The Effect of Hydroxyapatite-Chitosan Block Bone Graft on the Periodontal Regeneration in One-wall Intrabony Defect of Beagle Dogs

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A Dissertation Thesis

Submitted to the Department of Dental Science

and the Graduate School of Yonsei University

in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy of Dental Science

Sung-Koo Kim

April 2007

This certifies that the dissertation thesis

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April 2007

감사의 글

본 논문이 완성되기까지 부족한 저를 항상 격려해 주시고 사랑과 관심으로 이끌어 주신 최성호 교수님께 깊은 감사를 드립니다. 그리고, 많은 조언과 따뜻한 관심으로 지켜봐 주신 김종관 교수님, 채중규 교수님, 조규성 교수님, 김광만 교수님, 이용근 교수님, 김창성 교수님, 정의원 교수님께 진심으로 감사 드립니다.

연구 내내 많은 도움을 주신 치주과 교실원 여러분, 특히 채경준 선생님, 이중석 선생님께 고마움을 전합니다.

늘 변함없는 사랑과 헌신적인 도움으로 어려운 생활 속에서도 지팡이가 되어준 아내 태정과, 이제는 든든한 친구가 된 아들 병현에게 무한한 고마움의 마음을 전합니다.

오늘이 있기 까지 변함없는 믿음과 사랑으로 이해해 주시며, 물심양면으로 후원해 주신 어머님과 장인, 장모님께 감사의 마음을 드립니다.

마지막으로, 저의 마음속 영원한 정신적 기둥 이며, 하늘에서 항상 저를 지켜보고 계실 아버지께 이 논문을 바칩니다.

모든 분들께 진심으로 감사 드립니다.

2007년 6월

저자 씀

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Abstract

The Effect of Hydroxyapatite-Chitosan Block Bone Graft on the Periodontal Regeneration in One-wall Intrabony Defect of Beagle Dogs

This study evaluated a periodontal repair and a biomaterial reaction following implantation of a newly fabricated Hydroxyapatite-chitosan block bone and a chitosan membrane on the regeneration of one-wall intrabony defects in beagle dogs. The surgical control groups received a flap operation only, while experimental groups were treated with the Hydroxyapatite-chitosan block bone and/or a chitosan membrane. In new bone formation, there was a statistically significant difference between the chitosan membrane group and other treatment groups ($P < 0.05$) (Kruskal Wallis Test). The amount of new bone of the hydroxyapatite-chitosan block bone group and the Hydroxyapatite-chitosan block bone and chitosan membrane group was greater than control group, but there was no statistically significant different from the control group. Comparing the amount of new cementum with that of new bone in the four groups of our study, no cemental growth surpassed the bone growth. This result didn't suggest that new bone follows the coronal growth of new cementum, which disagreed with the results of previous studies. Therefore, the further study for the mechanism seems good to follow. The amount of new cementum of the hydroxyapatite-chitosan block bone group and/or the chitosan membrane group was greater than control group, but there was no statistically significant different from the control group. In chitosan membrane group, the amount of new bone formation was greater than other groups, which suggest that the potency of chitosan membrane induce the new bone regeneration. The results of the present study

did support the potential of chitosan in the guided tissue regeneration, but did not showed enough capacity to bear load. Therefore, the further studies to enhance the mechanical properties appear to be necessary.

KEY WORDS: Guided tissue regeneration, Hydroxyapatite-chitosan block bone, chitosan membrane

The Effect of Hydroxyapatite-Chitosan Block Bone Graft on the Periodontal Regeneration in One-wall Intrabony Defect of Beagle Dogs

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I. INTRODUCTION

The regeneration of bone has long been the critical issue in the field of periodontal and implant surgery. Many procedures have been developed for the purpose of promoting regeneration, including guided tissue regeneration and bone graft. All of them, however, have limitations.

Guided tissue regeneration (GTR) therapy using barrier membranes has been introduced to induce selective repopulation of undifferentiated cells that originate from the periodontal ligament, and has been shown to improve the periodontal regeneration in both animal and human experiments (Caffesse et al., 1993; Karring, 1983; Kim et al., 1998; Selvig et al., 1993). For periodontal defects, it has been suggested that the periodontal ligament cells and their migration potential are crucial for periodontal regeneration, and that regeneration might occur if the gingival connective tissue and epithelial cells are prevented from accessing the tooth surface.

Both the application of bone grafts, synthetic implant materials or inorganic bone graft materials in combination with GTR have been reported to favour the formation of bone (Dahlin et al., 1991; Alberius et al., 1992; Wetzel et al., 1995). Different types of biocompatible materials such as hydroxyapatites, calcium phosphates and inorganic bone graft materials have been used alone or in combination with GTR with varying results in terms of bone formation (Pinholt et al., 1991; Klinge et al., 1992; Fukuta et al., 1992; Hislop et al., 1993; Dahlin et al., 1991; Alberius et al., 1992; Wetzel et al., 1995).

The membrane barrier used in GTR should satisfy the following factors. It should be histocompatible, biocompatible and capable of space maintenance (Magnusson et al., 1988). It also should avoid cell migration and be easy to handle. Non-absorbable membrane ePTFE almost fulfills the factors mentioned above and is most widely used so far giving out good results (Nyman et al., 1982; Gottlow et al., 1986; Blumental, 1993; Becker et al., 1988; Pontoriero et al., 1988; Handelsman et al., 1991; Becker et al., 1993).

Chitosan has been reported to enhance the healing of injured connective tissue (Muzzarelli et al., 1988). Recently, a tissue engineering strategy has been suggested as a possible alternative to conventional regenerative therapy. Chitin is a natural polymer of N-acetylglucosamine, and is a component of the exoskeleton of a greatnumber of organisms such as shells and cuticles of arthropods including crustaceans and insects (Cabib, 1987). Chitosan has excellent potential as a structural base material for a variety of engineered tissue system (Madihally et al., 1999). Chitosan has been reported to enhance peridontal tissue regeneration (Madihally et al., 1999; Mukherjee et al., 2003; Park et al., 2003).

Chemical mediators or substances that enhance bone formation are thought to be conductive to periodontal regeneration. Among these materials, the influence of chitosan on bone regeneration is of a particular interest. The use of chitosan and mesh progressively improved the mechanical properties. These strong and cell-seeded hydroxyapatite cements may have potential for bone tissue engineering in moderate stress bearing applications (Michael et al., 2006). Chitosan takes an increasing interest for its non-toxic, immune enhancing, antimicrobial, and wound healing properties.

