Periodontal Regeneration Using the Mixture of Human Tooth-ash and Plaster of Paris in Dogs

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I. Introduction

The ultimate goals of periodontal therapy include not only the arrest of periodontal disease progression, but also the regeneration of structures lost to disease where appropriate. An appropriate periodontal regeneration should be restored original normal alveolar bone, periodontal ligament, and cementum which is destructed by the periodontal disease. Additionally, the periodontal ligament fibers must be anchored into the cementum[1,2]. However, periodontal healing following a conventional periodontal treatment occurred junctional epithelial attachment[3]. As a result, various materials such as bone replacement grafts, barrier membranes, and biologic modifiers currently used for the regeneration of periodontal tissue defects.

GTR technique is for the periodontal tissue regeneration by inducing the fibroblast or progenitor cells originated from PDL not allowing epithelium and gingival connective tissue ingrowth[4]. Non absorbable membrane has some problems in that early exposure of membrane and additional surgery for the removal of the membrane. It has been documented that bacterial infection caused by the early exposure of the membrane was one of the GTR failure factors and even if the GTR succeed, the amount of the tissue regeneration was decreased[5-7]. To overcome these problems, bioresorbable membrane was used, but bioresrobable membrane had some disadvantages in maintaining the space for

* "This study was supported (in part) by research funds from Chosun University, 2005"

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the tissue regeneration. However, when GTR using bioresorbable membrane combined with the bone graft materials was performed, more bone regeneration was observed comparing with the membrane only usage[8].

Heney et al.[9] demonstrated the amount of bone regeneration was dependent upon the gap space between tooth surface and the membrane. Several investigators have emphasized the gap space available during GTR procedure[10-12]. However, bioresorbable membrane did not provide a gap space because of the lack of rigidity. Therefore, space could be maintain by adding additional bone graft materials that could have retention of blood clot, and effect of osteoconduction or osteoinduction. Although autogenous bone grafts have been used widely, a wide variety of substitute materials have been experimented and used because autogenous bone graft materials have several disadvantages: limitation of graft volume available, donor site morbidity, and prolongation of the operation[13,14]. Among them, a mixture of tooth ash and plaster has been explored.

Tooth ash in 70 to 120㎛ was submitted for the domestic and international patency in 2003(Figure 1.). Tooth ash presents a problem in initial fixation and retention of the graft material. To overcome these problem, tooth ash is then mixed with an appropriate amount of plaster. Kim[15] et al have been suggested that the mixture of 2:1 weight ratio of tooth ash and plaster was proved to clinically effective. Since its development of implant material using the mixture of tooth ash and plaster, satisfactory results were seen in clinical application in some patients whose jaw bone defect, bone defect around an implant and sinus augmentation[16-19].

However, Few studies about periodontal tissue regeneration using the mixture of tooth ash and plaster have been reported. The purpose of this study is to evaluate the efficacy of the periodontal tissue regeneration pattern performing GTR with a mixture of tooth ash and plaster as bone substitute material in the treatment of class II furcation defects in dogs.

II. Materials and methods

1. Materials

This study was approved by the Animal Research Committee of Chosun University. For animal study, four adult dogs, weighing approximately 15㎏ each, were used to examination. The animals were good periodontal state without systemic disease. A resorbable membrane (Bio-Gide®, Swiss) and bone graft material using the mixture of tooth ash and plaster were used to examining for biological activity.

2. Methods

1) Animal preparation

Anesthesia in the animal was administered by intramuscular injection of Ketamine (0.1 ㎖/㎏, IM) and Xylazine-HCl (Rompun®, Korea Bayer, 0.1 ㎖/㎏, IM) into the gluteal region.

