The Effects of Chitosan Membrane Containing Tetracycline on Periodontal Healing of One-Wall Intrabony Defects in Beagle Dogs

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정의원 선생님과 채경준 선생님, 김태균 선생님 그리고 치주과 의국원 여러분들께도 많은 도움을 받았습니다. 감사합니다.

사랑과 격려로 학위과정을 마칠 때까지 보살펴 주시고, 성원해 주신 양가 부모님, 그리고 논문을 쓰는 동안 함께 해준 사랑하는 아들 재경, 그리고 무엇과도 바꿀 수 없는 소중한 아내 희성에게 이 논문을 바칩니다.

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Abstract

The Effects of Chitosan Membrane Containing Tetracycline on Periodontal Healing of One-Wall Intrabony Defects in Beagle Dogs

Background: Guided tissue regeneration (GTR) is an accepted therapeutic modality for the treatment of periodontal destructive lesions. However, incomplete regeneration or infection is often noted after GTR therapy. The purpose of this study was to evaluate the regenerative effects of chitosan membranes containing tetracycline (TC) applied to surgically created one-wall intrabony defects in beagle dogs.

Meterial & method: 4×4 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 defects either received chitosan membrane (CH group), chitosan membrane soaked in 0.5 % TC solution for 10 minutes (CH-TC0.5 group), chitosan membrane soaked in 1.0 % TC solution for 10 minutes (CH-TC1.0 group) or flap operation only (surgical control). The animals were sacrificed 8 weeks after the experimental surgery and comparative histologic and histometric examinations were done.

Result: The amount of junctional epithelium migration was 2.02±0.78 mm, 1.85±0.47 mm, 1.53±0.53 mm, and 1.72±0.15 mm in the surgical

control group, CH group, CH-TC0.5 group, and CH-TC1.0 group respectively. No significant differences were observed among the groups. The amount of connective tissue adhesion was 0.82±0.41 mm, 0.77±0.26 mm, 0.81±0.24 mm, and 0.59±0.28 mm in the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group respectively. Connective tissue adhesion did not show any statistically significant differences among the groups. The amount of new cementum regeneration was 1.53±0.52 mm, 1.75±0.27 mm, 1.99±0.41 mm, and 2.09±0.25 mm in the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively, with statistically significant difference between CH-TC1.0 group and the surgical control group ($p<0.05$). The amount of new alveolar bone regeneration was 1.19±0.68 mm, 1.53±0.17 mm, 1.77±0.45 mm, and 1.82±0.23 mm in the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively, with statistically significant difference between CH-TC1.0 group and the surgical control group ($p<0.05$).

Conclusions: The results suggest that CH-TC1.0 membrane may have beneficial effect on the regeneration of bone and cementum in one-wall intrabony periodontal defects of beagle dogs.

Key words: chitosan, tetracycline, membrane, regeneration, one-wall intrabony defect, cementum, alveolar bone.

The Effects of Chitosan Membrane Containing Tetracycline on Periodontal Healing of One-Wall Intrabony Defects in Beagle Dogs

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Ⅰ. *Introduction*

The ultimate goal of periodontal therapy is the regeneration of supporting tissues lost as a result of the disease process. Regeneration can be defined as the reproduction of a lost or injured part. It is the biological process by which the architecture and function of the lost tissue is completely restored.

The concept of periodontal regeneration includes the restoration of alveolar bone, periodontal ligament and cememtum. Although attempts have been made to regenerate lost alveolar bone and attachment apparatus, predictable success remains elusive. Many procedures like bone grafts (Kim et al., 1996; 1998), application of growth factor (Wikesjö et al., 1999; Choi et al., 2002) and guided tissue regeneration (GTR) (Becker et al., 1993) have been used, but there were some

limitations (Heiji et al., 1997).

GTR is based on the perception that given the right conditions, tissues for the most part are capable of self-reconstruction. For periodontal defects, periodontal tissues may regenerate depending on the cell types that migrate onto root surfaces, and that periodontal ligament cells are capable of regenerating periodontal tissues (Melcher, 1976). By using barrier membranes, GTR procedure allows periodontal ligament cells, bone cells, and cementoblasts to enter into the defect site first (Karring et al., 2000).

Various barrier membranes have been developed in periodontal surgery, including both non-resorbable and bioabsorbable type. Non-resorbable membranes require surgical removal, posing risks such as possible damage to newly-formed bone tissue through mechanical interruption or failure to achieve flap coverage over the new tissue.

Recently, there has been an increased interest on the investigation of bioabsorbable materials for barrier membranes which are biocompatible to the host, and do not require an additional surgical procedure to remove. (Yeo et al., 2005; Song et al., 2005). Among those, chitin and its derivative, chitosan (poly-N-acetyl glycosaminoglycan), has attracted particular attention.

Chitin is an important structural component of exoskeletion of invertebrates and cell walls of fungi (Sanford, 1989). Chitosan is a depolymerized and partially deacetylated derivative of chitin made by treating it with hot strong alkali (Tomihata et al., 1997). The resultant chitosan (1-4,2 -amino-2-deoxy-β-D-glucan) is a polycationic complex

carbohydrate with a structure similar to hyaluronic acid (Prudden et al., 1970). Biologically, chitosan is a biodegradable and nontoxic natural biopolymer and it is available in various forms including powder, gel, solution, and membrane (Graves and Cochran, 1994).

