The Effects of Chitosan nonwoven membrane on Periodontal Healing of 1-Wall Intrabony Defects in Beagle Dogs

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여 영 주

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Abstract

Objectives: This study was designed to evaluate the periodontal tissue regenerative effects of a chitosan nonwoven membrane applied to preclinical 1-wall intrabony defects surgically created in beagle dogs

Meterial & Method: 1-wall intrabony defects (4×4 mm) were surgically created in the bilateral mandibular second and fourth premolars. the surgical control group received a flap operation only, while the Resorbable membrane group was treated afterwards with a Resorbable membrane and the chitosan nonwoven membrane group were treated a chitosan nonwoven membrane. The subjects were sacrificed 8 weeks after surgery and a comparative histometric analysis was done.

Result: The amount of junctional epithelium migration in the surgical control group was 2.32±0.33 mm, 2.04±0.57 mm in the Resorbable membrane group, and 1.77± 0.70 mm in the chitosan nonwoven membrane group. No significant differences were observed among the treatments. The amount of connective tissue adhesion was 0.86±0.29 mm, 1.20±0.44 mm, and 1.12±0.18 mm in the surgical control, the Resorbable membrane, and the chitosan nonwoven membrane group, respectively. No significant differences were observed among the treatments. The amount of suprabony cementum regeneration was 0.31±0.05 mm, 0.24±0.09 mm, and 0.58±0.10 mm in the surgical control, the Resorbable membrane, and the

chitosan nonwoven membrane group, respectively. A significant difference in the suprabony cementum was observed between the surgical control group and chitosan nonwoven membrane group ($P<0.05$). The amount of intrabony cementum regeneration was 1.08±0.24 mm, 1.48±0.52 mm, and 1.68±0.15 mm in the surgical control. the Resorbable membrane, and the chitosan nonwoven membrane group, respectively. Significant differences were observed between the chitosan nonwoven membrane and the surgical control group ($P<0.05$). The amount of alveolar bone regeneration was 1.17±0.31 mm, 1.54±0.71 mm, and 1.81±0.16 mm, in the surgical control, the Resorbable membrane, the chitosan nonwoven membrane group, respectively. Significant differences were observed between the surgical control, and the chitosan nonwoven membrane group($P<0.05$).

Conclusions: The results demonstrate the beneficial effects of the chitosan nonwoven membrane to 1-wall intrabony defects of beagle dogs. 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 1-wall intrabony periodontal defects of beagle dogs.

Key words: chitosan nonwoven membrane (CNWM), One-wall intrabony defect, intrabony cementum, suprabony cementum.

The Effects of Chitosan nonwoven Membrane on Periodontal Healing of 1-Wall Intrabony Defects in Beagle Dogs

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1. Introduction

The ultimate goal of periodontal therapy is to regenerate the functional periodontium lost due to periodontal disease. To achieve this goal, various procedures or devices have been proposed including guided tissue regeneration, (Becker *et al*, 1992; Haney *et al*, 1993; Kim *et al.*, 1996, 1998a,b,; Trombelli et al., 1997) bone grafts, (Mellonig *et al.,* 1984) and the application of growth factors(Caffesse *et al.*, 1998; Wikesjö et al., 1999; Choi *et aL.,* 2002). Guided tissue regeneration (GTR) is based on the perception that given the right conditions, tissues for the most part are capable of self-reconstitution. For periodontal defects, it has been suggested that the periodontal ligament cells and their migration potential are crucial for periodontal regeneration, and that regeneration may occur if the gingival connective tissue and epithelial cells are

prevented from accessing to the tooth surface. Barrier membranes, placed at the time of reconstructive surgery, are commonly used to provide these conditions. GTR therapy was introduced to achieve a repopulation of the periodontal ligament fibroblasts and has been shown to promote periodontal regeneration. Chitosan is a derivative of chitin that is synthesized by treating it with hot strong alkali which results in abundant deacetylation of side chains. 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). It is biodegradable, non-toxic, and has a molecular weight of 800-1500 Kd (Sanford, 1989). Chitosan is available in a variety of useful forms including solutions, powders, flakes, gels, and films together with its unique chemical and biological properties, which makes it a very versatile biomaterial (Graves and Cochran, 1994).

The effect of chitosan on bone wound healing has been examined in various animal models. Malette et al. (1989) reported early evidence of enhanced radii bone regeneration in dogs. In their study, the chitosan-treated wounds healed by the marrow through the cortex. There is a report showing that chitosan ascorbate contributed to the reduction of tooth mobility and pocket depths as well as the enhanced capability of reconstructing the histoarchitectural tissue (Muzzarelli *et al.*, 1989). Muzzarelli et al. (1993) reported that gel-forming modified chitosans were more suitable for medical use than chitosan itself and had an osteoconductive potential of methylpyrrolidinone chitosan in bone repair

when applied after an apicoectomy and wisdom tooth avulsion. In another animal study, Muzzarelli et al. (1993) reported improved osseous healing of the defects created in the tibia of rabbits and femoral head of sheep. Klokkevold et al. (1996) reported that chitosan potentiates the differenciation of osteoprogenitor cells and may facilitate osteogenesis by interfering with the function of the cells that inhibit bone formation such as fibroblasts. Lee et al. (2000) reported that chitosan/Tricalcium Phosphate Sponges is feasible for use
as a scaffolding material to grow osteoblasts in a three dimensional structure for transplantation into a site for bone regeneration. Madihally et al. (1999) reported that chitosan has exellent potential as a structural base material for a variety of engineered tissue systems. Recently, Park et al. (2003) reported that chitosan enhanced the periodontal tissues regeneration.

