Reosseointegration of the mechanically disintegrated implants in dogs: a histometric analysis

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감사의 글

본 논문이 완성되기까지 부족하고 미흡한 저를 항상 격려해 주시고 사랑과 관심으로 이끌어 주신 정의원 교수님께 깊은 감사의 말씀을 드립니다. 그리고 바쁘신 와중에도 심사를 맡아주시고 많은 조언과 격려로 지도해주신 최성호 교수님, 이동원 교수님과 따뜻한 관심으로 지켜봐 주신 채중규 교수님, 조규성 교수님, 김창성 교수님, 박정철 교수님께 감사드리며, 특히 논문의 연구와 작성에 아낌없는 조언과 도움을 준 동기 이중석 교수에게도 감사의 말을 전합니다.

연구 내내 많은 도움을 주신 최연아 선생님, 이규안 선생님, 조아란 선생님을 비롯한 치주과 의국원들께도 감사의 말씀 전합니다.

그리고, 늘 조건 없는 사랑을 주시고 말없이 저를 믿어 주시는 사랑하는 가족들에게 진정으로 사랑과 감사의 마음을 전합니다.

마지막으로 한결 같은 모습으로 항상 나의 옆자리를 지켜주고 있는 아내 장혁임에게 사랑과 고마움의 마음을 전합니다.

모든 분들께 진심으로 감사드립니다.

2012년 6월

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The purpose of this study was to evaluate the re-osseointegration of the implants reinserted following mechanical breaking of osseointegration in dogs.

Rotationally mobile implants by using oversized drilling were bilaterally installed in five canine mandibles. After 4 weeks of healing period, immature osseointegration of the implant for the experimental group was intentionally broken by mechanical countertorque and reinserted at the same site, while the control group remained submerged without any surgical intervention. The animals were euthanized 4 weeks after breaking of osseointegration. Change of the implant stability quotient (ISQ) and Periotest™ value (PTV) were analyzed, and bone to implant contact (BIC, %) was histometrically measured.

The mean of PTVs at the end point were similar and were not statistically significant between the experimental and the control group. But in the mean of ISQ values and BIC, the experimental group showed significantly higher values than that of the control group.

Within the limitation of this study, it is concluded that the mechanically broken osseointegration can be successfully reosseointegrated, after unloaded and submerged healing for a certain period.

Key words: implant stability, breaking of osseointegration, mobile implant, reinsertion, re-osseointegration

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I. Introduction

Currently, high and rapid stability of the implant can be acquired even at the poor quality of bone thanks to the improved implant surface and thread design, which consequently make it possible to shorten the loading time. Clinical success of dental implants depends on osseointegration between bone and implant interface. In general, the extent of osseointegration is associated with the implant stability. If the initial stability of the implant is secure, immediate or early loading protocol can be successfully applied[1-4]. However, host factor like poor bone quality and surgical factor

like inconsistent drilling by a novice might lead to unstable implant fixation. In the previous study, absence of initial stability was considered as one of the major causes of implant failure in turned-surfaced implants[5]. However, when the rough surfaced implants were used, initially mobile implants also showed comparable osseointegration compared to normal press-fit implants after a certain period of healing[6-11]. Nevertheless, initially unstable implant should avoid immediate or early loading and is recommended to delay loading protocol[6].

It is important to know the degree of osseointegration for determination of loading time, especially in situation of initially mobile implants placed in poor quality of bone bed. In order to evaluate the extent of osseointegration, assessment of removal torque, or histological observation by biopsy and calculating bone-to-implant contact (BIC) would be most accurate[2,12]. However, since this method cannot be clinically applied to patients, other measuring tools are needed to determine the time point to load. Periotest™ value (PTV; Siemens AG, Benssheim, Germany) and resonance frequency analysis (RFA; Osstell Mentor, Integration Diagnostics AB, Göteborg, Sweden) have been used to measure the mechanical stability of the implant, and both are easily applicable noninvasive tools of measurement in clinic[13-15]. Some studies reported the detectability of PTV on bone resorption and correlationship with the degree of BIC[16,17]. However, there are wide range of variations according to the implant position and length, measuring angle, the distance between the implant and probe as well as host bone quality[18]. As another option, RFA has been clinically used,

which is displayed as the implant stability quotient (ISQ) from 1 to 100. It is known that values less than 45 indicate failure of the implant, whereas an ISQ value of about 60 to 70 indicates success[19].

