The effects of noncrystalline calcium phosphate glass on the healing of 1-wall intrabony defects in beagle dogs

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I. Introduction

The ultimate goal of periodontal therapy is the regeneration of periodontal tissue that has been lost due to destructive periodontal disease[1]. This requires new bone formation and new cementum formation accompanied by newly inserted functionally oriented fibers at a tooth site previously exposed to the oral environment.

Various surgical techniques, bone grafts, and guided tissue regeneration (GTR) have been used for periodontal regeneration. Bone grafts include autograft, allografts, xenografts, and synthetic grafts (bone substitutes). Autogenous bone grafts, by using intraoral or extraoral donor site, provide considerably favorable results to achieve periodontal regeneration. However, autografts require additional surgical procedures. This poses a problem since a large amount of donor tissue is often hard to obtain[2,3]. Allografts also result in problems like unreliable graft incorporation, immune response, and possible disease transmission. For these reasons, various kinds of synthetic bone substitutes have been investigated. Among these are hydroxyapatite, tricalcium phosphate, calcium carbonate, HTR polymer, and bioactive glass ceramics[4-8]. Because hydroxyapatite and tricalcium phosphate have no toxic reaction and have biocompatibility, they have been widely used for bone substitutes[9-14]. Yukna et al.,

Rabalis et al., and Meffert et al. reported based on

* This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea. (03-PJ1-PG1-CH08-0001)
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clinical studies that the use of hydroxyapatite resulted in attachment gain and pocket reduction[4,15,16]. On the other hand, Froum et al., Moskow et al., and Baldock et al. reported based on human histological studies that neither new bone formation nor attachment gain was observed around tricalcium phosphate and hydroxyapatite implants[17-19]. Therefore, clinically, hydroxyapatite and tricalcium phosphate have an advantage. Histologically, however, they act as biocompatible fillers that show limited new attachment.

Guided tissue regeneration (GTR) procedure has been established as a method to gain new attachment. Melcher suggested that periodontal tissues could regenerate depending on the cell types that migrate into the root surface, and that periodontal ligament cells are capable of regenerating periodontal tissues[20].

Some favorable current regenerative materials include autografts, allografts, bone substitutes, and GTR, which can be performed alone or in combination with these materials. Lekovic et al. and Nery et al. reported that combined use of synthetic bone grafts and GTR provided better results than using only synthetic bone grafts[21, 22]. On the other hand, Caffesse et al. reported that combination of GTR with synthetic bone grafts did not enhance periodontal regeneration[23].

Lee et al. recently fabricated calcium phosphate glass with Ca/P ratio of 0.6 using the CaO-CaF_2-P_2O_5-MgO-ZnO system[24]. This material, which has a noncrystalline structure and a low Ca/P ratio, can be expected to extend the application field to biomaterials for hard tissue repair. Low Ca/P ratio and amorphous states provide a great extent of dissolution and resorption that allows the fast ingrowth of surrounding bone.

The purpose of this study was to evaluate the effects of noncrystalline calcium phosphate glass and combined therapy with calcium phosphate glass and GTR on the regeneration of periodontal tissue in beagle dog.

II. Materials and Methods

1. Manufacturing calcium phosphate

Calcium phosphate glasses with Ca/P ratio of 0.6 were prepared from the system CaO-CaF_2-P_2O_5-MgO-ZnO. Mixed batches were melted in a platinum crucible at 1250° C and poured onto a graphite plate at room temperature. As-quenched glasses were ground using an alumina mortar. The particle size of the powdered sample was determined to be 200-500 ㎛.

2. Surgical protocol

Six (6) male adult beagle dogs were used for the experiment. All the teeth of the dogs were fully erupted and the periodontal tissues were in a healthy state. The distal side of the second premolar and the mesial side of the fourth premolar served as experimental sites.