2) Surgical procedure

The local anesthesia was performed in the both mandibular premolar region with 2% lidocaine HCl (Epinephrine 1: 80,000). A buccal crevicular and vertical incision was made and the flap was elevated subsequently to expose the alveolar bone. A intrabony defect measuring 4 ㎜ in apical, 4 ㎜ in mesiodistal and 4 ㎜ in buccolingual was made under the alveolar bone of the mandibular premolar with 4 ㎜ diamond round bur(Shofu Co., Japan). The notch was made with a 1/4 round bur in the root surface of the base of the defect as a reference point. The defect was filled a Bio-Gide® only as a control. For experimental group, the defects filled with a mixture of tooth ash and plaster and covered by a Bio-Gide®. The flap was closed and sutured. Antibiotics (Gentamicin sulfate, Korea, 0.1 ㎖/kg) was administered intramuscularly once daily for 5 days postoperatively. Oral rinsing with 0.12% chlorhexidine was performed twice a day for 2 weeks.

3) Histological examination

Two animals were sacrificed at 4 or 8 weeks after surgery in each. The specimen were fixed and decalcified in 10% formic acid for 2 weeks. They were processed routinely and were embedded in paraffin, The paraffin blocks were sectioned in 7 ㎛ thin slices and serial sections were made in 80 ㎛ mesiodistally. The sections were stained with Hematoxylin-Eosin. The sections were evaluated under a light microscope for detection of apical migration of junctional epithelium, the degree of inflammation, resorption of graft material, new cementum and bone formation, and periodontal ligament regeneration.

Ⅲ. Results

The results of histologic examination were as follows.

1. Control groups

A. at 4 weeks

Bio-gide® was completely degraded and the bony defect area was filled with connective tissue fiber. Large space was remained in the bifurcation area(Figure 2.).

B. at 8 weeks

New bone formation adjacent to the preexisting bone and soft tissue ingrowth were observed(Figs. 3-a, 3-b, 3-c, 3-7.).

2. Experimental groups

A. at 4 weeks

Bio-gide® was completely degraded and new bone was formed in the bony defect area. However, there was distinction between new bone and preexisting bone histolgically. Moreover, osteoclasts were observed around bone graft materials indicating active bone resorption. New vascularization was also shown(Figs. 4-a, 4-b, 4-c.).

B. at 8 weeks

New lamellar type trabecular bone formation and cementum and PDL regeneration was seen in the base of the in-

trabony pocket. New vascularization was also shown. The new bone formation was observed around the mixture of the tooth ash and Paster. Neither inflammatory cell, macrophage or giant cell were observed(Figs. 5-a, 5-b, 5-c, 5-d.).

Ⅳ. Discussion

Guided tissue regeneration(GTR) technique is for the periodontal tissue regeneration by inducing the fibroblast or progenitor cells originated from PDL not allowing epithelium and gingival connective tissue ingrowth[4]. Melcher[20] suggested that the type of periodontal regeneration was dependent on the phenotype of migration and division of progenitor cell populations in periodontal ligament after wounding. Also Nyman[21,22] et al. proposed that non-desirable types of tissue cells could be prevented from migrating into a wound by means of a membrane barrier and at the same time giving preference to those particular cells to repopulate the wound, which have the capacity to regenerate the desired type of tissue. Subsequently, it has been documented that new attachment occurred by the cells from periodontal ligament when the ingrowth of gingival epithelium and connective tissue did not allow[23,24].

GTR techniques have provided periodontal regeneration for the treatment of furcation defects, intrabony pocket, gingival recession or bone perforation[25]. GTR techniques utilizing a barrier membrane have been used for prevention of apical migration of epithelium and connective tissue. Nonresorbable e-PTFE membranes has been mostly used for periodontal tissue regeneration covered by the soft tissues in order to allow the bone regeneration to complete[4,26-30]. However, because the e-PTFE is a nonresorbable material, a second surgical procedure is necessary in order to remove it after 4 to 6 weeks. In addition, bacterial contamination with the early exposure of the membrane have been reported. And thus, several investigators suggested that bacterial contamination led to the adverse effect of the GTR technique[5,6,31]. Newly regenerated tissue beneath the membrane was mechanically traumatized during the second surgery. And also, the efficacy of GTR has been decreased when the membrane had not completely covered a regenerated tissue during second surgery[6,7].

