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# Histomorphometric analysis of an immediate non-functional loaded implant in dogs

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성견에서 비기능적 즉시부하후 임플란트 주위 조직형태계측학적 분석

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이 논문을 치의학 박사학위신청 논문으로 제출함.

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## Contents

Contents of Table ii
Contents of Figures iii
Abstract iv
I. Introduction 1
II. Materials & Methods 4
III. Results 8
IV. Discussion 12
References 16

## Contents of Table

Table 1. Experimental schedule

----- 7

Table 2. Histomorphometric results

----- 11

### Contents of Figures

**FIGURE 1.** Photomicrograph of Group 1. Woven bone is seen filling the peri-implant space. A small part of the implant is exposed in the right upper corner (arrow) (Villanueva osteochrome stain, original magnification X40).

----- 8

**FIGURE 2.** Photomicrograph of Group 2. Woven bone filled the peri-implant space. Foci of insufficient new bone formation are seen in the left-middle and right-lower portions of the implant (arrows) (Villanueva osteochrome stain, original magnification X40).

FIGURE 3. Photomicrograph of Group 2. Woven bone filled the peri-implant space. A focus of insufficient new bone formation is seen in part of the right portion of the implant (white arrows). The uppermost part of the implant (black arrows) is exposed (Villanueva osteochrome stain, original magnification X40).

국문초록

성견에서 비기능적 즉시부하후 임플란트 주위 조직형태계측학적 분석

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본 연구의 목적은 성견에서 비기능적 즉시부하후 임플란트 주위 조직형태학적 분석을 통 하여 즉시 부하의 효과를 알아보는 데 있다.

생후 8-9개월된 체중 12Kg 내외의 잡종 성견 5마리를 대상으로 하였다. 건강상태는 모두 양호하였으며, 동일조건하에 사육하였고 암수 구별없이 실험대상으로 사용하였다. 모든 성 견에서 하악의 우측을 Group 1(대조군)으로 지연 부하를, 좌측은 Group 2(실험군)으로 비기 능적 즉시 부하를 시행하였다. 직경 3.5mm, 길이 11mm인 RBM 처리된 double thread 형태의 임플란트(AVANA®, Busan, Korea)를 사용하였다. 식립 갯수는 1마리당 실험군과 대조군 4개 씩 총 40개를 식립하였다. 식립후 abutment로 cemented type을 이용하였다.

Group 1의 경우에는 88.23%의 골생성률을 보였고, Group 2의 경우에는 86.41%의 골생성율 을 나타냈으며, Group 1과 Group 2 간의 골생성률에 따른 통계학적인 유의성은 관찰되지 않 았다.

지연부하군과 즉시부하군의 신생골 형성능의 차이가 없어 임플란트 매식후 조기부하를 하 여도 무방한 것으로 판단된다. 이러한 치료 개념은 적절히 선택된 환자에서 성공적으로 이 용될 수 있으리라 사료된다.

iv

#### Introduction

Dental implantation has been benefited greatly from research into the biological characteristics of titanium. According to the existing protocol, to obtain predictable bone adhesion, loading should be avoided while the subperiosteum heals during two-stage surgery. However, the discomfort and anxiety that occur during the healing period are issues that must be resolved for both the physician and patient. Therefore, attempts have been made to apply immediate loading after surgery with the extensive support of clinicians.<sup>1</sup>

We are faced with two conflicting approaches to dental implantation: the traditional method requiring a long healing period and the ultrafast method of applying early loading. Although only a few years ago, immediate or early loading was considered possible only in areas with good bone quality, such as the anterior portion of the mandible, early loading is possible with a single implant even in areas with poor bone quality, such as the posterior portion of the maxilla. We therefore need to reevaluate the Branemark protocol.<sup>1</sup>

A healing period without loading is still considered a prerequisite for implant integration,<sup>2</sup> which leads to extended treatment periods, often with delayed functional improvement for the patient.<sup>3-6</sup> The elimination or reduction of the postsurgical interval between implant placement and implant loading is a

challenge for dental implantology. Early loading has been found to induce micromotion at the bone–implant interface, which may lead to fibrous encapsulation instead of direct bone apposition.<sup>4,5</sup>

Implants that were loaded immediately and implants that were not loaded immediately have been compared histologically in animal studies.<sup>7</sup> The histological findings from one animal study demonstrated that immediately loaded blade implants with hydroxyapatite (HA)-coated surfaces, when splinted together with teeth, form a direct bony interface similar to delayed loaded HA-coated blade implants in nonhuman primates.<sup>8</sup> Other study in dogs has found less BIC in the immediately loaded implant in comparison to delayed-loaded implants.<sup>9</sup>

Although great interest exists in the histological findings at the implant interface after immediate functional loading in humans, such analyses are very rare because of the few opportunities to examine the histology of implants retrieved from humans. The removal of implants following fracture or for other reasons (orthodontic and psychological) can provide extremely important scientific data on the bone response at the interface after different periods of healing. Furthermore, such histological data demonstrate that different kinds of implant system using various surfaces inserted in bone of different quality in the maxilla or mandible can be equally successful in establishing osseointegration. The biomechanical response of bone during loading is dependent on implant shape (cylindrical or tapered) and thread geometry, in addition to bone quality. A necessary requirement for long-term success after immediate functional loading is the primary stability and immobilization of the implant during and after surgery. The bone response at the interface of immediately loaded implants placed in humans is of clinical importance before adopting this treatment concept in routine daily practice.<sup>7</sup>

The purpose of this study was to investigate the effects of immediate non-functional loading by analyzing histomorphology around the implant tissues in dogs.

