The Effect of Collagen and Chitosan membrane coated with PLGA on Bone regeneration in Rat Calvarial Defects

Kun-Young Song

The Graduate School

Yonsei University

Department of Dental Science

The Effect of Chitosan and Collagen membrane coated with PLGA on Bone regeneration in Rat Calvarial Defects

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This certifies that the dissertation thesis

of Kun-Young Song is approved

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Thesis Supervisor Chong-Kwan Kim

_____

Jung-Kiu Chai

_____

Seong-Ho Choi

_____

Han-Sung Jung

_____

Yong-Keun Lee

The Graduate School

Yonsei University

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감사의 글

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

연구 내내 많은 도움을 주신 치주과 교실원 여러분, 특히 강남원 선생님, 정의원 선생님, 엄유정 선생님께 고마움을 전합니다.

그리고, 늘 아낌 없는 사랑과 헌신적인 도움으로 든든하고 따뜻한 버팀목이 되어준 사랑하는 아내와 항상 나의 얼굴을 미소 짓게 만드는 사랑하는 딸 재인과 개구쟁이 아들 동주에게 진정으로 사랑과 고마움의 마음을 전합니다.

마지막으로, 믿음과 사랑으로 이해해 주시고 항상 곁에서 든든하게 기도해주신 부모님과 장모님께 감사의 마음을 담아 이 논문을 드립니다.

하나님께 감사 드립니다.

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저자 씀

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Abstract

The effect of chitosan and collagen membrane coated with PLGA on bone regeneration in Rat Calvarial Defect

Resorbable membranes are being evaluated as potential candidates for periodontal and bone regenerative therapy. The objective of this study was to evaluate the effect of chitosan and collagen membrane coated with PLGA on bone regeneration in Rat Calvarial Defect.

A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium with the use of a saline cooled trephine drill[#]. After removal of the trephined calvarial disk, each PLGA coated collatape and chitosan membrane was applied to the defects. The animals were divided into 9 groups of 5 animals each and allowed to heal for 2 (5 rats) or 8 (5 rats) weeks. Each animal received one of three experimental conditions: PLGA 0.5%, PLGA 1%, PLGA 3%. The animals were sacrificed 8 weeks after surgery and comparative histometric analysis was done.

Surgical implantation of chitosan and collagen membranes resulted in enhanced local bone formation at both 2 and 8 weeks. Within PLGA coating examined, chitosan membrane did not exhibit an appreciable dose dependent response. Defect closure and new bone area were not significantly different in chitosan and collagen membranes group at 2 weeks. However, the defect closure and new bone in

collagen area were a significantly greater than those of the chitosan group at 8 weeks ($P<0.01$). The defect closures of the collagen membrane group were significantly greater than those of the chitosan group at 8 weeks ($P<0.01$).

In conclusion, collagen membrane coated with PLGA after 8weeks has a significant potential to induce bone formation in the rat calvarial defect model. Within the selected PLGA dose range and observation interval, there appeared to be no meaningful differences in bone formation.

Key Words: collagen membrane, chitosan membrane, dose response, PLGA coating, rat calvarial defect model

The Effect of Chitosan and Collagen Membrane coated with PLGA on Bone Regeneration in Rat Calvarial Defect

Kun-Young Song, D.D.S., M.S.D.

Department of Dental Science

Graduate School, Yonsei University

(Directed by Prof. Chong-Kwan Kim, D.D.S., M.S.D., PhD.)

I. Introduction

The main object of periodontal treatment is not only to relieve symptoms but also to regenerate the destroyed tissues (Stahll,1977). Periodontal regeneration means apposition of new cementum and new bone formation on diseased root surface with functional alignment of regenerated connective tissue fibers and upper gingival tissue regeneration. There are many approaches to regenerate inflammatory periodontal tissues. Root conditioning which enhances cell adhesive capacity, bone graft used for bony defect regeneration, guided tissue regeneration using specific cell migration and bone morphorgenic protein and other various growth factors are the methods used. Though clinical application of polypeptide growth factor and application of

biomedicine are also being used in regeneration therapy, the exact treatment method has not been founded yet.

The membrane barrier used in GTR should satisfy the following factors. It should be histocompatible, biocompatible and have capacity for space maintenance (Magnusson et al., 1988). It also must avoid cell migration and should be easy to handle. Non-absorbable membrane ePTFE almost fulfills the factors mentioned above and is most widely used till today 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). However, ePTFE needs 2nd surgery for removal therefore has a disadvantage of damaging immature tissues (Simon et al., 1994;1994;1995).

On the other hand, absorbable membrane does not need to undergo 2nd surgery and membrane exposure is rare (MacGinnis et al., 1998). But controlling the time of absorption is difficult and therefore could cause localized inflammatory reaction (Dahlin et al., 1988). In addition to the above disadvantages poor membrane stability at wet state causes space loss between the tooth and the membrane producing bad clinical results (Zitzmann et al., 2001).

To use absorbable membrane in GTR, the membrane should not only satisfy the above factors but also must not cause unwanted inflammatory reaction to newly regenerating tissues during absorption period. Also a absorbable membrane should be used in places where exact initial closure is possible since complete removal is

difficult when exposed (Becker et al., 1996).

