2010년도 2월
박사학위논문

가토에서 치아회분말과 치과용 연석고 혼합 이식재에 혈소판 풍부혈장, 조직접합제 혼합 매식 후 골형성 비교 평가

조선대학교 대학원

치의생명공학과

김 봉 균

가토에서 치아회분말과 치과용 연석고 혼합 이식재에 혈소판 풍부혈장, 조직접합제 혼합 매식 후 골형성 비교 평가

Histomorphometric analysis of bone healing in rabbits using tooth ash and plaster of Paris, platelet-rich plasma, and fibrin sealant

2010년 2월 25일

조선대학교 대학원

치의생명공학과

김 봉 균

가토에서 치아회분말과 치과용 연석고 혼합 이식재에 혈소판 풍부혈장, 조직접합제 혼합 매식 후 골형성 비교 평가

지도교수 김 수 관

이 논문을 치의학 박사학위신청 논문으로 제출함

2009년 10월

조선대학교 대학원

치의생명공학과

김 봉 균

김봉균의 박사학위논문을 인준함

위원장 조선대학교 교 수 정 재 헌 인

위 원 조선대학교 교 수 김 병 옥 인

위 원 서울대학교 교 수 김 영 균 인

위 원 조선대학교 교 수 김 학 균 인

위 원 조선대학교 교 수 김 수 관 인

2009년 12월

조선대학교 대학원

Contents

List of Tables

List of Figures

국문초록

가토에서 치아회분말과 치과용 연석고 혼합 이식재에 혈소판 풍부혈장, 조직접합제 혼합 매식 후 골형성 비교 평가

김 봉 균
지도교수 : 김 수 관
조선대학교대학원 치의생명공학과

본 연구의 목적은 치아회분말과 연석고 혼합 이식재에 fibrin sealant를 함께 이식한 경우의 신생골 형성을 비교 분석하여 골이식시 PRP와 fibrin sealat가 골형성에 미치는 효과를 평가하는 데 있다.

12마리의 가토에 형성된 48개의 골결손부를 12개씩 4개의 군으로 나누었다. 1군에서는 이식재를 이식하지 않았으며, 2군에서는 치아회분말과 연석고 혼합이식만을, 3군에서는 치아회분말과 연석고 혼합 이식재와 PRP, 그리고 4군에서는 치아회분말과 연석고 혼합 이식재와 fibrin sealant를 함께 이식하였다. 수술 후 4주와 8주에 각각 6마리씩의 가토를 희생시켜 조직학적 및 조직형태계측학적인 분석을 시행하여 다음과 같은 결과들을 얻었다.

1. 이식재를 이식하지 않은 대조군에 비해 이식재를 사용한 실험 1군, 2군, 3군 모두에서 유의한 골형성 증가 소견을 보였다.
2. 치아회분말과 연석고의 혼합재만을 이식한 실험 2군보다 PRP와 fibrin sealant를 혼합이식한 실험 3군, 4군에서 통계학적으로 유의할만한 골형성 증가 소견을 보였다.
3. 치아회분말과 연석고의 혼합재와 PRP를 혼합 이식한 실험 3군에 비해 fibrin sealant를 혼합 이식한 실험 4군의 신생골 형성이 다소 증가된 양상을 보였으나, 통계학적으로 유의할만한 차이는 없었다.

4. 모든 군에서 8주의 골형성율이 4주의 골형성에 비해 통계학적으로 유의할 만한 골형성 증가 소견을 보였다.

이상의 결과를 통해 볼 때 PRP와 fibrin sealant는 골이식재와 함께 이식에 사용한 경우 골 결손부에서의 골재생을 보다 효과적으로 유도하는 것으로 평가할 수 있으며, 분말형 이식재 사용시 형태 부여에 있어 보다 나은 조작성을 부여하므로 골 이식 수술시 유용하게 사용할 수 있을 것으로 생각된다.

I. Introduction

Autogenous bone used for the reconstruction of bone defects has advantages of early vascularization, excellent taking, safety from infectious diseases, and the absence of immunological rejection; thus, autogenous bone is a good bone graft material and produces the best prognosis. Nonetheless, shortcomings include a limited harvest, problems with the donor area, and resorption after grafting,[1] so allogenic bones, xenogenic bones, and synthetic bones have also been used, and many studies have been conducted using them.

Hydroxyapatite (HA) has been most widely used as a synthetic bone graft material.[2,3] HA has a chemical structure similar to teeth and bone, so its histocompatibility is excellent, and it not only plays a role as a scaffold to which bones can grow and invade, but also supplies ions such as calcium and phosphate. Therefore, it is advantageous in that it fuses well with adjacent bone tissues.[4] Nonetheless, this material has problems with processing and high cost.

