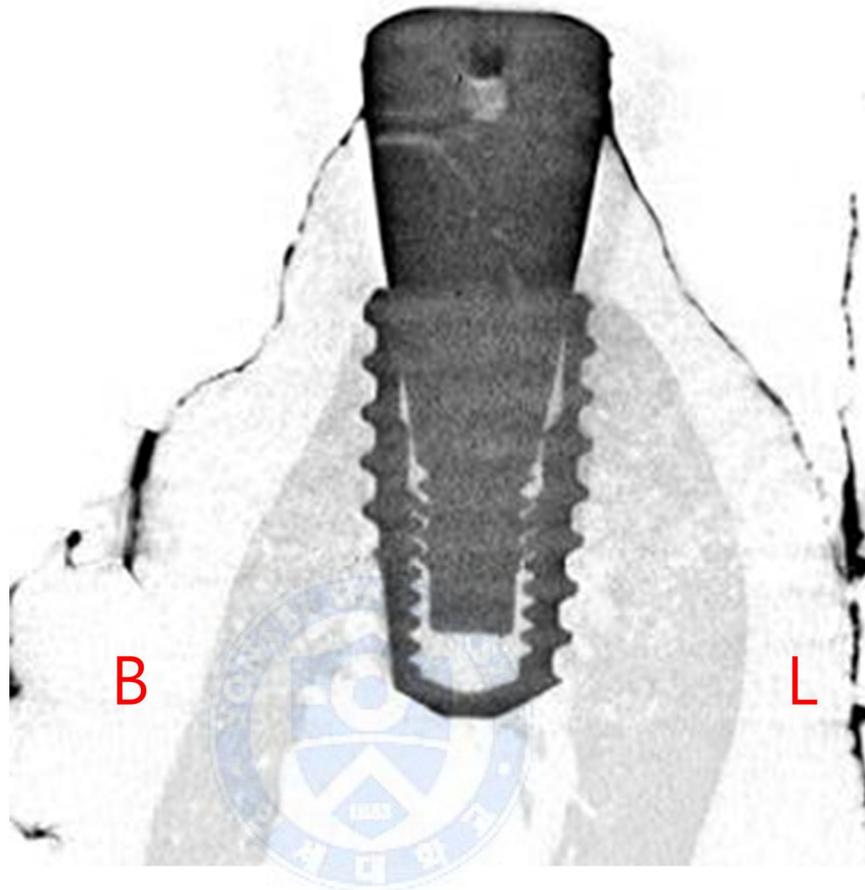
## **6. Microcomputed tomography**

Bone blocks containing one implant each were dehydrated in 70% ethanol and wrapped in Parafilm (SERVA Electrophoresis GmbH, Heidelberg, Germany) to prevent drying during tomographic examination. For quantitative 3D analysis, each specimen was placed vertically onto the sample holder of a Skyscan 1076 desktop x-ray micro-CT system (Skyscan, Kontich,

Belgium), with the long axis of the implant perpendicular to the scanning beam. A high-resolution image was obtained at 18  $\mu$ m voxel resolution.

Cone-beam reconstruction was then performed using Nrecon V1.4 (Skyscan). Thereafter, a constant region of interest (ROI) was defined along the length of the implant gap using CTAn V1.8 (Skyscan). The ROI included 32 pixels of bone surrounding the implant. For all images, a threshold was manually selected to isolate bone tissue and preserve the visualization of its morphology while excluding the implant material. The amount of BIC along the implant axis within the ROI was measured. Next, the amount of bone within the ROI was assessed by calculating the percentage of surface area inside the ROI occupied by bone. This area is referred to as the bone to implant volume (BIV).