The effect of chitosan on wound healing has been examined in various animal models. Park et al. (2003) demonstrated enhanced new bone and cementum formation and inhibition of the epithelial migration using chitosan/collagen sponge applied to one-wall intrabony defects in beagle dogs. In another study, effects of chitosan non-woven membrane on periodontal regeneration in beagle dogs was shown (Yeo et al., 2005). Klokkevold et al. (1996) reported that chitosan potentiated the differentiation of osteoprogenitor cells and might facilitate osteogenesis by interfering with the function of the fibroblasts that inhibit bone formation. Muzzarelli et al. (1989) reported that chitosan ascorbate would have enhanced capability of reconstructing the histoarchitectural tissue and contribute to the reduction of tooth mobility and pocket depths as well.

It would be preferable if the GTR membrane could deliver the antibiotic locally avoiding the systemic uptake in order to prevent early wound healing problem, such as infection. Cao et al. (2000) suggested that 25 % doxycycline-loaded resorbable membrane had a beneficial effect on osteogenesis in periodontal defects. Also Kurtis et al. (2002) suggested that polylactide/glycolide membranes with and without metronidazole might have a beneficial effect on periodontal regeneration.

Tetracycline (TC) is a potentially valuable antibiotic for its

broad-spectrum antibiotic activity against numerous periodontal pathogens. It inhibits human collagenase and bone resorption (Wikesjö et al., 1986; Golub et al., 1984; Roflom et al., 1993). In addition, it was reported to effect bone formation in periodontal defects (Marby et al., 1985). And it also can be used as a root demineralization agent. In another study, additional clinical attachment gain was noted when repeated local administration of minocycline ointment was used with GTR therapy (Yoshinari et al., 2001).

The aim of this study was to evaluate the periodontal tissue regenerative effects of chitosan membranes containing TC. For this purpose, one-wall intrabony defects, known for their unfavorable bone regeneration were surgically created in the beagle dogs and treated with the chitosan membrane containing TC.

Ⅱ. *Materials & methods*

A. Animals

Four male beagle dogs, 18 to 24 months old and weighting about 15 kg were chosen. The animals had intact dentition and healthy periodontium. Animal selection, management, surgical protocol, and preparation followed the routines approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals were fed a soft diet throughout the study to reduce possibility of mechanical interference during food intake.

B. Preparing a chitosan membrane containing tetracycline

In order to prepare the chitosan dope, biomedical grade chitosan (degree of deacetylation 98.0 %, MW 400,000) was dissolved in 2.0 % acetic acid with a concentration of 3.5 % (w/w). The dope was then filtered and de-aerated for further continuous spinning through a nozzle assembly with 0.1 mmø×3,050 holes using a metering gear pump.

The coagulation-bath was composed of 10 % NaOH solution. The bundle of filaments were neutralized and coagulated in the bath, and stretched approximately 20 %, rinsed in 100 ℃ water with the subsequent treatment of spinning oil emulsion and drying resulting in chitosan fiber product. The chitosan fibers were steam treated and

subjected to the stuffer box treatment in order to the fibers have the appropriate crimp level. These were then cut to 50 mm length staple fibers. The chitosan fibers were open with the opening machine, which were then carded by roll carding machine to manufacture a chitosan fiber web. Five or six layers of the manufactured webs were arranged, and subjected to an additional needle punching process to obtain the nonwoven fabrics. The thickness of the manufactured nonwoven fabrics was adjusted to 2 mm using a calender with the proper setting of the machine pressure.

Manufactured chitosan fabrics were soaked in 0.5 % or 1.0 % tetracycline solution for 10 minutes to adsorb tetracycline sufficiently and drying procedure was done for 24 hours at 40 ℃.

C. Experimental design

The animals in the surgical control group were given a flap operation only. The three experimental groups received treatment with the chitosan membrane (CH group), chitosan membrane soaked in 0.5 % tetracycline solution (CH-TC0.5 group), and chitosan membrane soaked in 1.0 % tetracycline solution (CH-TC1.0 group) respectively.

D. Surgical protocol

The surgical protocol was performed under general anesthesia induced by an intravenous injection of atrophin (0.04mg/kg:

Kwangmyung Pharmaceutical Ind. Co. LTD., Seoul, Korea), and the intramuscular induction with a compound of xylazin (Rompun, Bayer Korea Co., Seoul, Korea) and ketamin (Ketera, Yuhan Co., Seoul, Korea) followed inhalation (Gerolan, Choong-wae Pharmaceutical Ind. Co. LTD., Seoul, Korea). Routine dental infiltration anesthesia with 2% lidocaine hydrochloride with 1/80,000 epinephrine (Lidocaine HCl-Epinephrine inj, Kwangmyung Pharm., Seoul, Korea) was used at the surgical sites. The mandibular first and third premolars were extracted in advance of the experimental surgery, and the extraction sites were allowed to heal for 8 weeks.

At reconstructive surgery, the buccal and lingual mucoperiosteal flaps were elevated and 4×4 mm one-wall intrabony defects were created at the distal aspect of mandibular second premolars and the mesial aspect of mandibular fourth premolars. Following root planing, a reference notch was made with 1/4 round bur on the root surface at the base of the defect.