Additionally, maintenance is difficult in cases in which transplanted powder-type materials are used alone, which is also a shortcoming. A mixture of toothash and plaster of Paris (calcium sulfate hemihydrates), used in this study, are materials that have been developed to overcome such problems associated with synthetic bone. When teeth are burnt at high temperatures and prepared as a powder, graft materials including HA can be obtained; this is typically mixed with plaster of Paris to overcome fluidity.[5-7]

In previous studies,[8-16] it was shown that a mixture of toothash and plaster of Paris was a biocompatible resorbable material and the grafted toothash and plaster of Paris mixture underwent healing processes by oseoconduction, but osteogenesis and oseoinduction did not occur. This indicates that, compared with autogenous bones with osteogenesis and oseoinduction potential, the potential for new bone formation is low. There are problems if it is applied clinically to an area with a wide bone defect or to an area where bone

continuity is lost. One method that compensates for such shortcomings is the use of platelet-rich plasma (PRP).[3,17]

PRP contains growth factors that add oseoinduction potential to graft materials with only osteoconduction potential.[3,17,18] The effect of fibrin sealants, which have been widely used for hemostasis and the acceleration of wound healing during bone regeneration, has become a subject of interest,[9,14,16] and better results were obtained by endowing stability with PRP during bone grafting.[19]

Thus, in this study, we evaluated the effect of PRP and fibrin sealants on new bone formation after bone grafting.

Ⅱ. Materials and methods

1. Materials

Twelve New Zealand white rabbits, weighing 3.0-3.5 kg and that were older than one year were used, regardless of gender. Before experimentation, the animal protocol was evaluated and approved by the animal research committee at Chosun University to ensure that the policies, standards, and guidelines for the proper use, care, handling, and treatment of animals were observed. The health status of all animals was determined to be normal at the onset of the study.

Toothash and plaster of Paris were mixed in a ratio of 2:1 by weight. CTG (3.8% sodium citrate; Korea United Pharm Inc., Korea), Dirabine (thrombin; Korea United Pharm) and Calmia (10% calcium gluconate, Korea United Pharm) were used to prepare the PRP, and prefilled syringe-type fibrin sealant (Tissucol Duo Quick; Baxter AG, Wein, Austria) was used.

2. Experiment methods

a. Anesthesia of experiment animals

For the induction of general anesthesia, 0.2 ml/kg Zoletil® 50 (tiletamine/zolazepam, Virvac, France) and 0.25 mg/kg Rompun® injectable (xylazine hydrochloride, Bayer, Germany) were injected intramuscularly. Gentamicin sulfate (0.08 ml/kg; Daesung, Korea) was injected intramuscularly to prevent infections.

b. PRP preparation

Blood (9 ml) was collected from the rabbits by cardiac puncture using a syringe. The blood was mixed with 1 ml of 3.8 % sodium citrate to prevent coagulation. The blood was centrifuged (2,000g, 3min, Placon centrifuge; Oscotec, Korea). Red blood cells were separated from the blood after the first centrifugation, and a second centrifugation (5,000g, 5 min) was performed to collect the upper platelet-poor plasma (PPP) and the buffy coat layer. The upper PPP layer was discarded, and PRP was obtained by suspending the remaining lower layer. The PRP was prepared as a gel using a solution of 10% thrombin obtained prior to transplant and 10% calcium gluconate mixed at a ratio of 1:1.

c. Surgery

Hair on the cranial area of the rabbits was removed, the area was sterilized with betadine solution, and 2% lidocaine HCl (containing 1:100,000 epinephrine, Yuhan Corp., Korea) was injected subcutaneously. The skin and periosteum in the cranial area were incised, and the cranium was exposed by dissection. An 8-mm diameter bone defect was created on both sides, using a trephine drill.

In total, 48 bone defect areas were formed in the 12 rabbits, and they were divided into four groups of 12 each. Grafting materials were not transplanted in group 1, only the mixture of tooth ash and plaster of Paris was grafted in group 2, the mixed toothash and plaster of Paris together with PRP was grafted in group 3, and the mixture of toothash and plaster of Paris and fibrin sealant was grafted in group 4 (TmaterI). After performing the transplant, the periosteum was sutured using absorbmatersutures, and the skin was sutured using non-absorbable sutures.

