2009년 8월
박사학위논문

가토의 골 결손부에서 치아회분말과 항생제의 혼합 이식시 항생제 농도에 따른 골형성 비교 평가

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Effect of varying concentrations of tetracycline mixed with particulate dentin and plaster of Paris powders on bone formation in rabbit bone defects

2009년 8월 25일

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연구목적 : 본 연구에서는 최근 안정성과 유효성이 검증된 치아회분말을 이식재에 혼합하여 사용하는 항생제 농도에 따른 골형성에 대해 비교 평가하였다.

실험방법 : 가토의 두개골 골을 노출시킨 후 직경 8mm의 원형 크기로 trephine bur를 이용하여 전층으로 4곳(8mm 직경)의 두개골 결손부를 형성하여 대조군(Tetracycline를 혼합하지 않은 치아회분말)을 제외한 실험군 각각에 치아회분말과 plaster of Paris에 TC 50mg(실험 1군), TC 75mg(실험 2군), TC 100mg(실험 3군)을 혼합한 뒤 이식재를 결손부에 매식하고 골막을 흡수성 봉합사로 상부조직을 봉합하였다. 그 후 4주 및 8주 후 가토를 희생하여 각각의 실험군 및 대조군에서 조직을 채취하여 조직표본을 만들어 조직계측학적 평가를 실시하였다.

결 과 : 조직학적 관찰 결과 대조군과 비교하여 TC를 함유한 치아회분말의 사용은 조직학적으로 활발한 신생골 형성을 나타내었다. 50mg의 TC를 사용한 실험 1군에서 신생골 형성이 가장 활발하였고 다른 군들과 유의할만한 차이를 보였다. 실험 2군, 3군은 실험 1군에 비해 신생골 형성이 감소하는 양상을 보였다. 모든 군에

서 4주에 비해 8주에서 신생골 형성이 증가하기는 하나 유의할 만 차이는 없었다.

결　　론 : 이상의 결과에서 TC가 함유된 치아회분말은 임플란트 수복을 위한 골 결손부에서 적절한 골이식재로 사용할 수 있으며 TC 농도에 따라 신생골 형성이 증가할 수 있다고 볼 수 있다.

1. INTRODUCTION

Bone graft materials can be classified into three types: autogenous, allogenic, and alloplastic including commercialized heterogeous materials. Although autogenous grafts have been considered the gold standard for a long time, harvesting from the patient presents volume limitation problems and patient discomfort at the donor area. As a response to these shortcomings and concerns regarding allogenic bone, the use of bone substitutes or alloplastic materials has increased with positive results.[1,2]

Among the synthetic alloplastic materials, the ceramic series has the greatest reported usage. Hydroxyapatite, one of the calcium series, has been the subject of numerous studies.[3] Although this series was reported to be an ideal grafting material, it has many shortcomings which include manufacturing difficulty, cost and reduction in manufacturing capacity.[3] The use of ashed or calcinated natural bioceramics from natural sources presents an option to calcium phosphates obtained from reagent materials. Extracted teeth are ashed to remove organic substances leaving behind the inorganic substances hydroxyapatite and beta-whitelockite which are used as the major components of the graft material.[4]

Bone grafting materials have also been used in combination with tetracycline-HCl as an anti-bacterial agent in periodontal treatments.[5] Characteristics such as substantial attachment to the root surface,[6] anti-collagenous resolution effect[7] and condensing in ginigival crevicular fluid account for its efficacy in bone grafting.[8,9] Surgeons have used tetracycline-HCl mixed with bone graft materials for general implant

treatments and for bone graft associated with implants placements. It has been reported that the use of local administration of tetracycline-HCl accelerates the recovery of bone in the extraction area.[10] However, locally applied tetracycline-HCl has been reported to impede bone healing.[11,12] In cases where tetracycline-HCl was mixed with bone graft materials encouraging observations were obtained.[13,14] When freeze-dried bone was rehydrated with tetracycline-HCl, results indicated that tetracycline-HCl played a role in protecting localized tissues from infection.[15] The purpose of this study was to determine the effect of varying tetracycline-HCl concentrations mixed with particulate dentin and plaster of Paris powder on bone formation.