Different scaffold materials have been used with varying success to generate tissue-engineered bone formation in vitro. Ishaug et al. investigated bone formation in vitro by culturing stromal osteoblasts in a three dimensional, biodegradable poly(lactic-co-glycolic acid) foam (Ishaug et al., 1997). Chitosan/tricalcium phosphate sponges, transplanted into a site for bone regeneration, can be used as a scaffolding material to allow growth of osteoblasts in three-dimensional structure (Lee et al., 2000).

Chitosan nonwoven membrane is shown to accelerate the wound healing and infection control as well (Risbud et al., 2001; Wang et al., 2002; Mizuno et al., 2003; de Queiroz et al., 2003). Chitosan nonwoven membrane has been on the market for periodontal treatment. It has an embossed surface and a yellowish-white microporous structure, and was reported to encourage tissue attachment and first recovery. This membrane is absorbed via hydrolysis, and is eliminated through the Kreb's cycle as carbon dioxide and water, and is biodegraded completely in 6 months (Suk et al., 2002).

The placement of chitin gel in infrabony defects during periodontal surgery resulted in proper tissue integration followed by gradual resorption of the material without any acute inflammatory reactions (Muzzarelli et al., 1989).

However, chitosan has a low physical property leading to an improper use in the areas where it receives a lot of force. Several inherent disadvantages have been observed with these scaffold materials including weak structural integrity, variable degradation rates, inadequate tissue peneration, and host immune reactivity.

The aim of this study was to evaluate the regenerative effects of a chitosan membrane and/or calcium phosphate chitosan block bone applied to preclinical one wall defects surgically created in beagle dogs.

Ⅱ. MATERIAL AND METHODS

A. Materials

1. Animals

A total of six male beagle dogs, each weighing about 15 kg, were used in this study. The animals had intact dentition and a healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals were fed a soft diet throughout study, in order to reduce chance of mechnical interference with the healing process during food intake.

2. Hydroxyapatite/chitosan block bone

Hydroxyapatite/chitosan hybrid scaffold was manufactured by freeze-dried method. Chitosan solution was prepared by dissolving chitosan in 0.2 M acetic solution. Hydroxyapatite/chitosan solution was made by dissolving 10-70wt% hydroxyapatite in the chitosan solution. Hydroxyapatite/chitosan solution was poured in Φ 6 mm × 12 mm teflon mold. Above 5 hours, it was refrigerated in –70 ℃. It was freeze-dried under 6 mTorr by freezing dehydrator, above 3 days. The residual acetic acid was neutralized by 1 M NaOH solution, and then washed with the distilled water (above 3 times). The solvent was completely dried by freeze dry, during 3 days. Hydroxyapatite/chitosan hybrid scaffold was manufactured.

3. Chitosan membrane

Chitosan solution was prepared by dissolving 5 wt% of chitosan in 3 wt% acetic acid. The prepared chitosan solution was poured in mold, and feeze-dried. Then the solution was soaked in 5 wt% NaOH aqueous solution. It was washed with the distilled water (5-6 times). The chitosan membrane was constructed on 40℃ during 24

hours.

B. Experimental Procedures

1. Surgical procedures

Six male beagle dogs were used. 4X4 mm one-wall intrabony periodontal defects were surgically created bilaterally at the distal sides of the mandibular second premolars and mesial sides of the fourth premolars. The surgical control group received a flap operation only. The first group was treated with Hydroxyapatite/chitosan block bone. The second group was treated with chitosan membrane. The third group was treated with both Hydroxyapatite/chitosan block bone and chitosan membrane. The dogs were sacrificed at 8 weeks after the experimental surgery.

2. Histologic and histometric Analysis (Figure 1.)

Tissue blocks, which included teeth, bone, and tissue, were removed, rinsed in saline, then fixed in 10% buffered formalin for 10 days. After being rinsed in water, the block section were decalcified in 5 % formic acid for 14 days, and embedded in paraffin. Serial sections, 5 *u*m thick, were prepared at intervals of 80 μm. The four most central sections from each block were stained with hematoxylin/eosin (H-E) and examined using a light microscope. The most central section from each block was selected to compare histologic findings between groups. Computer-assisted histometric measurements were obtained using an automated image analysis system[††] coupled with a video camera on a light microscope[‡‡]. Sections were examined at 20x magnification.

[††] Image-Pro Plus®, Media Cybernetics, Silver Spring, MD, U.S.A

[‡‡] Olympus BX50, Olympus Optical Co., Tokyo, Japan

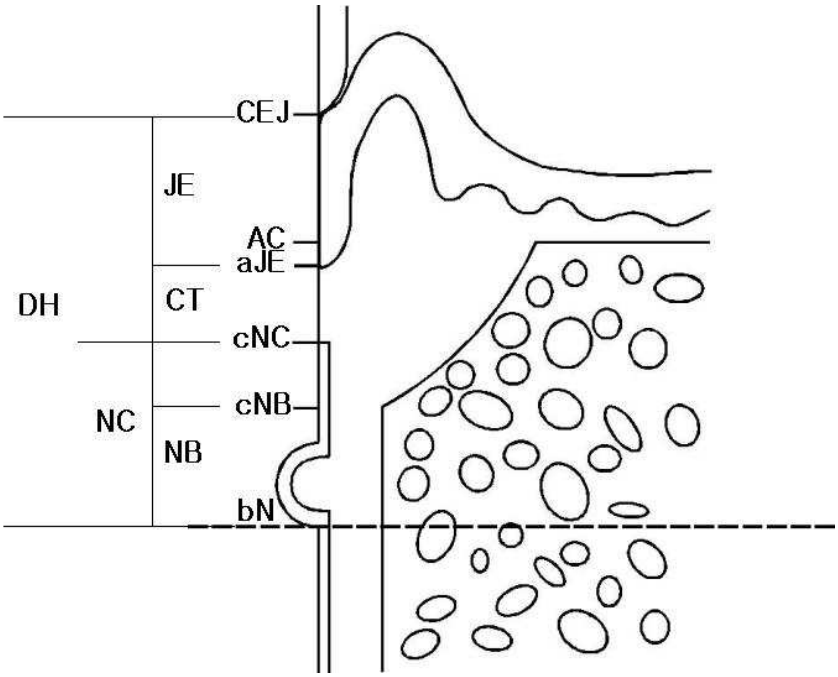

Figure 1. A schematic diagram depicting the experimental design, the landmarks and the parameters used in histometric analysis.

