The Effects of Hydroxyapatite-Chitosan Membrane According to Proportion on Bone Formation in Rat Calvarial Defects

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Abstract

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Absorbable membranes are being evaluated as potential candidates for periodontal and bone regeneration therapy. Absorbable membranes have poor membrane stability in the wet state and may cause space loss but do not need to undergo a second surgery, and membrane exposure is rare. Recently, interest in chitosan has increased due to its excellent biological properties such as biocompatibility, antibacterial effect, and rapid healing capacity. On the other hand, hydroxyapatite is used as a bone substitute in the fields of orthopedics and dentistry. The hydroxyapatite-chitosan(HA-CS) complex containing hydroxyapatite nanoparticles was developed for synergy of both biomaterials.

The objective of this study was to evaluate the effect of hydroxyapatite(HA)-chitosan(CS) membrane on bone regeneration in the rat calvarial defect.

Eight-millimeter critical-sized calvarial defects were created in 70 male Sprague-Dawley rats. The animals were divided into 7 groups of 10 animals and received either 1) chitosan(CS) 100% membrane, 2) hydroxyapatite(HA) 30%/CS 30%

membrane, 3) HA 40%/CS 60%, pressed membrane, 4) HA 40%/CS 60% membrane, 5) HA 50%/CS 50% membrane, 6) HA 50%/CS 50%, pressed membrane, or 7) a sham – surgery control. The amount of newly formed bone from the surface of the rat calvarial defects was measured using histomorphometry and following 2- or 8- week healing intervals.

Surgical implantation of the HA-CS membrane resulted in enhanced local bone formation at both 2 and 8 weeks compared to the control group. The HA-CS membrane would be significantly more effective than the chitosan membrane in early bone formation.

Further studies will be required to improve the mechanical properties to develop a more rigid scaffold for the HA-CS membrane.

In conclusion, concerning the advantages of biomaterials, the HA-CS membrane would be an effective biomaterial for regeneration of periodontal bone.

Key words: hydroxyapatite, chitosan membrane, rat calvarial defect model, bone formation

The Effects of Hydroxyapatite- Chitosan Membrane According to Proportion on Bone Formation in Rat Calvarial Defects

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I. Introduction

To achieve the regeneration of a lost periodontal attachment, various regenerative therapies have been advocated; these began with Melcher (1976), and then guided tissue regeneration (GTR) techniques were developed by Nyman *et al*. (1980) and Gottlow *et al*.(1986) A non-absorbable membrane, e-PTFE has an excellent cell-blocking effect and notable biocompatibility. However, e-PTFE requires additional surgery for removal and, therefore, has a potential disadvantage of damaging immature tissues(Simon *et al*., 1994; Simon *et al*., 1995).

On the other hand, the absorbable membrane does not require a second surgical procedure, and membrane exposure is rare. Aquirre *et al*. (1999) showed new bone

formation in bony defects using absorbable membrane. However, controlling the time of absorption is difficult and therefore could cause a localized inflammatory reaction (Dahlin *et al*., 1988). In addition to the above disadvantages, poor membrane stability in the wet state causes space loss between the tooth and the membrane, producing poor clinical results(Zitzmann *et al*., 2001). An absorbable membrane should be used in GTR in places where exact initial closure is possible, since complete removal is difficult when exposed (Becker *et al*., 1996). To solve this problem, many studies have been carried out on biodegradable membranes, and acceptable results were presented. The ideal membrane should be absorbable and should not require removal after the tissue regenerates; it also should block tissue migration effectively and resist inflammatory reaction. Lastly, it must have space-maintaining capacity.

Recently, interest in chitosan has increased due to its excellent biological properties such as biocompatibility, antibacterial effect, and rapid healing capacity. Chitin and chitosan (poly-N-acetyl glucosaminoglycan), a carbohydrate biopolymer extracted from chitin, are the second most abundant natural biopolymers next to cellulose. Chitin is a primary structural component of the exoskeleton of arthropods, (e.g. crustaceans), the cell wall of fungi, and the cuticle of insects. Chitin is a very stable polysaccharide and is a linear polymer of N-acetyl-D-glucosamine monomers joined in a 1,4β-glucosidic linkage (Aspinall, 1986). Chitosan is a derivative of chitin obtained by N-acetylating chitin (Sandford, 1989). As with polymers in general, enzymes can hydrolyze chitin and chitosan. The most effective enzyme for this process is lysozyme (Amano and Ito, 1978; Pangburn *et al*., 1982).

Although the healing effects of chitin and chitosan on mammalian wounds have been known for centuries, it was not until the 1960s that the ingredients were documented (Reynolds, 1960).

