The bone formation and healing effects of calcium phosphate glass cement in intrabony defects of beagle dogs

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This certifies that the Doctoral Dissertation
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감사의 글

이 논문이 완성되기까지 아낌 없는 조언과 격려로 지도해 주신 채중규 교수님께 감사를 드립니다. 또한, 동물실험의 경험이 부족한 저를 위해서, 실험의 진행과정을 도와 주신 정의원 교수님과 연세대 치주과 의국원 모두에게도 감사의 말씀을 드립니다. 그리고 논문의 주제부터 결론까지 전 과정을 컨트롤 해주시고, 논문이 학회지에 등재될때까지 감수해주신 최성호 교수님께 깊은 감사를 드립니다.

항상 곁에서 지켜봐 주시고 힘들 때 마다 버팀목이 되어주신 어머님과 장인어른, 장모님, 아낌없는 응원과 격려를 해준 사랑하는 누나, 형님들께 고마운 마음을 전합니다.

마지막으로 항상 밝은 미소로 옆을 지키며 아낌 없는 사랑과 조언으로 큰 힘이 되어준 나의 조선미 선생한테 사랑하는 마음을 전합니다. 감사합니다.

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ABSTRACT

The bone formation and healing effects of calcium phosphate glass cement in intrabony defects of beagle dogs

The aim of this study was to investigate the bone formation effect of amorphous calcium phosphate glass cement (CPGC) synthesized by a melting and quenching process.

In five male beagle dogs, 4x4mm 1-wall intrabony defects were created bilaterally at the mesial or distal aspect of mandibular second and fourth premolars. Each of the four defects was divided according to graft materials: CPGC with collagen membran e (CM), biphasic calcium phosphate (BCP) with CM, CM alone, or a surgical flap operation only. The dogs were sacrificed 8 weeks post surgery, and block sections of the defects were collected for histologic and histometric analysis.

There was significant difference in bone formation and cementum regeneration between the experiment and control groups. In particular, the CPGC and BCP groups showed greater bone formation than the CM and control groups.

In conclusion, CPGC was replaced rapidly with an abundant volume of new bone; CPGC also contributed slightly to regeneration of the periodontal apparatus.

Key words: Calcium phosphate glass cement, Bone formation, Bone grafting, 1-wall intrabony defect

The bone formation and healing effects of calcium phosphate glass cement in intrabony defects of beagle dogs

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I. INTRODUCTION

Regerative treatment of large periodontal bone defects remains a challenge for periodontists. Autologous bone grafting is considered to be the gold standard in reconstructive bone surgery due to its superior osteogenic potential compared to allogenic transplants. However, the availability of autogenous graft material is limited. Graft harvest lengthens operation time and can be associated with adverse effects, such as bleeding, pain, and infection. Allogenic bone material carries the risk of infectious disease transmission, including human immunodeficiency virus (HIV) or hepatitis.[1-2] Due to these drawbacks, inorganic composites are of special interest as a bone substitute. Synthetic grafts must be composed of a three-dimensional porous material to induce bone formation and osteoconduction.

Calcium phosphates (CP) have been received the most attention as synthetic agent, and they are widely used because of their good biocompatibility and osteointegrative properties.[3] Synthetic hydroxyapatite (HA) is one type of CP and can be anchored to native bone by the establishment of a physicochemical bond with living tissue.[4] Extensive research has been performed on CP over the past 20 years as a leading candidate of CP, and it has been used clinically in various forms due to its osteoconductive properties. Many investigators report that HA resulted in improved attachment and pocket reduction in clinical studies.[5]

However, the success of these materials is limited primarily due to inappropriate physical properties such as inadequate toughness, low elasticity, low resorbability, and lack of osteogenic properties.[6] Calcium phosphate glass (CPG) materials are known to have osteoconductive characteristics, serving as an active participatory template for the formation of new bone.[7] CPG is bioactive and have a chemical composition very similar to that of the mineral phase of bone. Recently, LeGeros and Lee reported on CPGC in a CaO-CaF2-P2O5-MgO-ZnO system, where it was observed to promote of bone-like tissue formation in vitro.[8] Moreover, CPGC is easily synthesized by a melting and subsequent quenching process and has a low viscosity in the molten state.[9] Accordingly, CPGC can offer stability in the repair of bone defects during the initial 6 weeks. Previous studies reported that CPGC has more desireable physical properties than BCP.[10] However, the bone formation and periodontal regeneration abilities of CPGC comparing with BCP have not been documented.

