Tissue integration patterns of non-crosslinked and crosslinked collagen membranes:

an experimental in vivo study

JIN XIANG

Department of Dentistry

The Graduate School, Yonsei University

Tissue integration patterns of non-crosslinked and crosslinked collagen membranes:

an experimental in vivo study

Directed by Professor Seong-Ho Choi

The Doctoral Dissertation

submitted to the Department of Dentistry

and the Graduate School of Yonsei University

in partial fulfillment of the requirements for the degree of

Ph.D. in Dental Science

JIN XIANG

December 2023

This certifies that the Doctoral Dissertation of JIN XIANG is approved.

Thesis Supervisor: Seong-Ho Choi

Ui-Won Jung

Jung-Seok Lee

Jae-Kook Cha

Jin-Young Park

The Graduate School

Yonsei University

December 2023

감사의 글

박사 과정과 학위논문을 마무리하며 이렇게 감사의 글을 쓰니 감회가 새롭습니다.배움에 대한 열의가 가득했던 연구실 선생님들과 조금이라도 더 많이 가르쳐 주시고 도와 주시려고 했던 교수님들 덕분에 지금까지 올 수 있었습니다.

먼저 부족함 많던 저에게 대학원을 시작할 수 있도록 기회를 주신 최성호 교수님, 교수님 덕분에 세상을 보는 시야를 넓힐 수 있었으며 더 많은 기회를 얻을 수 있게 되어 다시 한 번 진심으로 감사의 인사를 드리고 싶습니다. 그리고 박사 학위논문을 무사히 마 칠수 있도록 항상 성심성의껏 지도 해주시고, 인생에 있어서도 많은 조언을 해 주신 차재국 교수님 게도 진심으로 감사드립니다.교수님의 지도를 통해 연구가 무엇인지, 제계 부족한 점이 무엇이고 어떤 방향으로 나아가야 할 지에 대해 알 수 있었 습니다. 또한 논문을 완성할 수 있도록 이끌어 주신 박진영 교수님 깨도 진심으로 감사드립니다. 바쁘신 와중에도 심사를 해 주시며 아낌없는 조언과 지도를 해 주신 정의원 교수님, 이중석 교수님 께 도 깊이 감사를 드립니다.

마지막으로 언제나 흔들리지 않고 앞만 보고 달릴 수 있게 든든한 지원과 끊임없는 사랑으로 뒤를 지켜준 우리 엄마, 건강한 모습으로 오랫동안 제 곁에서 지켜봐 주시길 부끄럽지 않은 딸로서 살아갈 것을 약속 드립니다. 사랑하고 존경하는 엄마께 이 논문을 바칩니다.

2023년 12월

JIN XIANG 올림

TABLE OF CONTENTS

List of figures

List of tables

Abstract

Tissue integration patterns of non-crosslinked and crosslinked collagen membranes: an experimental in vivo study

JIN XIANG

Department of Dentistry
The Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

Purpose: Non-crosslinked and crosslinked collagen membranes are known to exhibit distinct degradation characteristics, resulting in contrasting orientations of the adjacent tissues and different biological processes. The aim of this study was to conduct a histomorphometric assessment of non-crosslinked and crosslinked collagen membranes regarding neovascularization, tissue integration, tissue encapsulation, and biodegradation.

Materials and Methods: Guided bone regeneration was performed using either a non-crosslinked (BG) or a crosslinked collagen membrane (CM) in 15 beagle dogs, which were euthanized at 4, 8, and 16 weeks (n=5 each) for histomorphometric analysis. The samples

were assessed regarding neovascularization, tissue integration, encapsulation, the remaining membrane area, and pseudoperiosteum formation. The BG and CM groups were compared at different time periods using nonparametric statistical methods.

Results: The remaining membrane area of CM was significantly greater than that of BG at 16 weeks; however, there were no significant differences at 4 and 8 weeks. Conversely, the neovascularization score for CM was significantly less than that for BG at 16 weeks. BG exhibited significantly greater tissue integration and encapsulation scores than CM at all time periods, apart from encapsulation at 16 weeks. Pseudoperiosteum formation was observed in the BG group at 16 weeks.

Conclusions: Although BG membranes were more rapidly biodegraded than CM membranes, they were gradually replaced by connective tissue with complete integration and maturation of the surrounding tissues to form dense periosteum-like connective tissue. Further studies need to be performed to validate the barrier effect of the pseudoperiosteum.

Keywords: Bone regeneration; Bone substitutes; Collagen; Cross-linking reagents

Tissue integration patterns of non-crosslinked and crosslinked collagen membranes: an experimental in vivo study

JIN XIANG

Department of Dentistry
The Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

I. INTRODUCTION

The principle of guided bone regeneration (GBR) can be primarily explained by the role of the barrier membrane. The barrier membrane covers a bone defect, thereby creating a secluded space for bone-forming cells to proliferate and regenerate the alveolar bone, and at the same time, it occludes the overlying soft tissues and prevents them from infiltrating the defect space. Resorbable collagen membranes are now the most frequently used barrier membranes for GBR [1](Sanz-Sanchez et al. 2015) due to the absence of a need for membrane removal, the ease of surgical manipulation and spontaneous healing after the incidence of membrane exposure. Nonetheless, the use of resorbable membranes is limited by their low capacity for space maintenance and rapid resorption rate [2](Bunyaratavej et al. 2001). Therefore, attempts have been made to slow the resorption

rate and prolong the duration of action.