Although both are useful as noninvasive tools to assess the implant stability, they have some limitations. PTV has wide range of variations in sensitivity according to the implant position and length, measuring angle, the distance between the implant and probe as well as host bone quality[18]. ISQ value may give information regarding success but cannot provide information whether implant can survival or not[20]. In this context, these devices alone might be inappropriate to predict exactly whether osseointegration is sufficient to bear screw tightening force and occlusal load or not. Even if the devices exhibited that the stability of implants is fine to load, clinical situation that premature excessive tightening of screw resulting in rotation of the implant by breaking osseointegration can be encountered. In this situation, clinicians have to decide whether the disintegrated implant should be in place or removed. Implant mobility has been divided three categories: 1) rotation mobility; 2) lateral or horizontal mobility; and 3) axial or vertical mobility[21]. Horizontal and vertical mobility generally means that the implant is encapsulated by fibrous tissue, which is regarded as implant failure to be removed[22]. On the other hand, if there was no sign of implant failure like inflammation and abnormal radiographic radiolucency during healing period, and acute rotational mobility occurs after screw tightening, clinicians are hard to decide whether to wait for re-integration of the implant or to remove

rotational implants.

The purpose of this study was to evaluate the re-osseointegration of the implants reinserted following mechanical breaking of osseointegration in dogs.

Ⅱ. Materials and methods

1. Experimental animals

Five male mongrel dogs, 18–24 months old and weighing about 30 kg, were used. All of the dogs had intact dentition and a healthy periodontium. Animal selection, management, and preparation, and the surgical protocols followed routine procedures approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

2. Implant design and surface

All implants (Haptite®, Dentis Co., Daegu, Korea) used in this study were provided by a courtesy of the Medi M institute and Dentis corporations. Thin hydroxyapatite (HA) layer (0.1–5 μm) was coated on the resorbable blast medium surfaced titanium implant surface using a super-high-speed blasting method[23]. The mean surface roughness was 1.83 μm. The implants were microthreaded in coronal part with 3.7 mm diameter and 8 mm in length.

3. Experimental design

A total of ten HA coated implants were bilaterally placed, which were divided into the experimental group and the control. All implants were inserted at the oversized drilled socket according to the previous experimental protocol[8]. The implants were submerged under unloaded circumstance for 8 weeks in the control group. On the other hand, in the experimental group, the implants were mechanically anti-rotated to break the immature osseointegration at 4 weeks postoperatively, and were repositioned to be healed without loading for another 4 weeks (figure 1)

4. Surgical procedures

Tooth extraction for induction of edentulous ridge and protocol of the loosened implant placement followed the previous experiment[8]. Briefly, following the extraction of all mandibular premolar, the edentulous alveolar ridges were allowed to heal for 8 weeks. A midcrestal incision and full-thickness mucoperiosteal flap was bilaterally made on the edentulous mandible. Implant site was prepared with a final oversized drill that was the same size as the fixture (3.7 mm diameter). The fixtures were manually inserted at the widened sockets in both sides of the mandible, and exhibited rotational and axial mobility by manually applied force. After connection of a healing abutment, PTV was measured. After measurement, the healing abutment

was replaced with a cover screw. ISQ value was not able to measure due to the error of the device, since the measuring device cannot be properly tightened to the loosened fixture. The flaps were sutured with a 4-0 resorbable suture material (Monosyn 4.0 Glyconate Monofilament, B. Braun, Tuttlingen, Germany), and the implants were submerged. The sutures were removed after 10 days.