DH: defect height JE: junctional epithelium migration

CT: connective tissue adhesion NC: new cementum regeneration

NB: new bone regeneration

3. Statistical analysis

Histometric recordings from the four sections from each defect were used to calculate the mean scores for each animal. All data were expressed as means ± standard deviation of the mean. Comparison between multiple groups and various conditions were analyzed using Kruskal-Wallis Test. Comparison between two groups and one condition was analyzed using Mann-Whiteny Test.

Ⅲ. RESULT

A. Clinical observations

Surgical procedures were uneventful and without complication in all dogs. Wound closure was successfully maintained throughout the experiment for all defects. Healing process was generally uneventful.

B. Histologic findings

In the control group, the apical migration of junctional epithelium was observed (Figure 3). The periodontal ligament fibers were generally oriented in a direction parallel to the root surface in suprabony area. Dense connective tissue was shown (Figure 4). No site showed signs of ankylosis. There was little or no sign of inflammatory cell infiltration. Above the apical notch, a small amount of new cementum and bone had formed along the root surface (Figure 3).

In the Hydroxyapatite/chitosan block bone group, all the Hydroxyapatite/chitosan block bone was resorbed (Figure 5, 6). In some parts of the slide, osteoblast and osteoids were observed around the bone marrow (Figure 7). The lamella bone could be distinguished and periodontal ligament fibers were observed between new bone and new cementum(Figure 7). In addition some inflammatory cell infiltration was in the connective tissue. The periodontal ligament fiber orientation was perpendicular to root surfaces around the reference notch area (Figure 7).

In chitosan membrane group, residual chitosan membrane remnants were observed at the connective tissue area (Figure 9, 11). In some parts of the slide, osteoblast and osteoids were observed around the bone marrow (Figure 10). The lamella bone could be distinguished and periodontal ligament fibers were observed between new bone and

new cementum (Figure 10). The inflammatory cell infiltration was lesser than other groups.

The chitosan membrane and the hydroxyapatite/chitosan block bone group revelaed similar results to that of the chitosan membrane or block bone group (Figure 12,13,14). The inflammatory cell infiltration into the connective tissue was greater than chitosan membrane group.

C. Histometric Analysis

Table 1, Table 2 and Figure 2 show the results of the histometric analysis. The amount of new alveolar bone formation in the chitosan membrane group was greater than those of the other groups, and there was a statiscally significant difference ($P < 0.05$). The amount of new cementum formation of chitosan membrane group was greater than those of the other groups, but there was no significant difference from other groups ($P > 0.05$). In apical migration of the junctional epithelium, no statistically significant difference was observed between the control group and the other groups ($P > 0.05$). The amount of connective tissue adhesion in surgical control group was greater than those of the other groups, but there was no significant difference ($P > 0.05$). (Tables 1, 2, Figure 2)

The amount of alveolar bone regeneration was (0.80 ± 0.18) mm in the control group, (1.20 ± 0.40) mm in the hydroxyapatite/chitosan block bone group, (1.85 ± 0.66) mm in the chitosan membrane group, and (1.22 ± 0.21) mm in the combination group, which showed a statisticcally significant difference for the chitosan membrane group. Chitosan membrane group was a statistically significant different from other treatment groups($P < 0.05$). The amount of cemental regeneration was (0.68 ± 1.23)

mm in the control group, (1.46 ± 1.58) mm in Hydroxyapatite/chitosan block bone group, (2.30 ± 0.63) mm in Chitosan membrane group, and (1.13 ± 0.90) mm in combination group. There was no statistically significant difference among 4 groups (P>0.05). The amount of epithelial migration was (2.68 ± 1.68) mm in the control group, (2.18 ± 1.56) mm in the hydroxyapatite/chitosan block bone group, (2.43 ± 0.56) mm in the chitosan membrane group, and (2.55 ± 0.77) mm in the combination group, which showed no statistically significant difference among the four groups (P>0.05). The amount of connective tissue adhesion was (1.22 ± 0.71) mm in the control group, (0.93 ± 1.46) mm in the hydroxyapatite/chitosan block bone group, (0.27 ± 0.36) mm in the chitosan membrane group, and (0.79 ± 0.83) mm in the combination group. There was no statistically significant difference among 4 groups (P>0.05) (Table 1).

In relative height percentage of the entire depth of defects, the results in each group are the following: The amount of junctional epithelium migration was (55.50 ± 21.74)% of the defect height in the control group, (46.41 ± 31.22)% in the experimental group I, (50.39 ± 4.37)% in the experimental group II and (60.28 ± 13.51)% in the experimental group III. There was no statistically significant difference among the four groups (P>0.05). The amount of connective tissue adhesion was (30.61 ± 22.80)% in the control group, (19.97 ± 31.25) % in experimental group I, (5.24 ± 6.71)% in the experimental group II, and (20.63 ± 21.93)% experimental group III, which showed no statistically significant difference among 4 groups (P>0.05). The amount of new cementum regeneration was (13.29 ± 23.12)% in the control group, (48.66 ± 41.37)% in experimental group I (47.24 ± 4.52)% in the experimental group II, and (27.33 ± 21.78)% experimental group III. In amount of new cementum

regeneration, which showed no statistically significant difference among 4 groups (P>0.05). The amount of new alveolar bone regeneration was (19.01 ± 8.40)% in the control group, (26.24 ± 9.10)% in experimental group I, (38.79 ± 11.44)% in the experimental group II, and (29.89 ± 7.92)% in experimental group III. Experimental group II was a statistically significant difference from the control group (P<0.05) (Table 2).

The illustration of periodontal healing in percentage of the defect height is shown in Figure 2.