Crosslinking of collagen fibers has been advocated to improve resistance to enzymatic degradation. Various physical and chemical crosslinking agents have been tested, such as ribose, glutaraldehyde, and ultraviolet rays; however, many of them have exhibited cytotoxicity and compromised biocompatibility [3](Gough et al. 2002). Recent advances have produced membranes with improved biocompatibility and endurance to degradation by utilizing bioinert crosslinking agents such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) [4, 5](Ahn et al. 2020; Park et al. 2015). Meanwhile, there is ample scientific evidence for the use of non-crosslinked collagen membranes, which are well known to show good biocompatibility and the ability to closely integrate with the surrounding tissues [6, 7](Omar et al. 2019; Park et al. 2022).

From an evaluation of the clinical data, it is difficult to determine whether crosslinking of the collagen membrane enhances the outcomes of GBR. A recent meta-analysis showed that crosslinked collagen membranes were more effective than non-crosslinked membranes when used for vertical ridge augmentation [8](Urban et al. 2019), whereas another meta-analysis reported no significant difference between the 2 types of collagen membranes concerning the gain in bone volume [9](Jimenez Garcia et al. 2017). Nevertheless, heterogeneity among studies means that this comparison is susceptible to bias. In addition, it is difficult to study membrane degradation clinically due to ethical issues. Therefore, a well-designed animal study is indicated to observe the healing pattern and tissue response surrounding the barrier membranes.

Most preclinical studies on resorbable membrane degradation and the tissue response have been performed using rat subcutaneous models [10-12](Naenni et al. 2020; Rothamel et al. 2005; Schwarz et al. 2006). Those studies showed that the rate of biodegradation was the fastest for non-crosslinked collagen membranes, although the amount of tissue integration and vascularization was the highest compared to other crosslinked collagen and synthetic membranes. Nevertheless, subcutaneous models lack

clinical representativeness, since in GBR application, the membrane is in contact with the alveolar bone, oral mucosa, and the bone substitute. In the literature, there is a surprising lack of well-designed animal studies comparing the degradation and healing pattern around non-crosslinked and crosslinked collagen membranes.

Therefore, the aim of this study was to conduct histomorphometric assessments of the non-crosslinked and crosslinked collagen membranes regarding neovascularization, tissue integration, tissue encapsulation, and biodegradation.

II. MATERIALS AND METHODS

1. Ethical statement and experimental animals

Fifteen beagle dogs were used for this study, aged 15–24 months and weighing 15 kg. The animals were raised under standard laboratory conditions (room temperature of 15°C–20°C and humidity of >30%) and fed a standardized soft diet throughout the study. The experimental approaches and protocols were authorized by the Institutional Animal Care and Use Committee, Yonsei Medical Center in Seoul, South Korea (approval no. 2016–0053), and was carried out according to the modified Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for research in preclinical settings.

2. Study materials

Collagen membranes

Two types of collagen membranes were compared in this study. Firstly, a non-crosslinked collagen membrane (Bio-Gide; Geistlich Pharma AG, Wolhusen, Switzerland) (BG) was composed of type I and III collagen derived from porcine peritoneum. This membrane has a bilayer structure consisting of a dense outer layer and a spongy inner layer. The second collagen membrane was a crosslinked collagen membrane composed of type 1 collagen, crosslinked using EDC (Collagen Membrane-P; Genoss, Suwon, Korea) (CM). This membrane had a uniform structure. Crosslinking was performed by immersing the membrane in 5 mM EDC solution at 4°C for 24 hours, followed by thorough washing and lyophilization (Figure 1).

Bone graft material

A collagenated-block bone graft material was used in this study (Mastergraft Putty, Medtronic, Minneapolis, MN, USA). This material is a synthetic biphasic calcium phosphate consisting of 15% hydroxyapatite and 85% β-tricalcium phosphate, which is equally distributed throughout ultra-purified resorbable type I bovine collagen.

3. Study design

In 15 beagle dogs, a chronic narrow ridge was induced on 1 side by extraction of the second, third, and fourth premolars. After 8 weeks of healing, GBR was performed on 2 sites unilaterally (Figure 2). The 2 experimental groups—i) the BG group and ii) the CM group— were randomly allocated to either the mesial or the distal site of the ridge. Animals were euthanized at 4, 8, and 16 weeks (n=5 for each period).

4. Surgical protocol

General anesthesia was induced using an intravenous injection of medetomidine (0.75 mg/kg; Tomidin, Provet Veterinary Products) and alfaxalone (2 mg/kg; Jurox). Inhalation anesthesia was performed using isoflurane (Forane, Choongwae Pharmaceutical, Seoul, Korea). Local anesthesia was performed by infiltration of lidocaine hydrochloride (1:100,000) (Kwang Myung Pharm Co., Ltd., Seoul, Korea). Supragingival scaling was performed prior to the extraction of the second, third, and fourth premolars. The buccal plates of the extraction sockets were removed using a tungsten carbide bur to create a narrow ridge.

After 8 weeks of healing, GBR was performed in a unilateral edentulous ridge. A crestal incision and 2 vertical incisions were made to raise a full-thickness mucoperiosteal

flap. The synthetic block graft materials were trimmed to the size of 6×5×5 mm and then applied to each bone defect (Figure 2). The graft materials were covered with either BG or CM trimmed to the size of 12×18 mm, followed by fixation of the membranes using 2 titanium pins (Membrane Pin; Dentium, Suwon, Korea) (Figure 1). The flap was advanced with periosteal releasing incisions, and tension-free primary closure was achieved with sutures (4-0 Monosyn, B. Braun, Melsungen, Germany). The specimens were euthanized at 4, 8, and 16 weeks postoperatively for histologic processing.