2. MATERIALS AND METHOD

2.1. Materials

The protocol was approved by the Animal Research Committee of Chosun University prior to its initiation.

2.1.1 Experimental animals

Twelve New Zealand white rabbits (males and females) raised under identical conditions for a certain time, older than one year (adult stage) and weighing 3 - 4 kg were used in this study. They were healthy prior to experimentation. Six animals were assigned to the 4 week group and six to the 8 week group.

2.1.2 Bone graft materials

Teeth in good condition were extracted from pigs, thoroughly cleaned with saline and ashed in a 950℃ furnace. The powders as particles of 100 mesh (0.149mm) in size were obtained using a motar and a pestle. The prepared pig's particulate dentin powder was mixed with plaster of Paris (Calcium sulfate hemihydrate, Gypsum Co., USA) at the 2:1 ratio in weight. All materials were sterilized with ethylene oxide prior to grafting and the graft materials were mixed with saline.

2.1.3 Antibiotics

The dose of tetracycline capsules (250mg, tetracycline hydrochloride, Chong Kun Dang Pharm, Seoul, Korea) was increased for each experimental group and mixed with the bone graft materials. The different

tetracycline-HCl concentration levels used is shown in Table 1.

2.2. Method

2.2.1 Anesthesia

Tiletamin and zolazepam (10mg/kg, Zoletil 50, Virbac Lab., France) and 2% zylaxine hydrochloride (3mg/kg, Rumpun, Bayer Korea Ltd., Korea) were inject intramuscularly into the rabbits. The animals were then immobilized. In the surgical area, the facial hair was bathed with alcohol and removed. The surgical area was sterilized with 10% Povidone-Iodine solution. In the cranial area, infiltration of anesthesia was performed using 2% hydrochloride lidocaine (Yuhan Co. Ltd., Seoul, Korea) containing 1:100,000 epinephrine for local hemostasis and the suppression of pain.

2.2.2 Generation of bone defect areas

An approximate 4 cm incision line was formed from the center of the ear root along the cranial median. Subperisteal dissection was carefully performed up to the upper margin of both orbitals so as not to injure the periosteum. Avoiding the exposed cranial suture line and paying attention so as not to injure the cerebral subdura, 2 circular defect areas per side were created using a Low speed hand piece attached to a 8mm trephine bur (3i, Biomet 3i^{TM}, Florida, USA). The defect size was 8mm in diameter and 2mm in thickness. A total of 4 defects were created in each rabbit.

2.2.3 Bone graft

Anitibiotics were mixed with particulate dentin and plaster of

Paris powder prepared in advance and grafted. After bone grafting, layer-to-layer suturing was performed using 4-0 vicryl (Ethicon, Johnson & Johnson, New Jersey, USA) for the periosteum and skin. In order to prevent infection in the surgical region after surgery, 1 ml gentamicin sulfate (0.1ml/kg, Deasung Gentamicin inj., Deasung Microbiological Labs.Co., Ltd, Uiwangsi, Korea) was intramuscularly injected once a day for 5 days.

2.2.4 Histomorphometric evaluation

At 4 and 8 weeks after implantation, the animals were sacrificed. The cranium of each rabbit was exposed and the implanted defect regions together with adjacent healthy bones were resected. The samples were immediately fixed in 70% alcohol for 6 days, dehydrated by alcohol washing and embedded in gylcol-metacrylate resin (Spurr Low-viscosity Embedding Media, Polysciences, Warrington, PA., USA). The polymerized samples were then sectioned using a high-precision diamond disc (Low speed diamond Wheel Saw 650, SBT, San Clemente, CA, USA). An initial 200㎛ in thickness section was obtained along the long axis and the section was finalized to 30㎛ thickness using a lapping and polishing machine (OMNILAP 2000, SBT, San Clemente, Ca., USA). For each sample, 2 slides were prepared and stained with Villanueva osteochrome bone stain (San Clemente, CA). Using a light microscope (Olympus BX 50, Tokyo, Japan), histomorphometric analysis was performed for each section.

