2011년 2월 석사학위논문 A comparative study on the bone formation capacity of autogenous tooth graft materials with toothash powder grafted to the tooth extraction socket of adult dogs 양 성 수

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목 차

표 목 차

도 목 차

국문초록

성견에서 발치와에 이식된 자가치아 이식재와 치아회분말의 골형성에 관한 비교 연구

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지도교수 : 문 성 용, DDS, MSD
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본 연구의 목적은 성견의 발치와에 최근에 개발된 자가치아 이식재와 치아회분말 이식재를 이식하여 자가치아 이식재와 치아회분말 이식재의 골 재생능력을 비교하고 자가 치아 이식재가 골 결손부에 이식재로 유용하게 사용될 수 있는지에 대해 평가하는데 있다.

성견 6마리를 대상으로 하였으며, 건강상태는 모두 양호하였고 치주조직은 염증이 없는 양호한 상태였다. 실험군은 이식재의 종류에 따라 3군으로 분류하였다; 발치와에 골 이식을 시행하지 않은 대조군 (n=12), 자가치아 이식재를 이식한 group 1 (n=12), 치아회분말 이식재를 이식한 group 2 (n=12), 희생시기에 따라 각각 4주군과 8주군으로 분류하였다. 평가는 Villanueva osteochrome bone stain을 시행한 후 광학 현미경(Olympus BX50, Tokyo, Japan)으로 관찰하였으며, 조직형태계측학적 분석을 시행하였다.

실험 1군(자가치아 이식재)에서는 4주에 발치와 주변으로 활발한 골형성이 관찰되었으며, 8주에는 이식재 주변으로 활발한 골형성이 관찰되었으며 신생골이 4주에 비해 증가하는 양상을 보였다. 실험 2군(치아회분말 이식재)에서는 4주에 발치와 변연부로 제한적인 신생골 형성이 관찰되었으며 발치와는 대부분 골이식재로 채워져 있었으며, 8주에는 4주군에 비해 이식재 주변 신생골이 증가하는 양상이 관찰되었다. 신생골 형성율을 비교한 결과 모든 군에서 4주에 비해 8주에서

유의성있는 증가가 관찰되었다.

이상의 결과로 미루어 보아 발치와에 골 재생술시 자가 치아 골 이식재가 다른 군에 비해 골형성이 우수하고 골형성 속도가 빠른 것으로 생각되었다.

Ⅰ. Introduction

For the reconstruction of the defective areas of hard tissues, autogenous bones, allogenic bones, xenogenic bones, and synthetic bones may all be used. The most commonly used bone graft material for hard tissue reconstruction is autogenous bones. Autogenous bones have been considered the gold standard of graft materials. Autogenous bone grafts form new bones through the three mechanisms: bone formation, osteoinduction, and osteoconduction; furthermore, they heal rapidly without rejection reactions. Nonetheless, they have shortcomings in that they require a second surgical area, they have a limited bone volume, bone resorption after the graft is unavoidable, and they cause a defect and discomfort in the donor area(1-3).

More recently, studies on the development of bone graft materials have been actively conducted, and demineralized freeze-dried allogenic bones, irradiated bones, autoclaved allogenic bones, and xenogenic bones have been used in clinics(4,5). Among these, the artificial synthetic material hydroxylapatite is a component consisting of the major skeletal structure of bones or teeth, and its biocompatibility and osteoconduction are good. Nonetheless, it has several shortcomings, notably in terms of preparation problems and high costs. In addition, in cases of powder-type materials alone, the restoration state of the bone defect area could not be stably maintained because of its fluidity (6-9).