Other studies since have suggested that chitosan enhances the formation of bone tissue. For example, Malette *et al*. (1986) presented early evidence of improved radii bone regeneration in dogs. In this case, the use of chitosan resulted in regeneration of the marrow through the cortex. The considerable healing effect of a chitosan gel for the wounds resulting from extraction or apicoectomy was also confirmed by radiography and a biopsy (Muzzarelli *et al*., 1993). Paik *et al.* (2001) reported that chitosan enhanced type Ⅰ collagen synthesis in the early stage, and facilitated differentiation into osteogenic cells in the human periodontal ligament fibroblasts *in vitro*. In addition, a chitosan/collagen sponge applied to one-wall intrabony defects surgically created in beagle dogs inhibited the apical migration of the epithelium and enhanced the growth of new bone and new cementum (Park *et al*., 2003).

Another biomaterial of interest is hydroxyapatite, which is a major component of human bone. Hydroxyapatite is used as bone substitute in the fields of orthopedics and dentistry because of its good osteoconductivity, bioactivity and biocompatibility (Salata *et al*., 1998). But hydroxyapatite is brittle and easy to fracture so it is difficult to mould into a specific shape. The hydroxyapatite-chitosan (HA-CS) complex, containing hydroxyapatite nanoparticles, was therefore developed to overcome the original disadvantage of hydroxyapatite (Guo X *et al.*, 2007 ; Zhang YF *et al*., 2007).

Although many materials are used to regenerate periodontal tissues, there is as yet no material that satisfies all conditions. This paper reports on the fabrication of the HA-CS membrane in diverse proportion. The objective of this study was to evaluate bone regeneration capacity of HA-CS membrane in rat calvarial defects.

II. Materials and Methods

1. Materials

1.1 Animals

This study included 70 male Sprague-Dawley rats (body weight 250-300g) maintained in plastic cages in a room with a 12 hour day/night cycle and an ambient temperature of 21°C. The rats were allowed free access to water and standard laboratory fool pellets. Animal selection, management, surgical protocol, and preparation followed the routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

1.2 Hydroxyapatite – Chitosan Membrane

Chitosan was dissolved in a 2% acetic acid solution, and then mixed with phosphoric acid solution. After Ca $(OH)_2$ was added to this solution, HA was able to be synthesized. At that time, HA proportion was regulated, and 0:100, 30:70, 40:60, 50:50 (HA weight: CS weight) HA-CS composites were developed.

After citric acid was added to each HA-CS composite, original solution for threading was developed. The HA-CS solution was filtered and threaded in a 10% NaOH solution and then washed and dried to develop the HA-CS membrane (Figure

2). Some of the membrane, HA 30 %/ CS 70 % and HA 50%/ CS 50%, were pressed for advanced mechanical properties. The size of HA nanoparticles was 20 – 100 nm. All HA-CS membranes passed the cytotoxic test, MTT assay, from the Department and Research Institute of Dental Biomaterials and Bioengineering, Yonsei University College of Dentistry, Seoul, Korea.

Table 1. Experimental design

Group		2 weeks	8 weeks
Control		5	5
Exp. 1:	Chitosan 100%	5	5
Exp. 2:	Hydroxyapatite 30%+ Chitosan 70%	5	5
Exp. 3:	Hydroxyapatite 30%+ Chitosan 70% , pressed	5	5
Exp. 4:	Hydroxyapatite 40%+Chitosan 60%	5	5
Exp. 5:	Hydroxyapatite 50%+Chitosan 50%	5	5
Exp. 6:	Hydroxyapatite 50%+ Chitosan 50%, pressed	5	5

Sum= 5×7×2 = 70 (Rats)

2. Methods

2.1 Surgical protocol

The animals were anaesthetized by an intramuscular injection (5mg/kg body wt.) of 4:1 solution of Zoletil[¶] and Rompun[††]. An incision was made in the sagittal plane across the cranium, and a full thickness flap was made, exposing the calvarial bone. A

standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium using a saline-cooled trephine drill[**]. After the trephined calvarial disk was removed, an HA-CS membrane was applied to each defect. The animals were divided into 7 groups of 10 animals each and were allowed to heal for 2 weeks (5 rats) or 8 weeks (5 rats). Each animal received either sham-surgery control in which no material was applied to the defect, a chitosan membrane, or an HA-CS membrane as the experimental condition (Table 1). The periosteum and skin were then closed and sutured with 4-0 Monosyn[##] sutures.

2.2 Histologic procedures

The animals were sacrificed by CO_2 asphyxiation at 2 or 8 weeks post-surgery. Block sections that included the experimental sites were removed. The samples were fixed in a 10% neutral buffered formalin solution for 10 days. The samples were then decalcified in 5% formic acid for 14 days, and embedded in paraffin. Serial sections, 5㎛ thick, were prepared at 80㎛ intervals, stained with hematoxylin/eosin (H-E), and examined using an optical microscope. The most central section from each block was selected to compare histological findings between groups.