The objective of this study was to evaluate the bone formatiom effect of CPGC in a system of CaO-CaF2-P2O5-MgO-ZnO and to determine the influence of CPGC on periodontal regeneration in 1-wall intrabony defects in mandible of beagle dogs by comparing CPGC performance with that of BCP.

II. MATERIALS AND METHODS

1. Animals

Five male beagle dogs (25kg~30kg), 18 to 24 month old were used. The animals had intact dentition and healthy periodontium. Animal selection,management, surgical protocol and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea

2. Experimental groups

Four defects were created in each dog, and one defect was assigned to each experimental group as follows:

CPGC group - defect filled with CPGC covered by collagen.

BCP group - defect filled with BCP covered by collagen.

CM group - defect covered by collagen membrane.

Control group - non-grafted.

3. Graft materials

BCP (Osteon™; Genoss.Co.Ltd., Suwon, Korea) is composed of 70% HA and 30% β -tricalcium phosphate (β-TCP). HA coated with β-TCP establishes an interconnected scaffold with a porosity of 300~500nm.

CPGC were prepared from the CaO-CaF2-P2O5-MgO-ZnO system. Mixed batches were melted in a platium crucide at 850℃ and poured onto a graphite plate at room temperature as quenched glasses were ground in an alumina mortar. Particle size of the powdered sample was 0.08 to 555.7μm (average size: 167.95μm). CPG was mixed with Na2CO3 powder in NaOH solution to maintain stability of shape ad control the setting time. The final product of CPGC was amorphous calcium polyphosphate.

CM (CollaTape®: Integra Lifesciences Co., Planisboro, USA) was fabricated using collagen obtained from bovine deep flexor (Achilles) tendon.

4. Surgical procedure

Surgery was performed under general anesthesia induced by intravenous injection of atropine (0.04 mg/Kg) and intramuscular injection of xylazin (Rompun, Bayer Korea, Seoul, Korea) and ketamine (Ketalar, Yuhan Co., Seoul Korea) followed by the administration of inhaled enflurance. Routine dental infiltration anesthesia (2% lidocaine with HCl epinephrine, 1:100 000, Kwangmyung Pharm., Seoul, Korea) was used at the surgical site. The mandibular 1st and 3rd premolar teeth were extracted in advance of the experimental surgeries, and the extraction sites were allowed to heal for 8 weeks. One-wall intrabony defects measuring 4x4mm were created bilaterally at the mesial aspect of mandibular 2nd and 4th premolar areas. A reference notch was made with a round bur on the root surface at the base of the each defect. In each dog, two defects were filled with CPGC or BCP then covered with CM. One defect was

not filled with graft material and was covered with CM. The other defect was treated by surgical flap operation only. The surgical flap was sutured with 5-0 resorbable suture material (Polyglactin 910, braided absorbable suture, Ethicon, Johnson & Johnson Int., Edinburgh, U.K.). Intramuscular antibiotics were administered for 3 days and daily dressing of 0.2% chlorhexidine solution (Hexamedin, 2% chlorhexidine, Bukwang Pharm. Co., Seoul, Korea) we applied for 7 days as postsurgical care. The suture was removed after 10 days. The animals were sacrificed 8 weeks after surgery and block sections of the defect sites were collected and prepared for histological and histometric evaluation (Figure 1).

5. Analysis

1) Histologic analysis

The block sections were fixed in 10% buffered formalin and decalcified with 5% nitric acid for 14 days. Paraffin wax blocks were made and sectioned in the mesio-distal direction with a thickness of 4μm. Each section was stained in hematoxylin-eosin (HE). General histological findings were observed with a stereoscope (LEICA MZFLⅢ, LEICA, WETZLAR, Germany) and microscope.