Therefore, beginning in 1992, in efforts to improve graft's maintenance, to obtain raw materials readily, and to reduce costs, Kim

et al.(10-12) made efforts to develop another artificial bone substitute using a mixture of tooth ash and plaster of Paris. Tooth ash powder can be obtained readily from teeth and is composed primarily of hydroxyapatite; plaster of Paris can be obtained easily and sterilized readily, has a low cost, and can be completely absorbed rapidly. Various studies have shown that toothash was combined with plaster of Paris at appropriate ratios that could improve the stability of tooth ash powder, of which the major component is hydroxyapatite, to accelerate bone healing. This mixture can be used as graft materials for bone defect areas (13). Nonetheless, use of this preparation was difficult because of infection risks.

Based on the above studies, methods that prepare the teeth extracted from patients as autogenous tooth graft materials.

In this study, in adult dogs, the bone generation capacities of autogenous bone graft materials and tooth ash graft materials were compared by grafting recently developed autogenous tooth graft materials and tooth ash materials to the extraction sockets. We also evaluated whether autogenous tooth graft materials could be applied usefully as graft materials for bone defect areas.

Ⅱ. Materials and methods

1. Experimental materials

(1) Experimental animals

Six adult dogs, 12-months-old and weighing approximately 15 kg, were used regardless of sex. Their health condition was good, and their gingival tissues were in good condition, showing no inflammation.

(2) Bone graft materials

a. Autogenous tooth graft materials

Two weeks prior to performing the bone grafts, both maxillary premolar teeth of adult dogs were extracted, and the preparation of autogenous tooth graft materials was consigned to the Korea tissue bank (Seoul, Korea). The particle size of graft materials was 0.5-1.0 mm.

b. Tooth ash powder graft materials

Two weeks prior to performing bone graft, both maxillary premolar teeth of the adult dogs were extracted, prepared as ash in a 950 ℃ Furnace, prepared as powder 0.149 mm in size, and used.

2. Experimental methods

(1) Numbering of adult dogs

Each adult dog was numbered with its own proprietary number from 1 to 6.

(2) Extraction of maxillary teeth for the preparation of

graft materials

For the induction of anesthesia, tiletamin and zolazepam (10 mg/kg, Zoletil 50, Virbac Lab., France), which are analgesics and anesthetics for animals, as well as 2% zylaxine hydrochloride (3mg /kg, Rumpun, Bayer Korea Ltd., Korea) were separately injected intramuscularly. For the purpose of local hemostasis and suppressing pain in the extraction area, infiltration anesthesia was performed with 2% hydrochloride lidocaine (Yuhan Co. Ltd., Seoul, Korea) containing epinephrine (1:100,000). Subsequently, the roots of the right and left maxillary first, second and third premolars were separated using a Stryker engine with a hand piece (Stryker® Corp., USA) and a fissure bur; they were then extracted and sutured according to conventional methods. The extracted teeth were sorted as 1-6 according to the number of the dogs and stored. The extracted teeth were divided to two groups for the preparation of autogenous tooth graft materials and tooth ash powder graft materials. To prevent postsurgical infection in the surgery area, 1 ml gentamicin sulfate (0.1 ml/kg, Deasung Gentamicin inj., Deasung Microbiological Labs. Co., Ltd, Uiwangsi, Korea) was injected intramuscularly once a day for 5 days.

Storage and treatment of the extracted teeth

a. Preparation of autogenous tooth graft materials: The teeth extracted from the adult dogs were stored in saline according to the number of the adult dogs, and autogenous tooth graft materials 0.5-1.0 mm in particle size were prepared by the Korea tissue bank (Seoul, Korea).

Preparation of tooth ash powder: By combining the teeth of 5 adult

dogs (excluding autogenous teeth of adult dogs). The tooth ash powder was prepared by cleaning the teeth well with saline, preparing them as ash in a 950 ℃ furnace, and powdered 0.149 mm in size.

(4) Classification of experimental groups

Three experimental groups were created according to the type of graft materials: the control group without any bone grafts in the tooth extraction socket (n=12), group 1, grafted with autogenous tooth graft materials (n=12), and group 2 grafted, grafted with tooth ash powder (n=12). The experimental groups were further divided into 4-week groups and 8-week groups according to the sacrifice times. Experiments were performed in the right mandible for the 8-week groups and in the left mandible for the 4-week groups.