2.3. Statistical analysis

A one-way ANOVA with Turkey pairwise multiple comparisons

was performed using the Statistical Package for the Social Sciences for Windows, version 16 (SPSS, Seoul, Korea). For comparisons of each group with time, the student t-test was used after validating that each data set was normally distributed. Statistical significance was established at $p<0.05$.

3. RESULTS

For the control group at 4 weeks after surgery, formation of a small amount of new bones was detected in the margin of the bone defect area (Fig. 1). 8 weeks after implantation, formation of new bones appeared only in the margin of the bone defect area (Fig. 2). There was no significant difference in new bone volume between the 4 and 8 week groups.

In experimental group 1, new bone formation was observed in the margin of the bone defect, as well as in the vicinity of the bone graft at 4 weeks. The amount of new bone formation was significantly enhanced at 4 weeks in experimental group 1 when compared to the control group and the other experimental groups (Fig. 3). 8 weeks after implantation, new bone formation was observed in themargin of the bone defect in the vicinity of the graft, and on the inside of the defect region (Fig. 4). Similar to the observations at 4 weeks, this group showed significantly enhanced bone formation at 8 weeks when compared to the control group and the other experimental groups. However, no significant difference was observed when comparing the new bone formation after 4 and 8 weeks within this group.

For experimental group 2, new bone formation was observed in the margin of the bone defect area, as well as in the vicinity of the graft. Although not significant, the amount of bone formation in this group at 4 weeks was observed to have increased when compared to the control group. However, the amount of new bone formation in this group at 4 weeks was significantly less was observed for experimental group 1 at 4 weeks (Fig. 5). At 8 weeks, new bone formation

was observed in the margin of the bone defect area, in the vicinity of the graft and on the inside of the defect area (Fig. 6). No significant difference was observed when comparing the new bone formation after 4 and 8 weeks within this group. However, similar to the observations at 4 weeks, increased new bone formation was observed at 8 weeks when compared to the control group, but the increase was not statistically significant. New bone formation observed for this group at 8 weeks was significantly less when compared to experimental group 1.

New bone formation in experimental group 3 was observed in the margin of the bone defect area. The amount of new bone formation in this group was comparable to the new bone formation in experimental group 2 with no significant difference between the two groups. However, new bone formation was observed to be significantly less for this group when compared to experimental group 1 at 4 weeks (Fig. 7). New bone formation was observed in the vicinity of the graft and on the inside the defect area at 8 weeks (Fig. 8). No significant difference was observed when comparing the new bone formation after 4 and 8 weeks within this group.

4. DISCUSSION

Recently, the placement of implants in the maxillo-mandibular edentulous area has become a general treatment procedure. Long-term prognosis of implant success is dependent on the presence of sufficient bone quality and bone volume in the vicinity of the implants. In many instances, a bone graft is required if either of these two factors are missing. Bone graft materials function as a scaffold forming a framework for bone to fill in around the bone defect area resulting from trauma, disease, or surgery.[16] In the restoration of a defect area caused by oral diseases or tooth extraction, grafting is often performedto preserve the width and height of the alveolar crest.

Tetracycline used in this study is a broad-spectrum antibiotic suppressing protein synthesized at the step of the binding of a transfer-RNA complex to a ribosome. It exerts bacteriostatic reaction to gram positive bacteria as well as gram negative bacteria.[17] The clinical efficacy of using this antibiotic as an adjunct to conventional mechanical therapy in patients with chronic periodontitis has been well documented and it has been shown effective in the elimination of periodontal pathogens. When applied in high concentrations at local site, tetracyleince-HCl has been reported to effectively suppress *P. intermedia* and *P. gingivalis* up to 90%.[18] It has also been used successfully to eliminate endotoxin in the dental root. Infections asso-ciated with implants have a clinical and microbiological similarity to pathogens associated with chronic periodontitis.[19,20] When implant perio-dontitis was treated with a local application of tetracycline-HCl, positive clinical and microbiological effects were reported due to its antimicro-

bial efficacy in reducing the number of *Prevotella intermedia/nigrescens, Fusobacterium sp., Bacteroides forsythus and Campylobacter rectus*.[21] Tetracyclines absorb to the dental surface, impede the activity of collagenase, interfere with the attachment of epithelial cells and downward proliferation and promote accelerated healing during bone graft. All of these properties were of special interest to the goals of this study, as well as findings indicating greater osseous fill when tetracyline was used in combination with allograft material.[6,7,13]