[¶] Zoletil20, Virbac Laboratoires, Carros, France

[††] Rompun®, Bayer Korea, Seoul, Korea

[**] 3i, Palm Beach Gardens, FL, USA

[##] Monosyn 4/0, Braun Aesculap AG & CO. KG, Tuttlingen

2.3 Histometric analysis

Computer-assisted histometric measurements were obtained using an automated image analysis system[‡‡] coupled with a video camera attached to an optical microscope[§§]. The sections were examined at 20x magnification. A digitizer was used to trace the defect outline versus new bone formation, and the percentage of bone fill was determined. The following histomorphometric parameters were measured for each sample.

- Defect closure (%): the distance (at each side of the defect) between the defect margin and the in-growing bone margin in mm. The percent defect closure may be obtained by subtracting this value from the total defect distance, then dividing by the total defect distance and multiplying by 100.
- Augmented area (mm^2): all tissues within the boundaries of the newly formed bone i.e. mineralized bone, fatty marrow, fibrovascular tissue/marrow, and residual biomaterial.

[‡‡] Image-Pro Plus®, Media Cybernetics, Silver Spring, M.D.

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

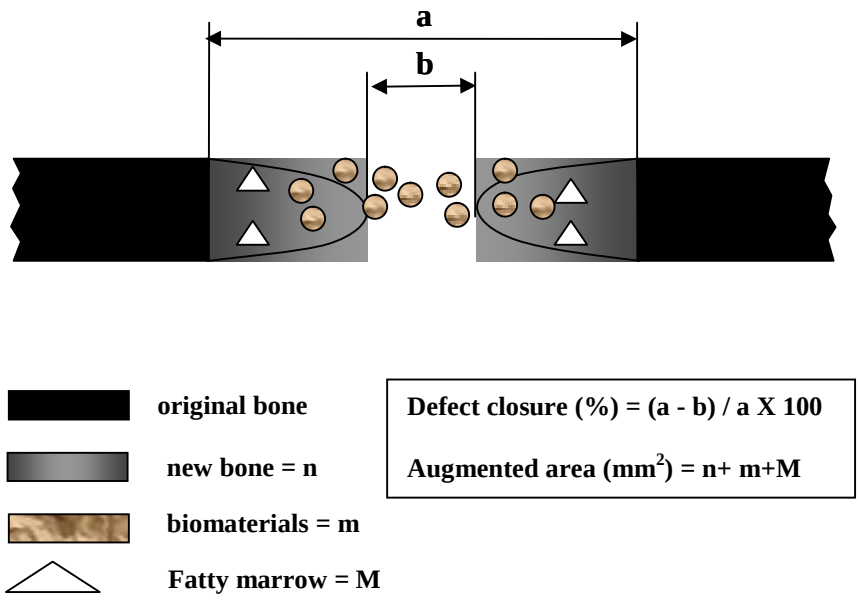

Figure 1. Schematic drawings of calvarial osteotomy defect showing histometric analysis.

2.4 Statistical Analysis

Histometric recordings from the samples were used to calculate mean and standard deviations (m±SD). One-way ANOVA was used to analyze the differences between treatment groups at each healing interval ($p<0.01$). For multiple comparisons of each healing interval, the LSD (Least Significant Difference) method was used ($p<0.05$).

For comparison between the 2- and 8-week healing interval within the same group, the statistical significance was determined by paired t-test ($p<0.01$).

III. Results

1. Clinical observations

Wound healing was generally uneventful and appeared similar for all nonpressed-membrane experimental groups (Exp. 1, 2, 4 and 5). In some pressed-membrane experimental groups (Exp. 3 and 6), slight inflammatory reaction was observed at 2 weeks, but inflammation was not detected at 8 weeks. Material exposure or other complications of the surgical sites were not observed.

2. Histologic observations

- Control

At 2 and 8 weeks post-surgery, defects filled with thin, loose connective tissue, with minimal new bone formation originating from the defect margins, were observed. The defect center had collapsed (Figure 3).

- Experimental groups

In both the chitosan-only and HA-CS membrane groups, 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. A large number of residual chitosan fibers

and hydroxyapatite particles were observed within the new bone at 2 weeks (Figure 10), but there appeared to be fewer of these at 8 weeks (Figure 11). Irrespective of the hydroxyapatite and chitosan dose levels, all defect sites exhibited bone formation, and volume was increased. At 8 weeks, the appearance of the new bone was more lamellar than that observed at 2 weeks.

Membrane remnants are composed of chitosan fibers and hydroxyapatite particles. The membrane remnants were surrounded by connective tissue. As the HA dose of membrane increased, the size of membrane remnants had decreased at 8 weeks.

3. Histometric analysis

Three animals were excluded from the histometric analysis due to technical complications in the histologic processing. Only limited new bone formation was observed in the controls.