(5) Extraction of the mandibular teeth and transplant of graft materials to the extraction socket

After the 2 weeks required for the preparation of autogenous tooth graft materials and tooth ash powder, tooth extraction was performed in 6 adult dogs using a method identical to the extraction performed in the right mandibular premolar for the preparation of the graft materials, and graft materials were transplanted to the mandibular extraction socket (8-week experimental group). After 4 weeks, using a method identical to the 8-week group, the left mandibular teeth were extracted, and graft materials were transplanted to the extraction socket (4-week experimental group).

a. The control group

Bone grafting to the extraction socket of the mandibular first premolar was not performed.

b. Group 1

Autogenous tooth graft materials prepared from the teeth of adult dogs were grafted to the extraction socket of the mandibular second premolars matching the number of the adult dogs (i.e., autogenous tooth graft material #1 was transplanted to adult dog #1).

c. Group 2

Tooth ash powder graft materials prepared from the teeth (excluding autogenous teeth) were grafted to the extraction socket of the mandibular third premolar (i.e., tooth ash powder A, prepared with the teeth that excluded the autogenous material, were transplanted to the number 1 adult dog).

After bone grafting, sutures were performed using 4-0 vicryl (Ethicon, Johnson & Johnson, New Jersey, USA). To prevent infections in the surgical area, after surgery, 1 ml gentamicin sulfate (0.1 ml/kg, Deasung Gentamicin inj., Deasung Microbiological Labs. Co., Ltd, Uiwangsi, Korea) was injected muscularly once a day for 5 days.

(6) Sacrifice of experimental animals and preparation of samples

After sacrificing the animals, both jaw bones, including the adjacent bones, were extracted, fixed in formalin, and dehydrated by alcohol washing. The samples were embedded in glycol-metacrylate resin (Spurr Low-viscosity Embedding media, Polyscience, Earrington, PPA, ISA). The polymerized samples were cut into sections 200 μm in thickness using a high-precision diamond disc (Low speed diamond wheel saw

650, SBT, San Clemente, CA, USA), and ultimately, they were polished to 30 μm in thickness using a lapping and polishing machine (OMNILAP 2000, SBT, San Clemente, CA, USA). Slides were prepared, stained with Villanueva osteochrome bone stain (San Clemente, CA, USA), and examined under a light microscope (Olympus BX50, Tokyo, Japan).

(7) Histomorphometric measurements and statistical analysis

To evaluate the regeneration of the bony defects, the surface of the extraction and the newly-formed new bones were calculated as percentages (new bone formation rate; NBFR) and evaluated.

Statistical analyses were performed using SPSS (ver.16) and the Mann-Whitney test. For the comparison of each group according to the time points, the distribution of each value within a normal distribution was confirmed with a one-sample Kolmogorov-Smirnov Test and analyzed by applying a paired t-test. Statistical analyses were performed at 95% significance level.

Ⅲ. Results

1. Histological findings

(1) Control group

a. 4-week group

Formation of immature new bones was initiated in the vicinity of the extraction socket(Fig. 4).

b. 8-week group

Limited formation of new bones in the vicinity of the extraction socket was observed(Fig. 5).

(2) Group 1 (autogenous tooth graft materials)

a. 4-week group

Active bone formation in the vicinity of the extraction socket was observed(Fig. 6).

b. 8-week group

Active bone formation in the vicinity of graft materials was observed, and a pattern of increased new bone growth relative to the 4-week group was observed(Fig. 7).

(3) Group 2 (tooth ash powder graft materials)

a. 4-week group

Limited formation of new bones in the boundary of the extraction socket was observed. The extraction socket was mostly filled with bone graft materials, and bone formation in the vicinity of the graft materials was observed(Fig. 8).

B. 8-week group

In comparison with the 4-week group, a pattern increased new bones growth in the vicinity of the graft materials was observed(Fig. 9).