Absorbable membrane made of collagen, polylactic acid, lactide and glycolide copolymer are developed until today and many researches on healing effects has been carried out (Wang et al., 1994;1996; Kay et al., 1997; Peleg et al., 1999; Araujo et al., 1998; Polson et al., 1995; Caffese et al., 1994; Simion et al., 1996; Bouchard et al., 1997). Among these, collagen aggregates platelet which accelerates stabilization and maturation of periodontal tissues in wounded sites and has a chemotatic effect on PDL cells inducing cell migration. It also available to prepare in various shapes and has a histologically acceptable property. However, due to rapid absorption period there could be a problem in space maintaining capacity thereby not suitable to use as a barrier.

Recently, interest on chitosan has been focused due to its excellent biological properties such as biocompatibility, antibacterial effect and rapid healing capacity. Chitosan is a derivertive made by treating with strong alkali and deacetlylization and has a molecular weight of 800-1500KD. It is biodegradable and has similar structural characteristics with glycosaminoglycan (GAG), specially hyaluronic acid which is largely founded in extracelluar matrix. Though collagen limits cell migration and disrupts regeneration, hyaluronic acid is thought to accelerate progenitor cell migration involved in tissue regeneration (Adzick et al., 1992). Moreover, chitosan inhibits fat absorption and helps in weight loss. It also controls cholesterol level and has an antimicrobiotic, anticarcinogenic and hemostatic effect (Brandenberg et al.,

1984; Kind et al.,1990; Klokebold et al., 1999; Muzzarelli et al., 1988;1989; Sandford et al., 1989). Earlier studies show that chitosan not only increases migration and differentiation of progenitor cells (eg. Osteoblast)but also inhibits cells like fibroblasts which disturbs bone formation resulting indirect increase in bone regeneration (Balassa et al., 1978; Klokkevold et al., 1996; Muzzarelli et al.,1993;1994; Ueno et al., 1999). Chitosan is known to accelerate cell migration and tissue maturation leading to would healing promotion. Chitosan could be used in various forms like powder, gel, film sponge, solution and etc. and adhesion to bioactive materials such as PDGF and BMP is easy. Therefore, chitosan could be clinically widely used in addition to bone substituant and barrier and many studies are being carried out in fields of dentistry and orthopedic surgery.

Among absorbable membranes, membrane composed of composite copolymer of polylactic acid and polyglycolic acid membrane was developed few years ago and its safety was proofed by using as suture material and surgical mesh from past. This is degraded and safely absorbed in the tissue and it is reported to have no antigen-antibody reaction or any inflammation except for minor tissue reaction during absorption. In 1994, Caffesse applied polylactic/polyglycolic copolymer (PLA/PGA) membrane to beagle dog and analyzed the absorption process of the membrane histomorphologically and histometrically, and compared tissue reaction with ePTFE membrane. At 1, 3 and 6 month after surgery, minimum inflammatory reaction took place, and new cementum integrated with collagen fiber was founded on exposed root

surface when both ePTFE membrane and PLA/PGA membrane were used.

Though many materials are used to regenerate periodontal tissues there is yet no material which satisfies all the conditions. On this basis, the object of this study is to allow the absorbable membrane to absorb adequately and maintain sufficient space by adding subsequent concentration of PLGA to collagen and chitosan membrane thereby finding out the effect of damaged periodontal tissue regeneration after applicating to mouse calvarium.

II. Materials & methods

A. Materials

1. Animals

Ninety male Sprague-Dawley rats (body weight 200-300g) were used in this study. They were maintained in plastic cages in a room with a 12 h-day/night cycle and an ambient temperature of 21°C, and were allowed *ad libitum* access to water and standard laboratory pellets. Animal selection and management, surgical protocol, and preparation followed the routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

Table 1. A figure of animal

	2 weeks	8 weeks
Control	5	5
Collatape only	5	5
Collatape (PLGA 0.5%)	5	5
Collatape (PLGA 1 %)	5	5
Collatape (PLGA 3 %)	5	5
Chitosan only	5	5
Chitosan (PLGA 0.5%)	5	5
Chitosan (PLGA 1%)	5	5
Chitosan (PLGA 3%)	5	5

Sum=5×9×2=90(Rats)

2 Membranes.

1) Collagen membrane

Collagen membrane¶ dried in drying machine for 24 hours after open.

2) Chitosan

Biodegradable chitosan membrane † (1-4, 2-amino,2-deoxy- β-D-glucosamine) was dried using distilled water three times at 50℃.

3) PLGA solution

PLGA* (poly[lactide-co-glycolide], 25:75.) was dissolved to methylene chloride

4) PLGA coating was carried out by precipitation method using solution above for 10 minutes and sterilized with E.O gas after coating.

¶ Collatape® Calcitek, Carlsbad, CA

† Texan MedTech, Korea

* Sigma, Co., U.S.A

B. Experimental Procedures

1. Surgical procedures

The animals were anaesthetized by an intramuscular injection (5 mg/kg body wt.) consisting of ketamine hydrochloride[§]. Routine infiltration anesthesia[*] was used at the surgical site. An incision was made in the sagittal plane across the cranium and a full thickness flap reflected, exposing the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium with the use of a saline cooled trephine drill[#]. After removal of the trephined calvarial disk, Each PLGA coated collatape and chitosan membrane was applied to the defects. The animals were divided into 9 groups of 5 animals each and allowed to heal for 2(5 rats) or 8(5 rats) weeks. Each animal received one of three experimental conditions: PLGA 0.5%, PLGA 1%, PLGA 3%. The periosteum and skin were then closed and sutured with 4-0 coated Vicryl violet.[**]