Recently, many investigations have been paid with more efforts to survey the effectiveness of using absorbable membranes for overcome these problems. As a result, extensive efforts had been employed to utilize bioresorbable membranes to achieve therapeutic purpose in clinical trials[7,32-35]. In clinical practice, the tissue regeneration barrier membranes are generally required to maintain their barrier functions for 4 to 6 weeks in order to secure the restoration of periodontal tissues[36].

Many aspects of technical development of bioresorbable membrane materials in GTR applications are focusing on the rigidity and degradation rate, with special emphasis on the easy clinical manageability. However, although bioresorbable membrane might be disturb tissue regenerate due to phys-

ico-chemical properties, it is reported that both types of barrier membranes employed in clinical settings have no differences in respect to the amount of new attachment[37,38].

However, when GTR using bioresorbable membrane combined with the bone graft materials was performed, more bone regeneration was observed comparing with the membrane only usage[8]. Bowers et al.[39,40] suggested that GTR with non-resorbable membrane combined with bone graft could expect better results in that more new attachment was formed when the GTR was performed with the demineralized freeze-dried bone(DFDB). Anderegg et al..[14] also reported that usage of the DFDB underneath the membrane had more new bone formation comparing with the membrane only cases. Additionally, Leonardis et al.[8] mentioned that the application of the bioresorbable membrane combined with bone graft material in GTR had better efficacy in periodontal tissue regeneration. This study also presents GTR using bioresorbable membrane with bone graft had more new bone formation and bone maturity than the membrane only control group.

On the other hand, some papers reported that the efficacy of regeneration by the PDL cells decreased because of the hinderance of the graft materials[39]. Caffesse et al.[40] reported that e-PTFE membrane with DFDB could not increase the periodontal attachment. Wallace et al.[41] also reported that there was no difference between GTR with e-PTFE membrane and GTR with e-PTFE membrane combined with the DFDB in the bifurcation area. This present study had consistent results with the previously mentioned Bowers[42,43] and Anderegg's study[14], in which bone graft material could support the barrier material and prevent its collapse, and eventually promote bone regeneration. In this study, experimental group showed more new bone formation than the control group, histologically. Moreover, better bone density and maturity was observed at 4 and 8 weeks in the experimental group.

In this study, mixture of tooth ash and plaster, mostly consisted of hydroxyapatite, was used as bone graft material. Stability and efficacy of the mixture of tooth ash and plaster have been proved since the mixture were developed in 1992[44-46]. Moreover, osteoconductability of the mixture of tooth ash and plaster was presented in several animal experiments[17-19,47]. Kim et al.[18] reported that when the mixture of tooth ash and plaster grafted in the implant bony defect area, bone-implant contact was successfully reconstructed. Kim et al.[19,48] also stated that mixture of tooth ash and plaster was effective and manageable bone substitutes after he treated mandibular defects area with the mixture of tooth ash and plaster in 10 patients. In addition, the mixture of tooth ash and plaster was documented as a good graft material for implant[48].

When GTR was performed with the combination of the membrane and bone graft, bone graft material could act as barrier for the gingival epithelium and connective ingrowth during the proliferation period of PDL originating cells. From these reasons, the experimental group had better bone formation results than the control group.

However, further study would be needed because the graft material could act obstacle for the migration and proliferation of the PDL originating cells.

Ⅴ. Conclusions

GTR was performed at the site of the surgically formed mandibular premolar bifurcaion area in dogs. The control group was applied only membrane. The experimental group was applied membrane with the mixture of the tooth ash and plaster.

The histopathologic results at 4 and 8 weeks were as follows:

In the 4 weeks control group, Bio-gide® was completely degraded and large space was existed in the furcation area.

In the 4 weeks experimental group, Bio-gide® was also showed fully absorption. Although new bone formation was observed, there was significant distinction between the preexisting bone and newly formed bone. Moreover, osteoclast was observed around bone graft materials indicating active bone resorption.