After 4 weeks of healing, breaking procedure was performed on the right side of the mandible. After a midcrestal incision and flap reflection, the implant was exposed and the ISQ value and PTV were measured. Then, the implant was loosened by applying a reverse torque with a ratchet. After the retrieved implant was returned to its original position, the ISQ value and PTV were measured, again. The flap was sutured and the implant was submerged again to be allowed to heal for 4 more weeks. On sacrifice of the animal, the implants were exposed to measure the ISQ value and PTV. Block sections that included segments of the implants were dissected for histological analysis.

5. Specimen preparation

The block sections were fixed in 10% neutral buffered formalin for 10 days. They were then dehydrated in ethanol, embedded in methacrylate, and sectioned in the mesiodistal plane using a diamond saw (Exakt, Apparatebau, Norderstedt, Germany). From each implant site, the central section was reduced to a final thickness

of about 20 μm. The sections were stained with hematoxylin-eosin. Histological analysis was performed using a microscope (DM-LB, Leica). After conventional microscopic examination, histometric measurements were made using an automated image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). Bone-to-implant contact (BIC; %) was calculated on the implant surface of the coronal micro threads and the apical macro threads.

6. Statistical analysis

The statistical analysis was performed using commercially available software program (SPSS 15.0; SPSS, Chicago, IL, USA). Histometric records from the calvarial defect samples were used to calculate the means (±SDs) of the group.

The data were examined with the Kolmogorov-Smirnov test for conformance to a normal distribution. Paired t-test was used to compare between control and experimental group, and to analyze the difference in parameters between baseline and 8 weeks healing period, and including osseointegration-breaking time point in experimental group.

Ⅲ. Results

1. Clinical findings

Surgical wound healing was uneventful during the experimental period, with no complications including wound dehiscence, severe swelling, or bleeding observed. All implants were well maintained during the postoperative periods.

2. RFA value (ISQ) and Periotest value (PTV)

In experimental group, the mean ISQ value was 77.00 at 4 weeks after implantation. After breaking procedure, the mean ISQ value decreased to 70.00 and then increased to 73.80 after the next 4 weeks of healing. The mean ISQ value in control group at 8 weeks after implantation was 68.80. The mean ISQ value of the experimental group showed significantly higher value than that of the control group ($p = 0.006$). ISQ values are shown in table 1.

In experimental group, the mean PTV was 22.20 at the time of implantation. At 4 weeks after implantation the mean PTV decreased to -3.80 and after breaking procedure the mean PTV increased to -0.80. After the next 4 weeks of healing, the mean PTV decreased to -5.40. In control group, the mean PTV at the time of

implantation was 12.60 and decreased to -5.20 after 8 weeks healing period. The differences in parameters between at baseline and at the end point were statistically significant both experimental group and control group ($p < 0.026$). In experimental group, the mean PTV at 'before breaking' and at the end point was statistically significant difference from at 'after breaking' ' ($p = 0.028$). The mean PTVs at the end point were similar and were not statistically significant between the experimental and the control group. PTVs are shown in table 2.

3. Histologic findings and histometric analysis

In histologic findings, we can see the mature bone lining the implant surface in the experimental group (fig. 4a-b, 5a). When the implant was retrieved, it was found that the groove between the threads was partly filled with bony substance. This bony substance formed by process of contact osteogenesis and had remodeled after 4 more healing period. And newly formed woven bone was found between lining bone to implant surface and the bone cut edge (fig. 4a-b, 5a). Primary spongiosa including trabecule and woven bone was present around the vascular units (fig. 4b, 5b). Osteoblasts lining the bone trabeculae and osteocytes were found within newly formed woven bone (fig. 4b, 5b). This may indicate that this new bone formed after breaking procedure. In the control group, we can see the borderline of the drilling margin which was present away from the tip of the implant threads (fig. 7a). Bone

apposition on the implant surface and newly formed bone extended from the cut bone surface filled the chamber (fig. 7a). The chamber was occupied with mature bone in microthread area (fig. 7a) and included also areas of bone marrow in contact with macrothread surface (fig. 7b,c).

The results from the histometric evaluation are presented in Table 3. The experimental group showed significantly higher BIC mean value than that of the control group ($p < 0.05$).