[§] Ketalar®, Yuhan Co., Seoul, Korea

[*] 2% lidocaine, 1:100,000 epinephrine, Kwangmyung Pharm., Seoul, Korea

[#] 3i, FL, USA

[**] Polyglactin 910, braided absorbable suture, Ethicon, Johnson & Johnson Int., Edinburgh,UK

2. Histologic and histometric Analysis

The animals were sacrificed by CO_2 asphyxiation at 2 and 8 weeks postsurgery. Block sections including the experimental sites were removed. Samples were fixed in 10% neutral buffered formalin solution for 10 days. Samples were decalcified 5% formic acid for 14 days, and embedded in paraffin. Serial sections, 5*u*m thick, were prepared at intervals of 80μm, stained with hematoxylin/eosin (H-E) and examined using a light microscope. The most central sections from each block were 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. A digitizer was used to trace the defect outline versus new bone formation, and a percentage of bone fill was determined. The following histomorphometric parameters were measured from each sample (Fig. 1);

- Defect closure (mm) : the distance between the defect margin and ingrowing bone margin.
- New bone area (mm^2) : all tissues within the boundaries of newly formed bone, i.e., mineralized bone and fatty marrow and fibrovascular tissue/marrow and residual biomaterial.

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

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

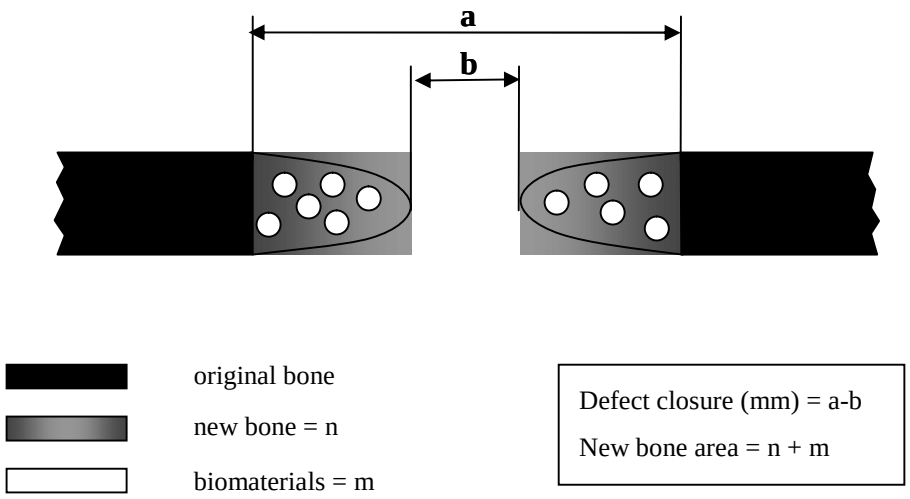

Figure 1. Schematic drawings of calvarial osteotomy defect showing histometric analysis

3. Statistical Analysis

Histomorphometric recordings and radiodensitometric recordings from the samples were used to calculate means and standard deviations (m±SD). To analyze the effect of both time and condition and to detect the interaction effect between time and condition, two-way analysis of variance was used ($P<0.05$). ANOVA and Post-hock test was used to analyze the difference between the groups at each time point ($P<0.05$). For the comparison between 2 and 8 weeks in a same group, statistical significance was determined by paired t-test ($P<0.05$).

III. Results

A. Clinical observations

Wound healing was generally uneventful and appeared similar for PLGA 0.5, 1, 3% experiments. Material exposure or other complications of the surgical sites were not observed.

B. Histologic observations

Control: At 2 and 8 weeks postsurgery, defects filled with thin loose connective tissue with a minimal amount of new bone formation originating from the defect margins were observed. The defect center had collapsed (Figure 1a).

Collagen groups: In both the collagen only and PLGA coated collagen, the defects were filled with loose or dense, fibrous connective tissue and limited new bone formation was observed at the defect margins at 2 weeks. But at 8weeks, the collagen groups were almost completely bridged with the new bone and appeared to be almost resorbed (Figure 5b). At 8 weeks the appearance of the new bone was more lamellar than that observed at 2 weeks. Irrespective of PLGA dose, all defect sites exhibited similar bone formation. At 2 weeks postsurgery, ACS fragments were observed embedded within the new bone without connective tissue intervention. No residual ACS could be detected at 8 weeks.

Chitosan groups: A large number of residual chitosan fibers were observed within

the new bone at 2 weeks and appeared to be less at 8 weeks without apparent differences between PLGA coating doses. Irrespective of dose, all defect sites exhibited bone formation, and volume was increased. Chitosan fibers showed round-stick like appearance and were surrounded by connective tissue. At 8 weeks the appearance of the new bone was more lamellar than that observed at 2 weeks. But compared to that of collagen group, new bone formation were less. At 2 weeks postsurgery, ACS fragments were observed embedded within the new bone without connective tissue intervention. No residual ACS could be detected at 8 weeks.

C. Histometric analysis

Tables 1-4 show the results of the histometric analysis. Only limited new bone formation was observed in the controls. Defect closure and new bone in collagen and chitosan was significantly different from that in controls.($P<0.05$)

Irrespective of PLGA coating dose, there was not a significant bone growth differences. New bone area and defect closure were not significant different between these two groups at 2 weeks. In defect closure, new bone area at 8 weeks, collagen group had a significantly greater value than chitosan group ($P<0.01$) and, there were no differences between the different PLGA dose level.

There were statistically significant differences between the results obtained at 2 and 8 weeks in collagen groups ($P<0.05$).