Table I. Defect preparation on parietal bone of rabbits (Unit: graft site)

Group	Grafting materials	4 weeks	8 weeks
Group 1 (Control)	No graft	6	6
Group 2	Toothash and plaster of Paris only	6	6
Group 3	Toothash and plaster of Paris + PRP	6	6
Group 4	Toothash and plaster of Paris + fibrin sealant	6	6

d. Tissue sample preparation

Six rabbits from each group were sacrificed at 4 and 8 weeks after surgery. Using the method described above, the cranium was exposed and samples including adjacent bone were collected using a disc. The samples were fixed immediately in 70% alcohol for 6days, dehydrated in alcohol, and embedded in glycol-methacrylate resin (Spurr Low-viscosity embedding medium (Polysciences, Warrington, PA., USA). The polymerized samples were sectioned into 200-m thick slices along the long axis using a highpre-cision diamond disc (low speed diamond wheel saw 650, SBT, San Clemente, CA, USA). Finally, 30m thick samples were prepared using a lapping and polishing machine (Omnilap 2000, SBT), and Villanueva osteochrome bone stain was applied.

3. Histomorphometric evaluation and statistical analysis

The prepared samples were examined under a light microscope (Olympus BX 50, Tokyo, Japan), and analyzed histomorphometrically.

A one-way ANOVA was performed using the Statistical Package for the Social Sciences for Windows, version 16 (SPSS, Seoul, Korea), followed by the Tukey method. After confirming that the data were normally distributed, Student's t-test was used to compare each group with time. A P value $<$ 0.05 was deemed to be statistically significant.

Ⅲ. Results

1. Histological findings

1) Group 1

a. Four-week group

A small amount of new bone formed in the margin of the bone defect area (Fig. 1).

b. Eight-week group

There was new bone formationin the margin of the bone defect area, and the amount of new bone had increased compared with the 4-week group.

2) Group 2

a. Four-week group

New bone formed in the margin of the bone defect area, and there was increased bone formation in comparison with the control group (Fig. 2).

b. Eight-week group

New bone formed not only in the margin of the bone defect area, but also in the vicinity of the graft materials, and there was increased bone formation when compared to the 4week control group (Fig. 3).

3) Group 3

a. Four-week group

New bone formed in the margins of the bone defect area, and unmineralized osteoid formed between the grafted materials (Fig. 4).

b. Eight-week group

New bone formed not only in the margins of the bone defect area, but also in the middle of the bone defect area, and there was residual bone graft material between the new bone (Fig. 5).

4) Group 4

a. Four-week group

New bone formed in the margins of the bone defect area, and unmineralized osteoid formed between the grafted materials, similar to group 3 (Fig. 6).

b. Eight-week group

New bone formed not only in the margins of the bone defect area, but also in the middle of the bone defect area.

2. Comparative histomorphological analysis

After 4 weeks, the new bone formed in the group 1 and groups 2, 3 and 4 were $0.046 \pm 0.008mm^3$, $0.198 \pm 0.036mm^3$, $1.465 \pm 0.078mm^3$, and $1.860 \pm 0.311mm^3$, respectively. Groups 2, 3 and 4 showed statistically significant new bone formation, compared with the group 1, and groups 3 and 4, in which used PRP and fibrin sealants were used, respectively, showed significant new bone formation, compared with group 2 (Table Ⅱ).

After 8 weeks, the new bone that formed in the group 1 and groups 2, 3 and 4 were $0.400 \pm 0.001mm^3$, $1.915 \pm 0.346mm^3$, $2.980 \pm 0.198mm^3$, and $3.170 \pm 0.368\ mm^3$, respectively. There was statistically significant new bone formation at 8 weeks, compared with 4 weeks, in all groups (Table Ⅱ).

Table Ⅱ. Comparison of new bone formation at 4 and 8 weeks (unit: mm^3)

	Group 1	Group 2	Group 3	Group 4
4 weeks	0.046±0.008	0.198±0.036*	1.465±0.078*,†	1.860±0.311*,†
8 weeks	0.400±0.001††	1.915±0.346*,††	2.980±0.198*,†,††	3.170±0.368*,†
P-value	0.001	0.02	0.01	0.41

*Statistically significant difference relative to Group 1 ($P < .05$).
†Statistically significant difference relative to Group 2 ($P < .05$).
††Statistically significant difference relative to 4 weeks ($P < .05$).