To facilitate examination of the soft-tissue border around implants, we diluted barium sulfate (E-Z-HD- barium sulfate powder; E-Z-EM Company Inc., New York, USA) with water at a 1:1 ratio and applied it to the soft tissue around implants (Oktay and Kilic, 2007). Soft-tissue height (S-ts height) was measured from the implant fixture–abutment junction to the top of the soft tissue on the buccal aspect. (Figure 4)



**Figure 4.** A view soft tissue boundary used with barium sulfate : micro CT analysis. B: buccal, L: lingual

## **7. Histological preparations and histomorphometric evaluation**

Tissues containing the implants were removed en bloc, fixed in 4% neutral-buffered formaldehyde, dehydrated using an ascending series of alcohol concentrations, and embedded in methyl methacrylate for undecalcified sectioning. Undecalcified cut and ground sections

containing the central parts of the implants were produced at a final thickness of 20  $\mu\text{m}$  using a macro-cutting and grinding system (Exakt 310 CP series; Exakt Apparatebau, Norderstedt, Germany). The sections were stained with Villanueva, and histomorphometric analysis was carried out using a light microscope (CX31; Olympus, Tokyo, Japan) with an image analysis system (Analysis TS Auto; Olympus) under 50 $\times$  magnification.

One examiner calculated BIC (%), defined as the length fraction (%) of mineralized bone in direct contact with the implant surface. Bone density (%), defined as the surface fraction (%) of the bone surface present in the area of the fixture thread, was also calculated. The analysis of BIC and bone density was confined to the neck portion of both implants types on the buccal and lingual sides. To evaluate bone loss and soft tissue height, the distances from the abutment–fixture junction to the first bone contact point and to the highest position of soft tissue were measured at the buccal side.

## **8. Statistical analysis**

The statistical analyses were performed using a software R, version 3.1.1 (<http://www.r-project.org>). The nonparametric repeated-measures analysis of variance method developed by Brunner and Langer (2000) was used to compare values between timepoints (4 and 8 weeks) and groups, and time  $\times$  group interactions. For each statistical comparison, only the model with the most precise outcomes (narrowest 95% confidence interval of the difference) was considered. Differences were considered significant when  $P$  values were  $<0.05$ .

### III. RESULT

No remarkable complication was observed during the healing period in either group (Figure 5). Two dogs had slight plaque accumulation, which was negligible. The procedure resulted in no open wound, infection, fracture, or lost implant. At sacrifice, all 24 implants were considered to be successfully integrated.



**Figure 5.** Intraoral photograph in 8 weeks after the implant surgery. No remarkable swelling, bleeding was found.

Median ISQ values for microthreaded and macrothreaded implants were 72.625 and 70.875, respectively. Resonance frequency testing revealed no significant difference between timepoints, groups, or interaction (Table 1). Radiographic evaluation revealed more bone loss in group 2 than in group 1 at both timepoints, but these differences were not significant (Table 1). Micro-CT analysis showed greater BIC values and soft-tissue height in group 2 than in group 1, and greater BIV values in group 1 than in group 2, at 4 and 8 weeks. However, these differences were not significant (Table 2). Histological analysis revealed no significant difference in BIC ratio, bone density, or bone loss. However, soft-tissue height was significantly greater in group 2 than in group 1 ( $P = 0.0004$ ; Table 3, Figure 6).

**Table 1.** Resonance frequency test and Intraoral radiographs data of value of ISQ, and bone loss (mm) for both implant groups at 4 and 8 weeks

		Group 1	Group 2	Time	Group	Time*group
		Median (Q1-Q3)	Median (Q1-Q3)			
RFA (ISQ)	4 weeks	74.75 (72-77.25)	69.5 (67-76)	0.5267	0.1476	0.2567
	8 weeks	72.125 (70.5-73.25)	70.875 (70.75-73.5)			
Bone loss (mm)	4 weeks	0 (0-0)	0.06 (0-0.37)	0.134	0.0895	0.7518
	8 weeks	0.105 (0-0.22)	0.28 (0-0.43)			