Each bilateral intrabony defects received one of the four experimental conditions: flap operation only (surgical control group), chitosan membrane (CH group), chitosan membrane soaked in 0.5 % TC solution (CH-TC0.5 group), chitosan membrane soaked in 1.0 % TC solution (CH-TC1.0 group). Experimental conditions were rotated among the defects sites in subsequent animals.

Membrane was adapted above the alveolar crest with approximately 3 mm extension over the defect bone margin. Gingival flap was adapted to cover the membrane and suturing was accomplished without tension.

The sutures were removed 7 days after surgery.

Post-surgery management included the administration of antibiotics intramuscularly (Tetracycline HCl, Chongkundang Pharmaceutical Co., Seoul, Korea), a soft diet and a daily topical application of a 0.12 % chlorhexidine solution (hexamedine, Bukwang Pharmaceutical Co., Seoul, Korea).

E. Histologic procedures

The animals were sacrificed 8 weeks after surgical procedure by an intravenous injection of concentrated sodium pentobarbital (Entobar, Hanlim Pharmaceutical Ind. Co. LTD., Seoul, Korea). Tissue blocks, which included teeth, bone, and tissue, were removed, rinsed in saline, then fixed in 10% buffered formalin for 10 days. Subsequently, the block sections were decalcified in 5 % nitric acid for 7 days and embedded in paraffin. Serial sections (5 ㎛ thickness) were made in the mesiodistal direction at interval of 80 ㎛. The four most central sections from each block were stained with hematoxyline/eosin and examination was performed by light microscopy.

F. Analysis

1. Histologic analysis

A PC-based digital image analysis system (Image-Pro Plus®, Media

Cybernetics, Silver Spring, MD, U.S.A) was used to analyze the experimental sites with regard to the junctional epithelium migration, the formation of new bone and new cementum, the absorption of the implanted material, the regeneration of the attachment apparatus, the arrangement of the connective tissue and the periodontal ligament fiber, root resorption, and ankylosis.

2. Histometric analysis (Fig. 1)

For the histometric analysis, both the cementoenamel junction (CEJ) and the notch were used as the reference points. The histometric parameters were:

▪ Defect height (DH) : the distance from the CEJ to the base of the reference notch.
▪ Junctional epithelium migration (JE) : the distance from the CEJ to the apical extension of the junctional epithelium.
▪ Connective tissue adhesion (CT) : the distance from the apical extension of the junctional epithelium to the coronal extension of cementium regeneration.
▪ Cementum regeneration (NC) : the distance from the base of the reference notch to the coronal extension of the newly formed cementum on the root surface.
▪ New alveolar bone regeneration (NB) : the distance from the base of the reference notch to the coronal extension of the newly formed

alveolar bone along the root surface.

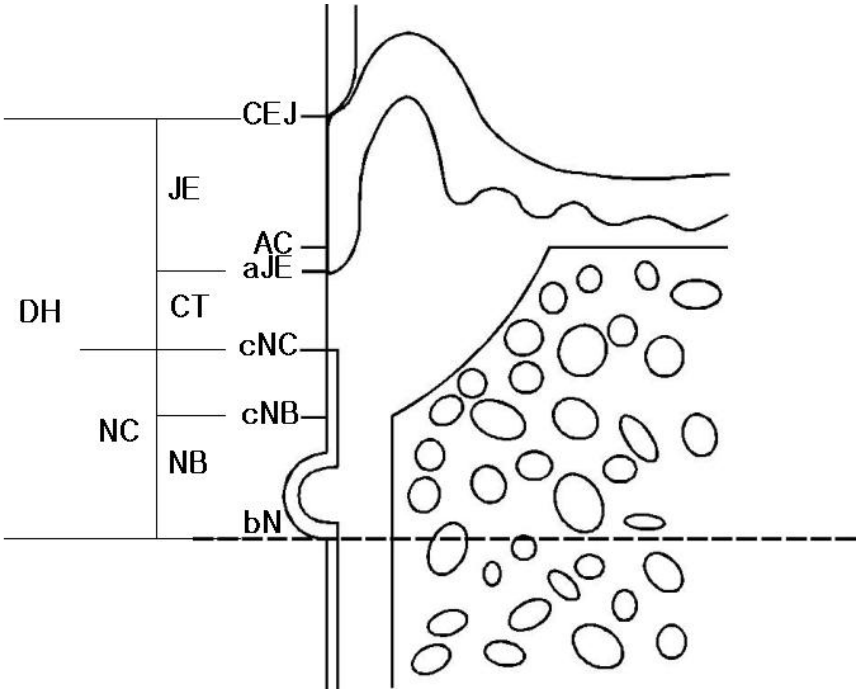

Fig. 1. A schematic diagram depicting the experimental design, the landmarks and the parameters used in histometric analysis.

CEJ: cementoenamel junction
AC: alveolar crest
aJE: apical extent of junctional epithelium
cNC: coronal extent of cementum
cNB: coronal extent of new bone
bN: base of the reference notch
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 block were used to calculate the mean score for each defect (mean±SD). The data were used to test for differences among the experimental group using the Kruskal-Wallis test. The Mann-Whitney U test was used for the comparison between the groups.