2) Histometric analysis

After conventional microscopic examination, computer-assisted histometric measure ments were obtained using an automated image analysis system (Image-Pro Plus®,

Media Cybernetics, Silver Spring, M.D.) coupled with a video camera mounted in a light microscope (LEICA DM-LB, LEICA, WETZLAR, Germany). The measuring parameters were as follows: the cementoenamel junction (CEJ) and the notch were used as reference points (bN) and the histometric parameters included defect height (DH), junctional epithelium (JE), connective tissue attachment (CT), cementum regeneration (NC) and bone regeneration (NB) (Figure 2).

3) Statistical analysis

Histomorphometric recordings from the four sections from each defect were used to calculate mean score for each animal. Statistical analysis was compared by the Kruskal-Wallis test. ANOVA Boneferroni method was used to evaluate the statistical sgnificance among the 4 groups ($p<0.05$).

III. RESULTS

1. Clinical findings

During the postoperative period, healing was uneventful for all animals. There were no sign of inflammation and no wound exposures.

2. Histological findings

Histologically, the junctional epithelium migrated apically and inflammatory cell infiltration was minimal in all defect sites. Connective tissue attachment was observed perpendicular to the long axis of tooth beneath the junctional epithelium. The periodontal ligament was organized with primarily irregular collagen fibers.

The CPGC group showed a large amount of new cementum and new bone formation, more than that seen in the control and CM groups. Resorption of graft material was detectable and several calcium phosphate remnants were surrounded by provisional connective tissue, which will eventually transform into bone. The regenerated bone tissue was embedded and formed within graft materials. The edge of the particles exhibited irregular features and the presence of multinucleated giant cells, indicating the resorption or dissolution process. In the mature new bone portion, osteoclasts observed on the surface of a secondary osteon within the bone trabeculae suggested an active remodeling process. New cementum that was thinner than the original cementum was observed along the root surface, extending to the level of regenerated

bone. Residual collagen membrane was not observed in the CPGC, BCP, or CM groups (Figure 3).

The BCP group showed newly formed woven bone and connective tissue around the remaining HA coated with β-TCP particles. There were no osteoblasts and osteoclasts around BCP particles adjacent to the defect base. However, loose connective tissue including undifferentiated cells and blood vessels was observed around coronal particles. Regenerated cementum and periodontal ligament space could be observed along the root surface, demonstrating that root resorption or root ankylosis had not occurred (Figure 4).

The CM group showed some regeneration of bone and comentum occuring along the root surface. Long junctional epithelium was formed more apically than in the other groups. The magnitude of resorption appeared greater in the root surface without cementum than in the root surface covered by new cementum (Figure 5).

The control group showed only rare regeneration of bone and cementum. A few inflammatory cells were observed at the surgical site. No ankylosis was observed (Figure 5).

3. Histometric findings

The results of histometric analysis are summarized in Table 1. The average defect height was not significantly different between groups.

There were significant differences in new bone height between the CPGC and BCP groups and the CM and control groups. In addition, new cementum height was higher

in the CPGC and BCP groups than in the CM and control groups. Epithelial attachment was highest in the CM group. The CPGC group showed less epithelial attatchment than the BCP group, but this difference was not significant.

IV. DISCUSSION

This study was designed to investigate bone formation and periodontal regenerative abilities of CPGC compared with BCP. For this purpose, we used 1-wall intrabony defects, which implies that the defect does not heal by itself during the lifetime of the animal. Without treatment (control group), less than 20% of bone was regenerated. Therefore, this model is useful for the evaluation of the regenerative effect of bone substitutes in dogs.