Table 2. Defect closure (group means ± SD; n=5, mm)

	2 weeks	8 weeks
Control	1.2 ± 0.2 ‡	1.3 ± 0.2 ‡
Collatape only	2.6 ± 1.0	5.7 ± 0.4 **‡
Collatape (PLGA 0.5%)	3.8 ± 1.5 *	7.3 ± 1.0 **†‡‡
Collatape (PLGA 1 %)	4.5 ± 0.9 *†	5.8 ± 1.4 **‡
Collatape (PLGA 3 %)	5.0 ± 2.1 **†‡	7.1 ± 2.0 **†‡‡
Chitosan only	3.1 ± 0.6 *	3.0 ± 0.8 *†
Chitosan (PLGA 0.5%)	4.1 ± 1.2 *	3.3 ± 0.8 *†
Chitosan (PLGA 1%)	4.0 ± 1.5 *	3.4 ± 1.2 *†
Chitosan (PLGA 3%)	3.0 ± 0.7 *	3.1 ± 1.2 *†

*: Statistically significant difference compared to control group (P<0.05) **(P<0.01)

†: Statistically significant difference compared to collagen group (P<0.05) ††(P<0.01)

‡: Statistically significant difference compared to chitosan group (P<0.05) ‡‡(P<0.01)

Table 3. New bone area (group means ± SD; n=5, mm^2)

	2 weeks	8 weeks
Control	0.3 ± 0.2 ‡	0.6 ± 0.2 ‡
Collatape only	1.3 ± 0.8 *	2.4 ± 0.5 *‡
Collatape (PLGA 0.5%)	1.4 ± 0.5 *	2.9 ± 0.4 **†‡
Collatape (PLGA 1 %)	1.6 ± 0.7 *	2.3 ± 0.9 *‡
Collatape (PLGA 3 %)	1.4 ± 0.3 *	3.9 ± 1.3 **†‡‡
Chitosan only	1.5 ± 0.4 *	1.5 ± 0.8 *
Chitosan (PLGA 0.5%)	1.5 ± 0.8 *	1.5 ± 0.5 †
Chitosan (PLGA 1%)	1.6 ± 0.6 *	1.0 ± 0.4 †
Chitosan (PLGA 3%)	1.1 ± 0.4	1.1 ± 0.3 †

*: Statistically significant difference compared to control group (P<0.01) **(P<0.01)

†: Statistically significant difference compared to collagen group (P<0.01) ††(P<0.01)

‡: Statistically significant difference compared to chitosan group (P<0.01) ‡‡(P<0.01)

IV. Discussion

The ultimate object of periodontal treatment besides symptom relief is to functionally regenerate damaged periodontal tissues. There are many methods introduced for regenerating damaged periodontal tissues. Starting with Melcher (1976) first, development of GTR technique by Nyman (1980) et al and Gottlow (1986) et al., non-absorbable membrane e-PTFE having excellent cell blocking effect and predominantly good biocompatibility is known to be the most effective material. Nevertheless, e-PTFE is not absorbed in the tissue, hence 2nd surgery for membrane removal is necessary. These results damage to newly formed immature periodontal tissues causing unfavorable effects which makes it not ideal membrane barrier.

Ideal membrane should be absorbable, block tissue migration effectively and have resistance to inflammatory reaction. Moreover, space maintaining capacity should be high.

According to Minabe (1991), if the membrane gets absorbed very early or causes inflammatory reaction while absorbing, it would harm newly formed tissues. Therefore 3-4 weeks after surgery is very important. Blumenthal (1991) also presented that absorbable membrane should maintain its function and not be absorbed till cell colonization, and inflammatory reaction should not affect the regenerated tissues. Gottlow (1993) introduced that membrane should be designed for biocompatibility, absorption period, space maintaining. Zellin (1995) et al.presented

that absorption period, space maintaining capacity and tissue reaction is important in absorbable membrane barrier. Caffesse (1997)et al. compared clinically between absorbable and non absorbable membrane in GTR. Aquirre et al (1999) showed new bone formation in bony defect using absorbable membrane.

The main disadvantage of absorbable membrane when it is exposed clinically is difficulty of membrane removal. If inflammation takes place membrane acts as a medium for inflammation and affect regenerated tissues below it. Therefore, strict control on plaque and smoking should be conducted. Though absorbable membrane does not need to undergo 2nd surgery for removal, the time taken for absorption becomes a problem. If the membrane is absorbed too early, regenerated tissues become immatured and amount of bone formation and level of attachment gain is reduced. On the other hand, if the membrane is absorbed too late, healing of the tissues is delayed and complications such as abscess could be formed.

To solve this problem many researches on biodegradable membrane was carried out and acceptable results were presented. Biodegradable membrane could be largely divided into natural and synthesized polymer. Natural polymer includes collagen, durameter, periosteum, connective tissue and etc. and synthesized polymers are mainly PLA (polylactic acid), PGA (polyglycolic acid) or compomer of PLGA. Chitosan is a natural polymer having similar bonding structure to human tissue. It is biodegraded in the tissues and has an excellent biocompatible property. Chitosan reduces fat absorption, controls cholesterol levels, increases connective tissue healing

capacity and has an antibiotic, antibacterial, anti-carcinogenic and hemostatic effect. Moreover, many studies proved the effect of chitosan on healing and bone formation process.