5. Histomorphometric measurements

Histomorphometric measurements were performed by a trained investigator (X.J.) who was blinded to the experimental groups, and outcomes were reviewed by 2 other investigators (J.K.C. and J.Y.P.). A semi-quantitative and descriptive score was given for the local tissue response at the implantation sites according to the ISO 10993-6 guidelines [10](Naenni et al. 2020) (Table 1). Quantitative measurements of the remaining membrane thickness and area were made using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA, USA) was used. The following variables were assessed for each sample:

- Remaining membrane thickness (mm): measured at 5 points equally dividing the defect site to obtain a mean value

- Remaining membrane area (mm^2): the area of the membrane remnants at the entire grafted site

- Neovascularization: the number of capillaries within the area of the membrane at the augmented site

- Encapsulation: the thickness of mature collagen surrounding the membrane

- Tissue integration: the degree of tissue growth into the membrane

- Pseudoperiosteum: the formation of periosteum-like tissues replacing the membrane, characterized by dense fibrous layer of connective tissues overlying a cellular layer

6. Statistical analysis

All measurement data are presented as mean ± standard deviation. Statistical analyses were performed using SPSS version 21.0 (IBM Corp, Armonk, NY, USA). Nonparametric tests were applied given the descriptive nature of the study. The Mann-Whitney U test and Wilcoxon signed rank test were used for inter-group and intra-group comparisons, respectively. The differences were deemed statistically significant when the P values were less than 0.05.

III. RESULTS

1. Clinical observation

Surgical wound healing was uneventful, without complications such as wound dehiscence, severe swelling, or bleeding.

2. Histological observation

Week 4

At week 4, membrane remnants of both BG and CM could be observed, although BG underwent substantially greater resorption than CM (Figure 3). Regarding angiogenesis, blood vessel formation was scantly evident within the membrane space of BG, whereas CM showed no signs of vascularization (Figure 4). BG was closely integrated with the surrounding connective tissues and interstitial matrix. In addition, the fibrous tissues surrounding BG appeared significantly thicker compared to those surrounding CM (Figure 3).

Week 8

At week 8, both CM and BG appeared to have been resorbed significantly (Figure 3). Regarding angiogenesis, the space once occupied by BG contained numerous blood vessels, whereas few blood vessels could be seen within the space of CM (Figure 4). It was evident that BG had been almost completely integrated with the surrounding tissues, which appeared to have greater maturity and thickness than at 4 weeks (Figure 3). For CM, the degree of tissue integration appeared similar to that at 4 weeks, and the surrounding fibrous tissues also showed similar maturity to that at 4 weeks.

Week 16

Even at 16 weeks, some of the samples showed unresorbed remnants of CM, which was still unintegrated with the surrounding tissues (Figure 3). Conversely, it appeared that BG was almost fully resorbed, with no signs of membrane remnants. Nevertheless, the membrane space of BG was occupied by a mature dense fibrous layer of connective tissue, which resembled the periosteum. It could be seen that the remaining superficial bone graft particles were entangled within this layer. Regarding angiogenesis, the pseudoperiosteum of BG was richly vascularized, whereas that of CM showed a scarce distribution of vessels (Figure 4).

3. Histomorphometric analysis

Neovascularization

At 4 and 8 weeks postoperatively, no statistically significant differences were found in the neovascularization score between CM and BG (P=0.053 and P=0.08, respectively) (Table 2). At week 16, the neovascularization score was significantly higher for BG than for CM (P=0.007). There was no significant difference in neovascularization scores between the different time points for both membranes (P>0.05).

Encapsulation

At weeks 4 and 8, the encapsulation scores of BG were significantly higher than those of CM (P=0.017 and P=0.042, respectively) (Table 2). However, at week 16, there was no significant difference between BG and CM (P>0.05). From weeks 4 to 16, the encapsulation score of both membranes tended to increase; however, the difference was not significant (P>0.05).

Tissue integration

The tissue integration score of the BG group was significantly higher than that of the CM group at weeks 4, 8, and 16 (P=0.004, P=0.021, and P=0.004, respectively) (Table 2). In the CM group, the tissue integration score significantly increased by week 16 compared to week 4 (P=0.046). In the BG group, the tissue integration score significantly increased from week 4 to week 16, and from week 8 to week 16 (P= 0.034 and P=0.041, respectively).

Remaining membrane thickness

BG was thicker than CM at week 4 (P=0.001) (Table 3). The thickness of BG and CM significantly decreased between 4 and 8 weeks (P=0.002 and P=0.001, respectively); however, there was no significant difference between 8 and 16 weeks (P>0.05).

Remaining membrane area

The remaining membrane area was higher in the CM group than in the BG group at week 16 (P=0.047) (Table 3); however, there were no significant differences at weeks 4 and 8 (P>0.05). In both groups, the remaining membrane area significantly decreased between 4 and 8 weeks, and between 4 and 16 weeks (Table 3); however, there was no significant difference between 8 and 16 weeks (P>0.05).

Pseudoperiosteum

Pseudoperiosteum was present only in 5 samples of the 16-week BG group.