Particulate dentin and plaster of Paris powder was the graft material mixed with tetracycline-HCl used in this study. Hydroxyapatite, the major component of this material, is the byproduct of ashing discarded extracted teeth. Because the ashing method is simple, this calcium phosphate can be manufactured individually which reduces manufacturing costs. It stores easily and reduces environmental pollution by recycling extracted teeth.[3] The stability and effectiveness of particulate dentin and plaster of Paris powder was increased by mixing it with dental plaster. Use of this mixed graft material was initiated in 1992 and has been the subject of many clinical studies.[3,4,22-25]

Dental plaster is readily used, easily sterilized, economical, and capable of absorbing completely and rapidly. It acts as a supporter of particulate dentin and plaster of Paris powder, minimizes the fluidity of the powder,[25] and provides an effective delivery system. Graft materials containing a mixture of particulate dentin and plaster of Paris powder and plaster have the following advantages: absence of noticeable foreign body reaction or inflammatory reaction, osseoinduction capacity, absorbability easy manipulation and low manufacturing cost. In addition, this mixed graft material has demonstrated to have direct contact with

new bones when mixed with saline for a short period.[4] Other advantages noted in an animal study were a lack of cytotoxicity and an absence of specific allergic actionst, suggesting that it could be used safely in vivo.[4]

The noted characteristics of both particulate dentin and dental plaster-make this mixed graft material a suitable candidate to be mixed with tetracycline-HCl and used in this study. After grafting, the particles of particulate dentin and plaster of Paris powder and tetracycline-HCl can be well maintained without dispersing, and particulate dentin and plaster of Paris powder and tetracycline-HCl can be placed readily and accurately in the graft site. The loss of graft materials during the healing period can be a major cause of inducing bone graft failure, thus making the maintainance of bone graft materials for a certain timecritical.

Drury et al.[26] observed that the use of tetracycline-HCl rehydrated freeze-dried allogenic bones resulted in new bone formation after 3 and 5 weeks implantation. This was 5 times greater than the amount of bone growth reported for free-dried allogenic bones mixed with saline. Al-Ali et al.[27] have shown that combining modified tetracycline-HCl doxycyclin with graft materials enables the graft materials to be better fused with adjacent bones.

However, these positive results are dependent upon the concentration of tetracycline-HCl used. At appropriate concentrations, the formation of new bone is increased. Rifkin et al.[28] reported that the minimal suppression concentration should be higher than 4~8 μg/ml. to achieve an antibiotic effect on pathogenic bacteria. But in order to suppress bone resorption. the concentration should be approximately 5~10 μg/ml.

Somerman et al.[29] reported that at the concentration of 50 mg/ml, the chemotaxis and proliferation of fibroblasts occurred actively, whereas concentrations higher than 50mg/ml appeared to be harmful to normal cell functions.

To examine the effect of varying concentrations of tetracycline-HCl mixed with particulate dentin and plaster of Paris powder on new bone formation, bone defects were created in the cranium of rabbits in this study. It was observed that the smallest volume of new bone formation occurred in the control group. In cases where tetracycline-HCl was added, new bone formation in the defects was augmented. However, when the concentration of tetracycline-HCl was increased above the appropriate level, there was a tendency for reduced new bone formation.

The findings of this study suggest that particulate dentin and plaster of Paris powder mixed with 50 mg/ml of tetracycline-HCl yields a significant increase in new bone formation and that levels above 75 mg/ml significantly reduces the amount of new bone formation. It also appears that increasing the implantation period from 4 weeks to 8 weeks does not result in significantly higher bone formation.