Collagen is well associated within the tissues and has an enhanced chemotactic effect on fibroblast to migrate upwards toward the membrane during the initial healing stage. It reduces membrane exposure and allows blot clot formation by platelet aggregation, thereby acting as a supporting body for initial angiogenesis and tissue formation. However, collagen is less likely used for barrier since its absorption rate is faster than its regeneration time.

Biodegradable membranes like PLGA are also usefully used in animal and human experiments. Studies show that it has no adverse effects during healing process and has a good periodontal regenerative capacity (Caffesse et al.,1997; Fleisher et al., 1995; Magnusson et al., 1994; Seo et al.,1999; Huh et al.,2000). But due to many inflammatory reactions during absorption period, usage as a membrane is currently reduced today.

Absorbable membranes used cannot be preferred from one and other since each of them has its own specific characteristic and advantage. In this study, each characteristic was equally considered to make an ideal membrane. The membrane used in the present study was prepared by using chitosan and collagen membrane with consequently different PLGA concentration coating to make different membrane stiffness. And this was used to find out the effect of space maintaining capacity on

bone formation.

The experimental model used in this study has been shown effective to evaluate the potential for bone formation (Schmitz et al., 1986; Caton et al., 1994; Kleinschmidt et al.,; Selvig et al., 1994; Freeman et al.,1973). We selected this model for the following reasons: 1) rats were readily available; 2) the surgical procedures on the rat calvarial bone are relatively simple to perform; 3) spontaneous healing would not occur at the control site (critical size defect); 4) the observations can be focused on the healing process of the bone, since there are no major nerves or blood vessels around the rat calvaria; 5) the calvarial defect model has many similarities to the maxillofacial region, as anatomically the calvaria consists of two cortical plates with a region of intervening cancellous bone similar to the mandible, and physiologically, the cortical bone in the calvaria resembles an atrophic mandible; 6) the preparation of the tissue specimens is easy; and 7) the parameters can be simply and accurately measured in each specimen.

In histometric analysis, the length and the area of new bone formation was compared. The measurement was done by using a computer program named Image Pro Plus program. The specimen was obtained from middle coronal section. The measurement of length of new bone formation is to compare the amount of cell migration. The more further the cells migrate, there is a high possibility for bone union. As the length growth of the cells increase, in considering the thickness, more bone formation could be predicted. Therefore, this could be said to be a good marker

for membrane's bone regenerative capacity.

The 1st and 2nd group using collatape and chitosan respectively showed a higher mean value than the control group at both 2 weeks and 8 weeks. The use of collatape only and PLGA coated did not show statistically significant difference. Changing concentration of coated membrane even did not show any difference.

Since the stiffness of collatape is low, PLGA was coated to increase the stiffness when concentration of PLGA was increased from 0.5% to 1%, and 3% there was 1% of significant difference. The results were similar for defect closure and new bone formation measurement.

In the 2nd group, the group with chitosan only and PLGA coating did not show significant statistical difference and changing concentration of coating also did not show a different result.

Comparison between defect closure measurement in 1st group and 2nd group at 2 weeks did not show a difference between collagen and chitosan but showed a significantly higher bone formation effect in collatape than chitosan. Even in measuring new bone formation area, there was no change at 2 weeks but had a higher value at 8 weeks.

At 2 weeks, collatape membrane was located below the epithelium surrounded by connective tissue without absorption while maintaining a reasonable form. There was few bone formation and no difference were seen in PLGA coated membranes. At 8 weeks, collatape absorption was certainly progressed and there was no inflammatory

cells infiltrated. The amount of new bone formation was increased but integration of surrounding tissues was not seen.

In the case of chitosan, there was an increase in volume and the external margin was well separated with the surrounding tissues. The particles composing chitosan was located as circular stick shape and each fiber was surrounded by connective tissue.

At 8 weeks, though absorption of chitosan was little progressed maintaining the its form, the entire size of the membrane seemed to be diminished. This shows that the density of chitosan components was reduced. Both chitosan and collatape did not show a difference with PLGA coated membrane and in concentration difference. PLGA coating is important in space maintaining in bone regeneration. The object of using PLGA was to increase the stiffness of membrane but unfortunately the results did not show much effect in this study. This may be due to low space maintaining capacity of the membrane and since there was no side effects such as inflammatory reaction, it could be thought that PLGA coating could be effective in bone formation when stiffer membrane is used in an another bone defect model.

Many studies presented that chitosan is effective in bone formation. But in this present study, the results prove that collatape is more effective. This was more clearly seen at 8 weeks than 2 weeks. Chitosan and collagen absorption was not seen at 2 weeks but at 8 week. Collagen was mostly absorbed forming new bone but partial absorption of chitosan seems to disturb bone regeneration. However, problem of rapid

absorption period, ability of maintaining membrane stability or any other disadvantage of collagen seem to be aided by PLGA coating. To make an ideal absorbable membrane, further studies using various coating materials with different concentration is needed and since amount of absorption is different among animals a human model with bone defect should be carried out.

V. Conclusion

The object of this study is to find out the effect of periodontal tissue regeneration on damaged tissues in rat calvarial defect by using collagen and chitosan membrane coated with 0.5, 1, 3% of PLGA respectively. The control group was designed by forming an 8mm bony defect on rat calvarian. Group 1 includes collatape only, collatape + 0.5% PLGA, collatape + 1% PLGA, collatape + 3% PLGA and Group 2 includes chitosan membrane only, chitosan membrane +0.5% PLGA, chitosan membrane+ 1% PLGA, chitosan membrane+ 3% PLGA, and each was sacrificed and observed at 2 weeks and 8 weeks. In this study, total of 90 mouse, with 5 in each experiment were used and defect closure (mm), new bone area (mm^2) were analyzed

1. In defect closure measurement, ACS and chitosan were more effective than control group at 2 weeks, but there was no difference in between chitosan and ACS. At 8 weeks, ACS and chitosan were also more effective than the control but ACS showed a higher bone formative effective.