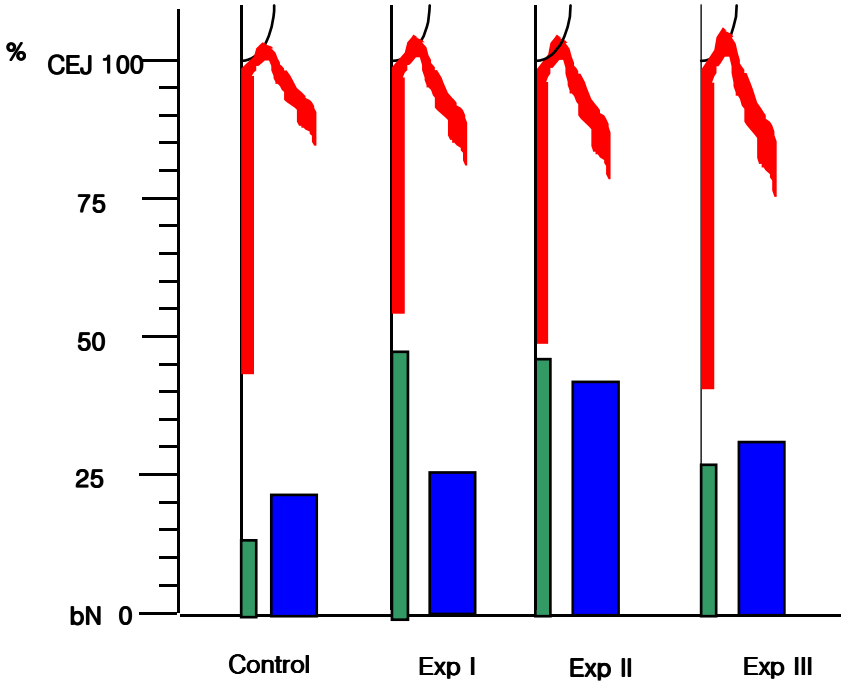

Figure 2. Periodontal healing illustrated in percentage of the defect height.

Control group: surgical control group received a flap operation only

Exp I : Hydroxyapatite/chitosan block bone group,

Exp II: the chitosan membrane group,

Exp III: Hydroxyapatite/chitosan block bone and chitosan membrane

CEJ: cemento-enamel junction, bN : base of the reference notch

Blue: new bone, Green: new cementum, Red: Junctional epithelium

IV. DISCUSSION

The ultimate goal of periodontal therapy is to regenerate the supporting tissue that was destroyed. Although, various procedures such as guide tissue regeneration (Kim et al., 1996; Kim et al. 1998; Moon et al., 1996; Trombelli et al., 1997), autografts (Schallhorn, 1972), other bone grafts (Mellonig et al., 1976; Mellonig, 1984; Kim et al., 1998a; Kim et al., 1998b), and the application of growth factor (Lynch et al., 1989; Becked et al., 1992; Wikesjö et al., 1999; Choi et al., 2002) has been already developed and used to help regeneration, each has its shortcomings.

The aim of this study was to evaluate the periodontal repair and the biomaterial reaction following implantation of a newly fabricated hydroxyapatite-chitosan block bone and chitosan membrane on the regeneration of 1- wall intrabony defects in the beagle dogs. Based on the results of Wikesjo et al's study, which found that naturally or ligature induced loss of attachment and surgically induced loss of attachment showed no difference in healing (Wikesjö, 1991).

In previous similar study, the tissue regeneration effect by chitosan was good. The inhibited apical migration of epithelium and increase in amount of new bone and new cementum suggest the potency of chitosan in inducing periodontal tissue regeneration (Park et al., 2003).

The chitosan nonwoven membrane has the potential to support the cementum and bone regeneration, possibly by providing the conditions needed for guided tissue regeneration in the one wall intrabony periodontal defects of beagle dogs (Yeo et al., 2005). The biocompatibility of the chitosan nanofiber membrane was confirmed, with enhanced bone regeneration and no evidence of an inflammatory reaction. The experiment shows that the novel biodegradable chitosan nanofiber membrane may be

useful as a tool for guided bone regeneration (Shin et al., 2005). Consequently, interest in the search for a new non-toxic, biodegradable material that would be free from any side effect has been growing. Among those considered, chitin and its extract, chitosan (poly-N-acetyl glucosaminoglycan), are attracting particular attention. It was reported that chitosan has biocompatibility with the host in a previous animal study and the host progressively reabsorbs chitosan almost 2 months after the treatment (Nakajima et al., 1986).

An ideal scaffolding material for bone tissue engineering should promote the expression of the osteoblastic phenotype. Calcium phosphate-chitosan composite scaffolds have been reported to support the proliferation and differentiation of seeded oseoblast cells as indicated by heigh alkaline phosphatase activities and the formation of mineralizes matrices (Lee et al., 2000).

Osteoblasts usually exhibit higher basal levels of alkaline phosphatase, a relatively early differentiation marker, than do cells that do not produce mineralized extraxellular matrix, such as fibroblasts (Sein et al., 1990). The in vitro engineering of bone tissue requires appropriate carriers that allow a three dimensional distribution of cells. Ishaug et al. suggested that a scaffold material used for bone formation should meet the following: Osetoblast proliferation and function were not affected by polymer foam size in the range of 150-710 μm increased over time for all constructs. Cell seeding density affected initial osteoblast attachment and proliferation rate, but differences became less significant over time with no measurable difference in function. Viable cells may be supported for only short distances into the 3-D matrices under static culture conditions. Achieving cell survival beyond the surface of large 3-D porous scaffolds may require altering culture conditions to improve delivery of

nutrients deep within the constructs while in vivo survival will depend on vascular in vasion of constructs(Ishaug et al., 1997).

A chitosan/collagen sponge applied to the preclinical 1-wall intrabony defects in beagle dogs was shown to have positive effects on periodontal tissue regeneration. The inhibited apical migration of the epithelium and the increase in new bone and new cementum suggests the potency of chitosan in inducing periodontal tissue regeneration (Park et al., 2003).

In the hydroxyapatite/chitosan block bone group of our study, it was not shown residual the hydroxyapatite/chitosan block bone remnant. The inflammatory cell infiltrated into the connective tissue (Fig 6,12). The hydroxyapatite/chitosan block bone group was not a statistically significant difference from control group. In new cementum regeneration, the chitosan membrane group was a statistically significant difference from other group. Our results are different from those of Park et al (Park et al., 2003). In this study, too much early it became the absorption. It appears that the hydroxyapatite/chitosan block bone was too much early absorbed and could not role of scaffold.

In this study, the chitosan membrane group was initially expected to show decidedly less migration of the epithelium compared to the surgical group and calcium phosphate chitosan block bone group. Howerever, the results showed no significant difference in the juctional epithelium migration among the groups. The amount of junctional epithelium migration was (2.68 ± 1.68) mm ((55.50 ± 21.74)% of the defect height) in the surgical control group, (2.18 ± 1.56) mm ((46.41 ± 31.22)% of the defect height) in the hydroxyapatite/chitosan block bone group, 2.43 ± 0.77mm ((50.39 ± 4.37)% of the defect height) in the chitosan membrane group, and (2.55 ± 0.77)

mm((60.28 ± 13.51)% of the defect height) in the combination group(the chitosan membrane group with the hydroxyapatite/chitosan block bone). There was no statistically significant difference among 4 groups (P>0.05)(Table 2).