The height of new bone formation was 1.90mm in the CPGC group and 2.42mm in the BCP group; this difference was not statistically significant. Likewise, the height of new cemetum was 1.34mm in the CPGC group and 1.70mm in the BCP group, which lacked statisticaly significance. These findings suggest that CPGC has an effect similar to BCP on the regeneration of periodontal tissues, including new bone and cementum. Previous studies found similar effects of CPGC on new bone formation. Nery et al. reported that the combination of calcium (Ca) and phosphate (P) in the ceramic implant enhanced repopulation of cells, new periodontal tissue attachment, and bone regeneration within the space of a periodontal osseous defect.[11] In other words, the activities of osteoblasts were promoted by calcium and phosphate ions which were separated during the melting process of graft material. Both CPGC and BCP used in this study were a combination of calcium and phosphate and thus would contributed to new bone formation and periodontal regeneration in the bony defects. However, the Ca/P raio is different between CPGC and BCP. The Ca/P ratio of

CPGC is about 0.6 and that of BCP ranges between 1.50 and 1.67.[12] This difference may result in different resorption rates of the two graft materials, because Ca2+ ions act as a network modifier in glass and secure bond strength between particles. As the Ca2+ ion portion increases, the resorption rate of glass decreases.[13] Therefore, when comparing Figures 3 and 4, the amount of remaining particles was smaller with CPCG than BCP after 8 weeks.

Generally, the barrier membrane is surgically removed after 4–6 weeks in guided tissue regeration (GTR) or guided bone regeneration (GBR). Connective tissue and bone regeneration may then occur within the bony lesion protected by the barrier.[14] Likewise, CPGC provides sufficient space for optimal wound stability while being resorbed after 8 weeks. This indicates that CPGC has the proper physical property to serve as graft material.

It should be mentioned that CPGC may be potentially useful in hard tissue surgery because of it's solubility behavior, since the solubility may be controlled by altering the chemial composition.[15] Again, CPG, which is the basis of CPGC synthesis, is associated with the release of biologically therapeutic molecules. CPG can dissolve some elements, oxides, or biological molecules that are insoluble or poorly soluble in glasses of other materials and crystalline compounds. Therefore, CPGC has the ability to not only promote new bone formation and periodontal regeneration but also works synergistically when synthesized with various materials. Many animal studies have demonstrated the ability of CPGC to function as a carrier of growth factor (GF) or human recombinant bone morphogenetic protein (rhBMP) and reported positive

results.[16-17] More studies are necessary to further clarify the potential applications of CPGC.

The findings of this study provide limited evidence of the excellent physical properties associated with CPCG. Future areas of interest might include determination of the resorption period and resorption rate of this compound. In addition, there should be further research to demonstrate of the application of CPGC for widespread uses.

V. CONCLUSION

CPGC was rapidly replaced with new bone, and the volume of new bone was abundant. CPGC also contributed slightly to regeneration of the periodontal apparatus.

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FIGURES

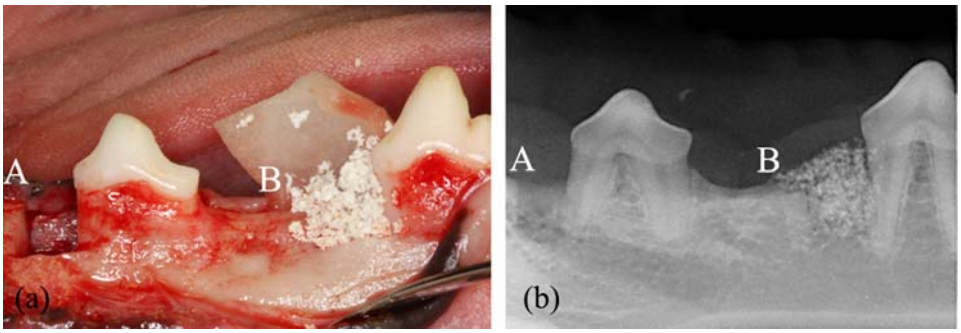

Figure 1. Clinical photograph (a) and radiograph (b) shows surgically prepared 1-wall intrabony defect at the mesial aspect of the second premolar and fourth premolar. (A: CM group or the control group, B: CPGC group or BCP group)