In the 8 weeks control group, new bone formation was observed adjacent to the preexsisting bone. Soft tissue ingrowth was also shown.

In the 8 weeks experimental group, new lamellar type trabecular bone formation and centum and PDL regeneration was seen in the base of the in the infrabony picket. New vascularization was shown.

From these results, the mixture of human tooth-ash and plaster of Paris can be considered as osteoconducting bone graft material. When GTR is performed using the mixture of human tooth-ash and plaster of Paris as bone graft material, more bone regeneration would be expected.

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사진 부도 설명(Ⅰ)

Figure 1. SEM picture of the tooth-ash.

Figure 2. Bio-gide® was completely degraded and large space was existed in the furcation area; H-E stain, magnification ×40.

Figure 3-a. New bone was observed in the superior part of the bifurcation area. No epithelial involvement; H-E stain, magnification ×40.

Figure 3-b. Bone grafting material remained; H-E stain, magnification×100.

Figure 3-c. Distinction between new bone and preexisting bone; H-E stain, magnification ×40.

Figure 3-d. Around bone grafting material. A few osteoclasts & active bone resorption around graft materials; H-E stain, magnification ×100.

Figure 4-a. New woven-bone formation adjacent to the preexisting bone was observed in the bony defects; H-E stain, magnification ×40.

Figure 4-b. There was soft tissue ingrowth; H-E stain, magnification ×100.

Figure 4-c. There was soft tissue ingrowth; H-E stain, magnification ×200.

Figure 5-a. New lamellar type trabecular bone is seen in the notch-the base of the infrabony pocket; arrow head. New vessel growth was seen; arrow; H-E stain, magnification ×40.

Figure 5-b. New lamellar type bone formation surrounding the remaining bone graft material is seen; H-E stain, magnification ×400.

Figure 5-c. The regeneration of the cementum and periodontal ligament were also observed in the base of the pocket; H-E stain, magnification ×100.

Figure 5-d. The regeneration of the cementum (arrow head) and periodontal ligament (arrow) were also seen in the base of the pocket; H-E stain, magnification ×200.

사진부도(Ⅰ)

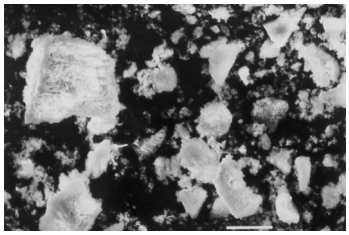


Figure 1.

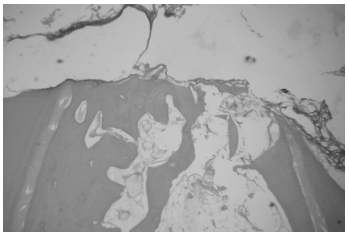


Figure 2.

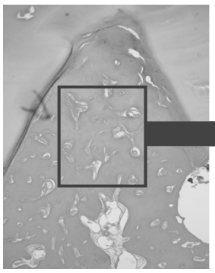
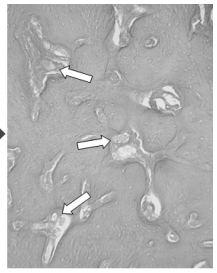


Figure 3-a.



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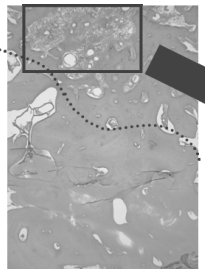

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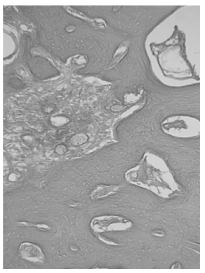

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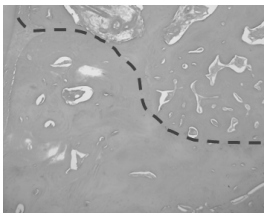
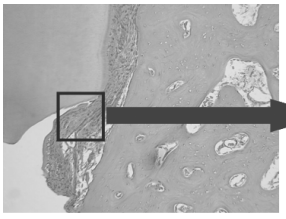


Figure 4-a.