RFA = resonance frequency analysis; ISQ = implant stability quotient; Q1 = 25<sup>th</sup> percentile; Q3 = 75<sup>th</sup> percentile

**Table 2.** Micro-CT data of value of BIC (%), BIV (%), and soft tissue height (mm) for both implant groups at 4 and 8 weeks

		Group 1	Group 2	Time	Group	Time*group
		Median (Q1-Q3)	Median (Q1-Q3)			
BIC (%)	4 weeks	52.066 (49.678-53.758)	54.562 (53.49-56.81)	0.5089	0.0633	0.0641
	8 weeks	53.403 (49.975-57.47)	53.989 (53.403-56.013)			
BIV (%)	4 weeks	44.403 (42.629-45.17)	44.232 (42.934-45.183)	0.4354	0.8185	0.6068
	8 weeks	45.663 (42.342-50.596)	45.597 (42.796-46.89)			
Soft-tissue height (mm)	4 weeks	1.899 (1.526-2.627)	2.251 (2.023-2.472)	0.4097	0.4484	0.8667
	8 weeks	1.527 (1.029-2.71)	2.153 (1.566-2.414)			

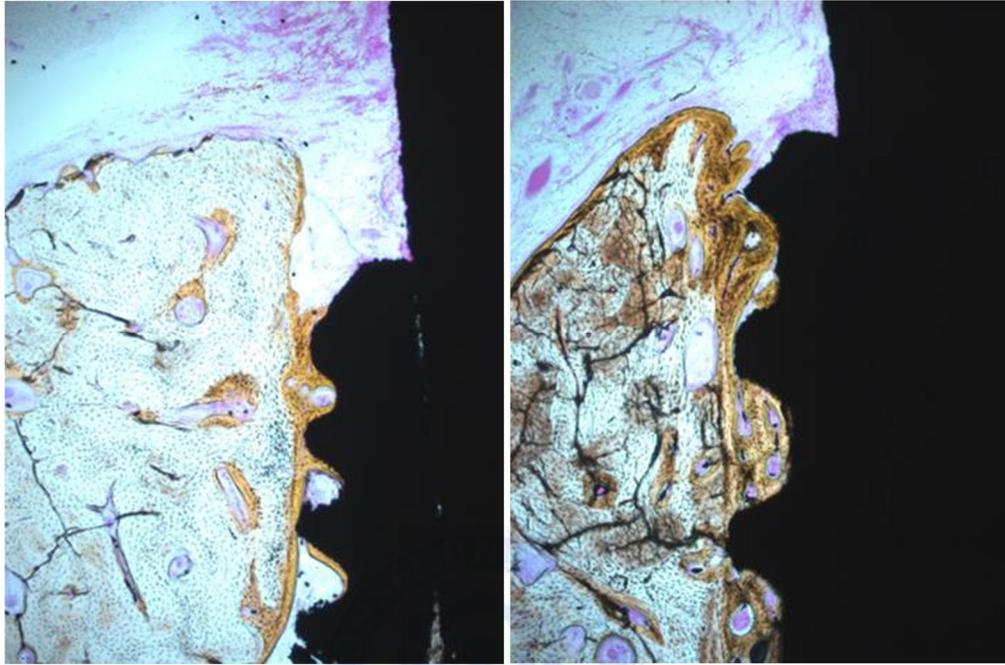
BIC = bone to implant contact; BIV = bone to implant volume; Q1 = 25<sup>th</sup> percentile; Q3 = 75<sup>th</sup> percentile.