IV. Discussion

The complex healing process on the implant surface following placement at the drilled socket has been thoroughly investigated[24,25]. Through a series of bone formation and maturation, rigid stability of the implant endurable a certain limit of load would be established. Mechanical engagement and press-fit at implant installation to stabilize the implant until completion of sufficient biologic union, have been considered as a prerequisite for osseointegration[26-28]. Therefore, the primary stability is a critical factor to determine the loading time. It has been recommended that unstable implant at placement should extend the unloaded healing time[6]. If premature excessive loading over bearing capacity is exerted, the immature osseointegration between implant and bone might break and the implant might rotate. In such a situation, the decision has been empirically made due to lack of evidence. In the present study, this clinical situation was simulated using the rotationally mobile implant in canine model[8], and the fate of the disintegrated implant was histologically evaluated. Four weeks of healing period was allowed to simulate the immature weak osseointegration. Jung et al. demonstrated comparable BIC with the control group at the same animal model[8].

In the present study, even at 4 weeks, the implants were successfully integrated into the bone bed, and exhibited favorable ISQ and PTV as consistent with the

previous study, in spite of lack of primary stability[8]. When the implant was retrieved by manual counter-torque, high resistance was felt. When the implant was reinserted, it was still unstable, and manually rotated. Nevertheless, following another 4 weeks of unloaded healing, all implants exhibited high ISQ and low PTV. The mean of PTV at the end point were similar and were not statistically significant between the experimental and the control group, but the mean of ISQ of the experimental group showed significantly higher value than that of the control group.

Interestingly, in terms of BIC, the experimental group was significantly higher than the control. When the implant was retrieved, it was found that the groove between the threads was partly filled with bony substance (figure 2). It might explain the reason of higher BIC in the experimental group. In the thread region, the bonding strength between bone and implant might be stronger than strength of the trabecular bone. If the bone fragment attached implant is repositioned, bony connection would be reestablished between the preexisting bone on the implant and bone bed, and new bone apposition would additionally take place on the vacant implant surface. This assumption is supported by the 'regional acceleratory phenomenon' that noxious stimuli like fracture and crush injury can promote osseous wound healing as a defensive mechanism[29]. It is expected that bleeding following disintegration of the implant might stimulate de novo bone formation[27].

Implant surface would influence the enhanced BIC during healing. Ivan et al. demonstrated that machined surfaced implants loosened by a reverse torque can be

reintegrated in a rabbit tibia[30]. All implants were placed in normal bone with mechanical engagement and were healed for 6 weeks. In general, the phylogenically lower animal has faster healing potential than the higher one. In the present study using a thin HA coated implant, 4 weeks of healing period was observed in dogs. In spite of relatively short period, it showed excellent mechanical and histological osseointegration. It might be attributed to the HA surface coated by a novel method, a SHS blasting process at a room temperature. Special interest has been paid to HA coating of dental implants, due to the compositional similarities between HA and natural bone and its biocompatibility with bone. However, the bonding strength to titanium surface, uniformity in thickness, and crystallinity are various according to the coating method. The coating technique used in the present study was developed to optimize the surface conditions of the implant to enable rapid secondary stability. It demonstrated that uniformly thin thickness (1 to 2 μm) of HA layer without deformation of its original roughness maintain was not exfoliated following removal of the firmly osseointegrated implant[23]. In addition, a high crystallinity (>95%) without amorphous HA and excellent wettability might promote osseointegration[23,31,32]. Therefore, moderately rough HA layer remained following removal of the implant would be associated with the results.

Within the limitation of small sample size, it is concluded that the mechanically broken osseointegration can be successfully reosseointegrated, after unloaded and submerged healing for a certain period.

V. Conclusion

Within the limitation of this study, it is concluded that the mechanically broken osseointegration can be successfully reosseointegrated, after unloaded and submerged healing for a certain period.