The aims of this clinical study were to evaluate the clinical effects of chitosan nonwoven membrane (CNWM) on the regeneration of periodontal tissue and compare the results to those obtained from the use of a Resorbable membrane (RM) and a flap operation only. For this purpose, 1-wall intrabony defects, which are known for their difficult bone regeneration were surgically created in the beagle dogs and treated with the CNWM. The healing process was recorded over an eight week period after surgery.

Materials and Method

A. Animals

Six 2-year-old Beagle dogs, approximately 15 kg, were used. The animals had intact dentition with a healthy periodontium. The Institutional Animals Care and Use Committee, Yonsei Medical Center, Seoul, Korea approved the selection of animals, management, surgical protocol, and preparation. The animals were fed a soft diet throughout the study, to reduce chance of mechanical interference with healing during food intake.

B. Chitosan non-woven membrane

a, Manufacturing Chitosan Fibres

In order to prepare the chitosan dope, biomedical grade chitosan (degree of deacetylation 100 %, MW 540,000, polydispersity 1.20) was dissolved in 2 % acetic acid with a concentration of 3 % (w/w). The dope was then filtered and de-aerated for further continuous spinning through a nozzle assembly with 0.1 mmø×1500 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. These

were then stretched approximately 20 % and rinsed in 100°C water with the subsequent treatment of spinning oil emulsion and drying resulting in chitosan fiber product. The titer of the chitosan fiber manufactured was 2 denier.

b, Manufacturing Needle-punched Nonwoven Fabrics made of chitosan fibres

The chitosan fibers were steam treated and subjected to the stuffer box treatment in order for the fibers to have the appropriate crimp level. These were then cut to 50 μm 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. (Department of Clothing & Textiles, Ewha Womans University)

C. Experimental design

The animals in the surgical control group were given a flap operation only. The two experimental groups received treatment with the

Resorbable membrane which composed of polyglycolide, d, 1-lactide/ glycolide, and poly-1-lactide and the chitosan nonwoven membrane respectively. (Biomesh® Samyang Co., Seoul, Korea)

D. Surgical protocol

The surgical procedure was performed under general anesthesia induced by an intravenous injection of atrophin (0.04 mg/kg: Kwangmyung Pharmaceutical Ind. Co., LTD. Seoul, Korea) and an intramuscular induction with a compound of xylazin (Rompun, Bayer Korea Co., Seoul, Korea) and ketamin (Ketrara, Yuhan Co., Seoul, Korea), followed by inhalation (Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea). Routine dental infiltration anesthesia (2 % lidocaine hydrochloride with 1/80,000 epinephrine) was used at the surgical sites. The mandibular first and third premolars were extracted prior to the experimental surgery, and the extraction sites were allowed to heal for 8 weeks.

For reconstructive surgery, the buccal and lingual mucoperiosteal flaps were elevated and 4×4 mm 1-wall intrabony defects were created at the mesial aspect of the mandibular second and fourth premolars and at the distal aspect of the mandibular second premolars. Following root planing, a reference notch was made with a 1/4 round bur on the root surface at the base of the defect. Each bilateral mandibular defect received one of the three experimental conditions: the chitosan nonwoven membrane, the

Resorbable membrane, and the surgical control sites. The flaps were repositioned and sutured with a 3-0 thread. The sutures were removed 7 days after surgery.

Post-surgical management included the administration of antibiotics intramuscularly (Tetracycline HCL, Chongkundang Pharmacuetical Co., Seoul, Korea), a soft diet, and the daily topical application of a 0.12 % chlorhexidine solution (Hexamedine, Bukwang Pharmaceuticals Co., Seoul, Korea).

E. Clinical and Histologic Procedures

The animals were euthanized 8 weeks after surgery by an IV injection of concentrated sodium pentobarbital. Block sections including the surgical sites were removed, rinsed in saline, and fixed in 10 % buffered formalin for 10 days. After rinsing in water, the block sections were decalcified in 5 % formic acid for 14 days, and embedded in paraffin. Serial sections, 5 μm thick, were cut in the mesial-distal direction. The four most central sections from each block were stained with hematoxylin/eosin and examined by optical microscopy.