These results are different from those of Park et al (Park et al., 2003). The high rate of migration indicates the wound instability of the 1-wall intrabony defects. Loss of tight adaptation of the chitosan membrane during the early postsurgical period may have contributed to the failure of controlling epithelium migration.This is not similar to the previous reports (Kim & Chai et al., 1998; Wikesjö et al., 1991).

In the hydroxyapatite/chitosan block bone, results differed from initial expectations. Since previous studies have reported that collagen has an ability to stabilize the fibrin-clot on the root surface by concentrating platelets on the site and subsequently constrain the apical migration of epithelium through their contact-inhibition effects (Beachey et al., 1979; Mason & Read, 1974; Ueno et al., 1999; Wikesjö & Nilvéus, 1990; Winter, 1974), it was expected that the migration of the epithelium would be largely impaired. Yet the outcome turned out controversial, the effect varying among the subjects. This outcome subsequently calls for further research involving more subjects.

The amount of connective tissue adhesion was (1.22 ± 0.71) mm ((30.61 ± 22.80)% of the entire depth of defects) in the control group, (0.93 ± 1.46) mm ((19.97 ± 31.25)% of the entire depth of defects) in the hydroxyapatite/chitosan block bone group, (0.27 ± 0.36) mm ((5.24 ± 6.71)% of the entire depth of defects) in the chitosan membrane group, and (0.79 ± 0.83) mm ((20.63 ± 21.93)% of the entire depth of defects) in the combination group. There was no statistically significant difference among 4 groups (P>0.05) (Table 2).

It is believed that the chitosan membrane affects the regeneration of cementum as a barrier membrane, which inhibits epithelial migration, inducing the differentiation of undifferentiated mesenchymal cells into cementoblasts, as well as by promoting the differentiation of osteogenic cells. This study distinguished between two types of cement regeneration. From the notch to the crest of new bone, the intrabony cementum showed an arrangement of cementoblasts. However, above the crest, the suprabony cementum with few cellular elements appeared to be a cementum-like substance with a parallel fiber adhesion and we could expect that the healing here be by the fibers with a gingival origin. The intrabony cementum with the perpendicularly inserted fibers is the true cementum that accompanies new attachment and is healed by the fibers from a periodontal ligament origin. This result, and previous reports by Barney et al. (1986) and Moon et al.(1996) confirms that new bone follows the coronal growth of new cementum. This suggests that cementum assists the formation of new bone and periodontal ligament fibers. But our results are not always similar to previous study for regeneration, such as membranes, bone grafts, and growth factors, in which new cementum increased only in equal amounts to new bone' (Blumental et al., 1986; Caffesse et al., 1987; Lynch et al., 1989; Mellonig. 1984; Quintero et al., 1982; Schallhorn et al. 1988).

The amount of cemental regeneration was (0.68 ± 1.23) mm ((13.29 ± 23.12)% of the entire depth of defects) in the control group, (1.46 ± 1.58) mm ((48.66 ± 41.37)% of the entire depth of defects) in the hydroxyapatite/chitosan block bone group, (2.30 ± 0.63) mm ((47.24 ± 4.52)% of the entire depth of defects) in the chitosan membrane group, and (1.13 ± 0.90) mm ((27.33 ± 21.78)% of the entire depth of

defects) in the combination group. There was no statistically significant difference among 4 groups (P>0.05) (Table 2).

In alveolar bone regeneation, the chitosan membrane group shows a significantly larger level of bone regeneration, compared with other groups. The amount of alveolar bone regeneration was (0.80 ± 0.18) mm ((19.01 ± 8.40)% of the entire depth of defects) in the control group, (1.20 ± 0.40) mm ((26.24 ± 9.10)% of the entire depth of defects) in the hydroxyapatite/chitosan block bone group, (1.85 ± 0.66) mm ((38.79 ± 11.44)% of the entire depth of defects) in the chitosan membrane group, and (1.22 ± 0.21) mm ((29.89 ± 7.92)% of the entire depth of defects) in the combination group. The chitosan membrane group was a statistically significant different from other treatment groups ($P<0.05$)(Table 2).

Comparing the amount of new cementum with that of new bone in four groups of our study, there was not more cementum growth than the bone growth. Our result is different from Park et al.(2003). In their resuts, cementum regeneration was significantly high only in the chitosan group. Their result suggests that new bone follows the coronal growth of new cementum. Park et al. suggests that new cementum stimulates the formation of new bone and periodontal ligament fibers, and the conspicuous increment of new cementum in the defects treated with chitosan indicates efficacy of chitosan in advancing cementum formation by inducing the differentiation of undifferentiated mesenchymal cells particularly into cementoblasts, as well as by promoting the differentiation of osteogenic cells. However, our results are different from their results. Therefore, the further studies for mechanism is necessary.

Newly formed bone trabeculae were generally lined with osteoblast like cells and osteoids, suggesting continued bone apposition after an 8 weeks observation period

(Siguardsson et al. 1994). The coronal thining of new bone apparent in all group confirms the reports that collagen is deficient in its ability to both make and maintain the space for cellular regeneration. In this study, only a small amount of new bone was coronally formed. This result verifies the reports that osteogenesis occurs only within the given space in periodontal defects and suggests the need for research into other carrier system.

Root resorption appears to be a common sequela of repair in experimental periodontal defects. Wikesjö et al. suggested that the initial resorption is caused by tissue remodeling during periodontal reconstructive surgery (Wikesjö et al., 1991). Most teeth in the present study exhibited superficial resorption, appearing more pronounced when the connective tissue was directly opposed to dentin rather than when the root surface was covered by cementum. This is in agreement with the suggestion that cementum matrix formation may prevent resorption

Ankylosis was not observed in any of the 4 groups. Ankylosis often occurs in faster sites of osteogenesis without the regeneration of the periodontal ligaments. Caffesse et al. noted that the periodontal ligament cells are far faster in repopulation the root surface than osteogenic cells (Caffesse et al., 1987). Similarly, these results revealed how the early colonization of defects by undifferentiated periodontal ligament cells prevented ankylosis.

The results of the present study did not appear to support the potential of chitosan to enhance bone formation, but still show low load bearing capacity. Therefore, the additional study to enhance the mechanical properties appears to be necessary.