IV. DISCUSSION

Although collagen membranes are known to lose their barrier function following rapid degradation, some studies have indicated that the membrane becomes closely integrated with the surrounding tissues to provide a continuous bioactive role in bone regeneration [13, 14](Cha et al. 2021; Omar et al. 2018). Therefore, in this study, BG and CM were applied in a GBR model at 3 sequential time points up to 16 weeks to assess the progression of vascularization, tissue integration, tissue maturation and membrane degradation. The main findings were i) BG exhibited greater neovascularization, tissue integration, and tissue maturation than CM at 4, 8, and 16 weeks, ii) almost complete biodegradation of both membranes occurred between 4 to 8 weeks, and iii) at 16 weeks, a thickened band of mature fibrous tissues was formed in the space once occupied by BG, whereas the membrane remnants were still present in the CM group.

The importance of angiogenesis in GBR has been emphasized since the introduction of resorbable collagen membranes [15](Schmid et al.1997). In the normal wound healing process, after the initial formation of a blood clot and granulation tissue in the defect space, the formation of a rich vascular supply has been shown to be crucial to osteoid formation and subsequent woven bone formation [16](Cucchi et al.2019). Although the vascular network is known to be mainly supplied by the alveolar bone at the defect site, an additional vascular supply from the overlying soft tissues may enhance healing and increase the rate of osteogenesis. Therefore, transmembraneous angiogenesis has been indicated to provide an additional healing source during the early formation of new bone by non-crosslinked collagen membranes [12, 17](Schwarz et al. 2006; Schwarz et al. 2008). In this study, vascularization of BG increased significantly from week 4 to week 16. The CM group showed delayed vascularization in comparison to BG, resulting in a significant difference in the number of vessels after 16 weeks. It could be assumed that the lack of

vascularization in the CM group might have been caused by the crosslinking of collagen. Related studies have also shown that neovascularization was negatively correlated with the extent of chemical cross-linking of the collagen matrix [12, 18](Schwarz et al. 2006; Thoma et al. 2012).

Tissue integration is an important criterion for absorbable barrier membranes to minimize immune responses and allow cellular proliferation around the membrane. Meanwhile, an integrated barrier membrane should ideally maintain the barrier function and cell occlusivity during the healing period. In this study, tissue integration was measured by the proximity between the barrier membrane and the surrounding tissues. BG was closely integrated with the surrounding tissues even at 4 weeks, whereas CM appeared to be separate from the adjacent tissues even at 16 weeks. These findings are similar to a previous study, in which increased levels of crosslinking were found to be negatively correlated with tissue integration [18](Thoma et al. 2012). Reduced tissue integration of crosslinked collagen membranes could be related to the release of toxic crosslinking agents from resorption of the membrane, which can alleviate negative tissue response. Compared with non-crosslinked collagen membranes, crosslinked collagen membranes with an extended absorption time showed significantly more adverse events and less bone regeneration [19](Tal et al. 2008). However, a preceding study using the current data showed that CM was superior to BG for new bone formation [20](Lee et al. 2020). This may be due to the fact that CM still maintained a certain spatial shape at week 16, while BG began to degrade rapidly at week 4, resulting in the displacement or loss of bone graft materials, affecting bone regeneration. This also indicates that the membrane obtained by chemical crosslinking has a moderately long degradation time and appropriate biocompatibility, but is not conducive to tissue integration and angiogenesis [11](Rothamel et al. 2005).

The existence of a periosteum-like structure in the BG group at week 16 was a key finding of this study. A human autopsy study [21](Cha et al. 2019) also found

periosteum-like structures at a site that had been grafted using BG, which seemed to last for more than 6 years after surgery. In this study, the tissue was found to contain small blood vessels, which could be assumed to supply blood to the defect site from the overlying tissues. In the BG group, the membrane appears to have been replaced by a highly vascular pseudoperiosteal tissue, which provided a biological barrier even after the collagen membrane was biodegraded. It may be speculated that this thickened layer of dense connective tissue might be the product of encapsulation and maturation of the tissues surrounding the integrated collagen membrane. This connective tissue structure should be studied further to reveal its biological activity in future studies.

This study had some limitations. Firstly, two commercially available barrier membranes with different collagen compositions and manufacturing techniques were used to represent crosslinked and non-crosslinked collagen membranes. Ideally, to properly assess the isolated effect of crosslinking, the same collagen membrane should be used with or without crosslinking. Secondly, the semi-quantitative and descriptive nature of the scoring system might have been subject to examiner bias. Measures were taken to prevent bias by blinding the examiners to the groups and involving multiple examiners to review the outcomes.

V. CONCLUSION

Although the BG membranes were rapidly biodegraded, they gradually became replaced by connective tissue with complete integration and maturation of the surrounding tissues to form dense periosteum-like connective tissue. However, the barrier function of BG membranes remains to be validated. In contrast, the CM membranes remained unresorbed longer and resulted in less vascularity and tissue integration than BG.

REFERENCE

1. Sanz-Sánchez I, Ortiz-Vigón A, Sanz-Martín I, Figuero E, Sanz M. Effectiveness of lateral bone augmentation on the alveolar crest dimension: a systematic review and meta-analysis. *J Dent Res* **2015**;94 Suppl:128S-42S.

2. Bunyaratavej P, Wang HL. Collagen membranes: a review. *J Periodontol* **2001**;72:215-29.

3. Gough JE, Scotchford CA, Downes S. Cytotoxicity of glutaraldehyde crosslinked collagen/poly(vinyl alcohol) films is by the mechanism of apoptosis. *J Biomed Mater Res* **2002**;61:121-30.