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Table 1. Defect Preparation (8mm, thickness 2mm) on Calvarial Bone of Rabbit (Unit : graft site)

Group	Grafting material	4 weeks	8 weeks
Control	Particulate dentin and plaster of Paris only	6	6
Experimental group 1	Particulate dentin and plaste of Paris + TC 50mg	6	6
Experimental group 2	Particulate dentin and plaste of Paris + TC 75mg	6	6
Experimental group 3	Particulate dentin and plaste of Paris + TC 100mg	6	6

*TC=Tetracycline HCl

Table 2. Statistical significance evaluation of each experimental group (p value)

		4 weeks	8 weeks
Control	Group 1	0.017	0.003
	Group 2	0.997	0.747
	Group 3	0.987	0.621
Group 1	Group 2	0.015	0.012
	Group 3	0.008	0.021
Group 2	Group 3	1.000	0.995

Table 3. Comparing of the new bone formation at 4, 8 weeks after experimentation (Unit : mm^3)

	Control	Group 1	Group 2	Group 3
4 weeks	0.70±0.67	2.40±0.69	1.11±0.69	1.20±0.50
8 weeks	1.45±1.00	2.74±0.82	1.64±1.05	1.59±0.37
P-value	0.194	0.209	0.689	0.742

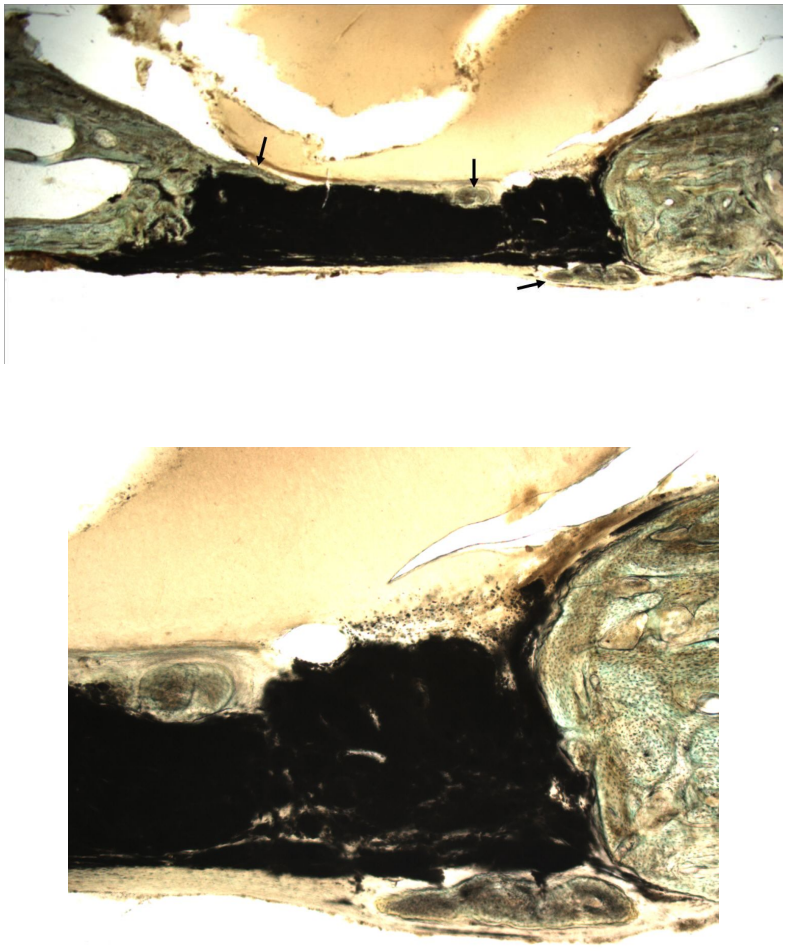

Figure 1. Control group at 4 weeks. Small amount of new bone formation (arrows) around the defect margin was identified, A: X40, B: X100.