#### Materials and Methods

#### STUDY ANIMALS

Five eight- to nine-month-old full-grown dogs weighing around 12 kg were used in the study. They were all healthy and reared under the same conditions. Animals of both genders were selected. Group I (control group) comprised those in which delayed loading was applied to the right side of the mandible, and Group II (experimental group) consisted of dogs in which immediate loading was performed on the left side of the mandible.

#### ANESTHESIA

After the dog was placed under general anesthesia with an intramuscular injection of 0.4 mg/kg xylazine (Rompun<sup>®</sup>, Bayer-Vetchem, Seoul, Korea) and 0.15 ml/kg ketamine (Ketara<sup>®</sup>, Yuhan, Seoul, Korea) in the thigh, 2% lidocaine was used to prevent bleeding and pain at the implant site.

#### **EXTRACTION**

Forceps were used to extract the 2<sup>nd</sup> to 4<sup>th</sup> premolars and 1<sup>st</sup> molar in the mandible in the control and experimental groups. The residual root and granulation tissue were removed completely at the time of extraction. A 12-week healing period was allowed in the control and experimental groups. The extraction in the experimental group was done 8 weeks after the extraction in

the control group to match the loading period. Suturing was not performed in all groups. Gentamicin (0.09 ml/kg) was injected intramuscularly for 5 days in all animals after extraction and implant placement to prevent infection.

#### IMPLANTS

Resorbable blast media (RBM)-treated double-threaded implants (Osstem Implant<sup>®</sup>, Busan, Korea) measuring 3.5 mm in diameter and 11 mm long were used in the study. Each animal received four implants in each group, for a total of 40 implants. Cemented type abutments were used after implantation.

#### IMPLANT PLACEMENT AND LOADING

Anesthesia was administered using the same method in all animals. The tissues were dissected to lift a periosteal flap using a periosteal elevator. Four implants were placed in each group. A cover screw was used to delay loading in the control group. After dental implantation, black silk was used for suturing. After radiography, gentamicin was administered to prevent infection. An 8-week period was allowed for bone healing and an abutment was placed after exposing the periosteum for loading. The same method was used in the experimental group, with an abutment placed for early loading following suturing. A temporary prosthesis was prepared using temporary resin. Occlusion was adjusted in all groups to avoid direct contact with the implants (Table 1). Histological and histomorphometric evaluation

An implant sample was obtained from bone blocks taken when the dogs were killed at 16 weeks after loading. The bone block including the implant portion was fixed in neutral formalin for 6 days immediately and then dehydrated in alcohol. It was embedded in glycolmethacrylate resin (Spurr's low-viscosity embedding media; Polyscience, Harrington, PA, USA). The polymerized sample was cut using a high-precision diamond disk (low-speed diamond wheel saw 650; SBT, San Clemente, CA, USA) in the axial direction of the implant in 200-µm slices. Finally, 30-µm-thick slices were prepared using a lapping and polishing machine (OMNILAP 2000; SBT). One slice of each implant was stained with Villanueva osteochrome bone stain (SBT) and observed under an optical microscope (BX50; Olympus, Tokyo, Japan).

#### Statistical analysis

A Mann–Whitney U-test was performed to evaluate statistical significance. Student's *t*-test was used for the histological evaluation. Values less than 0.05 were considered statistically significant.

Table 1. Experimental schee	dule
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Group		Schedule			
Group 1	12-week	Implant	Osseointegration	Load	Killed at 16
(Control)	healing period	placement	8 weeks	application	weeks
Group 2	8 weeks	12-week	No	Implantation	Killed at 16
(Experimental)	Extraction	healing period	osseointegration	and loading	weeks

#### Results

#### **GROUP** 1

Abundant new bone formation was observed with woven bone closely surrounding the implant screw. The implant was exposed in the top right (arrow; Fig 1).



**FIGURE 1.** Photomicrograph of Group 1. Woven bone is seen filling the peri-implant space. A small part of the implant is exposed in the right upper corner (arrow) (Villanueva osteochrome stain, original magnification X40).

#### GROUP 2

Abundant new bone formation was observed with woven bone closely surrounding the implant screw. The new bone formation was insufficient in the left middle and lower right portions (arrow; Fig 2). The implant was exposed overall (black arrow; Fig 3).