Dental infiltration anesthesia (2% lidocaine HCl) was used at the surgical site, after which both mandibular first and third premolar were extracted prior to the experimental surgeries. Surgical procedure was performed under general anesthesia (sodium pentobarbital) induced by intravenous injection. The extraction sites were allowed to heal for 2 months. After 2 months, 4×4 mm 1-wall intrabony defects were surgically created in the bilateral mandibular second and fourth premolars under the same general and local anesthesia (Figure 3). A total of 18 surgical sites for 6 beagle dogs (6 sites per group) were made. Following root planing, a reference notch was made with 1/4 round bur on the

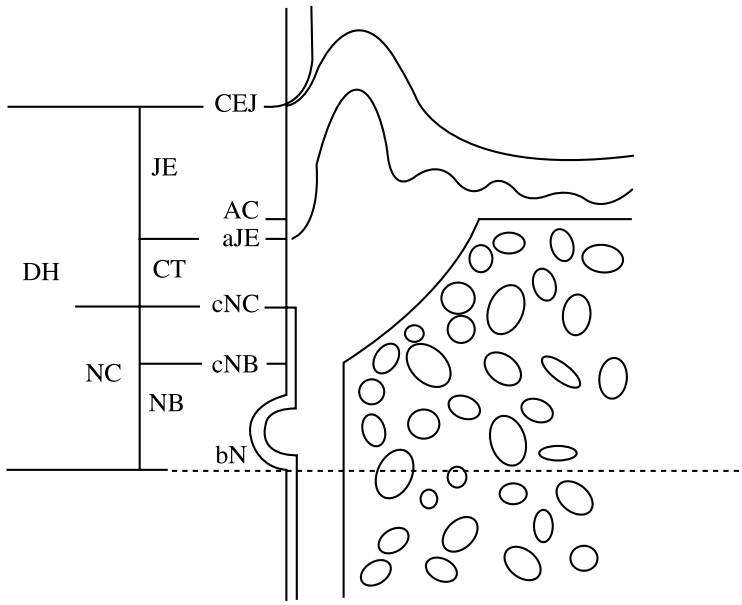

Figure 1. Schematic diagram depicting the experimental design, landmarks, and parameters used in the histomorphometric analysis.

CEJ: cementoenamel junction
aJE: apical extent of junctional epithelium
cNC: coronal extent of new cementum
DH: defect height
CT: connective tissue adhesion
NB: new bone regeneration
AC: alveolar crest
bN: base of the reference notch
cNB: coronal extent of new bone
JE: junctional epithelium migration
NC: new cementum regeneration

root surface at the base of defect.

3. Experimental design

Experimental group 1 was treated with calcium phosphate glass only. Experimental group 2 was treated with a combination of calcium phosphate and resorbable (Resolut®) membrane which was used for guided tissue regeneration. The control group went through a conventional flap operation. The subjects were sacrificed 8 weeks after operation.

4. Histologic findings

Specimens were fixed in 10% buffer formalin, decalcified in nitric acid, embedded in paraffin, sectioned mesiodistally making 4 specimens for each block, and stained with hematoxylin and eosin.

The following conditions were observed in the prepared specimens: apical migration of juctional epithelium, infiltration of inflammatory cells, resorption state of calcium phosphate glass, adhesion of connective tissue, new cementum and new bone formation, and periodontal ligament between new bone and new cementum

5. Histomorphometric analysis

The cementoenamel juction (CEJ) and the notch (bN) were used as reference points. Histometric parameters included defect height (DH), junctional

epithelium (JE), connective tissue adhesion (CT), cementum regeneration (NC), and alveolar bone regeneration (NB).

The histomorphometric recording from the 4 sections from each defect were used to calculate the mean score (Figure 2).

6. Statistical analysis

The Kruskal-Wallis test and Mann Whitney U test were used. Root resorption and ankylosis were scored when observed in one or more of the 4 sections for each tooth.

III. Results

1. Histologic findings

1) Control group

The junctional epithelium migrated apically. Connective tissue adhesion was observed parallel to the long axis of tooth beneath the junctional epithelium. Inflammatory cell infiltration was observed to be minimal in defect sites. New cementum and new bone were formed above the notch. Ankylosis was not observed in all teeth (Figures. 5-7).

2) Experimental group 1 (calcium phosphate)

Junctional epithelium migrated apically but not longer and more restricted than the control group. Connective tissue adhesion was observed parallel to the long axis of tooth beneath the junctional epithelium. No inflammatory infiltration was seen in the connective tissue. A large amount of new cementum was formed and the amount of new bone was a little more than that in the control group. New cementum formed near the notch was thick, but the formation gradually became thinner in the coronal direction. There were fibrous encapsulations above the new bone. Ankylosis was not observed in all teeth (Figures. 8-10).

3) Experimental group 2 (calcium phosphate + GTR)

Severe inflammatory cell infiltration was observed in the connective tissue. The other findings were similar to that of experimental group 1 (Figures. 11-13).