4. Ahn JJ, Kim HJ, Bae EB, Cho WT, Choi Y, Hwang SH, et al. Evaluation of 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide cross-linked collagen membranes for guided bone regeneration in beagle dogs. *Materials (Basel)* **2020**;13:E4599.

5. Park JY, Jung IH, Kim YK, Lim HC, Lee JS, Jung UW, et al. Guided bone regeneration using 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (EDC)-cross-linked type-I collagen membrane with biphasic calcium phosphate at rabbit calvarial defects. *Biomater Res* **2015**;19:15.

6. Omar O, Elgali I, Dahlin C, Thomsen P. Barrier membranes: more than the barrier effect? *J Clin Periodontol* **2019**;46 Suppl 21:103-23.

7. Park JY, Song YW, Ko KA, Strauss FJ, Thoma DS, Lee JS. Effect of collagen membrane fixation on ridge volume stability and new bone formation following guided bone regeneration. *J Clin Periodontol* **2022**;49:684-93.

8. Urban IA, Montero E, Monje A, Sanz-Sánchez I. Effectiveness of vertical ridge augmentation interventions: a systematic review and meta-analysis. *J Clin Periodontol* **2019**;46 Suppl 21:319-39.

9. Jiménez Garcia J, Berghezan S, Caramês JM, Dard MM, Marques DN. Effect of cross-linked vs non-cross-linked collagen membranes on bone: a systematic review. J Periodontal Res **2017**;52:955-64.

10. Naenni N, Lim HC, Strauss FJ, Jung RE, Hämmerle CH, Thoma DS. Local tissue effects of various barrier membranes in a rat subcutaneous model. *J Periodontal Implant Sci* **2020**;50:327-39.

11. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clin Oral Implants Res* **2005**;16:369-78.

12. Schwarz F, Rothamel D, Herten M, Sager M, Becker J. Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clin Oral Implants Res* **2006**;17:403-9.

13. Cha JK, Pla R, Vignoletti F, Jung UW, Sanz-Esporrin J, Sanz M. Immunohistochemical characteristics of lateral bone augmentation using

different biomaterials around chronic peri-implant dehiscence defects: An experimental in vivo study. *Clin Oral Implants Res* **2021**;32:569-80.

14. Omar O, Dahlin A, Gasser A, Dahlin C. Tissue dynamics and regenerative outcome in two resorbable non-cross-linked collagen membranes for guided bone regeneration: a preclinical molecular and histological study in vivo. *Clin Oral Implants Res* **2018**;29:7-19.

15. Schmid J, Wallkamm B, Hämmerle CH, Gogolewski S, Lang NP. The significance of angiogenesis in guided bone regeneration. A case report of a rabbit experiment. *Clin Oral Implants Res* **1997**;8:244-8.

16. Cucchi A, Sartori M, Aldini NN, Vignudelli E, Corinaldesi G. A proposal of pseudo-periosteum classification after GBR by means of titanium-reinforced d-PTFE membranes or titanium meshes plus cross-linked collagen membranes. *Int J Periodont Restor Dent* **2019**;39:e157-65.

17. Schwarz F, Rothamel D, Herten M, Wüstefeld M, Sager M, Ferrari D, et al. Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. *Clin Oral Implants Res* **2008**;19:402-15.

18. Thoma DS, Villar CC, Cochran DL, Hämmerle CH, Jung RE. Tissue integration of collagen-based matrices: an experimental study in mice. *Clin Oral Implants Res* **2012**;23:1333-9.

19. Tal H, Kozlovsky A, Artzi Z, Nemcovsky CE, Moses O. Long-term bio-degradation of cross-linked and non-cross-linked collagen barriers in

human guided bone regeneration. *Clin Oral Implants Res* **2008**;19:295-302.

20. Lee JT, Cha JK, Kim S, Jung UW, Thoma DS, Jung RE. Lateral onlay grafting using different combinations of soft-type synthetic block grafts and resorbable collagen membranes: an experimental in vivo study. *Clin Oral Implants* Res **2020**;31:303-14.

21. Cha JK, Sanz M, Jung UW. Human autopsy study of peri-implant dehiscence defects with guided bone regeneration: a case report. *Int J Periodont Restor Dent* **2019**;39:517-24.

TABLES

Table 1. Parameters evaluated during histopathological scoring of the samples

General tissue reaction	Scoring system
Pseudoperiosteum	−, absent; +, present in ≥10% of the target section; ++, present in ≥20% of the target section; and +++, present in ≥50% of the target section.
Neovascularization	The number of capillaries within the area of the membrane: 0, none; 1, minimal proliferation of capillaries; 2, formations of 4–7 capillary structures; 3, a wide band of capillaries; and 4, a substantial band of capillaries.
Encapsulation	Encapsulation was descriptively assessed based on the thickness of mature collagen surrounding the membrane and scored as follows: 0, none; 1, a narrow band; 2, a moderate band; 3, a thick band; and 4, a substantial band.
Tissue integration	The degree of tissue growth into the membrane: 0, none; 1, slight; 2, moderate; 3, marked; and 4, complete/severe.

Table 2. Results from the histological analysis.

Time point (weeks)	CM			BG		
	4weeks	8weeks	16weeks	4weeks	8weeks	16weeks
Pseudoperiosteum	-	-	-	-	-	++
Neovascularization	0±0	0.4±0.55	0.6±0.55	1.40±1.52	1.60±1.14	2.60±0.55*
Encapsulation	1±0	1.4±0.55	2.4±1.14	2±0.71*	2.2±0.45*	3.2±0.84
Tissue integration	0±0	0.4±0.55	0.8±0.45[b]	2.8±0.45*	2±1*	4±0 *,[b,c]

BG=Bio-Gide, CM=crosslinked collagen membrane.