Ⅲ. *Results*

A. Histologic observations

More apical migration of the junctional epithelium observed in the surgical control group than in the other groups (Fig. 2-A). There was minimal inflammatory cell infiltration in all the groups. The residual membranes were observed in the connective tissue of experimental groups (Fig 3-B, 4-A, 5-B). More bone regeneration was observed in the CH-TC1.0 group than in the surgical group (Fig. 5-A). The borderline between the new and the old bone was not distinguishable and the osteoblasts were densely arranged along the new bone surface. The amount of new cementum increased significantly along the root surface in the CH-TC1.0 group than in the other groups, and cementoblasts densely surrounded the new cementum. The cementum was thickest at the notch area and became thinner coronally. (Fig. 5-C). In the intrabony cementum layer, the cementoblasts were arranged closely and the fibers were embedded perpendicularly (Fig. 4-B, 5-C). On the contrary, in the suprabony cementum layer, the cementoblasts were rarely observed and the fibers showed parallel orientation. Therefore, it is believed that only the cementum layer with perpendicularly embedded fibers is the true cementum in the newly formed cementum. The periodontal ligament in the groups treated with GTR method was observed to have a more regular pattern and a

denser fiber arrangement than that of the surgical control group (Fig. 4-C, 5-C). The periodontal ligament in the surgical control was organized mainly with irregular, loose collagen fiber. All the groups maintained a good periodontal ligament space. Limited root resorption was observed in the majority of defects and appeared greater in the connective tissue immediately interfaced with root dentin than in the area covered by new cementum (Fig 2-B, 5-B). None of the specimens showed the sign of ankylosis.

B. Histometric observations (Table 1)

The average defect height (DH) was 4.35±0.15 mm, 4.36±0.14 mm, 4.28±0.13 mm, and 4.29±0.14 for the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. There was no significant difference among groups.

The junctional epithelium migration (JE) was 2.02±0.78 mm, 1.85±0.47 mm, 1.53±0.53 mm, and 1.72±0.15 mm for the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. There was no significant difference among groups.

The amount of connective tissue adhesion was 0.82±0.41 mm, 0.77±0.26 mm, 0.81±0.24 mm, and 0.59±0.28 mm for the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. There was no significant difference among groups.

The amount of cementum regeneration was 1.53±0.52 mm, 1.75±0.27 mm, 1.99±0.41 mm, and 2.09±0.25 mm for the surgical control group,

CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. The CH-TC1.0 group was significantly different from the surgical control group ($p<0.05$).

The amount of alveolar bone regeneration was 1.19±0.68 mm, 1.53±0.17 mm, 1.77±0.45 mm, and 1.82±0.23 mm for the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. The CH-TC1.0 group was significantly different from the surgical control group ($p<0.05$).

There was no significant difference between CH group, CH-TC0.5 group, and CH-TC1.0 group at any items.

Table 1. Histometric anaylsis (measurement in mm)

	control Mean±SD	CH Mean±SD	CH-TC0.5 Mean±SD	CH-TC1.0 Mean±SD
DH	4.35±0.15	4.36±0.14	4.28±0.13	4.29±0.14
JE	2.02±0.78	1.85±0.47	1.53±0.53	1.72±0.15
CT	0.82±0.41	0.77±0.26	0.81±0.24	0.59±0.28
NC	1.53±0.52	1.75±0.27	1.99±0.41	2.09±0.25 *
NB	1.19±0.68	1.53±0.17	1.77±0.45	1.82±0.23 *

DH : Defect height JE : Junctional epithelium migration

CT : Connective tissue adhesion NC : New cementum regeneration

NB : New bone regeneration

* : Statistically significant difference compared to the surgical control group ($p<0.05$).

Ⅳ. *Discussion*

The ultimate goal of periodontal therapy is to regenerate the destroyed supporting tissue including new acellular cementum attached to the underlying dentin surface, new periodontal ligament with functionally oriented collagen fibers inserting into the new cementum, and new alveolar bone attached to the periodontal ligament (Aukhil et al., 1986).

A variety of therapeutic methods, such as root planing, curettage, gingivectomy, and flap procedures including implantation of various materials into defects, have been used to attain periodontal regeneration. But regeneration of complete periodontium is limited. This results may be due to primary population of tissues with poor regenerative potential in the defect (Melcher, 1976).

Various bone grafts have been investigated to treat intrabony defects. However, autogenous bone has problems associated with limitation in harvesting. Decalcified freeze-dried bone (DFDB) has possibility of contamination and transmission of disease (Buck et al., 1989), and contains little bone morphogenetic proteins and has osteoconductive properties. Some previous studies dealt with bone grafts have shown that epithelium migrated apically between the root and adjacent tissues, inhibiting regeneration (Carranza et al., 1987; Kim et al., 1998; Heiji et al., 1997).

By using membrane, GTR method can induce selective repopulation of undifferentiated mesenchymal cells that originate from the periodontal ligament, and has been shown to improve the periodontal regeneration in

both animal and human experiments (Becker et al., 1991; Caffesse et al., 1988).

Recently, a new non-toxic, biodegradable materials for resorbable membrane that would be free from any side effects have been studied widely. (Park et al., 2003; Yeo et al., 2005). Among these materials, chitosan, a derivative of chitin (de-N-acetylated derivative of chitin) takes a increasing interest for its good biological and physical properties.

Chitin is one of the most abundant natural biopolymer, second only to cellulose. It has a similar chemical structure to cellulose, but contains components including acetyl groups, which make it more resistant to chemical attack. These groups decrease the solubility in general organic solutions and add a sour taste to it.