**Table 3.** Histomorphometrical data of value of BIC (%), Bone density (%), soft tissue height (mm), and bone loss (mm) for both implant groups at 4 and 8 weeks

		Group 1	Group 2	Time	Group	Time*group
		Median (Q1-Q3)	Median (Q1-Q3)			
BIC (%)	4 weeks	62.463 (51.24-62.865)	59.46 (58.1-59.94)	0.419	0.722	0.2636
	8 weeks	57.075 (55.75-59.64)	57.98 (55.73-60.34)			
Bone density (%)	4 weeks	41.702 (39.525-46.985)	47.695 (47.335-48.671)	0.5604	0.3101	0.5922
	8 weeks	47.03 (38.835-56.2)	46.74 (41.24-56.64)			
Bone loss (mm)	4 weeks	0.223 (0.096-0.312)	0.161 (0-0.324)	0.8074	0.2874	0.23
	8 weeks	0.253 (0.164-0.356)	0.112 (0.042-0.16)			
Soft-tissue height (mm)	4 weeks	2.362 (1.979-2.613)	2.993 (2.894-3.827)	0.2391	0.0004*	0.4707
	8 weeks	2.111 (1.348-2.613)	2.716 (2.2-3.254)			

BIC = bone to implant contact; BIV = bone to implant volume; Q1 = 25<sup>th</sup> percentile; Q3 = 75<sup>th</sup> percentile.

\* Significantly different between group1 and group2 ( $p < 0.05$ ).



**Figure 6.** x50 magnification view of Anyone microthread (left) and Anyone (right).

## IV. DISCUSSION

Microthreads in the crestal portion have been suggested to maintain marginal bone and soft tissue around implants. Microthreads transfer vertical load into a more compressive interface, creating less shear stress at the bone-implant interface (Hudieb et al., 2011). Surface area increases with decreasing thread pitch to produce more favorable stress distribution, as confirmed in many studies (Abrahamsson and Berglundh, 2006; Berglundh et al., 2005; Lee et al., 2007). However, these studies did not compare thread sizes; rather, they compared implants with microthreads with those lacking thread in the fixture neck area. According to Jung et al. (1996) and Song et al. (2009), thread location in the implant neck area can also affect the preservation of marginal bone.

Two recent clinical studies had different outcomes. Based on a clinical study of 59 patients, Vandeweghe et al. (2012) recommended a small thread pitch to allow positive stress distribution and limit the amount of bone loss. On the other hand, a clinical study of two implant types found that macrothreads in the neck portions of implants provide for marginal bone preservation similar to that achieved with microthreads (Kang et al., 2012). However, these clinical studies did not permit histological analysis and were limited to 2D radiographic analysis.

Clinical studies have demonstrated the negative effects, such as marginal bone resorption around implant fixtures, of non-threaded implant neck areas with smooth surfaces. Clinical and experimental studies have shown differences within peri-implant tissues by comparing non-threaded and microthreaded portions of fixture neck areas with rough surfaces. The effects of thread presence/absence and location on marginal bone have been confirmed and a

clinical study examining the additional factor of thread size has been progressed. This study compared the effects of implants with macrothreads and microthreads in the neck area with identical thread locations. Effects of thread size on peri-implant tissues were examined using resonance frequency analysis and the measurement of BIC and hard- and soft-tissue dimensions.

In the present study, all implants survived during the study period. Oral hygiene was stable in all six dogs, and no gingival problem such as bleeding or pus discharge was detected. Resonance frequency analysis produces ISQ values ranging from 1 to 100, with values <45 indicating implant failure and those of 60–70 indicating success (Sennerby and Roos, 1998). In the present study, both groups had more than 70. This indicates the success and stable fixation of the implants.

Although intraoral radiographic evaluation showed more marginal bone loss around macrothreaded than around microthreaded implants, this difference was not significant. This result indicates that macrothreading on the crestal portion of an implant fixture results in marginal bone preservation similar to that achieved with microthreaded implants.

High BIC values obtained from micro-CT image analysis are considered to indicate implant stability, which clinically enables functional dental reconstruction. BIV values provide information about the thickness of newly formed peri-implant bone (Bernhardt et al., 2012). In the present study, no difference in BIC ratio, BIV, or soft-tissue height was observed between groups. Barium sulfate has been used to expose soft-tissue deficiencies on radiographs and is an inexpensive, simple, and reliable method for evaluating and visualizing dentoalveolar structures (Oktay and Kilic, 2007).