Numbers represent the mean ± standard deviation of the parameters using the following scoring system: 0=none, 1=slight, 2=moderate, 3=marked, and 4=complete/severe. The general tissue reaction was evaluated using the following scoring system: −, not present; +, present in ≥10% of the viewed area; ++, present in ≥20% of the viewed area; and +++, present in ≥50% of the viewed area.

*: Significantly different from the other group at the same observation period according to Mann Whitney-U test. ($p < 0.05$)

a: Significant differences between week 4 and week 8 according to Wilcoxon signed rank test. ($p < 0.05$)

b: Significant differences between week 4 and week 16 according to Wilcoxon signed rank test. ($p < 0.05$)

c: Significant differences between week 8 and week 16 according to Wilcoxon signed rank test. ($p < 0.05$)

Table 3. Histomorphometric measurements at 4, 8 and 16 weeks after surgery.

	Time point(weeks)	4weeks	8weeks	16weeks
Thickness(mm)	CM	0.53±0.09[ab]	0.12±0.07	0.13±0.1
	BG	0.84±0.09[ab]*	0.3±0.19	0.14±0.05
Area (mm^2)	CM	2.16±0.48[ab]	0.21±0.18	1.16±0.79*
	BG	3.41±1.11[ab]	0.36±0.28	0.18±0.25

BG=Bio-Gide, CM=crosslinked collagen membrane.

Thickness: Remaining absorbable membrane thickness; Area: Remaining absorbable membrane area.

*: Significant different at the same observation period. (Statistical significance level was 5%, $p < 0.05$.)

a: Significant differences between week 4 and week 8 according to Wilcoxon signed rank test. ($p < 0.05$)

b: Significant differences between week 4 and week 16 according to Wilcoxon signed rank test. ($p < 0.05$)

c: Significant differences between week 8 and week 16 according to Wilcoxon signed rank test. ($p < 0.05$)

FIGURES

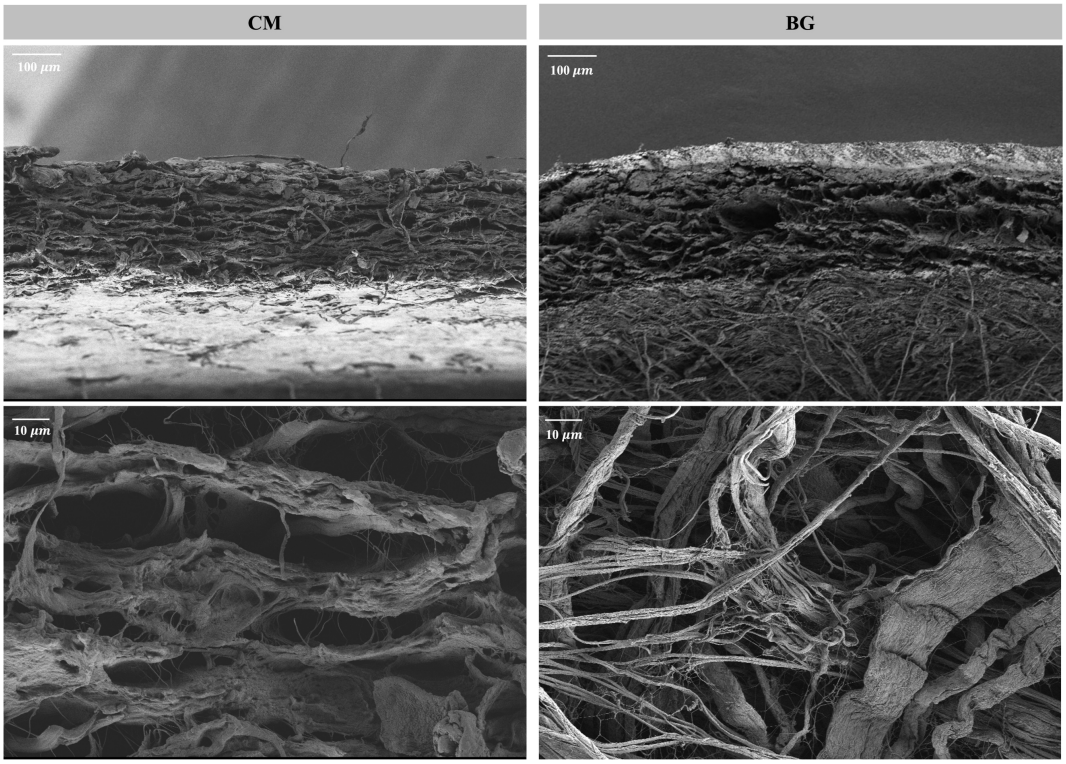

Figure 1. Scanning electron micrograph images of the collagen membrane

Scanning electron micrograph images of the non-crosslinked collagen membrane (Bio-Gide; Geistlich Pharma AG, Wolhusen, Switzerland) (BG) and the crosslinked collagen membrane (Collagen membrane-P; Genoss, Suwon, Korea) (CM) at ×100 and ×1,000 magnifications. CM: crosslinked collagen membrane, BG: Bio-Gide.