Histomorphometric comparative analysis

(1) The rate of new bone formation within the bony defect area

In regard to the new bone formation rate according to graft materials, in the 4-week group, new bone formation was significantly increased in groups 1 and 2 compared to the control group. Between group 1 and group 2, statistical significance in new bone formation was detected ($p<0.05$)

In the 8-week group, new bone formation was significantly increased in groups 1 and 2. Compared to group 2, new bone formation was significantly increased in group 1 ($p<0.05$).

Examining the rate of new bone formation according to the time points, in all groups, a pattern of increased growth in the 8-week groups compared to the 4-weeks groups was observed ($p<0.05$) (Table 1).

Table 1. New bone formation rate (NBFR) in extraction sites at 4 and 8 weeks (%)

	Control group	Group 1	Group 2
4 weeks	16.95±6.58	55.15±5.59[a,b]	30.25±2.61[a]
8 weeks	38.83±1.23[*]	67.43±12.33[a,b]	42.13±14.66[a]

[*]Statistically significant difference between 4-week group and 8-week group

[a]Statistically significant difference compared to the control group ($p<0.05$)

[b]Statistically significant difference compared to Group 2 ($p<0.05$)

Ⅳ. Discussion

In previous clinical studies, after the extraction of cysts, a mixture of tooth ash powder and plaster of Paris was grafted to the defect area and the follow-up observations have been reported for 10 patients (15,16). In animal experiments, a number of topics have been investigated, including comparisons with xenogenic bone graft materials (Bio-oss)(17), the healing process after grafting to the defect area in the vicinity of implants, applications in combination with platelet concentrations (18), the healing process of graft materials after the induction of osteoporosis (19), applications in combination with tissue adherence products (20), applications in combination with chitosan (21), and the healing process after osteoinduction regeneration surgery (22-24); through these and other studies, tooth ash powder has been confirmed to be a graft material with the good biocompatibility and good osteoinduction capacity. Nevertheless, its commercialization for clinical applications has been difficult because of various legal issues in Korea and other countries, including the absence of responsible administrative organizations (25).

More recently, methods have been developed to prepare the extracted autogenous teeth by up-to-date engineering techniques for bone grafts in identical patients. The genetic aspect of the extracted teeth of patient himself is identical. Thus, genetic and infection risks are absent, making this approach superior in terms of the patient's well-being and safety.

Kim et al.(26,27) analyzed the inorganic composition of autogenous tooth bone graft materials and reported the presence of HA excluding

Brushite: Ca/P=1.66, TCP(Tricalcium phosphate): Ca/P=1.50, ACP(Amorphous calcium phosphate): Ca/P=1.30-1.50, OCP(Octacalcium phosphate): Ca/P=1.33. The tooth crown area consists of HA, TCP, and ACP. The tooth root area consists of HA, TCP, and OCP. The area was thus confirmed to contain the calcium phosphate required for natural bone remodeling in human bodies. It has been reported that in cases in which the graft is applied to the bony defect area, bone healing and bone remodeling next to the autogenous bones was obtained. In addition, it has been reported that the results of the analysis of organic components showed that dentin and enamel contain several bone growth factors including type I collagen and BMP(Bone Morphogenetic Protein). Moreover, most organic substances are preserved in the graft materials because they are prepared by demineralization methods after the removal of contaminants by special engineering techniques.

However, despite reaching the commercial stage, studies on autogenous tooth graft materials are merely at level of clinical case reports. To answer the questions of dentists who still have doubts about the use of autogenous bone graft materials, and furthermore, to gain the academic acceptance already held by autogenous bones, allogenic bones and xenogenic bones, animal studies as well as additional prospective and retrospective studies are required. Therefore, this study was designed.

In our study, in the control group and group 2 (tooth ash powder graft materials) of the 4-week groups, slight new bone formation was observed in the extraction socket. In group 1 (autogenous tooth graft materials), on the other hand, active new bone formation was observed.