Tables

Table 1. ISQ values in experimental group and control group

(n=5, mean ± standard deviation)

	4 weeks		8 weeks
	Before breaking	After breaking	
Experimental	77.00 ± 5.15	70.00 ± 6.56	73.80 ± 5.85[§]
Control	NA	NA	68.80 ± 5.02

[§] Statistically significant difference from control group ($p = 0.006$)

Table 2. Periotest values in experimental group and control group

(n=5, mean ± standard deviation)

	Baseline	4 weeks		8 weeks
		Before breaking	After breaking	
Experimental	22.20 ± 16.41	-3.80 ± 1.79[§¶,]	-0.80 ± 2.28	-5.40 ± 0.89[§,¶]
Control	12.60 ± 2.61	NA	NA	-5.20 ± 0.84[§]

[§] Statistically significant difference from baseline ($p < 0.026$)

[¶] Statistically significant difference from 'after breaking' ($p = 0.028$)

Table 3. Bone-to implant contact percentage (BIC%) for microthread, macrothread and total area after 8 weeks healing period.

(n=5, mean ± standard deviation)

%	**Experimental**	**Control**
Microthread	64.35 ± 26.21[§]	45.48 ± 14.62
Macrothread	66.15 ± 20.33[§]	35.05 ± 15.75
Total	65.19 ± 22.48[§]	39.53 ± 13.67

[§] Statistically significant difference from control group ($p < 0.05$)

LEGENDS

Figure 1.

Schedule for surgical procedure

Extraction - 8 weeks healing period - implant placement

Experimental group (Right) : 4 weeks healing period - Breaking procedure

- another 4 weeks healing period

Control group (Left) : 8 weeks healing period

Figure 2.

The retrieved implant : The groove between the threads was partly filled with bony substance

Figure 3.

Overview of the ground section (mesio-distal plane) and depicting the landmarks for the histometric analysis: Mi- microthread area; Ma- macrothread area; TL- total area (original magnification x 12.5)

Figure 4.

Histologic findings from microthread area of breaking group

a) original magnification x 50, b) original magnification x 100

arrowhead = osteoblast; black star = osteoid; white star = newly formed woven bone

Figure 5.

Histologic findings from macrothread area of breaking group

a) original magnification x 50, b) original magnification x 100

arrowhead = osteoblast; black star = osteoid; white star = newly formed woven bone

Figure 6.

High magnification (x 200) from macrothread area of breaking group

a) Histologic photomicrograph from macrothread area of breaking group (x 200)

b) Polarized photomicrograph from macrothread area of breaking group (x 200)

arrowhead = boderline between lamellar bone and woven bone

Figure 7.

Histologic findings of control group

a) Histologic photomicrograph from microthread area of control group (x 50)

b) Histologic photomicrograph from macrothread area of control group (x 100)

c) Polarized photomicrograph from macrothread area of control group (x 100)