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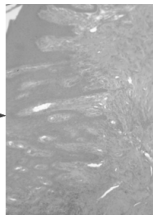

c.

Figure 5-a.

b.

Figure 5-c.

d.

-Abstract-

성견에서 치아회분말과 연석고를 이용한 치주조직재생술

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흡수성 차폐막을 이용한 조직 유도 재생술시 차폐막의 견고성으로 미루어 보아 재생을 위한 공간의 유지가 어려울 수 있다. 조직 유도 재생술과 함께 골이식술을 시행함으로써 공간 확보와 함께 적절한 혈병의 유지를 도모할 수 있고 이식된 골은 신생골 형성을 위한 핵으로 작용할 수도 있다. 최근에 사람의 치아회분말과 연석고를 혼합한 골이식재가 여러 연구를 통해 좋은 골이식재로 평가되었다. 본 연구에서는 성견 하악 소구치 2급 치근이개부위에 외과적으로 형성하여 흡수성 차폐막과 치아회분말-연석고 혼합 이식재를 이용한 조직유도재생술을 시행하여 치주 조직 재생의 양상을 조직학적으로 관찰하고자 한다.

생후 12개월에서 16개월 된 체중 15 Kg 내외의 성견 4마리를 이용하였다. 실험 재료로 생체흡수성 차폐막(Biogide®, Swiss)를 사용하였고, 골이식재로 치아회분말-연석고를 혼합매식 하였다. 양측 상악 소구치 부위에 변연 치조골하방에 4 ㎜ × 4 ㎜ × 4 ㎜, (깊이 × 근원심 × 협설폭경) 깊이로 골내낭을 형성하였다. 형성된 골내낭의 기저부위 치근 표면에 1/4 round bur로 notch를 형성하여 참고점으로 하였다. 무작위로 선택된 한 쪽의 결손부를 대조군으로 오직 생체 흡수성 차폐막을 사용하였고, 실험군으로 치아회분말-연석고와 생체 흡수성 차폐막을 결손부로부터 2 mm 이상 덮을 수 있도록 다듬어 결손부 위에 위치시킨 후 협측 판막을 덮고 봉합하였다. 4주 후 2마리, 8주 후 2마리를 희생시키고 통상의 방법으로 고정, 탈회, 포매의 과정을 거쳐 광학현미경으로 검경하였다.

그 결과,

1. 4주 대조군에서 Bio-gide®는 완전한 흡수를 보였고, 치근이개부내에는 큰 공간이 존재하였다.
2. 4주 실험군에서 역시 Bio-gide®는 완전한 흡수를 보였고, 골 결손부내에 더 많은 신생골 관찰되었다. 그러나 아직까진 기존골과 신생골간에 명확한 차이가 있어서 쉽게 구분할 수 있었다. 또한 골이식재 주변으로 파골세포가 다수 관찰되며 이로 미루어 보아 활발한 골흡수가 일어남을 알 수 있었다.
3. 8주 대조군에서 결손부내에서는 기존골에 인접하여 신생골 형성이 부분적으로 일어났으나 연조직 침입이 관찰되었다.
4. 8주 실험군은 신생골이 기존골과 매우 유사한 형태로 관찰되었고, 신생골 형성 부위에 신생 혈관 증식이 관찰되었다. 또한 골내낭 기저부위에서는 백악질과, 치주인대가 재생됨이 관찰되었다.

이상의 결과에서 치아회분말-연석고 혼합매식은 골재생을 위한 골전도성이 있는 재료로 사료되며, 이를 이용하여 치주조직재생술시 흡수성 차폐막과 병행하여 사용한다면 더 많은 골재생이 있을 것으로 기대된다.

주요어 : 치아회분말-연석고, 골재생, 치주조직재생