In the control group of the 8-week group, incomplete new bone formation in the vicinity of the extraction socket was observed. Nonetheless, most areas were still surrounded by trabecular tissues, and only partial bone formation was observed. In groups 1 and 2, a pattern of increased new bone formation and filling of the extraction socket with new bone trabeculae was observed.

Bone formation capacity in cases that used autogenous tooth graft materials was superior to that seen in cases that used tooth ash powder graft materials. This result may be due to the fact that in cases where bone graft materials were prepared from the teeth of other persons, to rule out genetic and infectious risk factors, organic substances were removed and only inorganic substances were preserved and used. On the other hand, in autogenous tooth bone graft material cases, graft materials were prepared by demineralization methods after the removal of contaminants by special treatments, and thus both inorganic substances and organic substances were preserved. Thus, the remodeling of the alveolar bone progressed more rapidly and better bone healing effects were observed.

In our study, autogenous tooth graft materials and tooth ash powder graft materials were transplanted, and histological findings at 4 weeks and 8 weeks were compared. We found that the bone formation capacity was better in cases that used autogenous tooth graft materials, and thus autogenous bone graft materials may be utilized usefully to repair bony defect areas.

Ⅴ. Conclusions

Autogenous tooth graft materials or tooth ash graft materials were grafted to the extraction sockets of both mandibles of adult dogs, and the animals were sacrificed after 4 weeks or 8 weeks. New bone formation in the bony defect areas were compared, and the following results were obtained.

1. In the control group, at 4 weeks, incomplete immature new bone formation was initiated from the margin of the vicinity of the extraction socket. At 8 weeks, limited new bone formation was observed in the vicinity of the extraction socket.

2. In group 1 (autogenous tooth graft materials), active bone formation in the vicinity of the extraction socket was observed. At 8 weeks, active bone formation in the vicinity of the graft materials was observed, and a pattern of increased new bone compared to the 4-week group was seen.

3. In group 2 (tooth ash powder graft materials), at 4 weeks, limited new bone formation in the boundary of the extraction socket was observed, and the extraction socket was filled mostly with bone graft materials. At 8 weeks, a pattern of increased new bone growth in the vicinity of graft materials compared to the 4-week group was observed.

4. The rates of new bone formation were compared, and it was found that in each group, significant increases were observed at 8 weeks vs. 4 weeks.

Based on these results, we conclude that during the bone regeneration process in the extraction socket, bone formation in autogenous tooth bone graft materials is superior to that seen in other groups, and bone formation rates may be faster.

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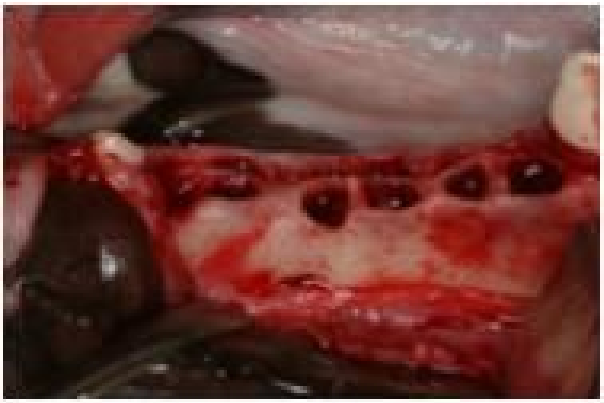