Irrespective of the HA-CS dose, there were no significant differences in defect closure eat either 2 or 8 weeks post-surgery. There was also no statistically significant difference between the results at 2 and 8 weeks in terms of defect closure.

However, one-way ANOVA revealed that new bone and augmented areas did show significant differences in each healing interval ($P<0.01$).

Tables 2 and 3 show the results of histometric analysis. New bone deposition between 2 and 8 weeks was significantly different in the Exp. 1 and Exp. 2 groups

(Figure 4, 5). Augmented areas were significantly decreased from 2 weeks to 8 weeks post-surgery in the pressed-membrane group (Figure 6, 9).

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

	2 weeks	8weeks
Control***	0.23±0.05	0.51±0.09
Exp 1	12.74±1.98*	15.06±2.74*
Exp 2	14.84±4.87*	10.38±2.06*¶
Exp 3***	23.74 ±2.84*	10.08±2.06*¶
Exp 4	14.00±3.02*	6.74±0.92*¶†ʃ
Exp 5	17.60±5.37*	6.45±0.60*¶†ʃ
Exp 6***	25.94±2.84*	6.88±3.55*¶†ʃ

*: Statistically significant difference compared to sham-surgery control group (P<0.05)
¶: Statistically significant difference compared to Exp 1 group (P<0.05)
†: Statistically significant difference compared to Exp 2 group (P<0.05)
ʃ: Statistically significant difference compared to Exp 3 group (P<0.05)
***: Statistically significant difference between 2 and 8 weeks (P<0.01)

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

	2 weeks	8weeks
Control***	0.22±0.05	0.49±0.10
Exp 1***	1.53±0.53*	2.88±0.11*
Exp 2***	1.84±0.34*	2.83±0.26*
Exp 3	2.22±0.46*¶	2.53±0.59*
Exp 4	2.27±0.48*¶	2.68±0.14*
Exp 5	2.44±0.14*¶†	2.64±0.13*
Exp 6	2.46±0.23*¶†	2.49±0.09*¶

*: Statistically significant difference compared to sham-surgery control group (P<0.05)
¶: Statistically significant difference compared to Exp 1 group (P<0.05)
†: Statistically significant difference compared to Exp 2 group (P<0.05)
***: Statistically significant difference between 2 and 8 weeks (P<0.01)

IV. Discussion

The main object of periodontal treatment is not only to relieve symptoms but also to regenerate the destroyed tissues. Many methods have been introduced for regenerating damaged periodontal tissues. Guided tissue regeneration (GTR) therapy was introduced in the 1980s to achieve a repopulation of the periodontal ligament fibroblasts, and was shown to promote periodontal regeneration. The membrane barrier used in GTR should be histocompatible, biocompatible and have the capacity for space maintenance (Magnusson *et al*., 1988).

Many absorbable membranes made of collagen or various kinds of polymers, including chitosan, have been developed to the present day, and many studies on their healing effects have been carried out (Wang *et al*., 1994; Wang *et al*., 1996; Kay *et al*., 1997; Peleg *et al*., 1999; Polson *et al*., 1995; Caffese *et al*., 1994; Bouchard *et al*., 1997).

Chitosan is known to accelerate cell migration and tissue maturation, leading to wound healing; therefore, many studies relating to this property are being undertaken in the fields of dentistry and orthopedic surgery. Chitosan could be adhesive to bioactive materials such as PDGF and BMP, and thus could be widely used clinically in addition to bone substitutes and barriers. The nano type of hydroxyapatite can also be attached to chitosan fibers.

Nano-sized HP may have other special properties due to its small size and huge

specific surface area. Webster *et al*. (2000) have demonstrated a significant increase in protein adsorption and osteoblast adhesion on the nano-sized ceramic. Studies have shown that nano-HA/chitosan composite scaffolds may serve as a good three-dimensional substrate for cell attachment *in vitro* and migration in engineered bone and periodontal tissue (Zhang YF *et al*., 2007). Some researchers experimented with a composite of chitosan and nano-HA paste, but it did not have porosity and could not be loaded with cells. Others have reported that fibrous scaffolds had a much greater surface-to-volume ratio than scaffolds with solid pore walls, which might have further increased protein adsorption capacity (Kong L *et al.*, 2005; Guobao W. and Ma PX, 2004).

In this study, we attempted to show the clinical efficacy of a newly-formed fibrous hydroxyapatite-chitosan (HA-CS) membrane in a rat model.

The experimental model used in this study has been shown to be effective for evaluating the potential for bone formation (Schmitz *et al*., 1986; Caton *et al*., 1994; Selvig *et al*., 1994). The rat calvarial defect model is convenient for examining bone regeneration because of its effective accessibility and lack of fixation requirements.