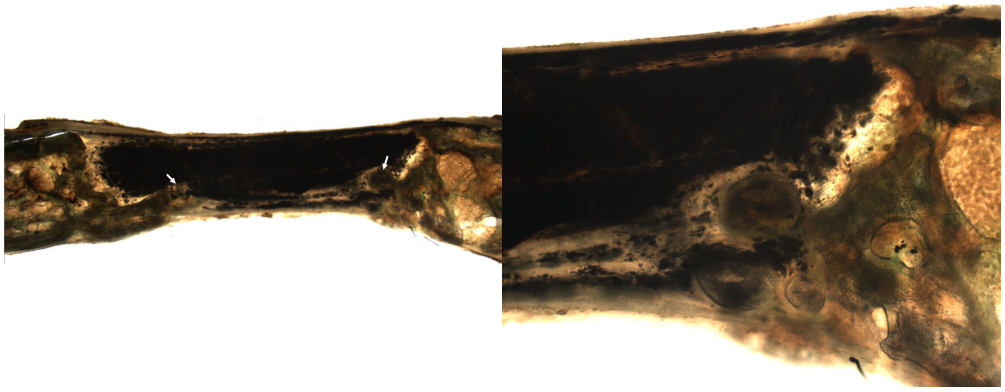

Figure 2. Control group at 8 weeks. New bone formation (arrows) around the defect margin was identified, A: X40, B: X100.

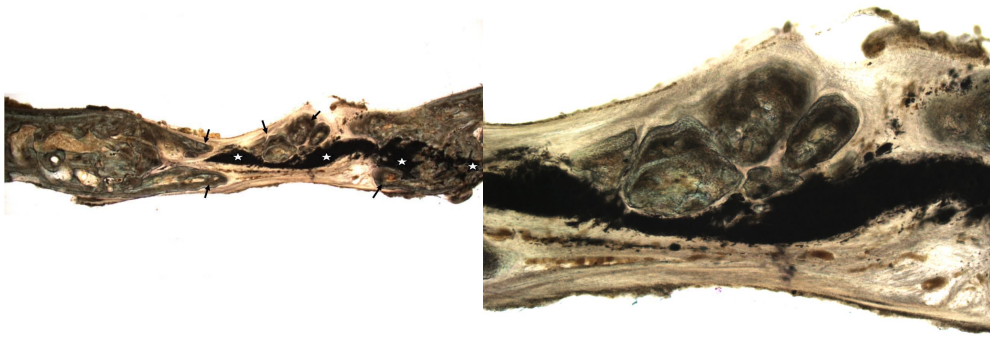

Figure 3. Experimental group 1 at 4 weeks. Increased amount of new bone formation (arrows) in between the graft materials (asterisks) as well as around the defect margin, A: X40, B: X100.

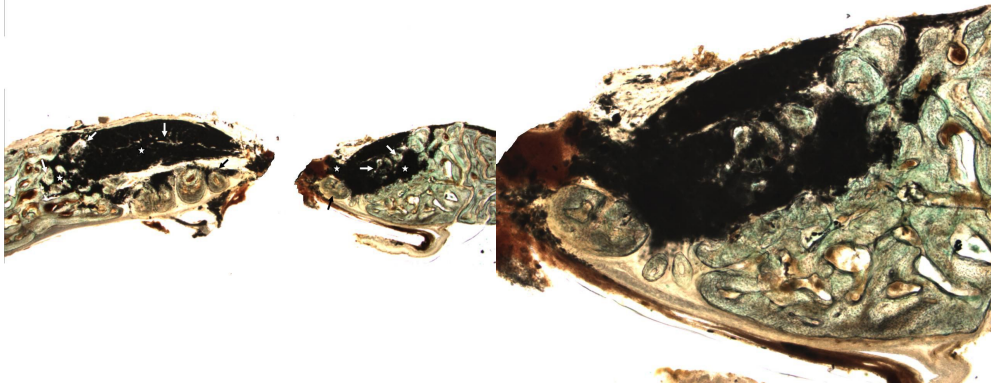

Figure 4. Experimental group 1 at 8 weeks. Increased amount of new bone formation (arrows) in between the graft materials (asterisks) as well as around the defect margin, A: X40, B: X100.