Fig. 1

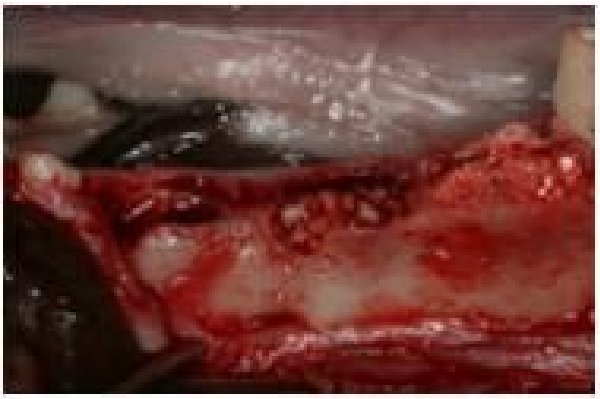
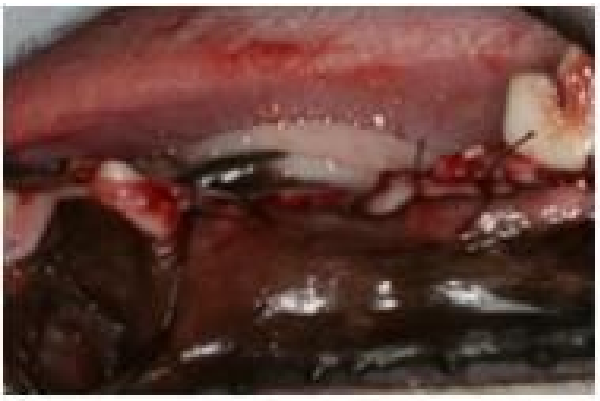


Fig. 2



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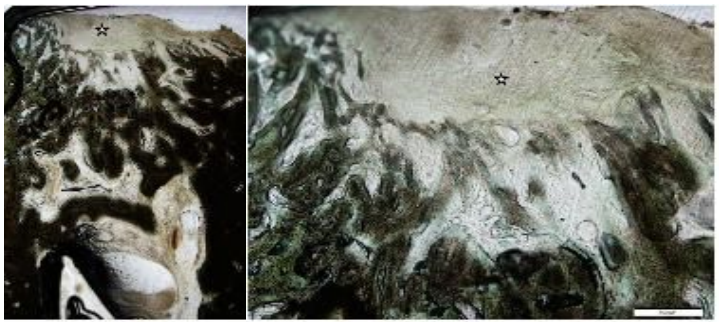

Fig 4. Control group at 4 weeks.
A: Low magnification shows defective new bone formation in the extraction site (open asterisk). (×12.5), B: High magnification shows defective new bone formation in the extraction site (open asterisk).(×40)

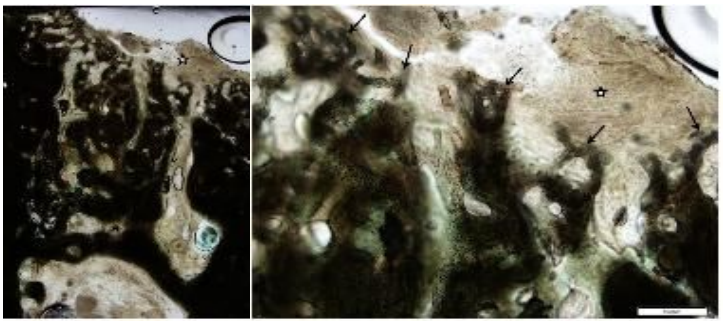

Fig 5. Control group at 8 weeks.
A: Low magnification shows limited new bone formation around the extraction site (open asterisk).(×12.5), B: High magnification shows limited new bone formation (arrows) around the extraction site (open asterisk).(×40)

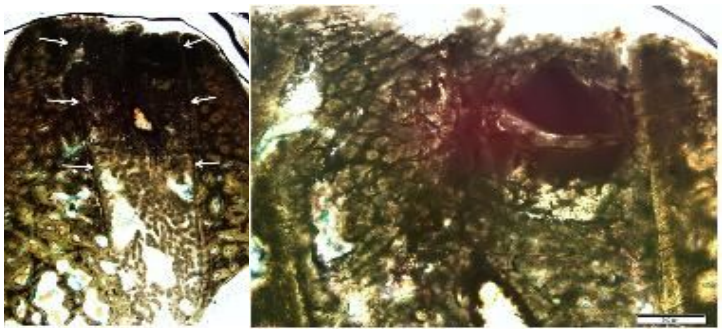

Fig 6. Group 1 at 4 weeks.