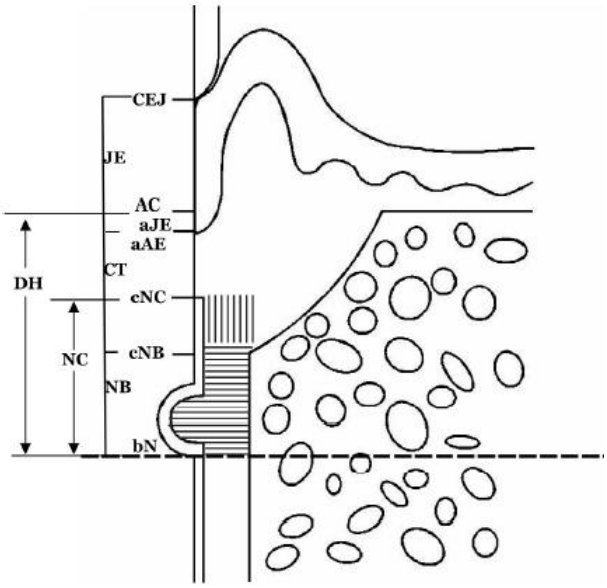

Figure 2. Schematic diagram depicting the landmarks and the parameters used in histometric analysis. The heights of new bone, new cementum, epithelial and connective tissue attachment in 4x4mm 1-wall intrabony defects were measured using an automated image analysis system.

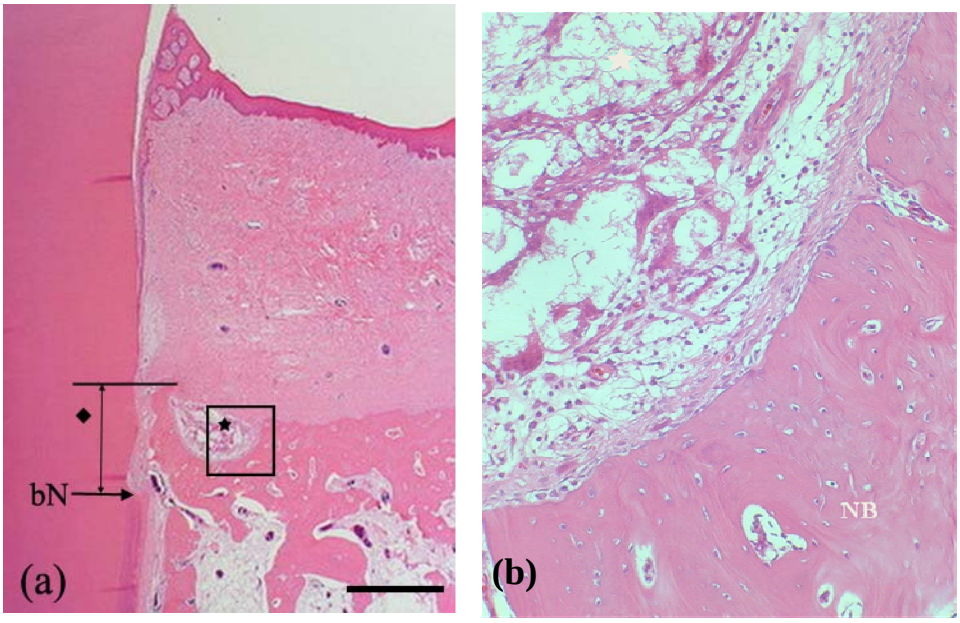

Figure 3. Surgical sections from the CPGC group.

(a) Histologic view of the CPGC group. Most particle were resorbed and ne w bone was formed above the notch. (Hematoxylin and eosin stainingg: original magnification x20; base of reference notch (bN): arrow; height of new bone: black diamond; CPGC particle: black star; bar = 2mm)

(b) Histologic view of magnified black square area (x200). Osteoblast-like cells were observed around remaining particles. Peripheral new bone was woven bone with isolated osteocytes. (Hematoxylin and eosin staining: original magnification x200; CPGC particle: black star; new bone: NB; woven bone: WB; bar = 0.1mm)