Ⅳ. Discussion

Osteoconduction is the term used when multiporous graft materials are transplanted next to bone. Capillary blood vessels, tissues in the vicinity of blood vessels, and bone precursor cells migrate to the multiporous space and bind to multiporous structures, together with the newly formed bone. Initially, fibrous vascular tissues begin to grow within the multiporous structures, and new bone tissue develops. Osteoconductive materials are not living cells, but passive scaffolds.[20] Bone and fibrous vascular tissue development is initiated in the presence of such passive scaffolds, and, ultimately, new bones replace them. Local controlling factors associated with osteoinduction include transforming growth factor-(TGF-), bone morphogenic protein, insulin-like growth factor, fibroblast growth factor, platelet-derived growth factor (PDGF), and interleukin-1.[21-23]

PRP releases several growth factors such as PDGF, TGF-1, and TGF2. Whitman et al.[24] described the preparation and use of platelet gels. These gels contains a high platelet concentration and can be used to successfully reconstruct the oral and maxillofacial area, the mandible, a fistula in the oromaxillary sinus, an alveolar cleft accompanying fistulas in the oral and nasal cavity, or used for implants. Marx et al.[25] introduced a method for obtaining platelet-rich plasma and reported that in a group treated with such platelet-rich plasma, bone growth rate increased from 1.62 to 2.16 times, and that there was an histomorphological increase in bone density. Anitua[26] used plasma rich growth factors in the extraction window of patients and reported excellent results radiologically and histologically.

Fibrin sealants are a fibrin adhesive system (FAS), used for the purpose of accelerating tissue adhesion and wound healing. The main component is fibrinogen and the system also contains thrombin, calcium chloride, and aprotinin. The FAS promotes the formation of fibrin clots.[27]

Fibrin induces bone regeneration by the osteoinduction phenomenon, and osteoinduction is revealed by the change of local host cells to ostoegenetic

cells.[28] Bsch et al.[29] reported that fibrin sealants accelerated sprouting of capillary blood vessels and, thus, connective cell formation was accelerated, which ultimately induced rapid new bone formation; fibrin sealants are directly involved in bone regeneration. Additionally, bone remodeling in the middle osteocyte area was accelerated when fibrin sealants were used in autogenous woven bones grafts.[30] In a study reported by Pflger et al.,[31] holes of various sizes were formed in a stainless steel cylinder and implanted, and the level of bone filling was substantially faster in the group that received fibrin sealants.

In this study, at 4 and 8 weeks, the PRP and fibrin sealant groups showed statistically significantly increased new bone formation, when compared with the other groups, which may reflect osteoinduction by growth factors and fibrin, as described above. At 4 and 8weeks, there was no significant difference in new bone formation between the PRP group and the fibrin sealant group, and in such cases, fibrin sealants are advantageous, due to the difficulty of collecting blood and its preparation. Additionally, as shown by previous studies, improved manipulation and the stability of graft materials following the use of fibrin sealants are more advantageous when applied to the oral cavity due to movements generated by phonation and mastication.

Ⅴ. Conclusions

Bone defect areas were formed in the cranium of rabbits, and using a mixture of toothash and plaster of Paris as graft materials, either platelet-rich plasma or fibrin sealants were transplanted together and tissues samples obtained after 4 and 8 weeks were analyzed histomorphometrically, and the following conclusions were formed.

1. There was a significant increase in new bone formation in the three groups in which graft materials were used, compared with the control group.
2. There was a significant increase in new bone formation in the group in which toothash and plaster of Paris and either PRP or fibrin sealants were used, compared with the groups that did not receive PRP or fibrin sealants.
3. There tended to be an increase in new bone formation in the group in which toothash and plaster of Paris, and fibrin sealants were used, compared with the group that used toothash and plaster of Paris and PRP; however, the difference was not statistically significant.
4. All groups at 8 weeks revealed a statistically significant increase in new bone formation when compared with those at 4 weeks.

REFERENCES

1. Mulliken JB, Glowacki J. Induced osteogenesis for repair and construction in the craniofacial region. Plast Reconstr Surg 1980;65:553-60.
2. Kim SG, Yeo HH, Kim YK. Grafting of large defects of the jaws with a particulate dentin-plaster of Paris combination. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:22-25.
3. Kim SG, Chung CH, Kim YK, Park JC, Lim SC. Use of particulate dentin-plaster of Paris combination with/without platelet-rich plasma in the treatment of bone defects around implants. Int J Oral Maxillofac Implants 2002;17:86-94.
4. Frame JW. Hydroxyapatite as a biomaterial for alveolar ridge augmentation. Int J Oral Maxillofac Surg 1987;16:642-55.
5. Kim SG, Kim HK, Lim SC. Combined implantation of particulate dentine, plaster of Paris, and a bone xenograft (Bio-Oss) for bone regeneration in rats. J Craniomaxillofac Surg 2001;29:282-8.
6. Kim SG. Bone grafting using particulate dentin. Key Eng Mater 2007; 342-343:29-32.
7. Kim SG, Chung CH, Kim YK. Grafting defects using a particulate dentin-plaster of Paris combination for implant placement: a case report. Hosp Dent (Tokyo) 2001;13:127-30.
8. Kim SY, Kim SG, Lim SC, Bae CS. Effects on bone formation in ovariectomized rats after implantation of tooth ash and plaster of Paris mixture. J Oral Maxillofac Surg 2004;62:852-7.
9. Choi DK, Kim SG, Lim SC. The effect of particulate dentin-plaster of Paris combination with/without fibrin glue in the treatment of bone defects around implants Hosp Dent (Tokyo) 2007;19:121-6.
10. Park SS, Kim SG, Lim SC, Ong JL. Osteogenic activity of the mixture of chitosan and particulate dentin. J Biomed Mater Res A 2008;87:618-23.
11. Kim SG, Kim YK, Park JS. Scientific evidence for autogenous tooth bone