In our histometric analysis, the length and the area of the new bone formation were compared. Measurements were taken by using computer software, called Image Pro Plus. Specimens were obtained from the middle coronal section. The length of new bone formation was measured to compare the amount of cell migration. The more the cells migrate, the higher the possibility of bone union. As the cells' length growth increases, in considering the thickness, more bone formation could be predicted.

Therefore, this could be regared to be a good marker for the membrane's bone regenerative capacity. Many rat studies have shown a significant increase in the area and density of newly formed bone when water-soluble oligo-chitosan was applied to a calvarial defect (Jung *et al*., 2000); and chitosan reconstituted with absorbable collagen sponge has significant potential to induce the regeneration of bone in rat calvarial critical-sized defects (Pang *et al*., 2005). A previous study showed the augmented area, including new bone, of a group treated with chitosan/absorbable collagen sponge at 2 weeks post-surgery was 6.19±2.03mm^2, and at 8 weeks post-surgery was 4.84±0.88mm^2. All experimental groups in the present study showed enhanced augmented area at both 2 and 8 weeks post-surgery.

Many studies suggested that BMP-2 is effective in bone formation (Hong SJ *et al*., 2006) and that the best carrier used for BMP-2 is collagen. In one of the studies (Song *et al*., 2005), the new bone area measured 2.4±0.5mm^2 at 8 weeks. This was almost identical to the present study.

New bone formation for the 50% HA dose groups (Exp. 5 and Exp. 6) at 2 weeks post-surgery was significantly greater than for the HA 0% group (Exp. 1) or HA 30% group (Exp. 2). There were significant differences between 2 and 8 weeks in new bone formation in HA 0% group (Exp. 1) and HA 30% group (Exp. 2). But Exp. 5 and Exp. 6, which received the high HA dose, were not statistically significant. As the HA dose increased, there was more new bone formation in the early healing period, 2 weeks after surgery, while there was no significant difference as time went on. This suggests that HA nanoparticles may resrorb quickly and induce new bone in the early

stage of healing.

Ideally, the bone substitute should conduct or induce bone formation at the same time as it is completely resorbed and substituted by bone tissue. Evidence from previous studies suggests that HA resorption can be mediated by cells (degradation by macrophages and osteoclasts) or by disintegration through the action of extracellular fluids (chemical dissolution) (Oonishi H *et al*., 1999; Manjubala I *et al.,* 2002). Our histologic study also showed the presence of multinuclear giant cells in close contact with the HA and chitosan surface and bone formation adjacent to the particles. Liljensten *et al.* (2003) stated that even for HAs considered absorbable, the resorption process is slow and its finalization is not well determined.

Levels of wound healing and bone formation were similar in the non-pressed membrane experimental groups. But in some pressed-membrane experimental groups, inflammation was observed at 2 weeks after surgery but had subsided at 8 weeks. Andrade *et al.* (2002) studied dense and porous HA cylinders and observed the fibrous tissue development surrounding dense implants and the direct contact of the new bone formed in the porous implants. Takeshita *et al.* (1997) used dense HA granules (300 to 600 μm) in bone defects created around osseointegrable implants and reported fibrous encapsulation of the granules. They concluded that dense HA granules negatively interfered with bone formation. Many other studies have reported improved bone-HA integration when the particles presented micro- and macropores. Our study also suggests that pressed-membrane with high proportion of HA particles in a unit area could cause inflammation in early healing period.

The augmented area of the pressed-membrane groups (Exp. 3 and Exp. 6) was significantly decreased after 8 weeks. As the HA dose increased, the augmented areas decreased at 8 weeks because the HA nanoparticles were absorbed earlier than the chitosan fiber.

From a histological view, it seems that the membrane lacked any major role as a scaffold. There was no statistical significance in defect closure that we could evaluate from length-growth of cells. Therefore, it appears that the HA-CS membrane does not stimulate cell migration to the center of defect. In summary, HA-CS membrane could collapse early in the healing period and seems to interfere in the formation of new bone in the central zone of surgical defects.

The pressed-membrane groups were developed primarily to overcome the weak mechanical properties of the conventional membranes and to be easy to handle. After the pressed-membrane absorbed water, however, there was no difference in handling from non-pressed, conventional membranes. Furthermore, pressed-membrane showed swollen shape at 2 weeks after surgery. To play a leading role in a scaffold, membranes should have improved mechanical properties in terms of water absorption.

V. Conclusion

The present study evaluated the effect of the hydroxyapatite-chitosan (HA-CS) membrane in rat calvarial defects. The animals were divided into 7 groups of 10 animals, and received either chitosan (CS) 100% membrane, various doses of the HA-CS membrane, or a sham – surgery control. The amount of newly formed bone on the surface of the rat calvarial defects was measured by histomorphometry, following healing intervals of 2 or 8 weeks.

1. Surgical implantation of the HA-CS membrane resulted in enhanced local bone formation at both 2 and 8 weeks compared to the control group.