V. CONCLUSION

This study evaluated the periodontal repair and biomaterial reaction following implantation of a newly fabricated hydroxyapatite/chitosan block bone and/or chitosan membrane on the regeneration of 1- wall intrabony defects in the beagle dogs. In chitosan membrane group, the amount of new bone formation was greater than the other groups, which suggests that the potency of chitosan membrane induce the new bone regeneration. The results of the present study did support the potential of chitosan membrane in the guided tissue regeneration, but did not showed enough capacity to regeneration of new cementum. Therefore, the further study is to be necessary.

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Table 1. Histometric Analysis: Height (mean ± standard deviation (mm))

	DH	NB	NC	JE	CT
Control	4.61 ± 1.16	0.80 ± 0.18	0.68 ± 1.23	2.68 ± 1.68	1.22 ± 0.71
Exp I	4.58 ± 0.42	1.20 ± 0.40	1.46 ± 1.58	2.18 ± 1.56	0.93 ± 1.46
Exp II	4.84 ± 1.11	1.85±0.66*	2.30 ± 0.63	2.43 ± 0.56	0.27 ± 0.36
Exp III	4.18 ± 0.53	1.22 ± 0.21	1.13 ± 0.90	2.55 ± 0.77	0.79 ± 0.83

Control group: surgical control group received a flap operation only

Exp I : Hydroxyapatite/chitosan block bone group.

Exp II : the chitosan membrane group.

Exp III : Combination group of Hydroxyapatite/chitosan block bone and chitosan membrane.

* a statistically significant difference from other group (P<0.05).

DH: defect height; JE: junctional epithelium migration; CT: connective tissue adhesion; NC: new cementum regeneration; NB: new bone regeneration

Table 2. Histometric Analysis (%)

	JE/DH	CT/DH	NC/DH	NB/DH
Control	55.50 ± 21.74	30.61 ± 22.80	13.29 ± 23.12	19.01 ± 8.40
Exp I	46.41 ± 31.22	19.97 ± 31.25	48.66 ± 41.37	26.24 ± 9.10
Exp II	50.39 ± 4.37	5.24 ± 6.71	47.24 ± 4.52	38.79 ± 11.44*
Exp III	60.28 ± 13.51	20.63 ± 21.93	27.33 ± 21.78	29.89 ± 7.92

Control group: surgical control group received a flap operation only

Exp I: Hydroxyapatite/chitosan block bone group.

Exp II: the chitosan membrane group.

Exp III: Combination group of Hydroxyapatite/chitosan block bone and chitosan membrane.

* a statistically significant difference from other group (P<0.05).

DH: defect height; JE: junctional epithelium migration; CT: connective tissue adhesion; NC: new cementum regeneration; NB: new bone regeneration

Figures

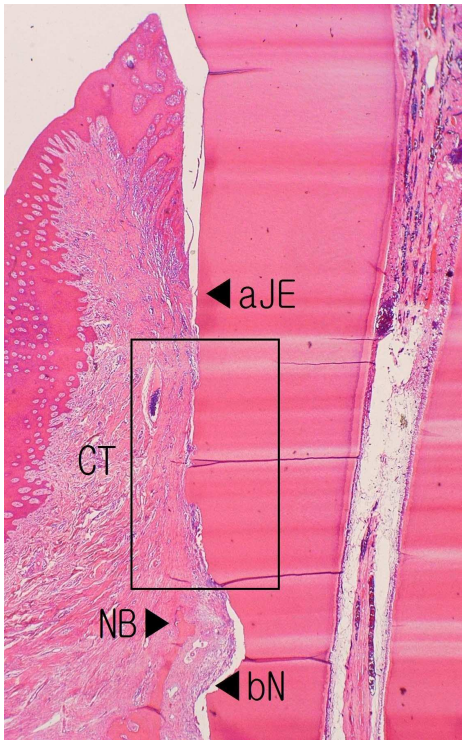


Figure 3. Control group (X20)

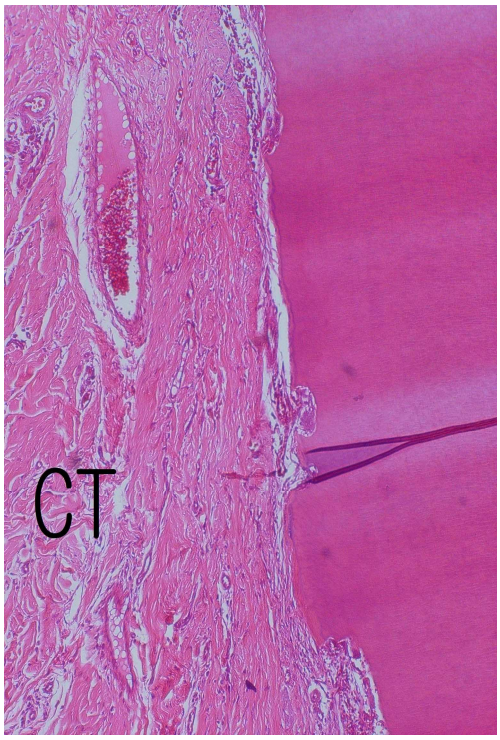
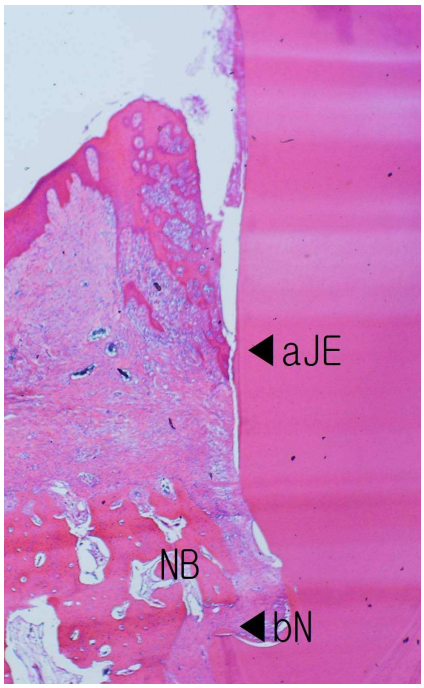


Figure 4. Control group (X100)

aJE : apical extent of junctional epithelium, bN : the base of the reference notch