F. Analysis

Histological Analysis

A PC-based image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD., USA) 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 materials, the regeneration of the attachment apparatus, the arrangement of the connective tissue and the periodontal ligament fiber, root resorption, and ankylosis.

Histometric Analysis

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

- Defect height : the distance from the CEJ to the base of the reference notch.
- Junctional epithelium migration : the distance from the CEJ to the apical extension of the junctional epithelium
- Connective tissue adhesion : the distance from the apical extension of the junctional epithelium to the coronal extension of cementum regeneration

- Cementum regeneraton : the distance from the base of the reference notch to the coronal extension of newly formed cementum on the root surface
- Intrabony cementum regeneation : the regenerated cementum where there are fibers inserted perpendicularly or where it is lined with cementoblast-like cells
- Suprabony cementum regeneraion: the regenerated cementum above the intrabony cementum regeneration, where the new cementum showed a parallel collagen fiber arrangement. It is not easily to distinguish between the cementoid tissue and the true cementum using optical microscopy.
- Alveolar bone regeneration : the distance from the base of the notch to the coronal extension of the newly formed alveolar bone.

Statistical Analysis

The histometric recording form 4 sections in each block were used to calculate the mean score and summary statistics (mean ± SD) based on the mean scores were used to further analyze the difference between the experimental conditions using the Kruskal-wallis test.

A Mann-Whitney U test was used to make a comparison between the groups. In addition, the root resorption and ankylosis was scored as being present if it was observed in one or more of the four sections for each tooth.

Results

Histological observations

There was more apical migration in the extension of the junctional epithelium observed in the surgical control group than in the other groups (Fig. 3A). There was minimal inflammatory cell infiltration in all the groups. The residual RM was observed in the connective tissue, and there were peculiar foreign body reaction and giant cell infiltration (Fig. 4B). More bone regeneration was observed in the CNWM group than in the other groups (Fig. 3A, 4A, 5A). The borderline between the new and the old bone was indistinguishable, but the osteoblasts were densely arranged along the new bone surface (Fig. 4C, 5C, 5D). New cementum grew more prominently along the root surface in the CNWM group than in the other groups, and cementoblasts densely surrounded the new cementum (Fig. 5B, 5D). Moreover new cementum increased in thickness coronally to apically (Fig. 5C). The periodontal ligament in the CNWM group was observed to have a more regular pattern and a denser fiber arrangement than that of the surgical control group (Fig.3B, 4C, 5C). In the intrabony cementum layer, it was observed that the cementoblasts were arranged closely and the fibers were embedded perpendicularly (Fig 5C, 5D). In addition, a thin layer of cementum was observed above this cementum area. In the suprabony cementum layer, the cementoblasts were rarely observed and the fibers showed parallel

orientation (Fig. 5B). 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 surgical control was organized mainly with irregular, loose collagen fibers (Fig 3B). The fibers were embedded perpendicularly to the new bone, and there appeared to be more new cementum in the CNWM group and RM group, which exhibited a denser fiber arrangement than in the control group (Fig.3B, 4C, 5C, 5D). All the groups maintained a good periodontal ligament space, and showed no evidence of ankylosis and resorption.

Histometric Observations

The defect height averaged (Mean±SD) 4.86±0.24, 4.88±1.11, and 4.48±0.56 mm for the defects receiving the CNWM, the RM, and the surgical control group, respectively, with no significant differences between the treatments.

The junctional epithelium was approximately 1.77±0.70 (36.4 %), 2.04± 0.57 (41.8 %), 2.32±0.33 (51.8 %) mm for the CNWM, the RM, and the surgical control group, respectively, with no significant differences between the treatments.

The amount of connective tissue adhesion was 1.12±0.18 (23.0 %),

1.20±0.44 (24.6 %), 0.86±0.29 (19.2 %) mm for the CNWM, the RM, and the surgical control group, respectively, with no significant differences between the treatments.

The amount of cementum regeneration was 2.26±0.23 (46.5 %), 1.72±0.55 (35.2 %), 1.39±0.21 (31.0 %) mm for the CNWM, the RM, and the surgical control group, respectively. The CNWM group was significantly different from the surgical control ($p<0.05$).

The amount of intrabony cementum regeneration was 1.68±0.15 (34.6 %), 1.48±0.52 (30.3 %), 1.08±0.24 (24.1 %) mm for the CNWM, the RM, and the surgical control group, respectively. The CNWM was significantly different from the surgical control ($p<0.05$).

The corresponding amount of the suprabony cementum regeneration was 0.58±0.10 (11.9 %), 0.24±0.09 (4.9 %), 0.31±0.05mm (6.9 %) mm for the CNWM, the RM, and the surgical control group, respectively. The CNWM group was significantly different from the surgical control ($p<0.05$).

The amount of alveolar bone regeneration averaged 1.81±0.16 (37.2 %), 1.54±0.71 (31.5 %), and1.17±0.31 (26.1 %) mm for the CNWM, the RM group, and the surgical control group, respectively. The CNWM group was significantly different from the surgical control group ($p<0.05$).