Chitosan is prepared by hot strong alkali treatment with chitin, which results in abundant deacetylation of the side chains. Chitosan has structural similarity to the glycosaminoglycans, especially hyaluronic acid, which are easily found in the extracellular matrices of many tissues. Hyaluronic acid is believed to facilitate the migration and differentiation of progenitor cells, which promote tissue regeneration (Gallagher et al., 1986). Chitosan invigorates important cellular processes by stimulating the growth factors (Varki, 1996). In addition, the chito-oligomer has been reported to initiate the formation of hyaluronic acid (Hitz et al., 1996). Chitosan not only induced osteogenesis by conjoining the growth factors to stimulate osteoblast differentiation but also indirectly facilitated regeneration by interfering with the adhesion and proliferation of the cells that inhibit bone formation such as fibroblasts (Klokkevold et al., 1996).

In addition to its biological merits, chitosan has good physical properties such as availability in a variety of forms (Tomihata and Ikata, 1997). Furthermore, it is also easy to combine chitosan with other materials (Ito et al., 1991).

The use of tetracycline (TC) offers the prospect of accelerating the normal healing process (Victor et al. 1986). And in vitro studies, TC is highly effective for the majority of periodontopathic microorganisms in juvenile periodontitis (Becker et al., 1991; Demolon et al., 1993) and refractory periodontitis (Silverstein et al., 1988). Bjorvatn et al. (1971) immersed freshly extracted molars of rats in TC solution, which caused a marked stimulation of alveolar bone formation after replantation. In similar study, high concentration of locally applied TC might have osteogenic effect in addition to its antibiotic action (Hars et al., 1972).

In this study, surgically created one-wall intrabony defects were used. In many investigations, one-wall intrabony defects were known as unfavorable configuration for natural osteogenesis. More walls mean better osteogenesis, because increase in the number of walls brings about an ample supply of osteogenic cells (Mellonig, 1984). Furthermore, if the number of walls in defect is increased, the firm fixation of the implanted materials and easy suturing is possible, which assists in suppressing epithelial migration.

The use of surgical defects was decided based on a previous report showing that the healing process showed no difference between the surgical defects and the attachment loss caused by natural disease or ligation (Wikesjo et al., 1990; 1991a; 1991b). An artificial method also

helps keep the initial experimental conditions of the control and the experimental group almost identical, which heightens the credibility of the experiments (Haney et al., 1993).

The observation period in the histologic studies in animals has varied from 2 weeks to 3 or 6 months. Eight weeks healing interval has been required to evaluate regeneration effect of implanted biomaterials in previous studies (Kim et al., 1998; Polson et al., 1982; Sigurdsson et al., 1995), and Choi et al. (2002) reported that there was no significant difference in bone regeneration by BMP between 8 and 24 weeks interval. Therefore, in the present study, 8 weeks healing period was taken up to observe the initial healing process.

The biodegradable, nonwoven CH, CH-TC0.5, and CH-TC1.0 membranes are easy to manipulate, and have a porous structure that provides pores for the ingrowth of regenerative cells, which results in an increase of cell differentiation and vascular infiltration. The membrane allows the coagulum to adhere on the root surface to be repopulated by cellular elements derived from the periodontal ligament and alveolar bone, while the gingival fibroblasts and epithelial cells are excluded. In previous studies, it was reported that a nonwoven membrane served as an excellent medium for various osteoconductive substances, and provided the structure needed for the in growth of regenerative cells, especially osteoprogenitor cells (Blumenthal et al., 1986).

Usually, groups treated with membranes were expected to show less migration of junctional epithelium compared to control group (Becker et

al., 1993). But in this study, there was no significant difference in the junctional epithelium migration among four groups. Because membranes prepared in the present study were somewhat lack in stiffness, loss of tight adaptation of membrane around the root surface in early wound healing period was occurred, resulting in the failure of controlling epithelial migration.

In terms of connective tissue adhesion, no significant difference was seen among the groups. But CH group, CH-TC0.5 group and CH-TC1.0 group showed tighter distribution of collagen fibers with functional arrangement, while the surgical control group had loosely distributed collagen fibers, showing random to parallel orientation.

The amount of new cementum regeneration was 1.53±0.52 mm, 1.75±0.27 mm, 1.99±0.41 mm, and 2.09±0.25 mm in the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. New cementum regeneration was significantly higher in the CH-TC1.0 group compared with the control group ($p<0.05$). It is thought that the CH-TC1.0 membrane effects 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. The regenerated cementum observed to be cellular from the notch to the alveolar crest and was generally covered by cementoblast-like cells and cementoid with perpendicular fiber insertion. However, the newly formed cementum above the alveolar crest was observed as a thin strip along the root surface with few cellular elements and parallel fiber

arrangement. This result suggests that the suprabony site in a periodontal defect is healed by the fibers of gingival origin, while the infrabony site is healed by the fibers of periodontal ligament origin (Barney et al., 1986). This difference in wound healing accounts for why suprabony site is more likely to lose structural, functional continuity in the case of a relapse.