CT: connective tissue, NB: new bone



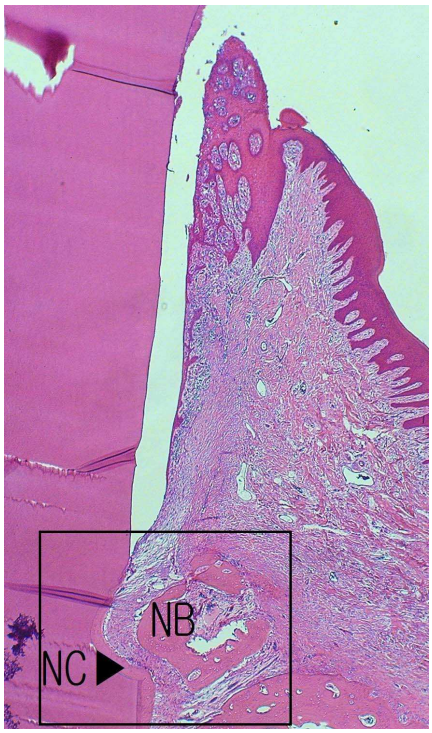


Figure 5. Hydroxyapatite/chitosan block bone(X20) Figure 6. Hydroxyapatite/chitosan block bone (X20)

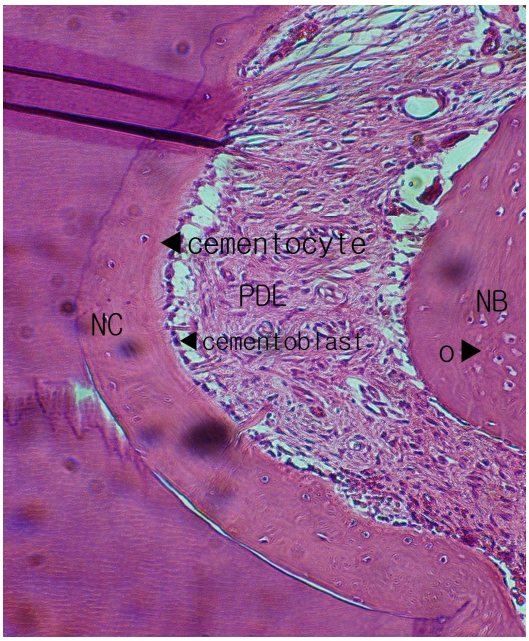


Figure7. Hydroxyapatite/chitosan bone (x200)

NB: new bone, NC: new cementum , O:osteocyte, PDL: periodontal ligament

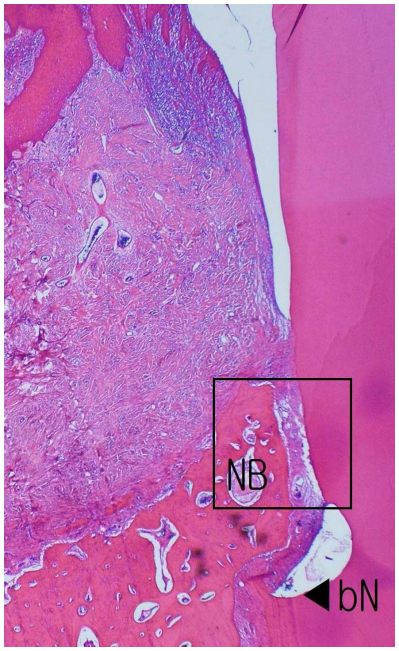


Figure 8. Chitosan membrane (X20)

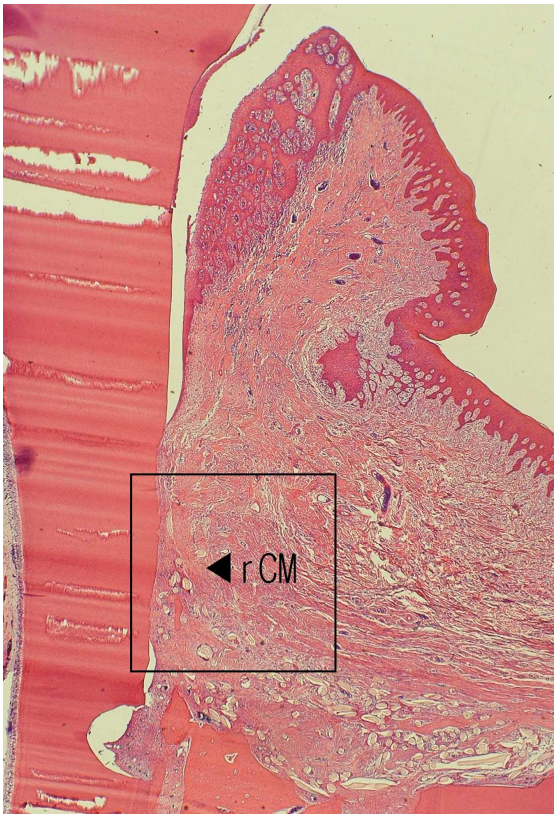


Figure 9. Chitosan membrane (X20)

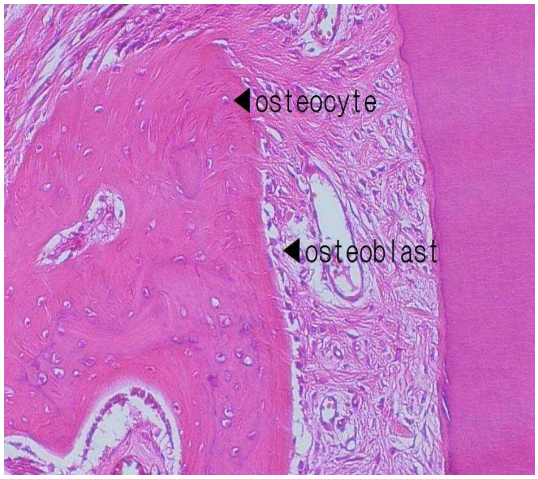


Figure10. Chitosan membrane (X100)

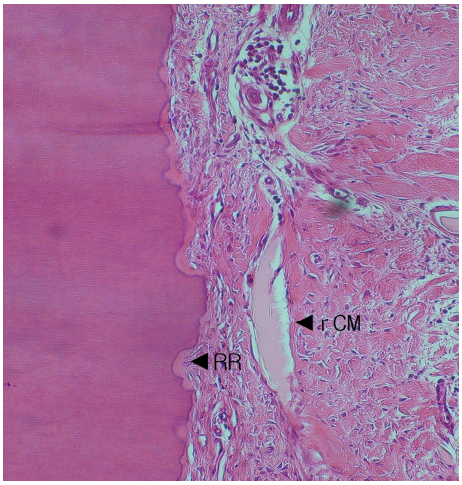


Figure 11. Chitosan membrane (X100)