Discussion

Regeneration of the lost periodontal structures is the ultimate goal of periodontal therapy. Previous studies have suggested that the primary source of cells that repopulate the root would determine the result. The only possibility for regeneration appears to come from the periodontal ligament acting as a source for cell proliferation.

Guided tissue regenerative (GTR) therapy using barrier membranes have been introduced to induce the selective repopulation of undifferentiated cells that originate from the periodontal ligament and has been shown to improve the periodontal regeneration in both animals and humans (Selvig *et al.*,1993; Kim *et al.*, 1998; Caffesse *et al.*, 2000; Karring *et al.*, 2000). GTR therapy proposes that specific cells in addition to the migration potential of these cells are responsible for the healing outcome following periodontal reconstructive therapy and excluding the connective tissue of the gingival flap from the root surface is of critical importance.

Various sources for the reconstruction of the alveolar bone have been used in periodontal therapy, including autograft bone, bone derivatives, and bone substitutes, such as calcium-based ceramics, bioactive glasses, synthetic polymers, and resorbable polyesters. However, autogenous bone has problems associated with limitations in the amount of grafts that can be procured, while decalcified freeze-dried bone (DFDB) contains little bone morphogenic protains. DFDB has osteoconductive

properties, and problems associated with possible contamination, transmission of disease and displacement from the graft site due to pressure (Mima *et al.*, 1983; Mellonig,1984). Therefore, a new non-toxic, biodegradable material that would be free from any side effects has attracted increasing interest. Among those considered, chitin and its extract, chitosan (poly-N-acetyl glucosaminoglycan), have attracted particular attention.

Various animal models have been used to examine periodontal tissue regeneration, e.g., 1-, 2-, and 3-wall intrabony defects and horizontal bone loss, which include through and through furcation involvement. More walls mean better osteogenesis, since an increase in the number of walls brings about an ample supply of osteogenic cells (Mellonig, 1984). Furthermore, the increasing number of walls in a defect facilitates the firmer fixation of the implantation materials and easier suturing, which in turn assists in suppressing epithelial migration. This study used 1-wall intrabony defects, which are known to be difficult cases for natural osteogenesis, to explore the efficacy of CNWM as a potential implantation material and a barrier membrane in periodontal regeneration. Wikesjö et al. (1991) reported that there was no difference in the healing process between the surgical defects and the attachment loss is caused by natural disease or ligation. The use of surgical defects was decided based on a previous report showing that an artificial method helps keep the initial conditions of the control and the experimental group almost identical, which heightens the credibility of

the experiments (Haney *et al.*, 1993).

Chitin is one of the most abundant natural biopolymers, 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 a derivative of chitin that is prepared by a hot strong alkali treatment, 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 migraion 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). Klokkevold et al.(1996) reported that chitosan not only induces osteogenesis by conjoining the growth factors to stimulate osteoblast differetiation but it also indirectly facilitates regeneration by interfering with the adhesion and proliferation of the cells that inhibit bone formation such as fibroblasts.

In addition to its biological merits, chitosan is a superior implantation material because of its versatile physical properties as well as availability in a variety of forms-such as solutions, powders, flakes, gels, sponges, fibers and films (Totinmihata and Ikata, 1997). Furthermore, it is also

easy to combine chitosan with other materials. Ito et al. (1990,1991) developed self-hardening mixtures of chitosan and hydroxyapatite as well as chitosan and β-tricalcium phosphate, and proposed their use as a bone filling paste of edentulous ridges. Biomedical grade chitosan used to prepare CNWM. The bundle of chitosan filaments were stretched and rinsed in 100°C water and a spinning oil emulsion. The manufactured chitosan fiber was subjected to a stuffer box reatment to manufacture the chitosan fiber web. 5 or 6 layers of the manufactured webs were arranged to obtain the non-woven fabrics. The thickness of the manufactured nonwoven fabrics were adjusted to 2 mm.

In this study, in the CNWM group, it was expected that the migration of the epithelium would be restrained and would be smaller than the two other groups. However, there was no significant difference in junctional epithelium migration observed among groups. In the previous reports, a high rate of migration was observed in the surgical control group, which indicates the wound instability of the 1-wall intrabony defects.

There is no significant difference in the connective tissue adhesion among the groups. In the CNWM and RM groups, the two value of each group was somewhat similar. The RM and CNWM groups had a tighter distribution of collagen fibers and a section of them were in a functional arrangment, while the surgical control group had collagen fibers that were loosely distributed, with their orientation varying from random to parallel adjacent to the roots.