graft material (AutoBT). J Kor Dent Sci 2009;3:42-5.
12. Kim SG, Choi YO, Kim YK. Histologic evaluation of peri-implant defects with a particulate dentin-plaster of Paris combination and bioresorbable membrane barriers: a preliminary study. Hosp Dent (Tokyo) 2004;16:15-8.
13. Na TH, Kim SG, Yoon JH, Lim SC. Effect of the bone regeneration of the mixture of human or bovine toosh-ash and the plaster of Paris in rats. J Korean Maxillofac Plast Reconstr Surg 2004;26:334-40.
14. Kim SG, Kim SH, Lim SC, Bae CS. Effect of fibrin sealant on early bone healing with tooth ash and plaster of Paris in ovariectomized rats. Key Eng Mater 2007;330-332:1281-4.
15. Kim SG, Park OJ, Lim SC, Bae CS. Effect of high local concentrations of antibiotics on early bone formation with tooth ash and plaster of Paris in the ovariectomized rat. Key Eng Mater 2007;330-332:1311-4.
16. Kim WB, Kim SG, Lim SC, Kim YK, Park SN. Effect of Tisseel™ on bone healing with particulate dentin and plaster of Paris mixture. Oral Surg Oral Med Oral Pathol Oral Radiol Endod (in press)
17. Kim SG, Kim WK, Park JC, Kim HJ. A comparative study of osseo-integration of Avana implants in a demineralized freeze-dried bone alone or with platelet-rich plasma. J Oral Maxillofac Surg 2002;60:1018-25.
18. Aghaloo TL, Moy PK, Freymiller EG. Evaluation of platelet-rich plasma in combination with anorganic bovine bone in the rabbit cranium: a pilot study. Int J Oral Maxillofac Implants 2004;19:59-65.
19. Kania RE, Meunier A, Hamadouche M, Sedel L, Petite H. Addition of fibrin sealant to ceramic promotes bone repair: long-term study in rabbit femoral defect model. J Biomed Mater Res 1998;43:38-45.
20. Cornell CN. Osteoconductive materials and their role as substitutes for autogenous bone grafts. Orthop Clin North Am 1999;30:591-8.
21. Bessho K, Tagawa T, Murata M. Comparison of bone matrix-derived bone morphogenetic proteins from various animals. J Oral Maxillofac Surg 1992;50:496-501.

22. Steinbrech DS, Mehrara BJ, Saadeh PB, Greenwald JA, Spector JA, Gittes GK, Longaker MT. Hypoxia increases insulinlike growth factor gene expression in rat osteoblasts. Ann Plast Surg 2000;44:529-34.
23. Hammacher A, Hellman U, Johnsson A, Ostman A, Gunnarsson K, Weste-rmark B, Wasteson A, Heldin CH. A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. J Biol Chem 1988;263:16493-8.
24. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alterna-tive to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 1997;55:1294-9.
25. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638-46.
26. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999;14:529-35.
27. Schlag G, Redl H. Fibrin sealant in orthopedic surgery. Clin Orthop Relat Res 1988;227:269-85.
28. Mulliken JB, Kaban LB, Glowacki J. Induced osteogenesis--the biological principle and clinical applications. J Surg Res 1984;37:487-96.
29. Bösch P, Braun F, Eschberger J, Kovac W, Spängler HP. [The action of high-concentrated fibria on bone healing] Arch Orthop Unfallchir 1977; 89:259-73.
30. Bösch P, Lintner F, Braun F. [Autologous cancellous bone grafting in rabbits using a fibrinogen adhesive system] Wien Klin Wochenschr 1979;91: 628-33.
31. Pflűger G, Bösch P, Grundschober F, Kristen H, Plenk H Jr, Schider S. [Investigation of bone growth into porous metal implants] Wien Klin Wochenschr 1979;91:482-7.