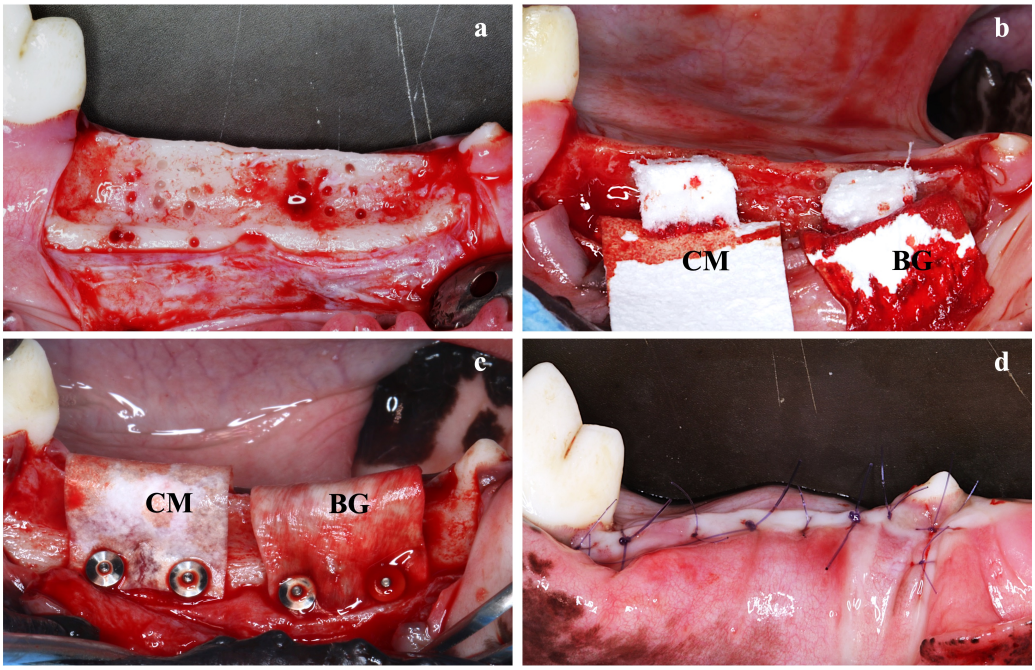

Figure 2. The surgical procedure.

(a) A full-thickness flap was elevated, and the alveolar bone bed was decorticated. (b) A collagenated synthetic bone block was placed in each experimental site (Mastergraft Putty; Medtronic, Minneapolis, MN, USA). (c) The bone substitutes were covered by 2 different membranes according to group allocation; left: crosslinked collagen membrane, right: a non-crosslinked collagen membrane, and fixed with 2 titanium pins. (d) Primary closure was achieved.

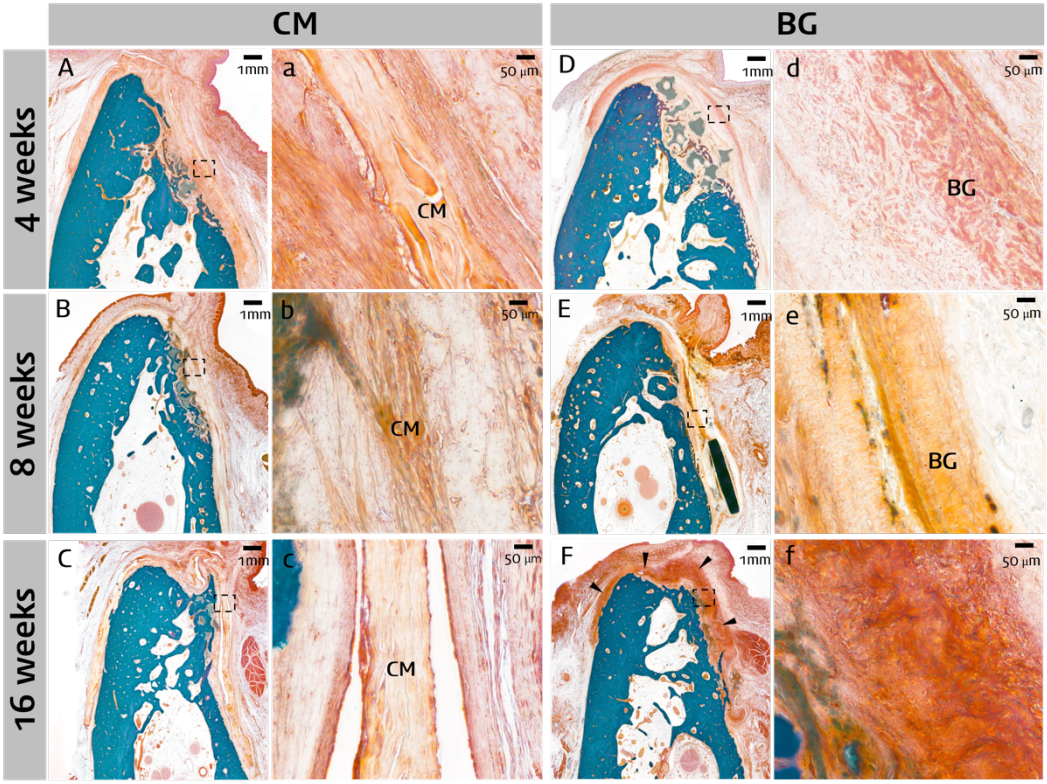

Figure 3. Histological images obtained at 4, 8, and 16 weeks.

(A-F) Low-magnification images from the CM and BG groups. The black triangles indicate the pseudoperiosteum. Histological images obtained at 4, 8, and 16 weeks. (a-f) High-magnification images of the areas highlighted in panels (A-F). CM: crosslinked collagen membrane, BG: Bio-Gide.