The amount of alveolar bone regeneration measured approximately 37.2%, 31.5%. and 26.1% of the defect height for the CNWM, RM, and the surgical control groups, respectively (Table 1). The CNWM group showed a significantly high level of regeneration, compared to the surgical control groups. The osteoblasts were arranged along the new bone surface. A 60% increase in bone regeneration in the chitosan group was reported by Mellonig et al. (1998) who applied decalcified freeze-dried bone to 1-wall defects, and by Kim et al. (1998) who applied calcium sulfate into 3-wall defects. 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, particularly osteoprogenitor cells (Blumenthal *et al.,* 1986). The newly formed bone was frequently lined with osteoblasts and osteoids. Therefore, continued new bone formation was expected after an 8-week observation period (Sigurdsson *et al*., 1994). Since the use of 1-wall defects reduced the capacity of space making, a smaller amount of new bone was formed in this study than in previous studies. In this study, the CNWM group showed significantly more bone regeneration than the other groups. Coronal thinning of new bone was observed in the all groups. These membranes were deficient in their ability to both make and maintain the space for cellular regeneration.

CNWM is easy to manipulate, it is biodegradable, and has a porous structure that provides pores for the in growth of regenerative cells,

which results in an increase in cell differentiation and vascular infiltration. The membrane allows the coagulum adhering to 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 the three groups in this study, cementum regeneration was significant only in the CNWM group. The amount of new cementum was measured to be approximately 46.5 %, 35.2 %, and 31.0 % of the defect height for the CNWM, the RM, and the surgical control groups, respectively, with a significant difference between the CNWM group and the surgical control group ($p<0.05$) (Table 1). It is believed that the CNWM 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. This study distinguished between the 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 parallel fiber adhesion and was healed 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. In a comparison of the amount of new cementum with that of new bone in all three groups, there was more cementum growth than the bone growth.

This outcome differs from the results from other procedures for regeneration, such as membranes, bone grafts, and growth factors, in which new cementum increased only in equal amounts to new bone. (Quintero *et al.,* 1982; Mellonig, 1984; Blumental *et al*., 1986; Caffesse *et al.*, 1988,; Schallhorn *et al*., 1988; Lynch *et al*., 1989) The conspicuous increment of new cementum in the defects treated with chitosan indicates the 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. It is believed that the intrabony cementum with the perpendicularly inserted fibers is the true cementum that accompanies new attachment. More intrabony cementum was found in the CNWM group than in the other groups. Therefore, the CNWM group appears to have some effect on the maturing cementum. However, a longer-term study will be needed to make a final evaluation.

Residual RM particles were observed in the connective tissue. Peripheral giant cell infiltration and foreign body reactions were noted. However, CNWM particles were not observed. It was reported that chitosan has biocompatibility with the host in a previous animal study

(Nakajima *et al.*, 1986). In addition, chitosan is progressively reabsorbed by the host almost 2 months after the treatment (Nakajima *et al.*, 1986).

Ankylosis often occurs in the sites of fast osteogenetic development without the regeneration of the periodontal ligaments (Schroeder *et al.*, 1992). This was not observed in any of the three groups. Caffesse et al. (1987) reported that ankylosis might occur if the primary proliferation of cells from the bone takes place. Therefore, these results revealed that the speed of the periodontal ligament cells in repopulating the root of the defects determines whether or not ankylosis occurs.

Consequently, CNWM therapy effectively contributed to the formation of new bone and new cementum in the 1-wall intrabony defects. However, further studies of the effects of CNWM as well as a more effective delivery system for chitosan are needed.

Conclusions

A CNWM applied to preclinical 1-wall intrabony defects in beagle dogs was shown to have beneficial effects on periodontal tissue regeneration. The increase in new bone and new cementum suggests the potency of the CNWM in inducing periodontal tissue regeneration.

1. The amount of junctional epithelium migration in the surgical control group was 2.32 ±0.33 mm, 2.04 ±0.57 mm in the Resorbable membrane group, and 1.77 ± 0.70 mm in the chitosan nonwoven membrane group.

No significant differences were observed among the treatments.

2. The amount of connective tissue adhesion was 0.86 ± 0.29 mm, 1.20 ± 0.44 mm, and 1.12 ± 0.18 mm in the surgical control, the Resorbable membrane, and the chitosan nonwoven membrane group, respectively.

No significant differences were observed among the treatments.

3. The amount of suprabony cementum regeneration was 0.31 ± 0.05 mm, 0.24 ± 0.09 mm, and 0.58 ± 0.10 mm in the surgical control, the Resorbable membrane, and the chitosan nonwoven membrane group, respectively.

A significant difference in the suprabony cementum was observed between the surgical control group and chitosan nonwoven membrane group ($P<0.05$).

4. The amount of intrabony cementum regeneration was 1.08±0.24 mm, 1.48±0.52 mm, and 1.68±0.15 mm in the surgical control. the Resorbable membrane, and the chitosan nonwoven membrane group, respectively.

Significant differences were observed between the chitosan nonwoven membrane and the surgical control group ($P<0.05$).

5. The amount of alveolar bone regeneration was 1.17±0.31 mm, 1.54±0.71 mm, and 1.81±0.16 mm, in the surgical control, the Resorbable membrane, the chitosan nonwoven membrane group, respectively.