arrowhead = line demarcating drilled margin

Figures

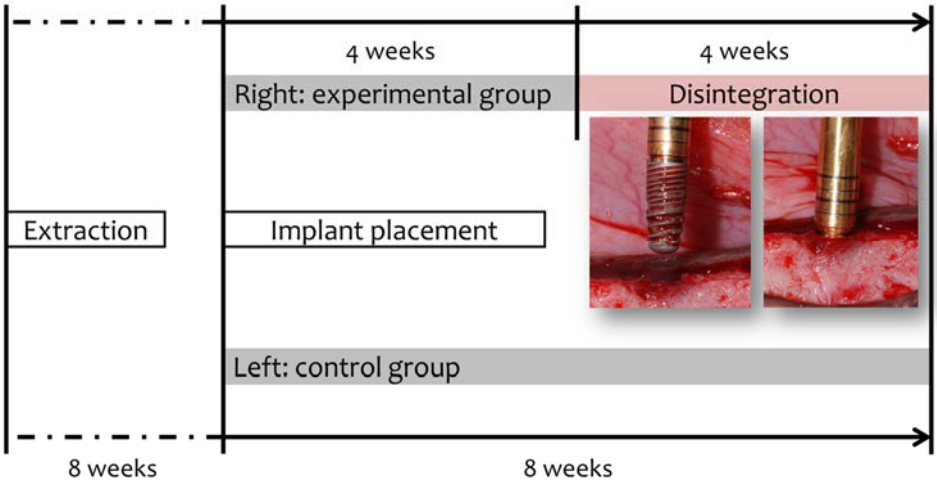

Figure 1

Figure 2

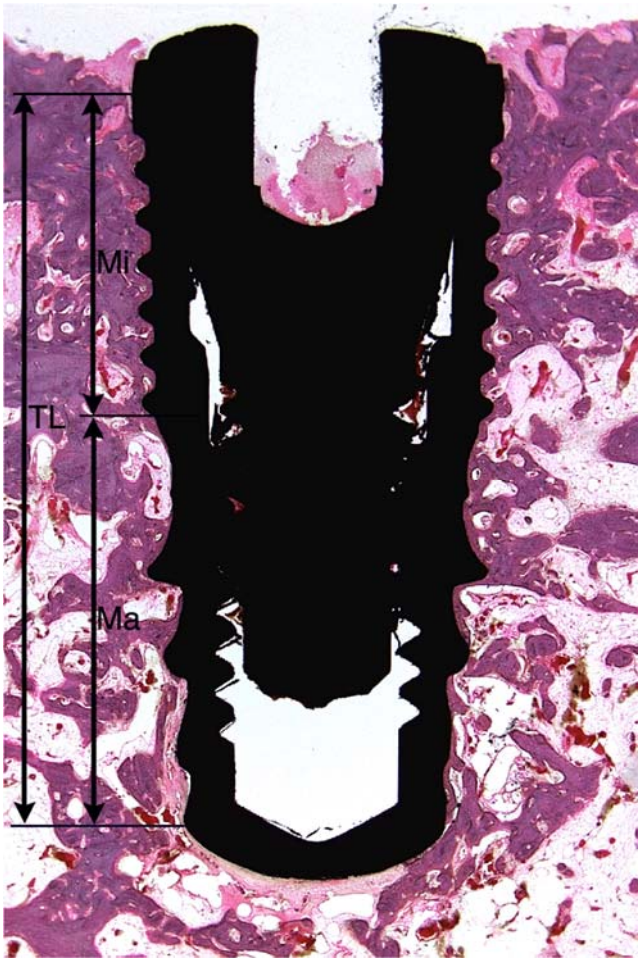

Figure 3

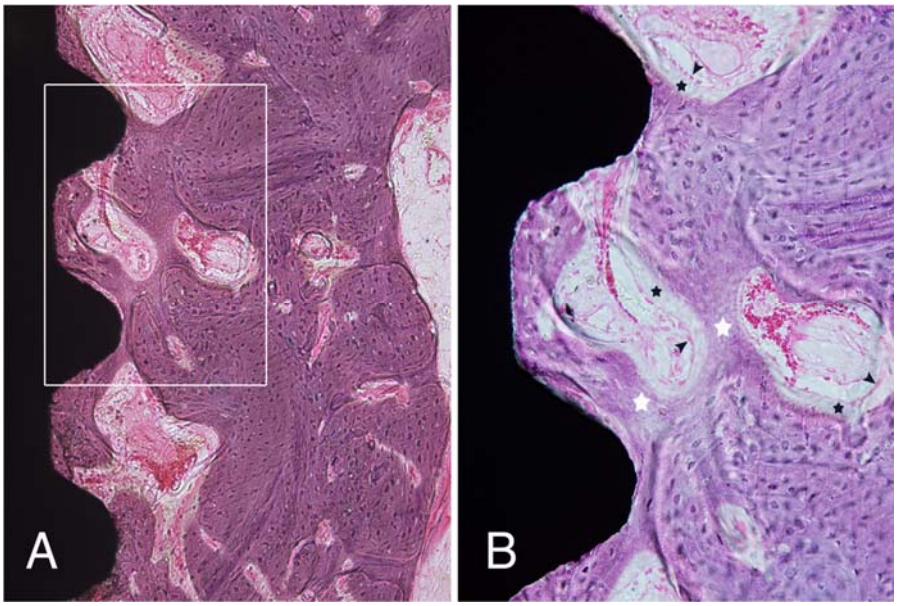

Figure 4

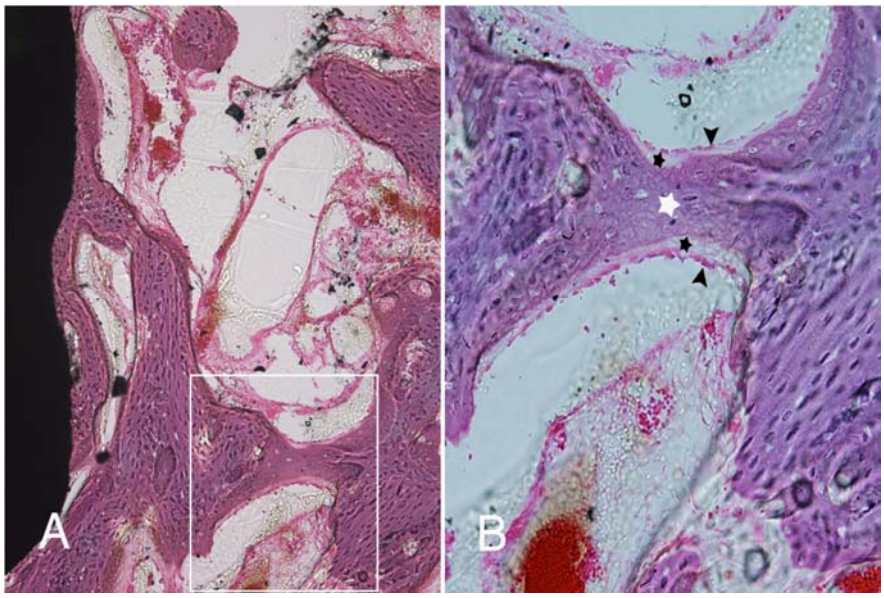

Figure 5

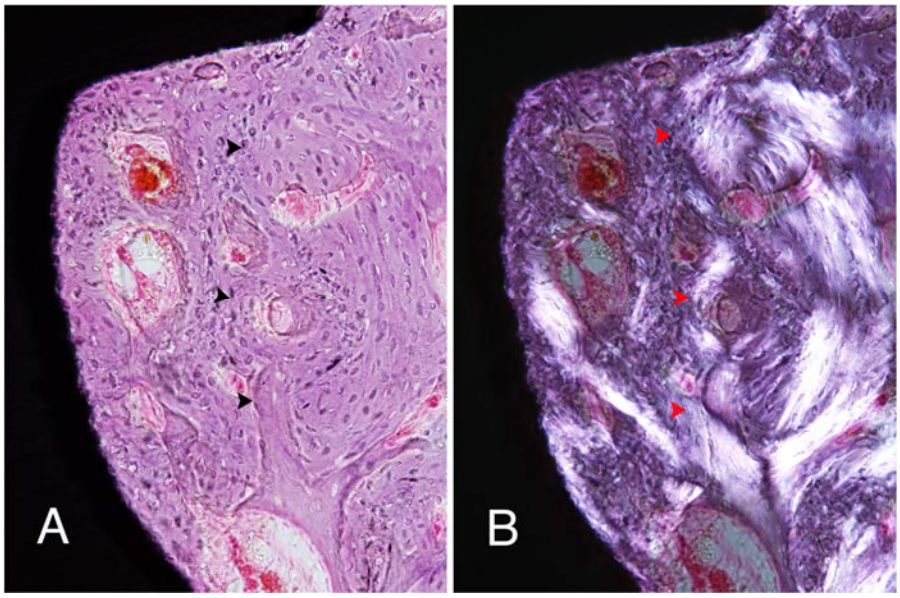

Figure 6

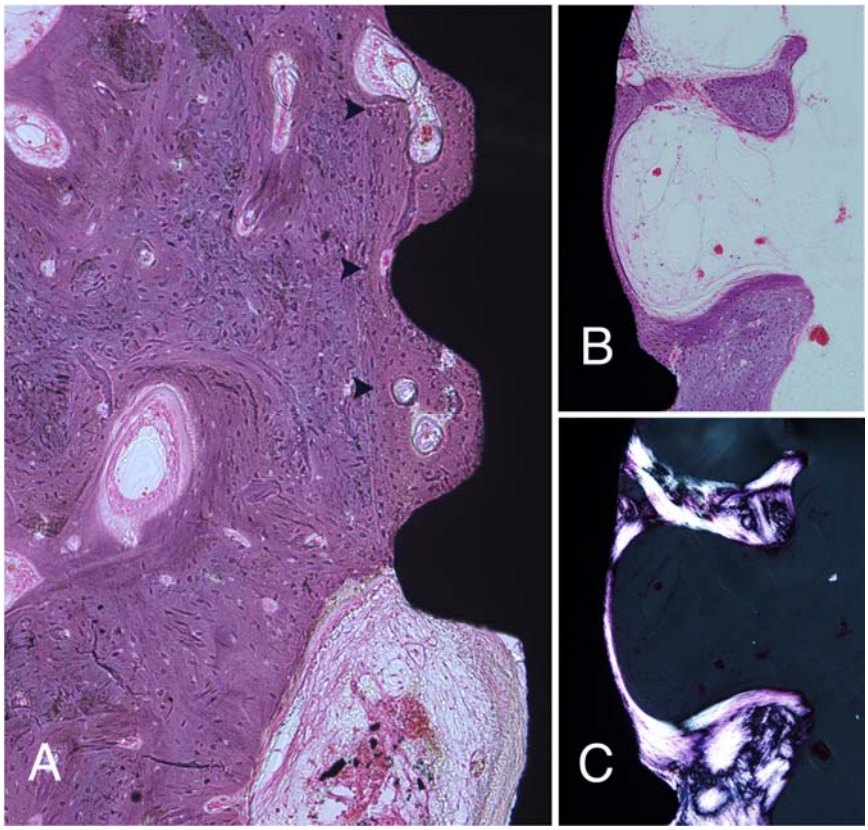

Figure 7