2. Histomorphometric analysis

1) Defect height

The defect height was measured at 4.82 ± 0.45 ㎜ in the control group, 4.61 ± 0.71 ㎜ in experimental group 1, and 4.92 ± 0.62 ㎜ in experimental group 2. There was no statistically significant difference in defect height among the 3 groups.

2) Junctional epithelium migration

Junctional epithelium migration was 30.90 ± 9.92% of the defect height in the control group, 24.08 ± 9.12% in experimental group 1, and 38.68 ± 12.22% in experimental group 2. There was no statistically significant difference regarding junctional epithelium migration among the 3 groups.

3) Connective tissue adhesion

Connective tissue adhesion was 36.38 ± 9.03% of the defect height in the control group, 26.96 ± 4.23% in experimental group 1, and 27.87 ± 9.70% in experimental group 2. There was no statistically significant difference regarding connective tissue adhesion among the 3 groups.

4) New cementum formation

New cementum regeneration was 32.92 ± 10.51% of the defect height in the control group, 49.16 ± 12.70% in experimental group 1, and 39.62 ± 12.14% in experimental group 2. A statistically significant difference in new cementum regenera-

Table 1. Histomorphometric analysis (%)

	control		exp1		exp2		
	mean	S.D	mean	S.D	mean	S.D	P value
JE/DH	30.90	9.92	24.08	9.12	38.68	12.22	0.121
CT/DH	36.38	9.03	26.96	4.23	27.87	9.70	0.184
NC/DH	32.92	10.51	49.16*	12.07	39.62	12.14	0.049
NB/DH	27.24	7.49	43.51	13.34	36.47	15.11	0.057

* Statistically significant difference compared to control group, P〈0.05 (n=6).

DH: defect height JE: junctional epithelium migration

CT: connective tissue adhesion NC: new cementum regeneration

NB: new bone regeneration

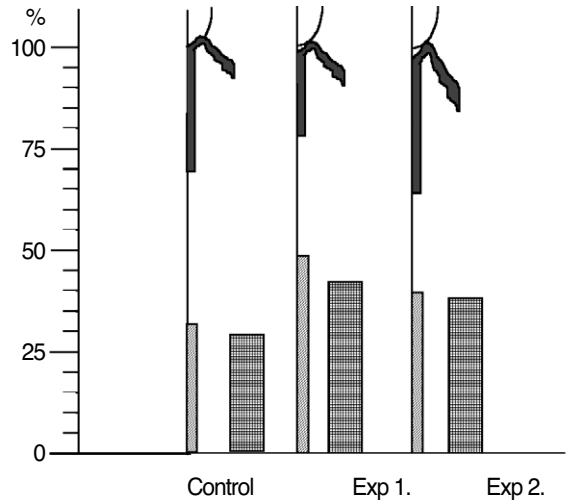

Figure 2. Periodontal healing illustrated in percentage of the defect height.

tion was observed between the control group and experimental group 1 (p〈0.05).

5) New bone formation

New bone regeneration was 27.24 ± 7.49% of the defect height in the control group, 43.51 ± 13.34% in experimental group 1, and 36.47 ± 15.11% in experimental group 2. There was no statistically significant difference regarding new bone formation among the 3 groups.

IV. Discussion

Many investigations about bone substituting materials for treatment of periodontal disease have been made. Inducing new bone formation and new cementum formation are ideal characteristics for the bone substituting materials used in the periodontal defect. They should have biocompatibility and economic efficiency and should also be easy to use.

Recently, autogenous bone grafts and synthetic bone grafts have been popularly used. Autogenous bone grafts provide good clinical and histological results. However, they require additional surgical procedure and a large amount of donor tissue is often hard to obtain. Therefore, many synthetic bone graft materials have been developed until the most recent year.

Melcher suggested that periodontal ligament cells are capable of regenerating periodontal tissues[20]. Nyman et al. showed new cementum regeneration with principal fiber insertion originating in the periodontal ligament cells using a cellulose acetate filter (milipore filter)[25,26]. Following this study, Gottlow et al. proposed the term "guided tissue regeneration" (GTR) for the procedure, which had shown regeneration in several intrabony defect cases using ePTFE membranes[27].