Significant differences were observed between the surgical control, and the chitosan nonwoven membrane group ($P<0.05$).

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Figure Legends

Fig. 1. Schematic diagram depicting the landmarks and parameters used in histomorphometric analysis.

Fig. 2. Periodontal healing illustrated as percentage of the defect height. AC=alveolar crest.

Fig. 3-A. photomicrograph of a surgical control section showing epithelial downgrowth, connective tissue adhesion and new bone formation above the notch (magnification×20)

Fig. 3-B. fiber adhesion showing a parallel orientation along the new cementum in the supracrestal region. The periodontal ligament was mainly organized with irregular, loose collagen fiber (×100)

Fig. 4-A. photomicrograph of the RM showing epithelial downgrowth, CT adhesion, new bone formation, residual membrane (×20)

Fig. 4-B. residual membrane (×100)

Fig. 4-C. new periodontal ligament mediating the attachment to the new intrabony cementum and new bone above the notch (×100)

Fig. 5-A. photomicrograph of the chitosan non-woven membrane showing new bone formation above the notch (×20)

Fig. 5-B. fiber adhesion showing a parallel orientation along the suprabony cementum in the suprecrestal region (×100)

Fig. 5-C. the periodontal ligament with densely arranged fibers (×100).

Fig. 5-D. perpendicularly oriented new periodontal ligament mediating attachment to the new intrabony cementum and new bone (×400)

Table 1. Histometric Analysis (measurements in mm)

($p<0.05$)

	Control group	BM group	CNWM group
	Mean ± SD	Mean ± SD	Mean ± SD
DH	4.48±0.56	4.88±1.11	4.86±0.24
JE	2.32±0.33	2.04±0.57	1.77±0.70
CT	0.86±0.29	1.20±0.44	1.12±0.18
NC	1.39±0.21	1.72±0.55	2.26±0.23*
IBC	1.08±0.24	1.48±0.52	1.68±0.15*
SBC	0.31±0.05	0.24±0.09	0.58±0.10*
NB	1.17±0.31	1.54±0.71	1.81±0.16*

DH : Defect height
JE : Junctional epithelium migration
CT : Connective tissue adhesion
NC : New cementum
IBC : Intrabony cementum
SBC : Suprabony cementum
NB : New bone

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

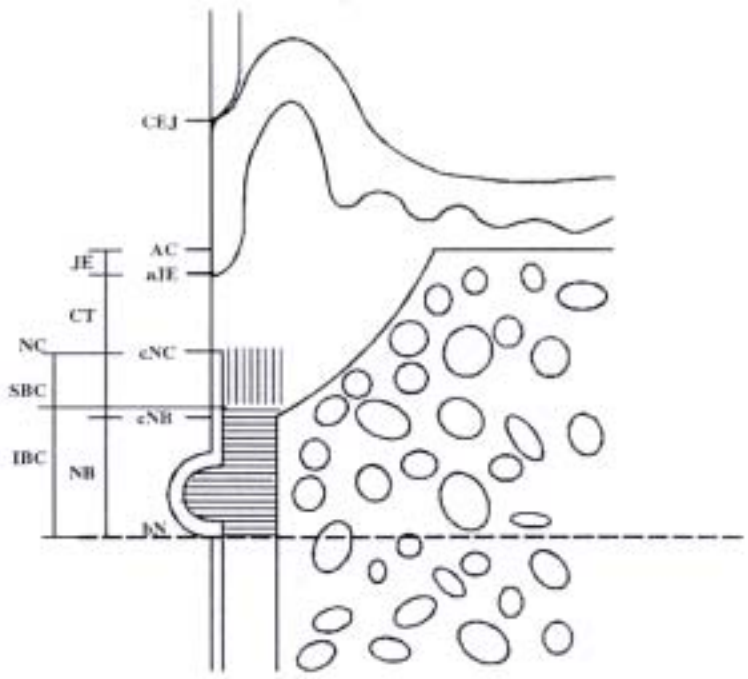

Figure 1. Schematic diagram depicting the landmarks and parameters used in histomorphometric analysis.

CEJ : Cemento-enamel junction
AC : Alveolar crest
aJE : apical extent of Junctional epithelium migration
cNC : coronal extent of New 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
SBC : Suprabony cementum regeneraton

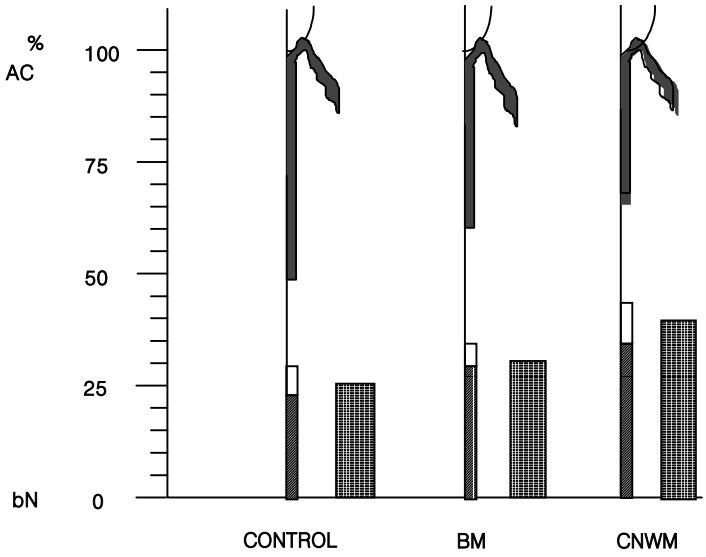

Figure 2. Periodontal healing illustrated as percentage of the defect height.