RR: root resorption, rCM : residual chitosan membrane

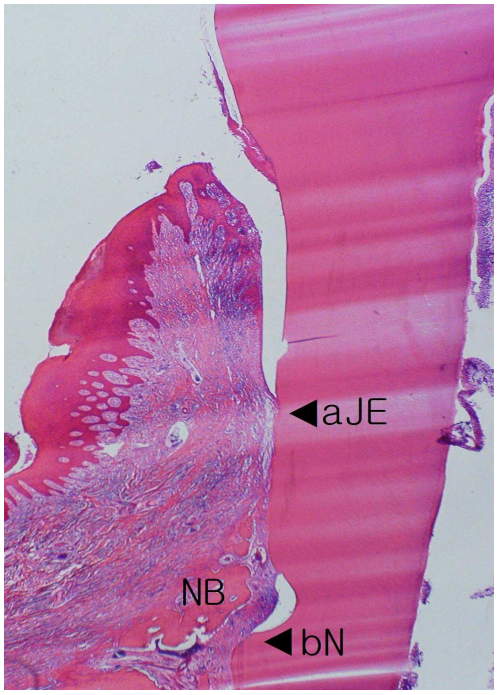


Figure 12. Combination (X20)

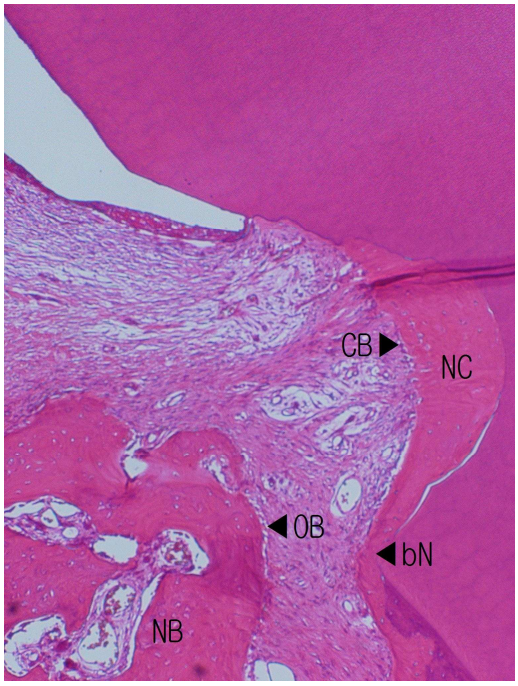


Figure 13. Combination (X100)

aJE : apical extent of junctional epithelium, Bn : the base the reference notch

NC: new cementum, NB: new bone, OB:osteoblast, CB: cementoblast

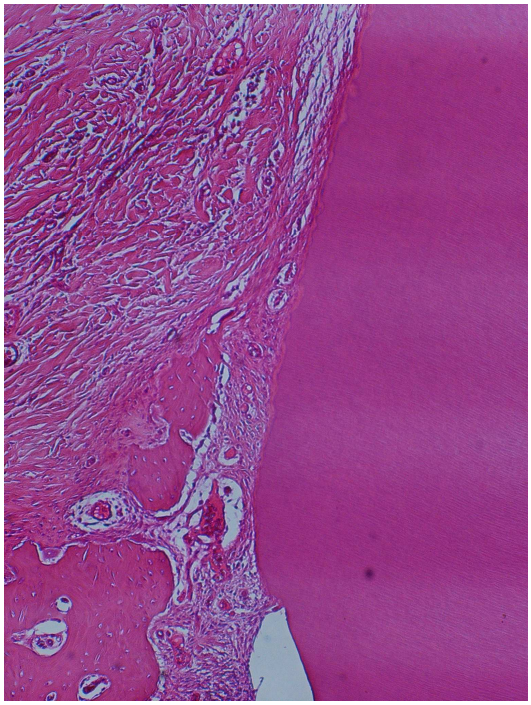


Figure 14. Combination (X100)

국문요약

성견 1 면 골 결손부에서 하이드록시아파타이트-키토산 block bone 과 키토산 차단막 의 치주조직 재생에 대한 연구

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김 성 구

키토산을 이용한 재료는 치주 조직 재생 및 신생골 형성에 효과적인 물질로 알려져 왔다. 이 연구는 새로 개발된 Hydroxyapatite-chitosan block bone 과 Chitosan membrane 을 이용하여, 성견 1 면 골내낭에서의 치주조직 재생에 미치는 영향을 조사하였다. 4X4mm 1 면 골 결손부에 Hydroxyapatite-chitosan block bone 을 이식한 후 조직유도재생술을 시행한 군과, Chitosan membrane 을 이용하여 조직유도재생술을 시행한 군, 그리고 두 가지를 모두 사용한 군(combination 군)을 치은박리소파술만 시행한 군과 비교하여 다음과 같은 결과를 얻었다. Chitosan membrane 을 사용한 군은 Hydroxyapatite-chitosan block bone 만을 이용한 군 혹은 Chitosan membrane 과 함께 사용한 군 그리고 치은박리소파술만 사용한 군에 비해 신생골 형성에 있어서 통계학적으로 유의성 있는 증가량의 차이를 보였다 ($P<0.05$) (Kruskal Wallis test). 신생백악질과 신생골의 생성량은 연관성이 있었다. 기존의 연구결과들은 신생골 생성량이 신생백악질의 생성량을 따르는 양상이었으나 이번 연구결과는 일정하지 않았으므로, 이에대한 연구가 필요하다고 사료된다. Hydroxyapatite-chitosan block bone 만을 사용한 군과 이것을 Chitosan membrane 과 함께 사용한 군은

치은박리소파술만 사용한 군에 대해 신생골과 신생백악질의 생성에 있어서 수치상 우월적 차이를 보였으나 통계학적 유의성 있는 차이를 보이진 않았다 ($p>0.05$). 이러한 연구결과로 볼 때, Hydroxyapatite-chitosan block bone 보다는 Chitosan membrane 이 조직 유도 재생에 보다 긍정적인 효과를 보인다는 것을 알 수 있다. 이는 그간, 발표된 충전방식의 키토산을 이용한 이식재의 결과와 다소 상충되는 결과로 판단된다. 따라서, 조직재생에 대한 키토산을 이용한 충전식 접근은 앞으로 연구가 더욱 필요하다고 판단된다. Chitosan membrane 은 신생골형성에 있어서 양호한 결과를 얻을 수 있었다. 그러나, 임상에서 적용되기 위해서는 앞으로 연구가 더욱 필요하다고 사료된다.

핵심되는 말: 조직유도재생, 하이드록시아파타이트 키토산 블록형 골, 키토산 차단막