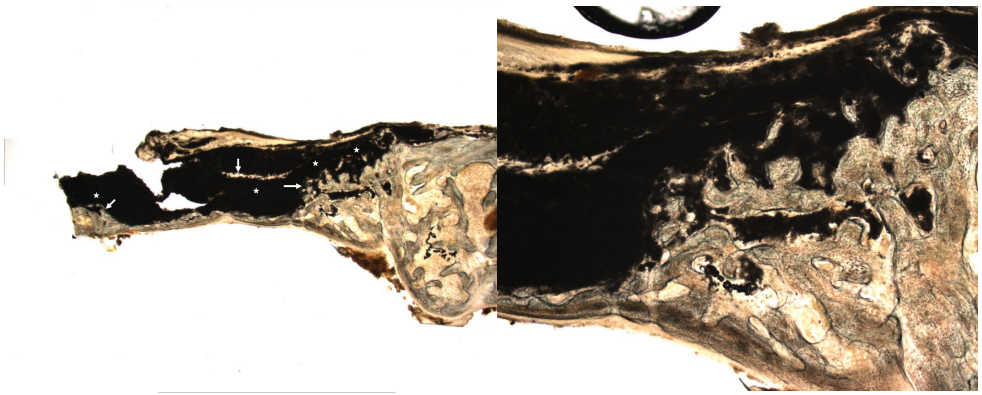

Figure 5. Experimental group 2 at 4 weeks. Increased amount of new bone formation (arrows) in between the graft materials (asterisks) as well as around the defect margin, A: X40, B: X100.

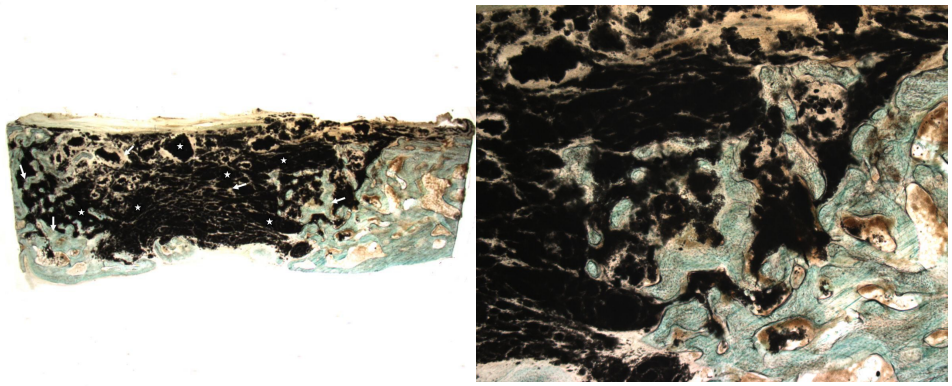

Figure 6. Experimental group 2 at 8 weeks. Large amount of new bone formation (arrows) in between the graft materials (asterisks) was demonstrated, A: X40, B: X100.

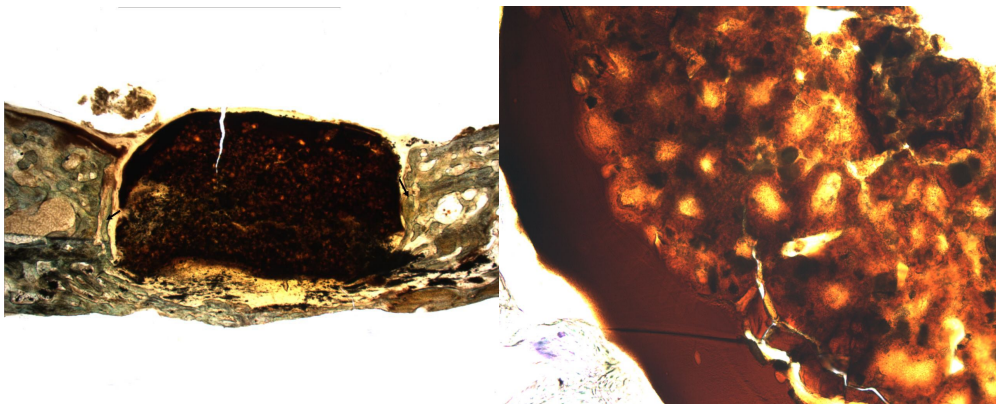

Figure 7. Experimental group 3 at 4 weeks. New bone formation around and in between the graft materials (chip) was identified in the darker defect area. Small amount of new bone formation (arrows) around the defect margin was demonstrated, A: X40, B: X100.