AC = Alveolar crest.

bN = base of Notch

■ : Junctional epithelium migration

□ : Suprabony cementum

▨ : Intrabony cementum

▦ : New bone

Figures

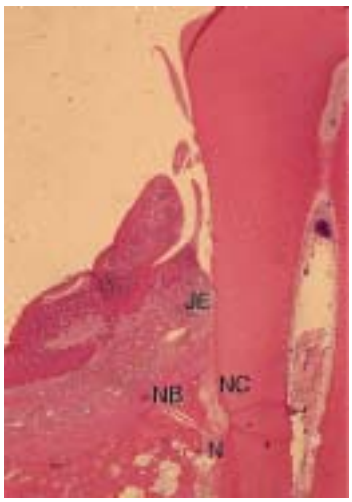

Fig. 3-A.

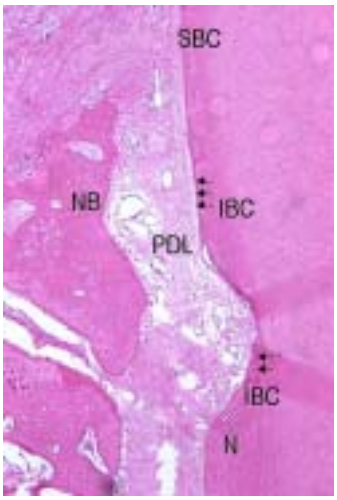

Fig.3-B.

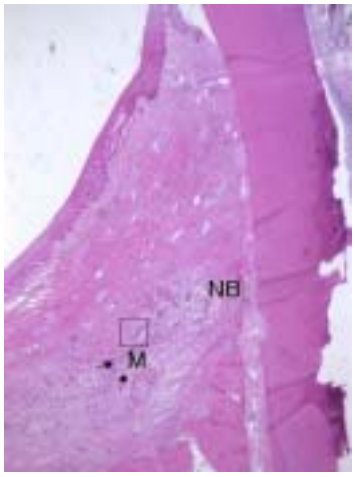

Fig. 4-A.

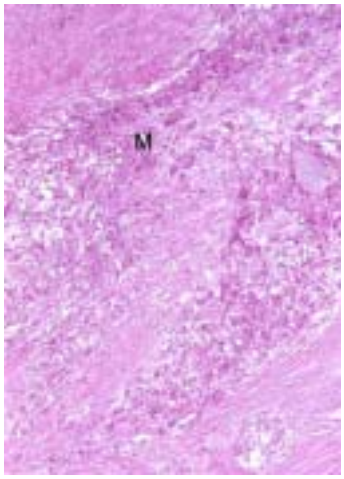

Fig. 4-B.

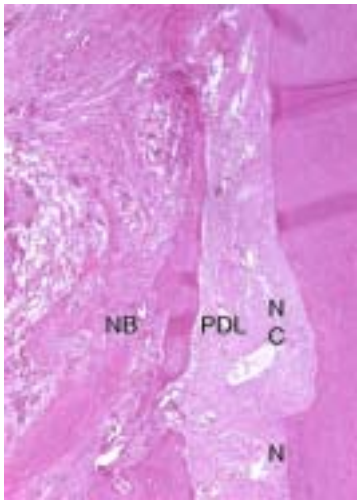

Fig. 4-C.

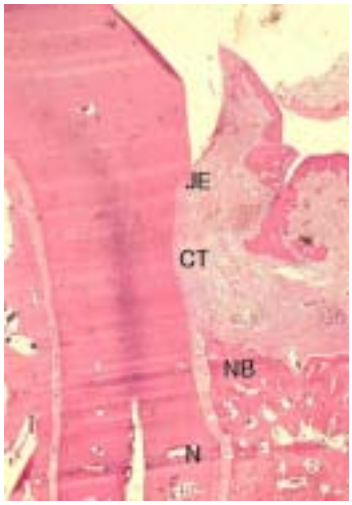

Fig. 5-A.