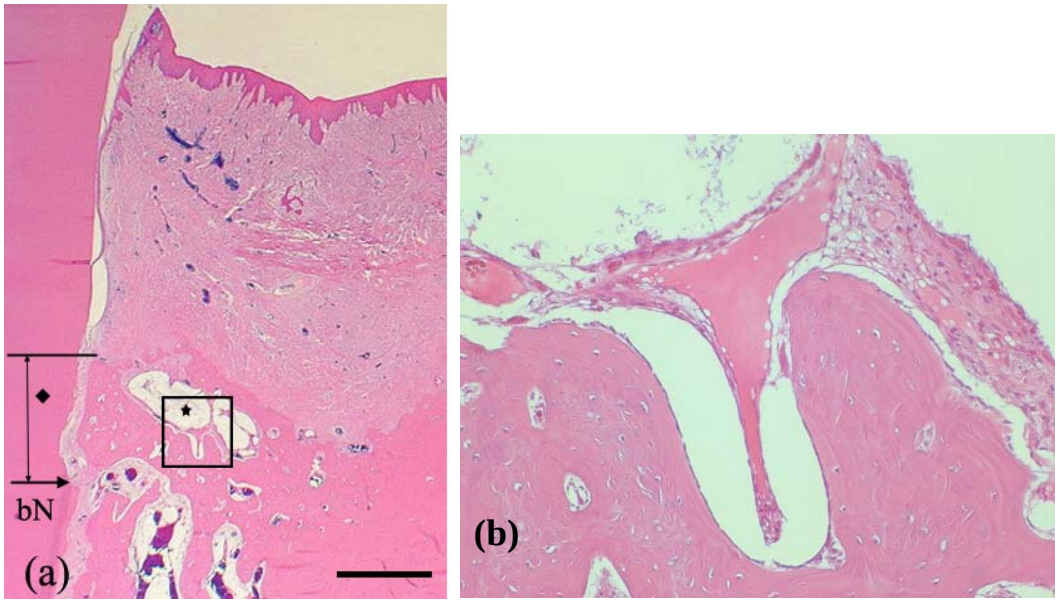

Figure 4. Surgical sections from the BCP group.

(a) Histologic view of BCP group. There were more remaining particles than in the CPGC group. New bone was formed above the notch. (Hematoxylin and eosin staining: original magnification x20; base of reference notch (bN): arrow; the height of new bone: black diamond; BCP particle: black star; bar = 2mm)

(b) Histologic view of magnified black square area (x200). Multi-nucleated giant cells were arranged around particles and woven bone was formed. (Hematoxylin and eosin staining: original magnification x200; BCP particle: black star; new bone: NB; woven bone: WB; bar = 0.1mm)

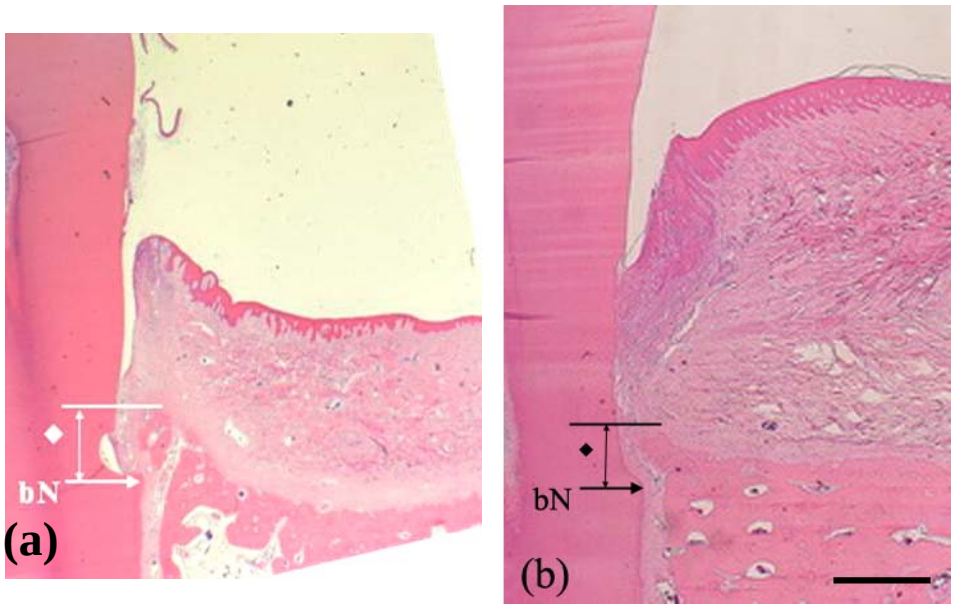

Figure 5. Surgical sections from the CM group and the control group.