A: Low magnification shows active new bone formation (arrows) in the extraction site.(×12.5), B: High magnification shows active new bone formation (arrows) in the extraction site. Graft materials (triangles) are visible in the extraction site.(×40)

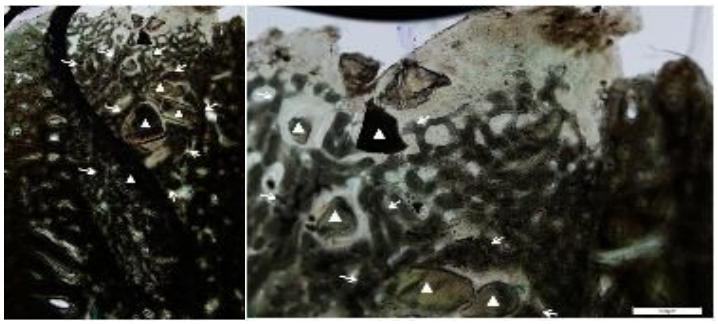

Fig 7. Group 1 at 8 weeks.

A: Low magnification shows active new bone formation (arrows) around the graft materials (triangles) in the extraction site.(×12.5), B: High magnification shows active new bone formation (arrows) around the graft materials (triangles) in the extraction site.(×40)

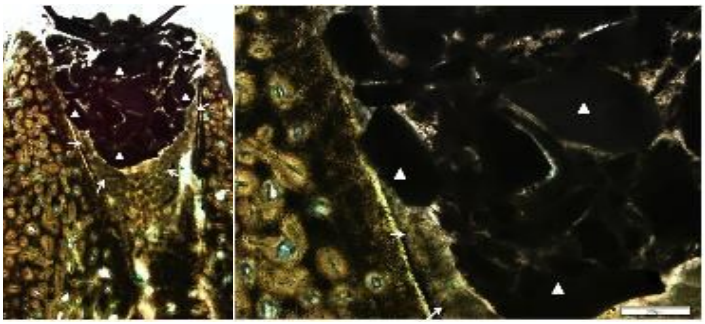

Fig 8. Group 2 at 4 weeks.
A: Low magnification shows limited new bone formation (arrows) around the defect margin. Most of the extraction defect was filled in with graft materials (triangles).(×12.5), B: High magnification shows graft materials (triangles) with limited new bone formation (arrows).(×40)

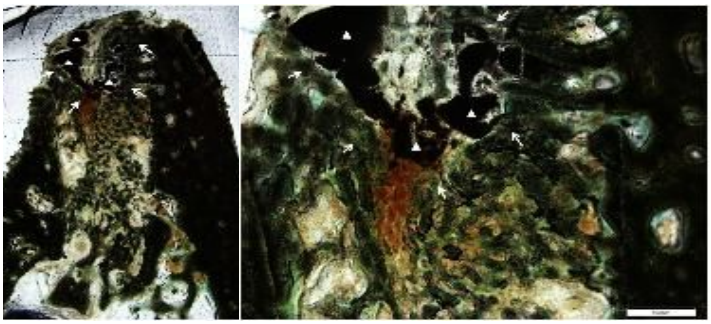

Fig 9. Group 2 at 8 weeks.
A: Low magnification shows increased new bone formation (arrows) around the graft materials (triangles) in the extraction site.(×12.5), B: High magnification shows increased new bone formation (arrows) around the graft materials (triangles).(×40)

저작물 이용 허락서

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논문제목	한글 : 성견에서 발치와에 이식된 자가치아 이식재와 치아회분말의 골형성에 관한 비교 연구				
	영어 : A comparative study on the bone formation capacity of autogenous tooth graft materials with toothash powder grafted to the tooth extraction socket of adult dogs				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2011 년 2 월 일

저작자: 양 성 수 (서명 또는 인)

조선대학교 총장 귀하