2. The HA-CS membrane with high dose level of HA would be significantly effective than that of low dose level of HA in early bone formation.

3. Further studies will be required to improve the mechanical properties for development of a more rigid, pressed scaffold for the HA-CS membrane.

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Legends

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

Figure 2. Photograph of hydroxyapatite-chitosan membrane

Figure 3. Representative photomicrographs of control group at 2(a) and 8 (b) weeks postsurgery. At both 2 and 8 weeks, the augmented area was covered with dense connective tissue. Arrows indicate the margin of defect. Minimal new bone formation was observed. (HE stain: an original magnification X20) (HE stain: an original magnification X20)

Figure 4. Representative photomicrographs of chitosan membrane group. (a) At 2 weeks, the defects were filled with loose or dense, fibrous connective tissues. (HE stain: an original magnification X20) (b) At 8 weeks, chitosan membrane was slightly resorbed. There was an increase in new bone volume. (HE stain: an original magnification X20)

Figure 5. Representative photomicrographs of hydroxyapatite 30%/ chitosan 70% membrane group. (a) At 2 weeks, there was almost no resorption of membrane and there was slight new bone formation.(HE stain: an original magnification X20) (b) At

8 weeks, chitosan and hydroxyapatite particles were a little resorbed. (HE stain: an original magnification X20)

Figure 6. Representative photomicrographs of hydroxyapatite 30%/ chitosan 70%, pressed membrane group. (a) At 2 weeks, inflammatory cells infiltrate the defect sites. (HE stain: an original magnification X20) (b) At 8 weeks, inflammation was subsided and new bone formation was moderate. (HE stain: an original magnification X20)

Figure 7. Representative photomicrographs of hydroxyapatite 40%/ chitosan 60% membrane group. (a) At 2 weeks, limited new bone formation was observed at defect margin. (HE stain: an original magnification X20) (b) At 8 weeks, HA-CS membrane resorbed obviously. (HE stain: an original magnification X20)

Figure 8. Representative photomicrographs of hydroxyapatite 50%/ chitosan 50% membrane group (a) At 2 weeks, there was almost no resorption of membrane and there was slight new bone formation.(HE stain: an original magnification X20) (b) At 8 weeks, HA-CS membrane resorbed obviously.(HE stain: an original magnification X20)

Figure 9. Representative photomicrographs of hydroxyapatite 50%/ chitosan 50%, pressed-membrane group (a) At 2 weeks, photograph shows that center of membrane has collapsed. (HE stain: an original magnification X20) (b) At 8 weeks, the defect was filled with new bone-like cartilage. (HE stain: an original magnification X20)

Figure 10. Representative photomicrographs of hydroxyapatite 40%/ chitosan 60%, membrane group at 2 weeks postsurgery. The area of new bone is wider than low-dose HA level groups. (HE stain: an original magnification X100)

Figure 11. Representative photomicrographs of hydroxyapatite 50%/ chitosan 50%, pressed-membrane group at 8 weeks postsurgery. The appearance of new bone was more lamellar than at 2 weeks. Osteoblast-like cells and very few giant multinucleated cells could be detected at the periphery of the margin. (HE stain: an original magnification X100)

Figures

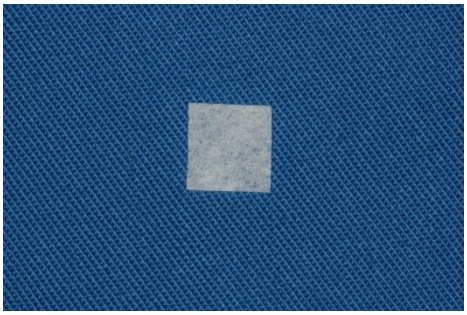
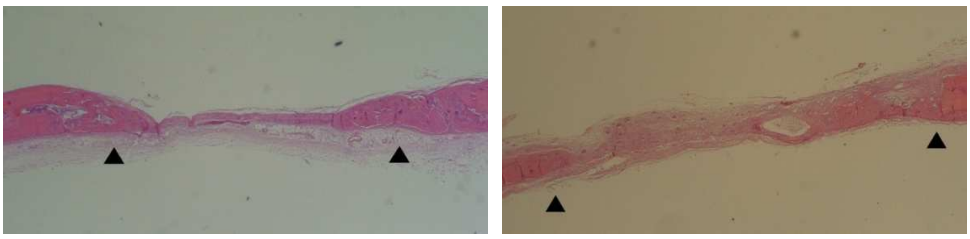
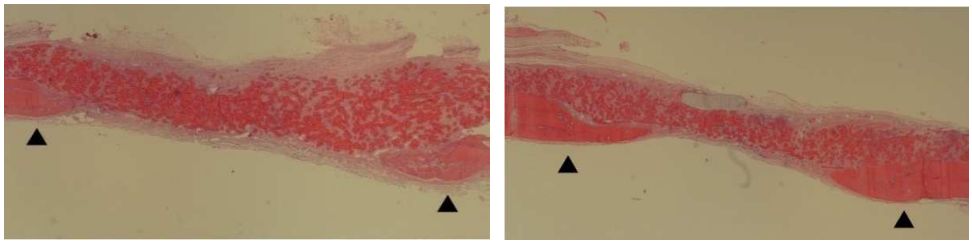


Figure 2. Hydroxyapatite-chitosan membrane.