The amount of new bone regeneration was 1.19±0.68 mm, 1.53±0.17 mm, 1.77±0.45 mm, and 1.82±0.23 mm in the surgical control group, CH group, CH-TC0.5 group, and CH-TC1.0 group, respectively. In new bone regeneration, the CH-TC1.0 group showed significantly higher level of regeneration, compared with the surgical control group ($p<0.05$). This result suggests that soaking chitosan membrane with 1.0 % TC solution showed positive effects on bone regeneration. However, proper concentration of TC solution for optimal result could not be determined in this study. Besides the antibacterial effects of TC, the possible mechanisms of TC-loaded membrane caused extensive new bone formation were suggested that cell originated form periodontal ligament or bone might be differentiated into bone-forming cell, namely osteoblast by the TC-loaded membrane-created healing environment. and TC might initiate demineralization on the bone surface layer, which would probably resulted in the release of some osteogenic factors, such as transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), or bone morphogenetic proteins (BMPs), into the surrounding tissues to trigger the bone induction effects. TC-loaded membrane also segregated the cellular periosteum from the fibrous periosteum, which

might result in the vigorous activation and proliferation of osteoblasts in the cellular periosteum. But there still are many questions to be clarified, which could not be explained in the present study protocol. The boundary between the new bone and old bone was indistinguishable and newly formed bone surface was frequently lined with osteoblast-like cells and osteoids where continued bone apposition after 8-week observation period was predictable. The amount of alveolar bone regeneration measured in groups treated with GTR modality was somewhat lesser than that of previous study dealt with bony defects similar to present defect design (Park et al., 2003; Song et al., 2005; Yeo et al., 2005). Because membranes used in this study did not have sufficient stiffness for maintaining defect space and no bone graft material was implanted in the defect, partial collapse of membranes might have occurred and resulted in decrease of periodontal regeneration rate.

Residual membrane particles were observed in the connective tissue. but peripheral giant cell infiltration and foreign body reactions were not noted. It was reported that chitosan has biocompatibility with the host in a previous animal study (Nakajima et al., 1986).

In this study, most cases showed root resorption, appearing more pronounced when the connective tissue was in direct contact with dentin than the root surface covered by cementum or cementiod.

Ankylosis often occurs in the sites of fast osteogenetic development without the regeneration of the periodontal ligaments (Card et al., 1987), and not observed in any of the four groups. Usually, periodontal

ligament cells are far faster in repopulation at the root surface than osteogenic cells.

Overall, The results suggest that surgical application of chitosan membrane soaked with 1.0 % tetracycline solution could promote the regeneration of alveolar bone and cementum in intrabony periodontal defects. However, further studies to improve physical properties of membranes is required. Also, Investigation for more effective delivery system and the specific application protocol for clinical use is needed.

Ⅴ. *Conclusion*

1. The average defect height (DH) was 4.35±0.15mm, 4.36±0.14 mm, 4.28±0.13 mm, and 4.29±0.14 for the surgical control group, the CH group, the CH-TC0.5 group, and the CH-TC1.0 group, respectively. There was no significant difference among groups.

2. The junctional epithelium migration (JE) was 2.02±0.78 mm, 1.85±0.47 mm, 1.53±0.53 mm, and 1.72±0.15 mm for the surgical control group, the CH group, the CH-TC0.5 group, and the CH-TC1.0 group, respectively. There was no significant difference among groups.

3. The amount of connective tissue adhesion (CT) was 0.82±0.41 mm, 0.77±0.26 mm, 0.81±0.24 mm, and 0.59±0.28 mm for the surgical control group, the CH group, the CH-TC0.5 group, and the CH-TC1.0 group, respectively. There was no significant difference among groups.

4. The amount of cementum regeneration (NC) was 1.53±0.52 mm, 1.75±0.27 mm, 1.99±0.41 mm, and 2.09±0.25 mm for the surgical control group, the CH group, the CH-TC0.5 group, and the CH-TC1.0 group, respectively. The CH-TC1.0 group was significantly different from the surgical control group ($p<0.05$).

5. The amount of alveolar bone regeneration (NB) was 1.19±0.68 mm, 1.53±0.17 mm, 1.77±0.45 mm, and 1.82±0.23 mm for the surgical control

group, the CH group, the CH-TC0.5 group, and the CH-TC1.0 group, respectively. The CH-TC1.0 group was significantly different from the surgical control group ($p<0.05$).

Surgical application of CH-TC1.0 membrane could promote the regeneration of alveolar bone and cementum in intrabony periodontal defects. The increase in new bone and new cementum suggests the potency of the CH-TC1.0 membrane in inducing periodontal tissue regeneration.

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Figure Legends

Fig. 2-A Photomicrograph of a surgical control section showing epithelial downgrowth, connective tissue adhesion and new bone formation above the notch. (magnification×20)

Fig. 2-B Photomicrograph of a surgical control section showing loose and parallel fiber orientation along the root surface in the supracrestal region (arrow headed). root resorption was noted. (×400)

Fig. 3-A Photomicrograph of the CH section showing epithelial downgrowth, CT adhesion, new bone formation. (×20)

Fig. 3-B Photomicrograph of the CH section showing the residual membrane particle. (×100)

Fig. 4-A Photomicrograph of the CH-TC0.5 section showing epithelial downgrowth, CT adhesion, new bone formation, residual membrane. (×20)

Fig. 4-B Photomicrograph of the CH-TC0.5 section. Fibers are embedded perpendicularly to new bone and new cementum. (×100)

Fig. 4-C Photomicrograph of the CH-TC0.5 section. The periodontal ligament with well developed vessels. (×400)