Figure legends

Fig. 1. Group 1 at 4 weeks. Limited new-bone formation (arrows) around the defect margin (A: ×20). New bone formation (arrows) around the defect margin (B: ×100).

Fig. 2. Group 2 at 4 weeks. Limited new-bone formation (arrows) occurred around the defect margin (asterisks) (A: ×20). New-bone formation (arrows) around the defect margin (asterisks) (B: ×40).

Fig. 3. Group 2 at 8 weeks. New-bone formation in the central portion (arrows) as well as the defect margin (A: ×20). New-bone formation (arrows) intermingled with implanted chips (asterisks) around the defect margin (B: ×40).

Fig. 4. Group 3 at 4 weeks. Limited new bone around the defect margin and unmineralized osteoid formation between the implanted chips (A: ×20). Unmineralized osteiod formation was seen between the implanted chips (asterisks) (B: ×100).

Fig. 5. Group 3 at 8 weeks. Trabecular new bone formation occurred in the central portion and at the defect margin. Implanted chip debris (arrows) between new bone trabeculae (A: ×20). Well-formed trabecular bone with fragmented implanted chips (arrows) (B: ×40).

Fig. 6. Group 4 at 4 weeks. Limited new-bone around the defect margin and unmineralized osteiod formation between the implanted chips (A: ×20). Unmineralized osteiod formation was seen between the implanted chips (asterisks) (B: ×100).