The bone graft materials used in a combined therapy with membranes were expected to not only induce bone formation but also act as clot-stabilizer. They could provide the matrix upon which the cells could be allowed to differentiate into osteoblast. A non-resorbable type membrane was used at first, but it had disadvantages such as additional surgical operation, danger of infection, and requirement of careful skill. Consequently, a resorbable type membrane was developed. Calcium phosphate and its derivatives have been studied among the synthetic bone grafts. Meffert et al., Kenney et al., and Mora et al. reported based on human clinical studies that hydroxyapatite showed pocket reduction and increase of attachment leve[14,11,28]. Froum et al. reported that hydroxyapatite implants showed pocket reduction clinically but there was no histological evidence of new attachment, cementogenesis, and osteogenesis[17]. Stahl et al. reported that hydroxyapatite acted as a well tolerated filler[29]. Baldock et al. and Stahl et al. reported that tricalcium phosphate did not show increased new attachment, cementogenesis, and osteogenesis histologically[14,19]. Hashimoto et al. reported that biphasic calcium phosphate (βtricalcium phosphate + HA) had osteoconductive potential. This potential could be related to degradation by macrophage phagocytosis as noted in the clinical and histological observation of monkeys[30]. The difference of many alloplasts is caused by the rate of resorption before new bone regenerates. This rate of resorption is influenced by physical properties such as surface area, form of product, and crystallinity. Chemical properties such as Ca/P ratio, elemental impurities, and pH of surrounding area also affect the rate of resorption[24]. Lee et al. recently fabricated new calcium phosphate glasses. These calcium phosphate glasses used in this experiment had Ca/P ratio of 0.6. They had 200-500 ㎛ particle size and had a noncrystalline structure. Consequently, calcium phosphate glasses accelerated new bone regeneration. An increase in the number of intrabony pocket lead to a more achievable periodontal regeneration[31].

In this study, experiments were conducted to evaluate the effect of noncrystalline calcium phosphate glasses on 1-wall intrabony defect in beagle dogs. This kind of defect is generally difficult for periodontal regeneration. The experiment combined the use of GTR and calcium phosphate glasses also. The membrane was expected to stabilize the calcium phosphate glasses and contribute to their initial fixation. Because there were some differences of defect height in every group, the migration of junctional epithelium, connective tissue adhesion, new cementum regeneration, and new bone regeneration were shown to be the ratio of defect height for accuracy.

For the migration of junctional epithelium, no statistically significant difference was observed among the 3 groups. However, experimental group 1 looked more restricted than the control group. In spite of using GTR to restrain the migration of junctional epithelium apically, experimental group 2 did not show statistically significant difference with the control group. However, it did exhibit a more apical migration of junctional epithelium than control group. It was supposed that this was the reason the membrane caused inflammation and failed initial fix-

ation.

With regard to connective tissue adhesion, there was also no statistically significant difference among the 3 groups, but the experimental groups looked more restricted than the control group. The amount of new alveolar bone formation in experimental group 1 was higher than that of the control group (experimental group 1: 16%, P=0.057). However there was no statistically significant difference among the 3 groups. The difficulty in maintaining the forming shape in the experimental site could be due to the high flowability and unresorbable fibrous encapsulated glasses, which disturb the bone ingrowth into the surrounding tissue.

With regard to new cementum regeneration, there was a statistically significant difference between the control group and experimental group 1. Calcium phosphate glasses were found to be effective in cementum regeneration. Thick formation of new cementum was observed near the notch, and it gradually became thinner in the coronal direction. This result showed that good agreement in the new bone followed the coronal growth of new cementum, suggesting that the cementum helped the formation of new bone and periodontal ligament fibers. Lee et al. recently reported that noncrystalline calcium phosphate glass was effective in cementum regeneration[32]. The results of this study were similar to Lee's findings. Ankylosis was not observed in all 3 groups, considering that ankylosis often occurred in the site of fast osteogenetic development without the periodontal regeneration.

In summary, Calcium phosphate glasses used in this study restrained the migration of junctional epithelium and effectively increased new cementum and relatively new bone.(P=0.057)

Further studies are required to reduce the flowability in order to stabilize the calcium phosphate glasses in the periodontal wound.

V. Conclusions

Periodontal therapy promotes the regeneration of periodontal tissue that has been lost due to destructive periodontal disease. This requires new bone formation and new cementum formation accompanied by newly inserted fibers at a tooth site previously exposed to oral environment.

This study evaluated the effect of noncrystalline calcium phosphate glasses and combined therapy with calcium phosphate glass and GTR on the regeneration of periodontal tissue in beagle dog.