Histological analysis also revealed no significant difference between groups in BIC ratio, bone density, or bone level. However, significantly greater soft-tissue height was observed

around macrothreaded than around microthreaded implants. According to Albrektsson and Johansson (1991), 50% BIC is needed to ensure implant stability. The results of the present study (58.71% in group 1, 59.36% in group 2) are thus satisfactory. Regarding marginal bone loss, all histological slides showed that the top of the buccal bone was higher than the BIC initiation points, most of which were at the first implant threads. This finding explains the importance of the location of the first thread.

Although microthreaded implants have more contact surfaces than do macrothreaded implants, this difference did not affect bone preservation. This finding may be explained by the surface modification technique. Titanium implants with nanostructured calcium-coated surfaces may induce strong bone integration by improving osseointegration (Sun Young Lee et al., 2012; S. Y. Lee et al., 2012). In the present study, histological, but not micro-CT, analysis showed greater soft tissue height in association with microthreaded than with macrothreaded implants. Considering that many studies have used one or two histological sections per implant, section selection may strongly influence the results. In addition, the 2D nature of histological slide sectioning may affect the inherent discrepancy. Moreover, we observed no difference in group  $\times$  time interaction between groups.

This study had several limitations. The study sample was small, and the study period did not allow the observation of long-term effects, such as those of the prosthetic procedure and occlusal loading. Despite these limitations, the study findings suggest that in many aspects, under identical conditions, implants with macrothreads and those with microthreads in the neck area have similar effects on peri-implant tissues. Furthermore, no clinical difference is expected between the use of implants with macrothreads, which have a simpler design, and those with microthreads.

## V. CONCLUSION

In conclusion, no difference in peri-implant hard or soft tissues was observed according to thread size in the implant neck area



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## 국문요약

# 임플란트 경부의 나선선의 크기에 따른 임플란트 주위 경조직과 연조직의 효과: 동물실험

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이 동물실험의 목적은 BIC와 경조직, 연조직의 관점에서 임플란트 경부의 나선선의 크기에 따른 임플란트 주위 조직의 효과를 BIC와 경조직, 연조직의 관점에서 알아보기 위함이다. 6마리의 비글견의 소구치들과 제1대구치(P2,P3,P4와 M1)의 발치 3개월 후에 실험적 임플란트가 식립되었다. 치유된 발치와에 두 가지 임플란트 (Anyone microthread®와 Anyone®)가 식립되었다. 임플란트 식립 후 4 주째, 8 주째 시점에서 Resonance frequency testing, 구강 내 방사선학적 분석, micro-computed tomography, 조직계측학적 분석이 시행되었다. 치유기간 동안 두 군에서 모두 합병증을 보이지 않았다. Resonance frequency testing에서 두 군 간의 통계학적 유의성은 없었다. 구강 내 방사선학적 분석에서 Anyone®군에서 Anyone Microthead®군

보다 더 많은 골흡수를 보였지만 통계학적 유의성은 없었다. Micro-CT 분석에서 BIC, BIV 값, 그리고 연조직 높이에서 두 그룹 내에 유의성 있는 차이는 없었다. 조직학적 분석에서 BIC의 비율, 골밀도나 골소실에서 두 그룹 내에 유의성 있는 차이는 없었다. 하지만, 연조직의 높이는 Anyone®군에서 Anyone Microthead®군 보다 유의성 있게 높게 나타났다. (P=0.0004). 이 번 연구결과, 나사선의 크기는 임플란트 주변 경조직 및 연조직에 영향을 미치지 않는 것으로 나타났다.



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핵심단어: 동물실험, 골 임플란트 상호반응, 연조직 임플란트 상호반응, 컴퓨터단층촬영 영상, 형태학적 분석