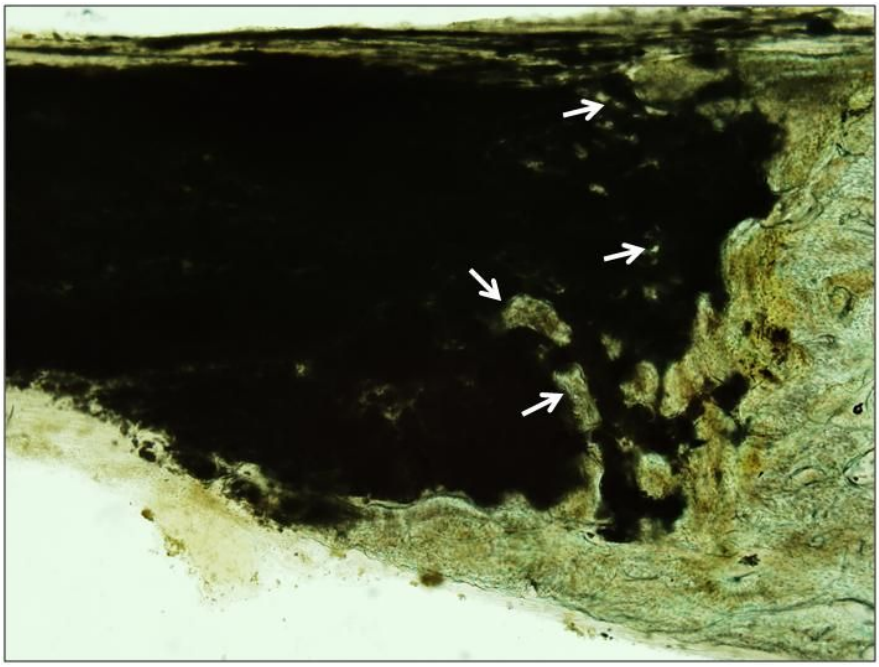

Fig. 1

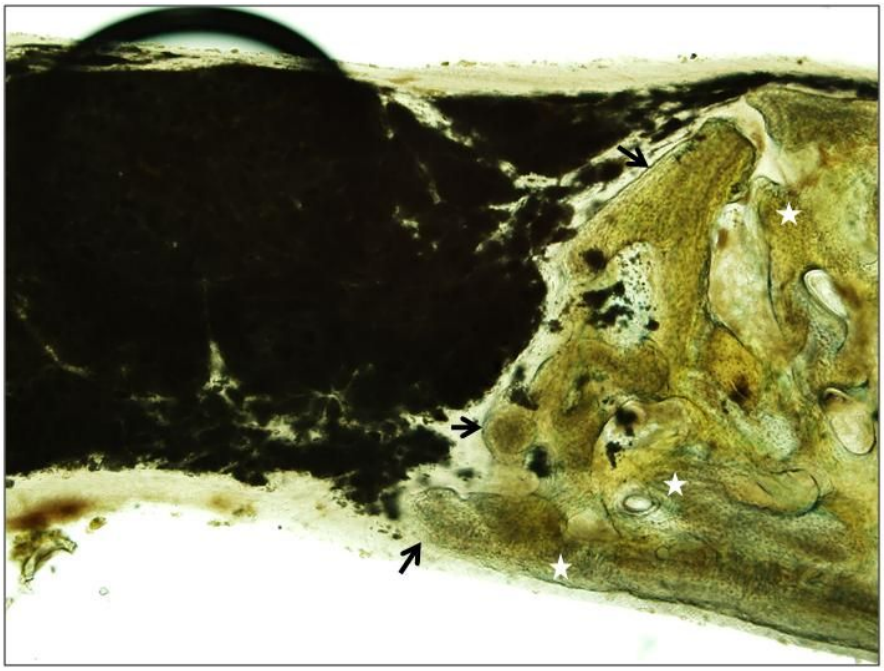

Fig. 2

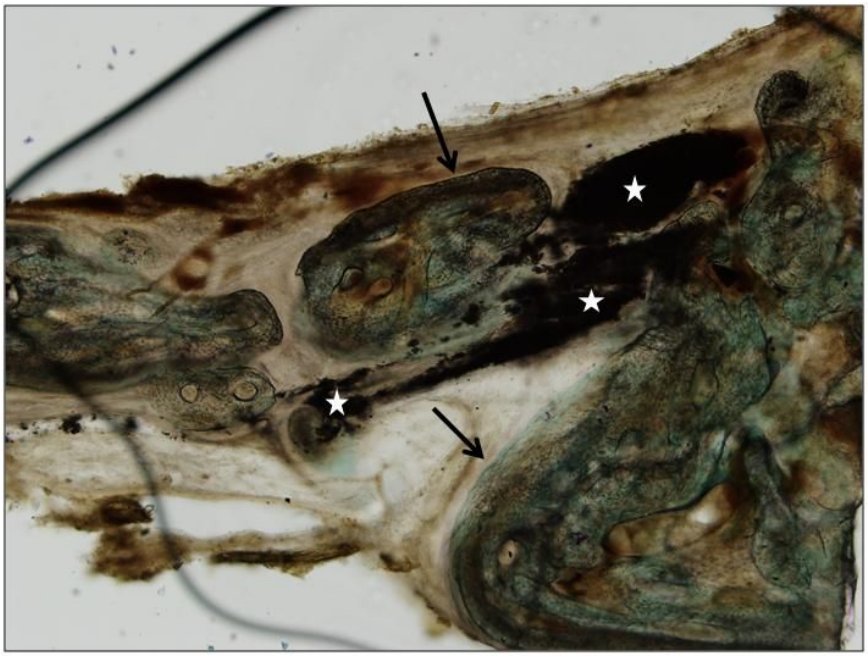

Fig. 3

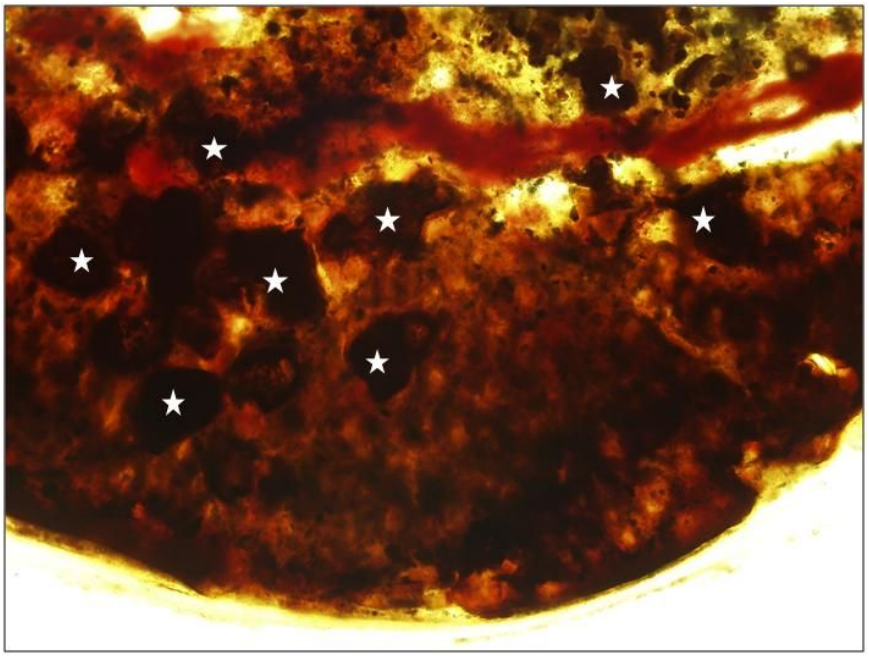

Fig. 4

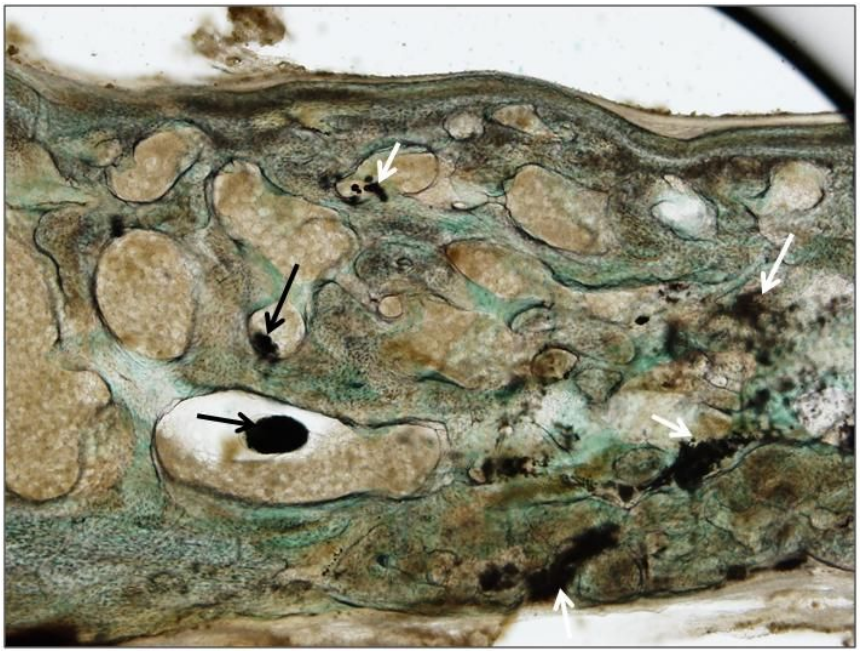

Fig. 5

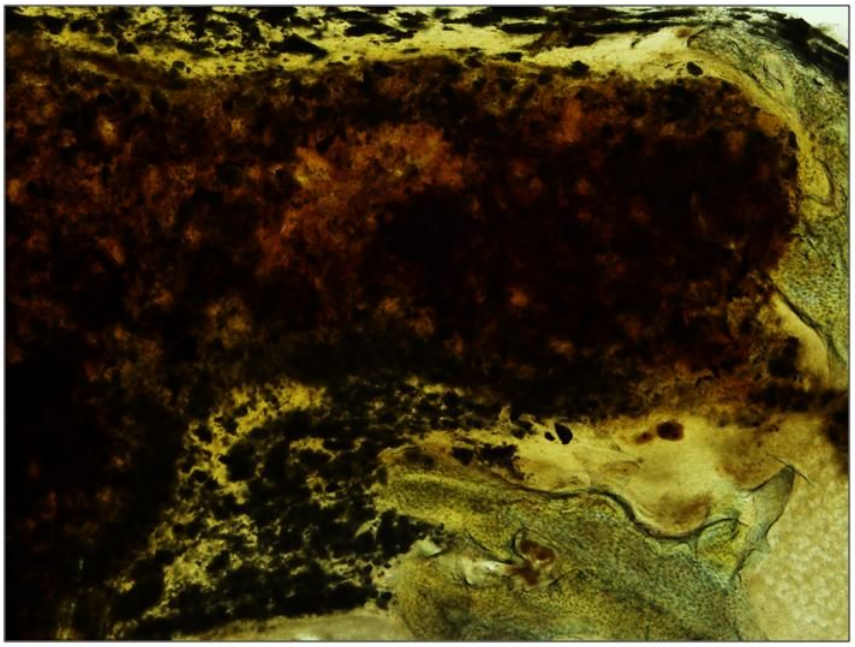

Fig. 6

저작물 이용 허락서

학 과	치의생명공학과	학 번	20077560	과 정	박사
성 명	한글 : 김 봉 균 한문:金 奉 均 영문: Kim, Bong-Kyun				
주 소	강원도 원주시 개운동 원주현대홈타운스위트 101-104 103동1301호				
연락처	E-MAIL : drbongdal@naver.com				
논문제목	한글 : 가토에서 치아회분말과 치과용 연석고 혼합 이식재에 혈소판 풍부혈장, 조직접합제 혼합 매식 후 골형성 비교 평가				
	영어 : Histomorphometric analysis of bone healing in rabbits using tooth ash and plaster of Paris, platelet-rich plasma, and fibrin sealant				

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( )

2010 년 2 월

저작자: 김 봉 균 (서명 또는 인)

조선대학교 총장 귀하