Eighteen (18) intrabony defects, with 4x4 ㎜ 1-wall intrabony defects, were surgically created in the mandibular second and fourth premolars of 6 beagle dogs. The control group carried out a conventional flap operation. Experimental group 1 was treated with calcium phosphate glasses only and experimental group 2 was treated with combination of calcium phosphate glasses and GTR. The subjects were sacrificed 8 weeks after the operation and a histological examination was done. The results were as follows:

1. The junctional epithelium migration was 30.90 ± 9.92% of the defect height in the control group, 24.08 ± 9.12% in experimental group 1, and 38.68 ± 12.22%in experimental group 2. There was no statistically significant difference among the groups.
2. Connective tissue adhesion was 36.38 ± 9.03% of the defect height in the control group, 26.96 ± 4.24% in experimental group 1, and 27.87 ± 9.70% in experimental group 2. There was no statistically significant difference among the groups.
3. New cementum regeneration was 32.92 ± 10.51% of the defect height in the control group, 49.16 ± 12.70% in experimental group

1, and 39.62 ± 12.14% in experimental group 2. There was a statistically significant difference between the control group and experimental group 1.

4. New bone regeneration was 27.24 ± 7.49% of the defect height in the control group, 43.51 ± 13.34% in experimental group 1, and 36.47 ± 15.11% in experimental group 2. There was no statistically significant difference among the groups.

The calcium phosphate glasses used were observed to restrain the migration of junctional epithelium. They also increased the formation of new cementum and relatively new bone.

VI. Reference

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사진부도 설명

Figure 1. Schematic diagram depicting the experimental design, landmarks, and parameters used in the histomorphometric analysis.

Figure 2. Periodontal healing illustrated in percentage of defect height.

Figure 3. Surgically created 1-wall defect; the formation of 1-wall defect with 4x4mm in second and fourth premolar.

Figure 4. Calcium phosphate graft and GTR
Calcium phosphate graft only and combination of calcium phosphate and GTR in 1-wall defects.

Figure 5. Control (H-E×20). Apical migration of junctional epithelium and new bone regeneration above the notch.

Figure 6. Control (H-E×100). Connective tissue adhesion observed parallel to root surface; inflammatory cell infiltration minimal in connective tissues.

Figure 7. Control (H-E×100). New bone and new cementum formation.

Figure 8. Experimental 1 (H-E×20). Junctional epithelium migration was more restricted than the control; no inflammatory cells infiltrated the connective tissue.

Figure 9. Experimental 1 (H-E×100).
Connective tissue adhesion observed parallel to root surface.

Figure 10. Experimental 1 (H-E×100)
Large amount of new cementum and new bone formation; fibrous encapsulations observed above the new bone.

Figure 11. Experimental 2 (H-E×20).
New cementum and new bone formation.

Figure 12. Experimental 2 (H-E×100)
Severe inflammatory cell infiltration observed in the connective tissue.

Figure 13. Experimental 2 (H-E×100)
Thick new cementum formation near the notch becomes thinner in the coronal direction.
(N:notch, JE:junctional epithelium, CT:connective tissue, NB:new bone, NC:new cementum, MP:material particle)

사진부도 (I)

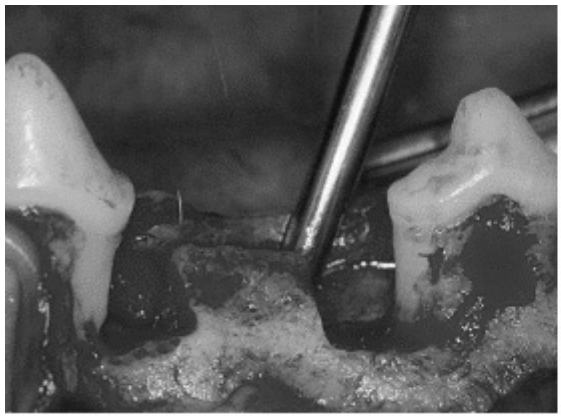

Figure 3. Surgically created 1-wall defect.