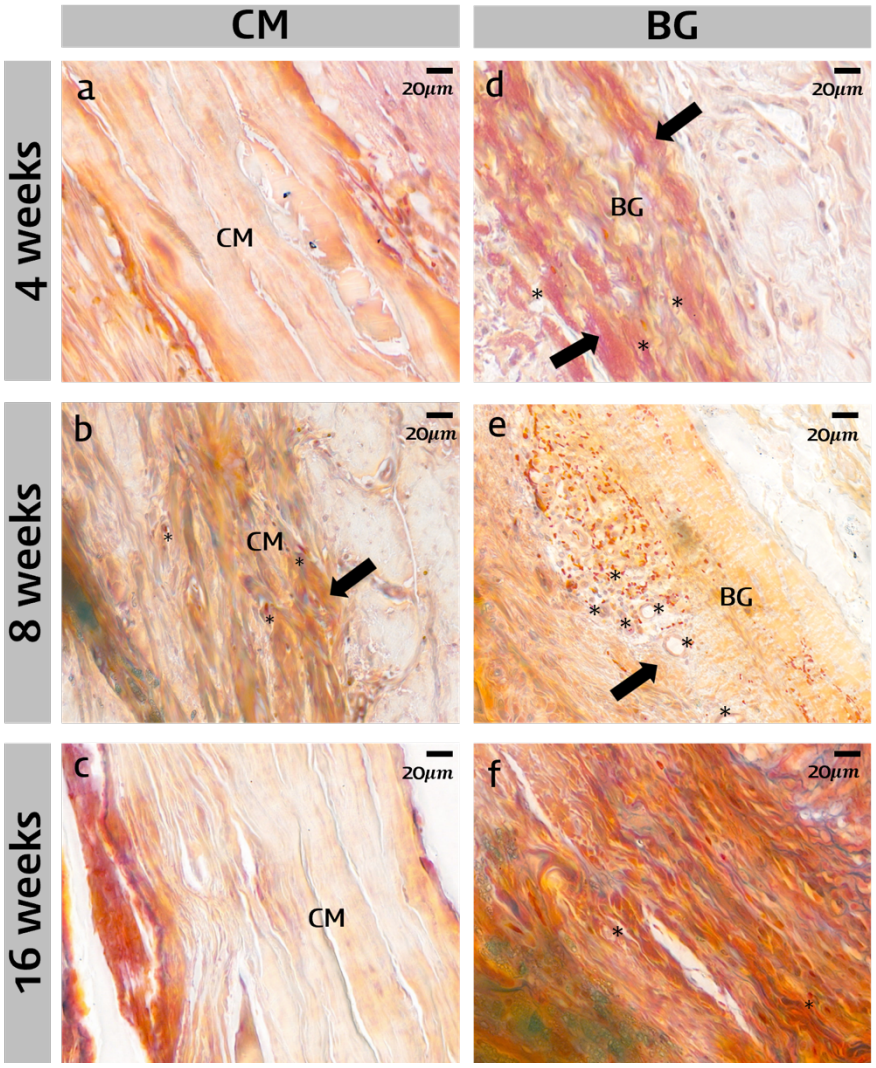

Figure 4. High-magnification images of a representative sample from each group.

Neovascularization is indicated by an asterisk. The black arrows indicate tissue integration of the surrounding connective tissues with the membrane. CM: crosslinked collagen membrane, BG: Bio-Gide.

국문요약

가교 및 비가교 콜라겐 차단막의 조직학적 융화 양상 전임상 비교 연구

<지도교수 **최성호**>
연세대학교 대학원 치의학과
JIN XIANG

목적:비가교화 및 가교화 된 콜라겐 차단막은 분해 특성의 차이로 인해 서로 다른 생물학적치유 양상을 나타낸다. 이 연구의 목적은 비가교화 및 가교화 된 콜라겐 차단막을 혈관화, 주변 조직과의 융화 및 캡슐화, 생분해와 관련하여 조직학적으로 비교 평가하는 것이다.

방법: 15마리의 비글개에서 비가교화(BG) 또는 가교화 된 콜라겐 차단막(CM)을 사용하여 골유도재생술을 시행하였고 4주, 8주, 16주에 5마리씩 안락사 하였다. 신생 혈관의 수, 조직 융화 및 캡슐화의 정도, 잔존 차단막의 면적, 가성골막 형성의 여부를 조직학적으로 평가하였다. 이후 비모수적 통계방법을 사용하여 군간, 기간별 비교가 이루어졌다.

결과: 16주차에 잔존 차단막의 면적은 CM 군이 BG 군 보다 유의하게

컸다. 반대로, 신생혈관 점수는 BG 군이 CM 군보다 유의하게 컸다. BG는 16주차의 캡슐화 점수를 제외하고 모든 기간에서 CM보다 훨씬 더 높은 조직 융화 및 캡슐화 점수를 나타냈다. BG 군에서 16주차에는 가성골막의 형성이 관찰되었다.

결론: 비가교화 콜라겐 차단막은 가교화 된 차단막보다 빠르게 흡수되지만, 치밀한 결합 조직으로 대체되고 주변 조직과 긴밀히 융화되어 골막과 유사한 조직을 형성한다. 새롭게 형성된 가성골막의 역할을 검증하기 위해서는 추가 연구가 수행되어야 한다.

핵심되는 말: 골 재생; 골 대체물; 콜라겐; 가교 시약