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국문요약

성견에서 기계적인 힘에 의해 분리된 임플란트의 재유착 : 조직계측학적 분석

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이 진 우

이 실험의 목적은 임플란트가 기계적인 힘에 의해서 골유착이 깨진 후, 그 임플란트를 다시 재위치시켰을때 임플란트와 골이 재유착하는지에 대해서 성견에서 실험을 통해서 평가하는 것이다.

5마리 개의 제2소구치 부위 양쪽으로 큰 직경의 드릴링을 시행하여 회전하며 움직이는 임플란트를 식립하였다. 4주의 치유기간 후에 실험군에서 의도적으로 기계적인 힘을 가하여 미성숙한 골유착을 깬 후 같은 자리에 임플란트를 재위치시켰다. 반면 대조군은 어떤 외과적인 시술 없이 매몰시킨 채로 유지되었다. 동물들은 골유착을 깨는 시술 4주 후에 안락사시켰다.

임플란트 안정성 지수와 페리오테스트 수치의 변화가 분석되었으며 임

플란트와 골의 접촉정도 (BIC %)가 조직계측학적으로 분석되었다.

실험의 마지막에 측정된 실험군과 대조군에서의 페리오테스트 수치의 평균값은 유사하였으며 통계학적으로 유의한 차이가 없었다. 그러나 임플란트 안정성 지수와 BIC 값에서는 실험군이 대조군보다 통계학적으로 유의할 정도로 큰 값을 보였다.

한계를 가진 본 연구를 통해서, 기계적인 힘에 의해 깨진 골유착은 어느 정도의 기간 동안 외력이 가해지지 않도록 유지된 채 치유된다면, 성공적으로 재유착이 일어난다는 결론을 얻을 수 있었다.

핵심되는 말: 임플란트의 안정성, 골유착의 깨짐, 움직이는 임플란트, 재삽입, 재유착