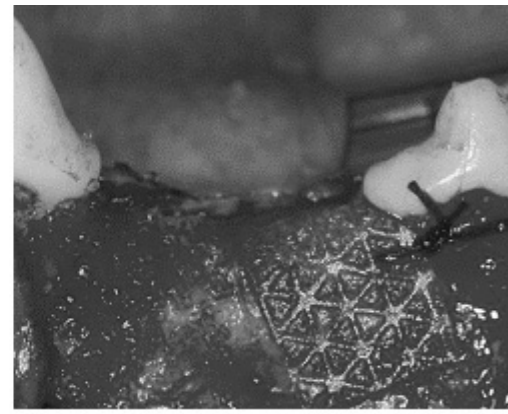

Figure 4. Calcium phosphate graft and GTR

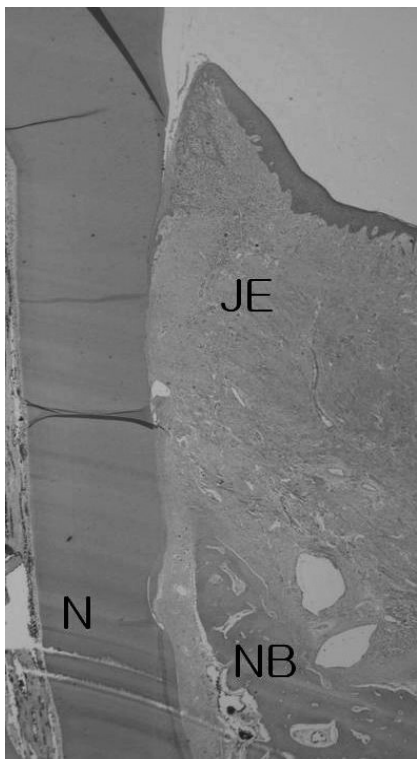

Figure 5. Control (H-E×20).

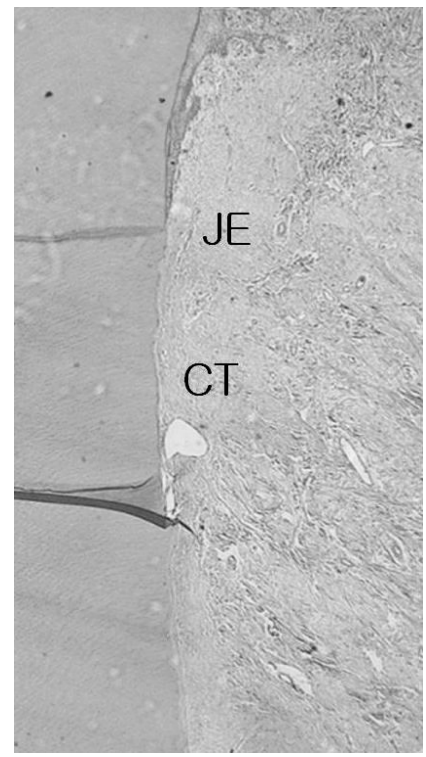

Figure 6. Control (H-E×100).

사진부도 (Ⅱ)

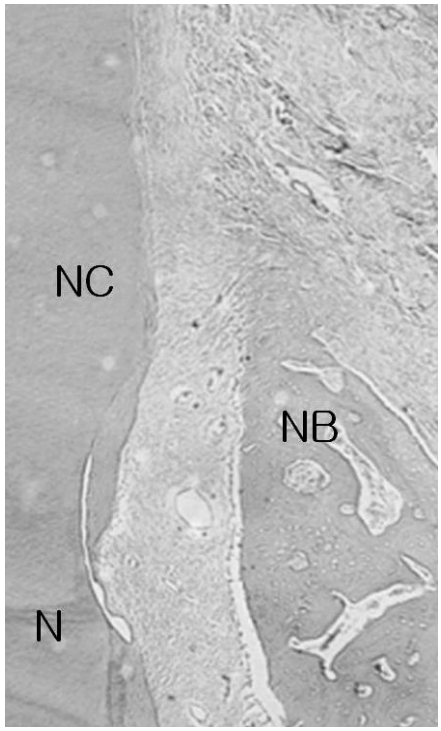

Figure 7. Control (H-E×100).