Fig. 5-A Photomicrograph of the CH-TC1.0 section showing epithelial downgrowth, CT adhesion, new bone formation, residual membrane. (×20)

Fig. 5-B Photomicrograph of the CH-TC1.0 section. Suprabony cementum formed a thin strip along the root surface and fiber

adhesion showed parallel orientation along the newly formed cementum in the supracrestal region. Root resorption was observed. (×100)

Fig. 5-C Photomicrograph of the CH-TC1.0 section showing perpendicularly oriented new periodontal ligament mediating attachment to the new intrabony cementum and new bone. (×400)

Figures

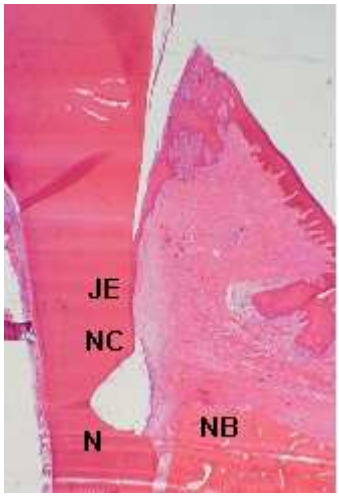
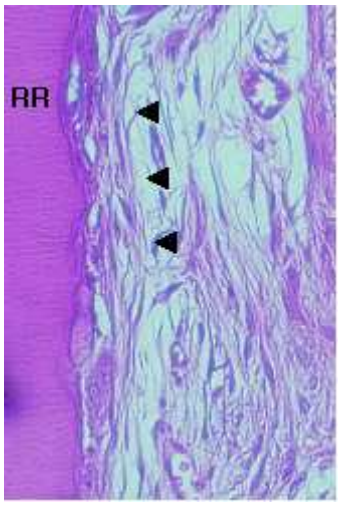


Fig. 2-A



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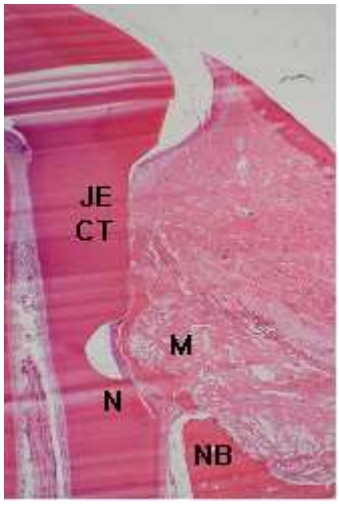

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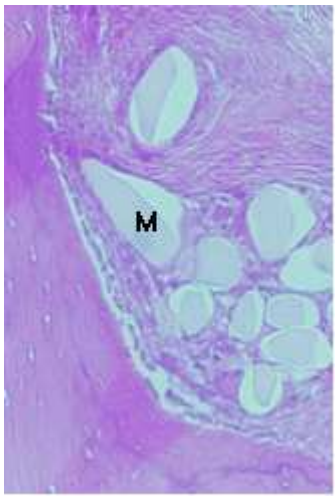

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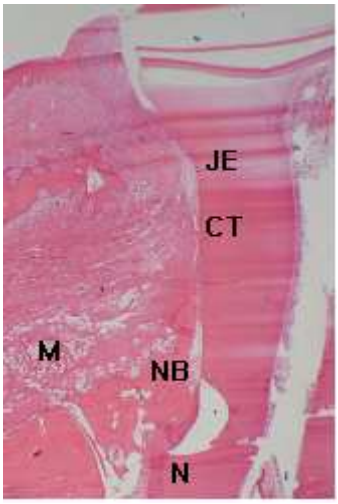


Fig. 4-A

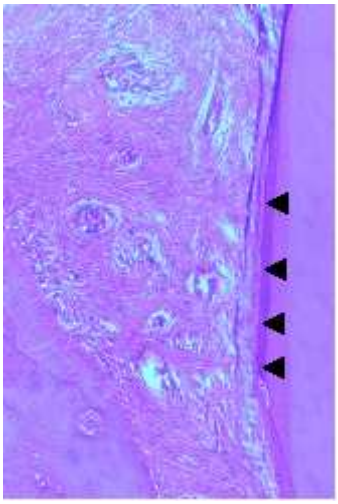


Fig. 4-B

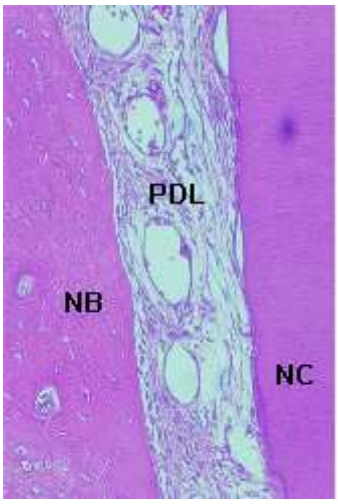


Fig. 4-C

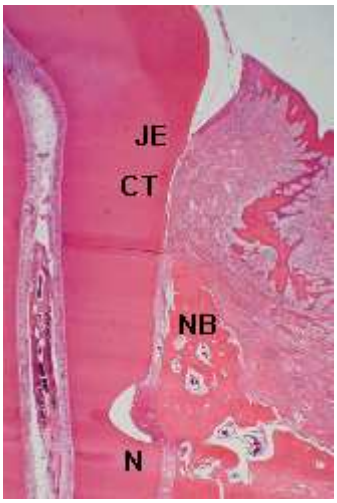
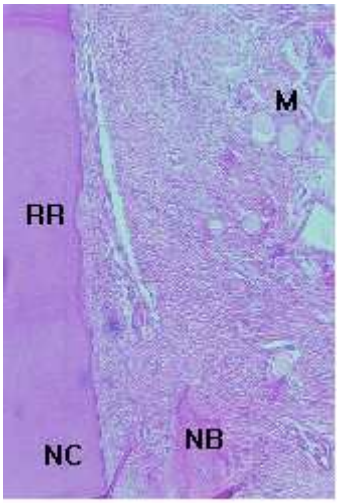