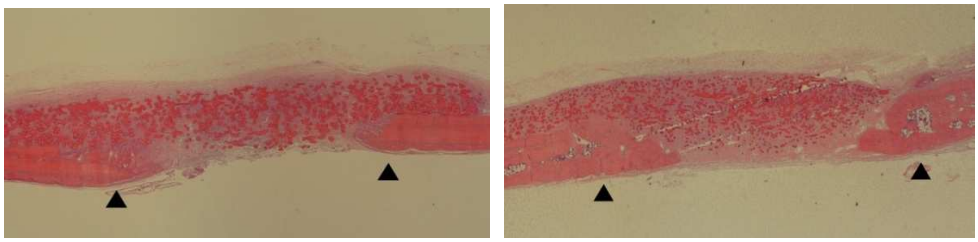
(a) **(b)**

Figure 3. Control at 2 and 8 weeks postsurgery (X20).



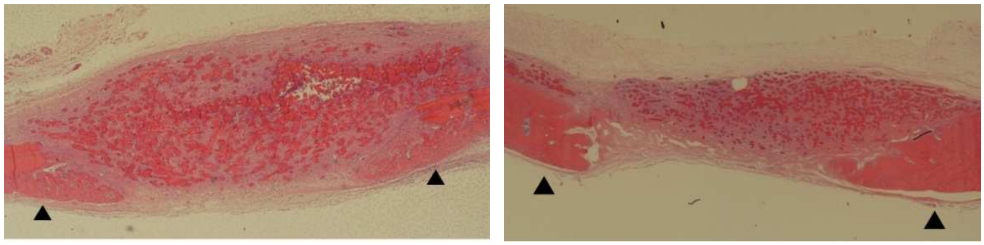
(a) **(b)**

Figure 4. Exp-1: chitosan membrane group at 2 and 8 weeks postsurgery (X20).



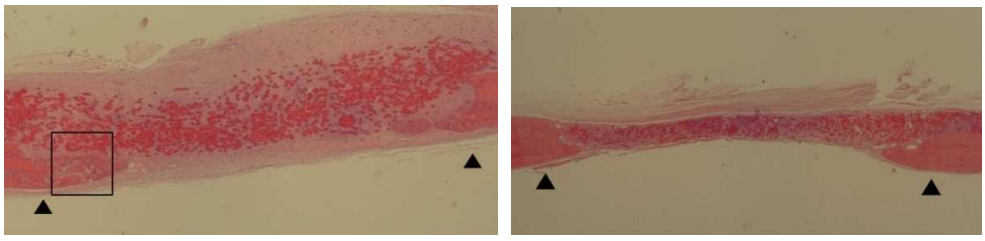
(a) **(b)**

Figure 5. Exp-2: Hydroxyapatite 30%/ chitosan 70% membrane group at 2 and 8 weeks postsurgery (X20).



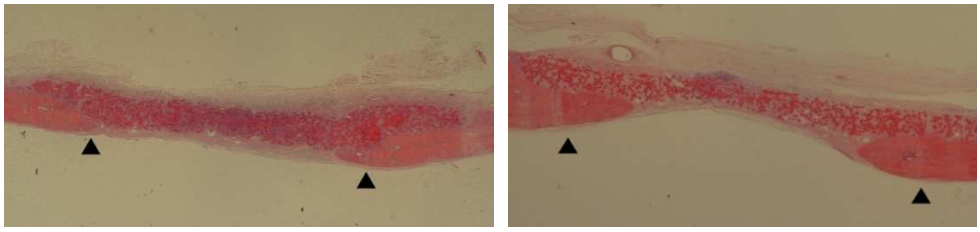
(a) **(b)**

Figure 6. Exp-3: Hydroxyapatite 30%/ chitosan 70%, pressed membrane group at 2 and 8 weeks postsurgery (X20).



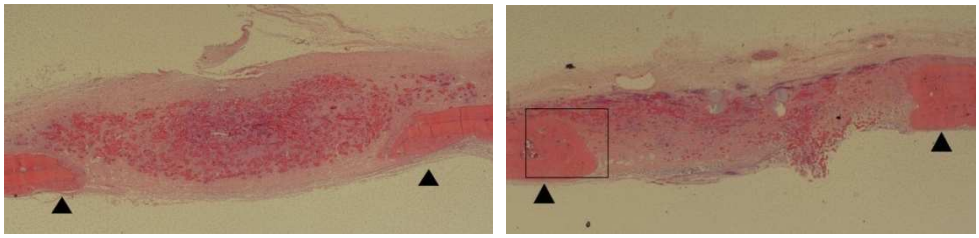
(a) **(b)**

Figure 7. Exp-4: Hydroxyapatite 40%/ chitosan 60% membrane group at 2 and 8 weeks postsurgery (X20).