2. In new bone area measurement, ACS and chitosan were more effective than control group at 2 weeks, but there was no difference in between chitosan and ACS. At 8 weeks, ACS and chitosan were also more effective than the control but ACS showed a higher bone formative effective.

3. PLGA coating in chitosan did not show much effectiveness on bone regeneration, while collatape showed 1, 3% of defect closure at 2 weeks and 1% of difference at 8 weeks. In new bone area, 0.5, 1% of difference was shown at 8 weeks.

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Figures

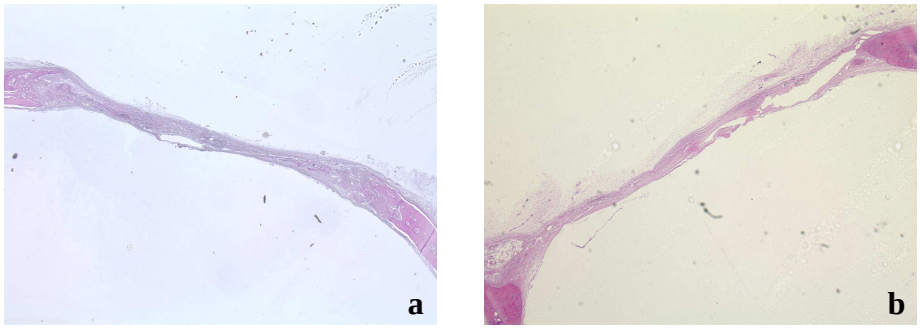

Figure 2. Representative photomicrographs of defect sites receiving control at 2 and 8 weeks postsurgery. (a, b ×20).

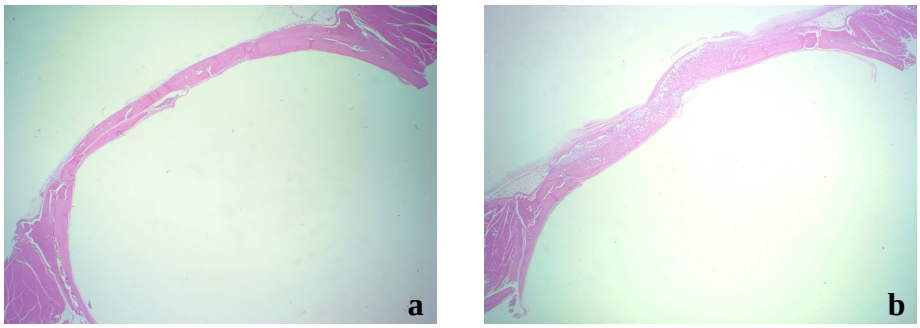

Figure 3. Representative photomicrographs of defect sites receiving collatape only at 2 and 8 weeks postsurgery. (a, b ×20).

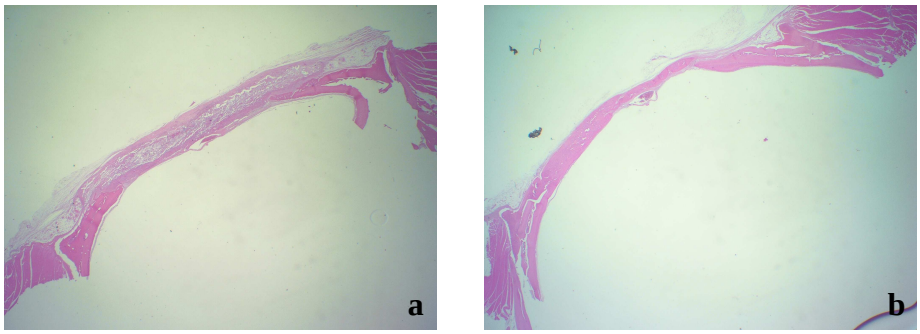

Figure 4. Representative photomicrographs of defect sites receiving collatape coated PLGA 0.5% at 2 and 8 weeks postsurgery. (a, b ×20).

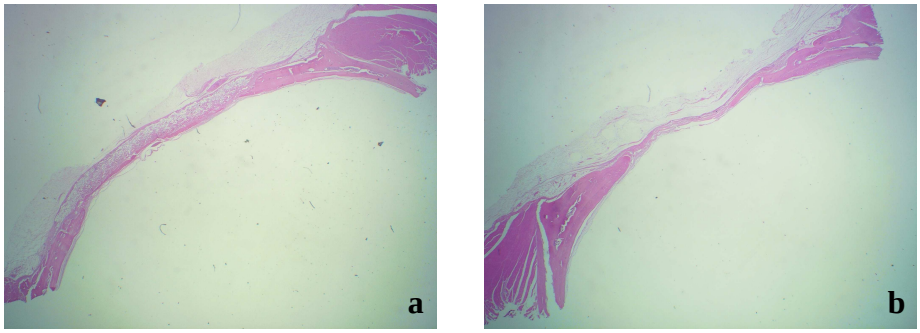

Figure 5. Representative photomicrographs of defect sites receiving collatape coated PLGA 1% at 2 and 8 weeks postsurgery. (a, b ×20).