Fig. 5-A



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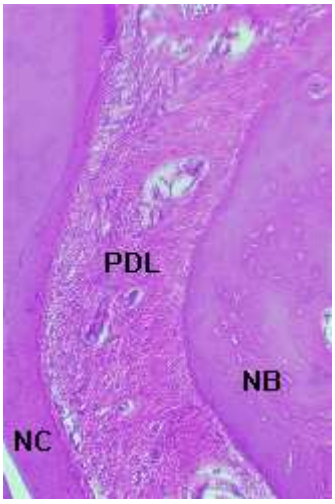

Fig. 5-C

국문요약

성견의 *1*면 골내낭에서 테트라싸이클린 함유 키토산 차단막이 치주조직 치유에 미치는 영향

(지도교수 최 성 호)

연세대학교 대학원 치의학과

한 광 희

연구배경: 치주 치료의 궁극적인 목표는 파괴된 치주조직을 구조적, 기능적으로 재생시키는데 있다. 이를 위해 조직유도 재생술, 골 이식술, 성장인자적용등과 같은 다양한 재생 술식들이 시행되어져 왔다. 이중 차단막을 이용한 치주조직 재생술은 오랜 기간에 걸쳐 실험되고 임상에 적용되어져 왔으며 최근 생분해성 차단막의 재료로써 생체적합성이 우수하고 항균 작용과 창상 치유에 효과적인 키토산에 대한 다양한 연구가 활발히 진행되고 있다.

이 연구의 목적은 테트라싸이클린을 함유한 키토산 차단막을 beagle dog에 외과적으로 형성한 1면 골내낭에 적용하여 치주조직의 재생에 미치는 영향을 평가하는 것이다.

재료 및 방법: Beagle dog에서 하악 양측의 제 2 소구치의 원심면과 제 4 소구치의 근심면에 4×4 mm 크기의 1면 골내낭을 외과적으로 형성하고, 임의로 위치를 선택하여 치은 박리 소파술만 시행한 군을 대조군으로, 치은박리 소파술 후 키토산 차단막을 적용한 군을 실험 1군 (CH 군)으로,

치은박리 소파술 후 0.5 % 테트라싸이클린 용액에 10분간 침지시킨 키토산 차단막을 적용한 군을 실험 2군 (CH-TC0.5 군)으로, 치은박리 소파술 후 1.0 % 테트라싸이클린 용액에 10분간 침지시킨 키토산 차단막을 적용한 군을 실험 3군 (CH-TC1.0 군)으로 설정하여 실험하였다. 실험동물은 술 후 8주에 희생시키고 조직학적 검사를 시행하였다.

결과: 접합상피의 치근단 이동량은 대조군, 실험 1군, 실험 2군, 실험 3군에서 각각 2.02 ± 0.78 ㎜, 1.85 ± 0.47 ㎜, 1.53 ± 0.53 ㎜, 1.72 ± 0.15 ㎜로 나타났으며, 각 군 간에 통계적인 유의차는 없었다. 결합조직 유착의 길이는 대조군, 실험 1군, 실험 2군, 실험 3군에서 각각 0.82 ± 0.41 ㎜, 0.77 ± 0.26 ㎜, 0.81 ± 0.24 ㎜, 0.59 ± 0.28 ㎜로 나타났으며, 각 군 간에 통계적인 유의차는 없었다. 신생백악질 형성은 대조군, 실험 1군, 실험 2군, 실험 3군에서 각각 1.53 ± 0.52 ㎜, 1.75 ± 0.27 ㎜, 1.99 ± 0.41 ㎜, 2.09 ± 0.25 ㎜로 나타났으며, 대조군과 실험 3군 사이에 유의성 있는 차이를 보였다 ($p<0.05$). 신생골 형성은 대조군, 실험 1군, 실험 2군, 실험 3군에서 각각 1.19 ± 0.68 ㎜, 1.53 ± 0.17 ㎜, 1.77 ± 0.45 ㎜, 1.82 ± 0.23 ㎜로 나타났으며, 대조군과 실험 3군 사이에 유의성있는 차이를 보였다 ($p<0.05$).

결론: CH-TC1.0 차단막을 외과적으로 형성한 치조골 결손부에 적용시킨 경우 신생 백악질 및 치조골의 형성이 촉진된 것으로 분석되며, CH-TC1.0 차단막의 사용은 치주 조직 재생에 유용한 적용 방법으로 사료된다.

핵심 되는 말 : 키토산, 테트라싸이클린, 1면 골내낭, 치주 조직 재생, 신생 백악질, 신생골