**FIGURE 2.** Photomicrograph of Group 2. Woven bone filled the peri-implant space. Foci of insufficient new bone formation are seen in the left-middle and right-lower portions of the implant (arrows) (Villanueva osteochrome stain, or iginal magnification X40).



**FIGURE 3.** Photomicrograph of Group 2. Woven bone filled the peri-implant space. A focus of insufficient new bone formation is seen in part of the right portion of the implant (white arrows). The uppermost part of the implant (black arrows) is exposed (Villanueva osteochrome stain, original magnification X40).

#### Histomorphometric result (Table 2)

The bone formation ratio in Groups 1 and 2 was 88.23 and 86.41%, respectively. No significant difference in new bone formation was observed in the two groups.

### Table 2. Histomorphometric results

Rate of new bone formation (%)
$88.23 \pm 7.15$
$86.41 \pm 9.63$

P > 0.05 (0.615)

#### Discussion

The success with dental implants in the mandible suggests the possibility of other surgical and prosthodontic treatments.<sup>10</sup> Patients prefer implants that allow early or immediate loading because they want both an early recovery of mastication and hastened cosmetic recovery.

Immediate loading has recently become one of the main research topics in restorative dentistry. Following the developments of the immediate loading concept by Ledermann et al,<sup>11</sup> a variety of related clinical approaches have been reported.<sup>12-14</sup> The main objective of immediate loading is to achieve high mechanical stability, while avoiding micromovement during the course of osseointegration.<sup>15</sup> The success of immediate loading depends on bone quality and quantity, as well as on the healing potential of bone. With low bone density, the implant should be fixed by waiting for active formation of the implant-sheath bone with no loading for 3 to 6 months post-implantation.<sup>16</sup>

The development of mineralized bone at the interface of immediately loaded implants depends on two key factors. First, micromovement of the implant must be avoided to create a static environment for bone healing. Second, the appropriate biological principles must be used to avoid the formation of connective tissue and to achieve close bone-to-implant contact.<sup>17-19</sup>

The Periotest value (PTV) is a useful parameter for predicting the micromotion of implants and natural teeth. A histological investigation of beagle dogs

- 12 -

suggested that the immediately loaded implant in poor-density bone is more successful when it is splinted to a proximal tooth or an implant with a PTV less than +5 is fixed firmly and the micromotion is less than 30  $\mu$ m. The compromised health of the implant is restored to the optimum condition by splinting it to a healthy proximal tooth. When the implant is supported with cortical bone (buccal, lingual, and basal) immediately after installation, the implant is fixed in the alveolar bone rigidly when the micromotion is less than 30  $\mu$ m. The bone contact-free area becomes covered with implant-sheath bone, even with immediate loading.<sup>16</sup>

Regarding immediate loading protocols, Akagawa et al<sup>20</sup> and Sagara et al<sup>9</sup> compared early loaded screw implants with non-loaded implants in preclinical models. Although not quantified, both studies reported greater marginal bone loss for the early loaded group after 3 months of loading. It was suggested that the high crestal bone loss was a specific feature of the early loading protocols.

Romanos et al<sup>7</sup> reported that immediately loaded implants retrieved from humans showed a high percentage of BIC after loading periods of 2 and 10 months in a histological and histomorphometric analysis. This observation was independent of the implant system used, and the implants examined had a screw-geometry and rough surfaces designed to promote new bone formation at the initial stages of healing during loading. According to the literature, an osseointegrated, clinically stable implant is associated with a BIC of at least 25%.<sup>21</sup> This is dependent on the implant form, the surgical procedure, the loading forces involved, and the loading period. Therefore, we investigated the rate of peri-implant bone formation rather than the rate of BIC. The rate of peri-implant bone formation was the area of newly formed bone divided by the area outside the implant thread, expressed as a percentage.

Romanos and Nentwig<sup>22</sup> used Ankylos implants in monkeys and reported that the rate of BIC did not differ significantly between no-load and immediate-load groups at 50.20 and 64.25%, respectively. Piatelli et al<sup>17</sup> found similar results in monkeys fitted with titanium plasma spray (TPS)-coated implants, in which the rate was 55.8% in the no-load group and 75.0% in the immediate-load group. Zubery et al<sup>23</sup> obtained rates of 58.4 and 50.0% in no-load and immediate-load groups, respectively, in dogs fitted with interim implants.

Because the rate of bone metabolism in animals is about twice that in humans,<sup>24</sup> caution should be exercised when applying our results to humans.<sup>25</sup> Froum et al<sup>26</sup> reported a BIC percentage of 40 to 72% in humans. Ledermann et al<sup>11</sup> also reported high percentages of BIC (70–80%) after examining four immediately loaded mandibular implants retrieved from a person after 12 years of function.

In this study, we obtained rates of new bone formation of 88.23 and 86.41% in the control and immediate loading groups, respectively. The difference between the two groups was not significant. As no significant difference was seen in new bone formation between the delayed and immediate loading groups, early loading might be possible after implant placement. This treatment concept may prove successful for daily clinical practice in properly selected cases.

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