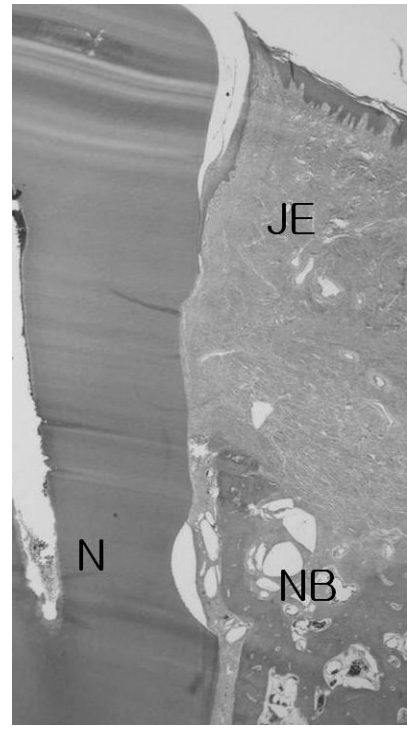

Figure 8. Experimental 1 (H-E×20).

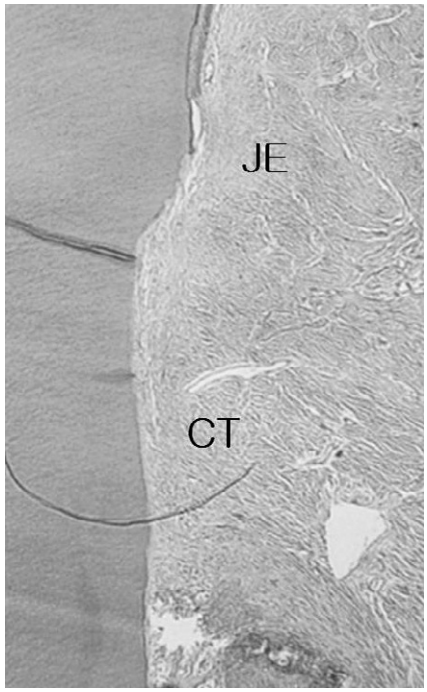

Figure 9. Experimental 1 (H-E×100).

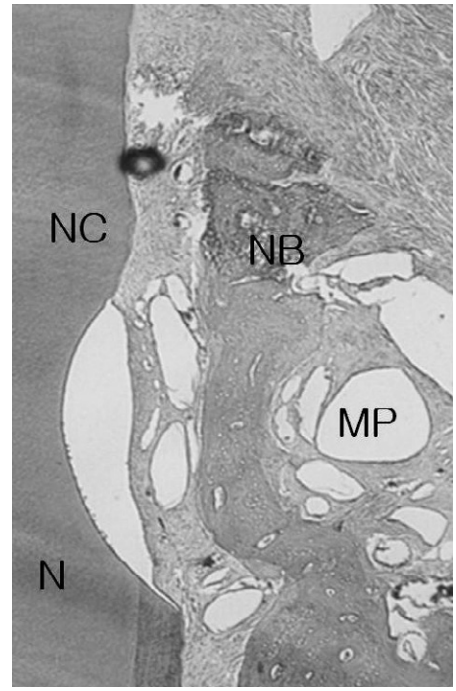

Figure 10. Experimental 1 (H-E×100).

사진부도 (III)

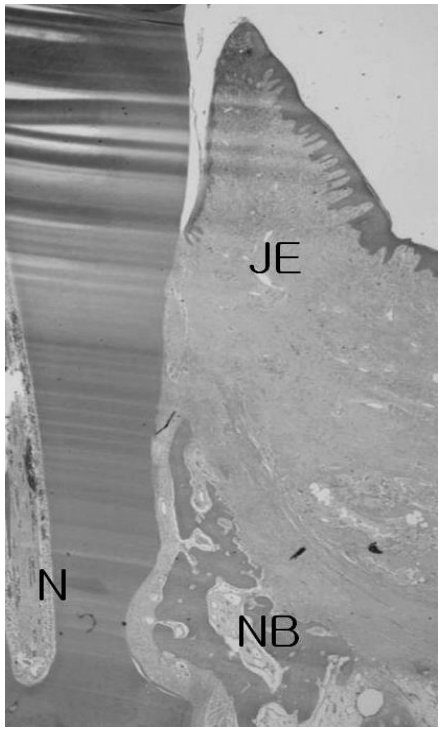

Figure 11. Experimental 2 (H-E×20).