(a) Histologic view of the CM group. A small amount of new bone was formed above the notch. Thick connective tissue was present. (Hematoxylin and eosin staining: original magnification x20; base of reference notch (bN): arrow; height of new bone: black diamond; bar = 2mm)

(b) Histologic view of the control group. There was very little new bone above the notch. Long junctional epithelium was observed and inflammatory cell infiltrate was relatively strong. (Hematoxylin and eosin staining: original magnification x20; base of reference notch (bN): arrow; height of new bone: black diamond; bar = 2mm)

TABLE

Table 1. Comparison of histometric analysis between groups (mean±SD in mm)

	CPGC group	BCP group	CM group	Control group
Defect height	5.09±0.58	5.33±0.81	5.54±0.59	5.04±0.51
New bone height	1.90±0.44*§	2.42±1.34*§	1.09±0.26	0.70±0.32
New cementum height	1.34±0.73*	1.70±1.09*	1.17±0.65	0.95±1.34
Epithelial attachment	1.88±1.02§†	2.51±1.10	3.13±0.94*	1.62±0.88§†
Connective tissue attachment	1.55±0.75	0.89±0.55	0.97±0.96	1.13±1.70

*statistically significant difference from control (p<0.05)

§statistically significant difference from CM group (p<0.05)

†statistically significant difference from BCP group (p<0.05)

CPGC: calcium phosphate glass cement, BCP: biphasic calcium phosphate, CM: collagen membrane

국문요약

성견 일벽성 치주 결손부에 이식한 calcium phosphate glass cement 의 신생골 형성 효과

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이 승 범

현재 이용되는 생체재료들 중에서, 생체용 세라믹은 그 구성성분을 변화시킴으로써 생체 활성도에 변화를 유도할 수 있기 때문에 높은 주목을 받아왔다. 생체용 세라믹의 흡수율과 조성을 신생골이 형성되는 과정에 거치는 광화 시기의 조성과 유사하도록 변화시킬 수 있다. 이번 연구의 목적은 일련의 연구를 통해 변화시킨 calcium phosphate glass 를 기반으로 새롭게 개발한 calcium phosphate glass cement 를 성견의 일벽성 치주 결손부에 이식함으로써 신생골 형성능을 알아보고자 하였다.

성견에서 4 곳의 결손부를 외과적으로 형성함으로써 실제 임상에서 일어날 수 있는 모형을 재현하여 실험하고자 하였다. 양측 하악 제 2 소구치 원심면과 제 4 소구치 근심면에 폭 4.0mm, 깊이 4.0mm 의 결손부를 형성하였다. 두곳의 결손부에 calcium phosphate glass cement 또는 biphasic calcium

phosphate 를 이식하고, 나머지 결손부에는 collagen membrance 만 피개하거나, 대조군으로 설정하였다. 8 주 후의 조직학 소견및 조직계측학적 결과를 분석하였다.

조직계측학적으로, 신생골의 형성량과 신생 백악질의 형성량에 있어서는 골이식재를 이식한 실험군이 대조군이나 음성 대조군에 비해 많게 나타났다. 조직 시편상 관찰결과, calcium phosphate glass cement 를 이식한 실험군이 biphasic calcium phosphate 를 이식한 실험군에 비해 이식재의 잔존량이 적게 나타났다.

결론적으로, 새롭게 개발된 calcium phosphate glass cement 는 치유기 동안 형성된 신생골로 빠르게 대체되며, 이번 실험에 제한적이나마 신생골 형성 및 신생 백악질 형성을 도모하였다.

핵심되는 말 : 생체 재료, calcium phosphate glass cement, 골 형성, 골 이식, 1 벽성 치주 결손부