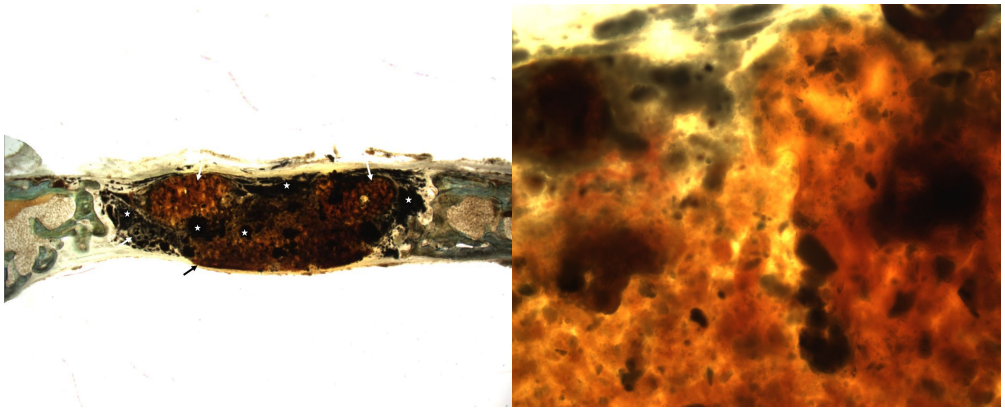

Figure 8. Experimental group 3 at 8 weeks. Increased amount of new bone formation (arrows) around and in between the graft materials (asterisks) was demonstrated, A: X40, B: X100.

ABSTRACT

Effect of varying concentrations of tetracycline mixed with particulate dentin and plaster of Paris powder on bone formation in rabbit bone defects

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Objectives : Recently, the bone graft in bone defect for implant placement has been generalized. Some surgeons are using graft materials mixed with tetracycline (TC) antibiotics for prevention of the infection around the graft materials but there are few investigations on the effect of the antibiotics on the bone formation. Hence, in this study, we were to compare and assess the bone formation according to the concentration of antibiotics mixed with graft materials.

Study design : Cranial center of rabbit was sectioned and exposed. Then 4 circular defects of 8mm-diameter withfull thickness were made using the trephine bur on the cranium. The subjects were divided into the experimental groups (particulate dentin and plaster of Paris mixed with the TC) and the control group (particulate dentin and plaster of Paris without TC). The experimental groups were subdivided into the experimental group 1 (with 50mg of TC); the experimental group 2 (with 75 mg of TC); and the experimental group 3 (with 100mg of TC). The graft materials

were placed into the defect area and the soft tissue was closed with the absorbable suture. 4 and 8 weeks after the experiment, the rabbits were sacrificed and the tissues samples from each group were histometrically assessed.

Results : The histological analysis showed that the experimental group presented more active new bone formation than the control group. The experimental group 1 with 50mg of TC showed the most active new bone formation. But the experimental group 2 and 3 with more TC showed less new bone formation than the experimental group 1. No significant difference was found between The 4 week- and 8 week- results showed no significant difference.

Conclusion : The particulate dentin and plaster of Paris including TC was considered to be an appropriate bone graft material in bone defects for implant placement. Further clinical investigations were necessary.

저작물 이용 허락서

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논문제목	한글 : 가토의 골 결손부에서 치아회분말과 항생제의 혼합 이식시 항생제 농도에 따른 골형성 비교평가				
	영어 : Effect of varying concentrations of tetracycline mixed with particulate dentin and plaster of Paris powders on bone formation in rabbit bone defects				

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( )

2009 년 8 월 일

저작자: 오 선 영 (서명 또는 인)

조선대학교 총장 귀하