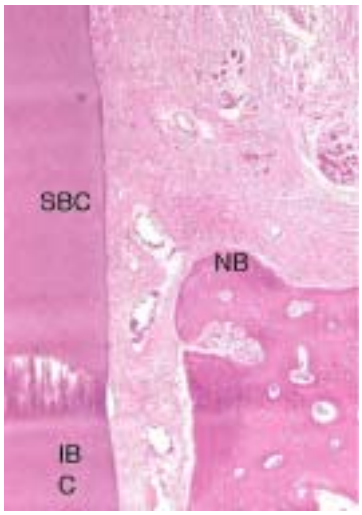

Fig. 5-B.

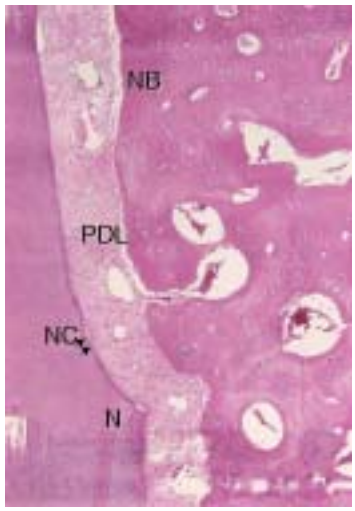

Fig. 5-C.

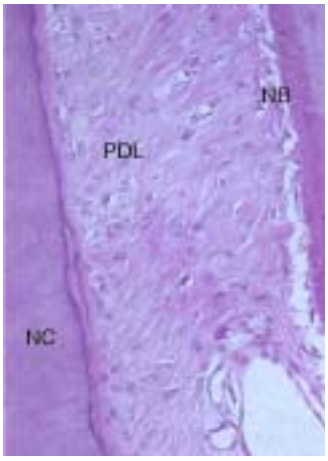

Fig. 5-D.

국문 요약

키토산 부직포가 성견의 1면 골내낭에서 치주조직 치유에 미치는 영향

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여 영 주

치주조직의 재생이란 치주질환으로 파괴된 지지조직들이 치주인대 조직의 분화에 의해 신생 골, 신생 백악질을 형성하고 새로운 치주인대 섬유가 수직으로 매입되어 구조적, 기능적으로 재형성된 치유형태를 말한다. 파괴된 치주조직의 재생을 위해 다양한 골이식술과 치주조직유도재생술이 행해지고 있으며, 키토산은 생체적합성이 우수하고 생분해되며, 항균 작용과 창상 치유 촉진에 효과적인 기능을 가지므로 다양하게 연구, 응용되고 있다.

이 연구의 목적은 키토산을 이용한 생체 분해성 차단막 (키토산 부직포)을 beagle dog 에 외과적으로 형성한 1-wall defect에 적용하여 치주조직의 재생에 미치는 영향을 평가하는 것이다.

재료 및 방법 : Beagle dog 의 하악 양측의 제 2 소구치의 근원심 면과 제4 소구치의 원심면에 4×4 mm 크기의 1-wall defect를 외과적으로 형성 후, 대조군에는 임의로 선택된 위치에 치은 박리 소파술만 시행하고, 한 실험군에는 Resorbable membrane (Biomesh®)를 이용하여 조직 유도 재생술

을 시행하고, 나머지 실험군에는 키토산 부직포를 이용하여 조직 유도 재생술을 시행한다. 실험 동물은 술 후 8주에 희생시키고, 조직학적 검사를 시행한다.

결과 : 새로운 백악질 형성은 2.26 ± 0.23 mm, 1.72 ± 0.55 mm, 1.39 ± 0.21 mm 로 각각 CNWM군, RM군, 대조군에서 측정되고, CNWM군과 대조군은 통계적 유의치를 보였다. ($p<0.05$). 신생골 하방의 백악질 형성은 CNWM군, RM군, 대조군에서 1.68 ± 0.15 mm, 1.48±0.52 mm, 1.08 ± 0.24 mm로 각각 측정되고, 역시 CNWM군과 대조군에서 통계적 유의차를 보였다 ($p<0.05$). 신생골 상방의 백악질 형성은 CNWM군, RM군, 대조군에서 각각 0.58 ± 0.1 mm, 0.24 ± 0.09 mm, 0.31±0.05 mm로 측정되고, CNWM군과 대조군에서 통계적 유의차를 보였다 ($p<0.05$). 신생골 형성은 1.81 ± 0.16 mm, 1.54 ± 0.71 mm, 1.17 ± 0.31 mm로 측정되고 역시 CNWM군과 대조군에서 통계적 유의차를 보였다. 몇몇 표본에서 흡수되지 않은 RM이 관찰되었다. 치근 유착은 관찰되지 않았다.

결론 : 이 연구에서 치조골 결손부에 적용시킨 키토산 부직포는 신생 백악질과 신생골 형성을 촉진 시켰다고 평가된다. 키토산 부직포의 사용은 키토산의 골유도 효과 (osteo-inductive effect)를 포함한 치주 조직 재생 능력과 GTR의 효과를 함께 기대할 수 있는 효과적인 적용 방식으로 사료된다.

핵심되는말 : 키토산 부직포, 1-면 골내낭, 치주재생, 신생골, 신생백악질