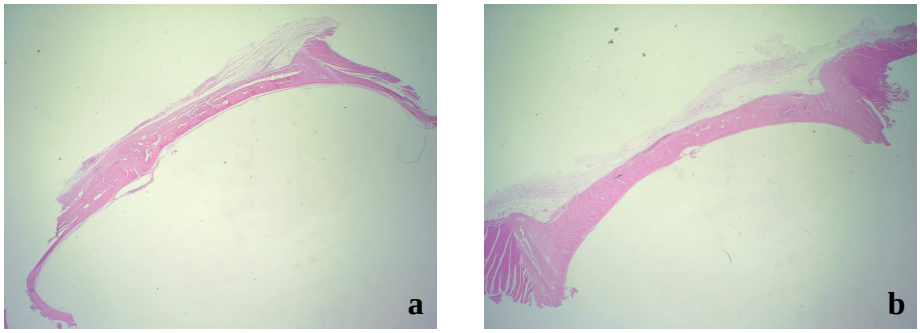

Figure 6. Representative photomicrographs of defect sites receiving collatape coated PLGA 3% at 2 and 8 weeks postsurgery. (a, b ×20).

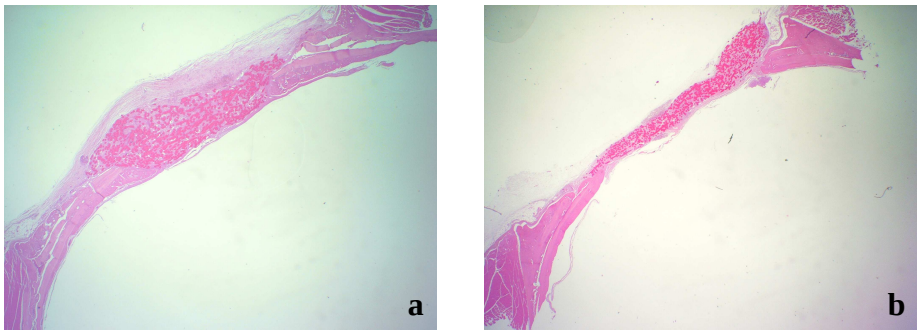

Figure 7. Representative photomicrographs of defect sites receiving chitosan only at 2 and 8 weeks postsurgery. (a, b ×20).

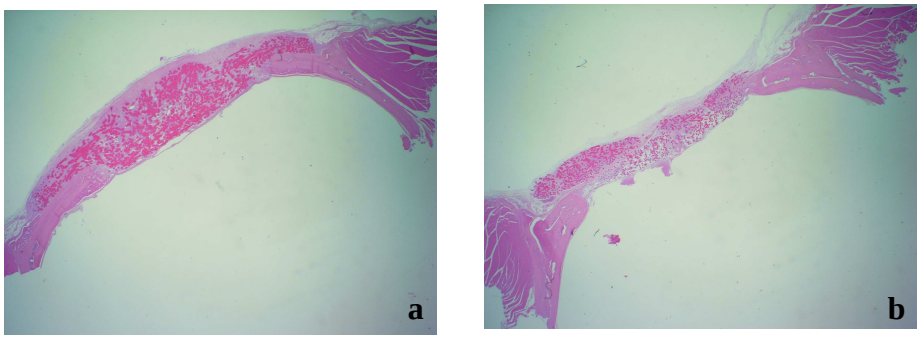

Figure 8. Representative photomicrographs of defect sites receiving chitosan coated PLGA 0.5% at 2 and 8 weeks postsurgery. (a, b ×20).

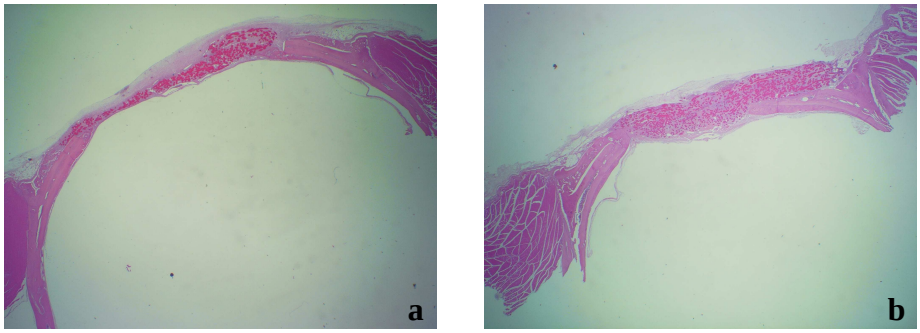

Figure 9. Representative photomicrographs of defect sites receiving chitosan coated PLGA 1% at 2 and 8 weeks postsurgery. (a, b ×20).

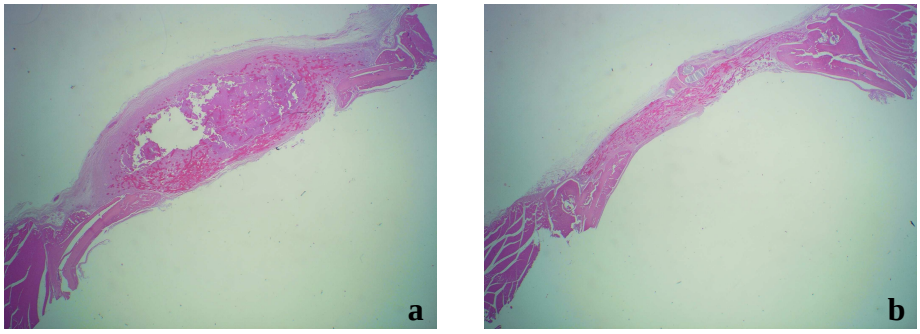

Figure 10. Representative photomicrographs of defect sites receiving chitosan coated PLGA 3% at 2 and 8 weeks postsurgery. (a, b ×20).