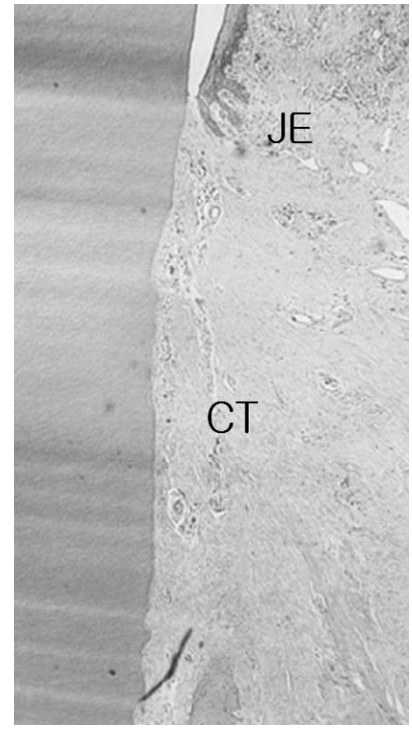

Figure 12. Experimental 2 (H-E×100).

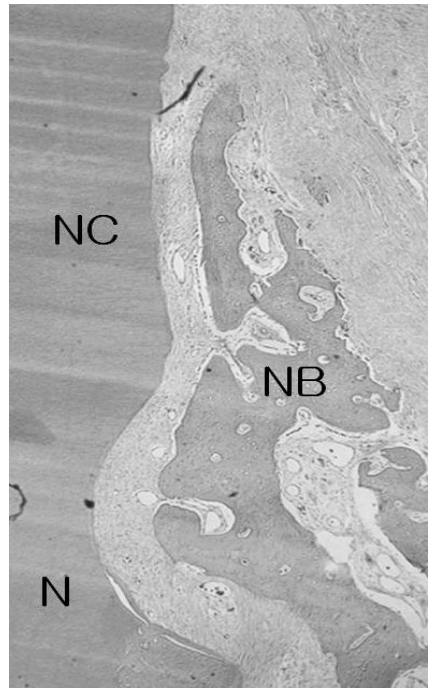

Figure 13. Experimental 2 (H-E×100).

-국문초록-

비결정성 calcium phosphate가 성견의 1면 골내낭에서의 치주조직 재생에 미치는 영향

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연구목적 : 이번 실험의 목적은 외과적으로 형성된 성견의 1면 골내낭에 새롭게 제조된, Ca/P 비율이 0.6인 비결정성 calcium phosphate를 적용하였을 때 치주조직의 재생에 미치는 영향을 평가하는 것이다.

연구방법 : 6마리 성견의 양측 하악 제2소구치의 원심면, 제4소구치의 근심면에 외과적으로 1면 골내낭을 형성하여 치은박리소파술을 시행한 부위를 대조군으로, calcium phosphate만을 이식한 부위를 실험 1군, calcium phosphate와 GTR을 동반한 부위를 실험 2군으로 설정하고 실험하여 술 후 8주에 치유결과를 조직학적으로 관찰하였다.

결론 :

1. 접합상피의 치근단 이동은 대조군에서 결손부 깊이의 30.90 ± 9.92%, 실험 1군에서 결손부 깊이의 24.08 ± 9.12%, 실험 2군에서 결손부 깊이의 38.68 ± 12.22%로 나타났으며 대조군과 실험 1,2군간에 통계적 유의차는 없었다.
2. 결합조직 유착은 대조군에서 결손부 깊이의 36.38 ± 9.03%, 실험 1군에서 결손부 깊이의 26.96 ± 4.24%, 실험 2군에서 결손부 깊이의 27.87 ± 9.70%로 나타났으며 대조군과 실험 1, 2군간에 통계적 유의차는 없었다.
3. 신생백악질 형성은 대조군에서 결손부 깊이의 32.92 ± 10.51%, 실험 1군에서 결손부 깊이의 49.16 ± 12.70%, 실험 2군에서 결손부 깊이의 39.62 ± 12.14%로 나타났으며 대조군과 실험 1군간에 통계적 유의차가 있었다.
4. 신생골 형성은 대조군에서 결손부 깊이의 27.24 ± 7.49%, 실험 1군에서 결손부 깊이의 43.51 ± 13.34%, 실험 2군에서 결손부 깊이의 36.47 ± 15.11%로 나타났으며 대조군과 실험 1, 2군간에 통계적 유의차는 없었다.

이상의 결과에서 볼 때, calcium phosphate glasses는 신생골 형성에는 통계적으로 유의차는 없었지만 상당히 증가된 양상을 보였고 신생백악질 형성에는 크게 기여함을 알수 있었다.

주요어 ; 칼슘포스페이트, 골이식, 1면 골내낭, 치주재생