(a) **(b)**

Figure 8. Exp-5: Hydroxyapatite 50%/ chitosan 50% membrane group at 2 and 8 weeks postsurgery (X20).



(a) **(b)**

Figure 9. Exp-6: Hydroxyapatite 50%/ chitosan 50%, pressed membrane group at 2 and 8 weeks postsurgery (X20).

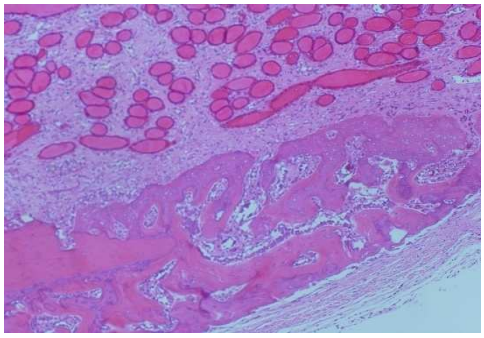


Figure 10. Exp-4, 2 weeks (X100).

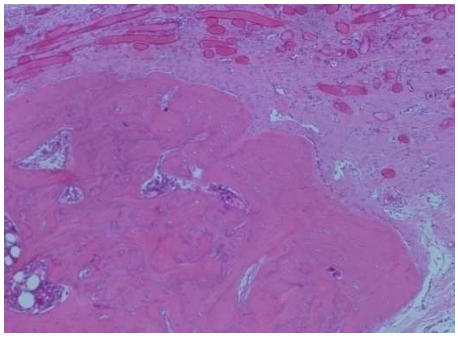


Figure 11. Exp-6, 8 weeks (X100).

국문요약

비율이 다른 Hydroxyapatite-chitosan 차단막이 백서 두개골 결손부 치유에 미치는 영향

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신 정 아

차단막을 이용한 조직유도재생술이 골재생을 위한 방법으로 제시되어 왔다. 흡수성 차단막은 흡수속도 조절이 어렵고 염증반응의 우려가 있으나, 비흡수성 차단막에 비해 2 차수술을 하지 않아도 된다는 장점이 있다. 한편, 키토산(chitosan, CS)은 생분해가 가능하고 독성이 없는 중합체로서 생체적합성이 우수한 것으로 알려져 있으며 치주 병소에 적용하는 연구가 지속되고 있다. Hydroxyapatite(HA)는 인체의 뼈를 구성하는 성분 중 하나로, 치과와 정형외과 분야에서 활용되고 있는 골대체제이다. 딱딱하고 부러지기 쉬운 HA 의 단점을 보강하기 위해 나노입자 형태로 변형시킨 후,

키토산과의 시너지 효과를 얻고자 HA-CS 복합섬유를 개발하였다. 본 연구에서는 백서 두개골에 다양한 농도의 HA-CS 차단막을 이식하여, HA-CS 의 비율에 따른 골형성량을 비교하고자 하였다. 70 마리의 백서를, 1) 키토산 (CS) 100% 차단막, 2) HA 30%/CS 70% 차단막, 3) HA 40%/CS 60% 압축 차단막, 4) HA 40%/CS 60% 차단막, 5) HA 50%/CS 50% 차단막, 6) HA 50%/CS 50% 압축 차단막, 7) 아무것도 이식하지 않은 대조군 그룹으로 나누어. 각각의 차단막을 백서 두개골의 8 ㎜ 원형 결손부에 이식하였다. 이를 술후 2 주, 8 주에 희생하여 치유 결과를 조직학 및 조직계측학적으로 비교 관찰하였다. 그 결과 HA-CS 차단막은 rat calvarial defect 에서 대조군에 비해 많은 골형성을 나타내었다. 또한 HA 함유량이 가장 많은 실험 5 군과 6 군에서는, HA 함량이 적은 실험군에 비해 초기 2 주차 골생성량이 유의하게 더 많았다.

결론적으로, HA-CS 차단막은 생체재료의 장점을 가지고 있으며, 치주조직의 재생치료에 효과적으로 사용될 수 있을 것으로 보인다. 그러나, HA-CS 차단막이 확실한 scaffold 역할을 할 수 있기 위해서는, 향후 기계적 물성의 개선을 위한 연구가 더 필요하다.

핵심되는 말: 수산화인회석(hydroxyapatite), 키토산부직포, 백서 두개골 결손부, 신생골 형성