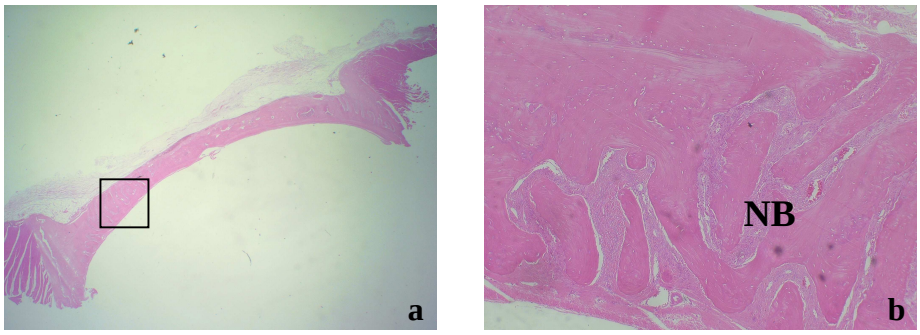

Figure 11. Representative photomicrographs of defect sites receiving collatape coated PLGA 3% at 8 weeks postsurgery.
(a: ×20, b: ×100) (NB: New bone)

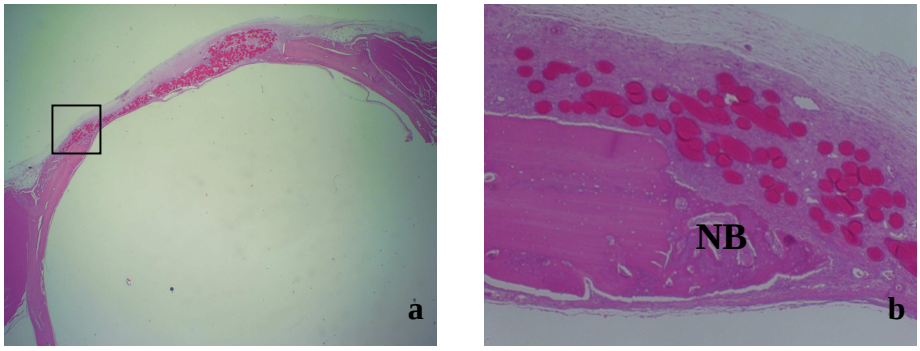

Figure 12. Representative photomicrographs of defect sites receiving chitosan coated PLGA 1% at 2 weeks postsurgery.
(a: ×20, b: ×100) (NB: New bone)

국문요약

백서 두개골 결손부에서 PLGA로 코팅된 collagen막과 키토산 부직포가 골재생에 미치는 효과

< 지도교수 **김 종 관** >

연세대학교 대학원 치의학과

송 건 영

골재생을 위해 여러가지 방법이 제안되고 그 중 비흡수성 차단막을 이용한 조직유도 재생술이 효과적으로 사용되어 왔으나 2차 수술이 필요하므로 미성숙 신생조직에 기계적 손상을 가한다는 단점이 있다. 이에 비해 흡수성 차단막은 2차 수술은 필요없으나 흡수속도 조절이 어렵고 염증반응을 막기 힘들다. 그래서 다양한 재료의 흡수성차단막이 제안되고 있으나 모든 것을 만족시키는 것은 찾기 힘들다. 이 연구에서는 백서 두개골 결손부에 콜라젠막과 키토산부직포에 각각 PLGA를 농도를 달리 코팅하여 차단막으로 사용함으로서 골재생에 미치는 효과를 비교 분석하였다. 대조군, 실험1군 (콜라젠막, PLGA 0.5%, 1%, 3% 코팅한 콜라젠막) 실험2군 (키토산 부직포, PLGA 0.5%, 1%, 3%로 코팅한 키토산 부직포) 모두 9군에 각각 5마리씩 총 90마리의 백서를 사용하여 2주, 8주에 나누어 희생하였다.

조직계측학적으로 볼 때 결손부 폐쇄와 신생골 형성량에서 대조군에 비해 각 실험군은 유의성 있는 차이를 보였고 2주에서 실험1군과 2군의 차이는 없었으나 8주에서는 콜라젠 그룹이 키토산 부직포 그룹보다 유의성 있는 효과를 보였다. 또한 PLGA 코팅한 것은 농도별 차이가 없었다.

백서두개골 결손부에서 콜라젠막, 키토산 부직포, 그리고 각각에 PLGA 코팅한 것은 모두 골재생에 효과를 보였고 콜라젠이 8주에서 키토산보다 더 좋은 결과를 나타냈으나 PLGA를 코팅한 것은 별다른 효과를 나타내지 못했고 농도 별 차이도 없다고 생각된다. 이상의 결과에서 볼 때 콜라젠과 키토산 막은 흡수가 빠르고 공간 유지능력이 떨어지므로 이를 극복하기 위해 PLGA 코팅을 한 경우 콜라젠과 키토산막의 강도를 높이고 흡수를 지연시켜 치조골 재생을 증진시키므로 임상 적용에 효과적이라고 생각된다.

핵심되는 말: 신생골 형성, 골결손 폐쇄, 콜라젠막, 키토산부직포, PLGA 코팅, 백서 두개골 결손부