The histologic evaluation of the Biodegradability and Tissue response of the absorbable collagen Sponge(ACS) following implantation on the 3 wall intrabony defects.

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감사의 글

이 논문의 처음 시작 단계부터 완성에 이르기까지 자상한 관심과 가르침을 주신 김종관 교수님께 깊은 감사의 마음을 전합니다. 바쁘신 와중에고 논문 교정과 심사를 맡아주신 채중규 교수님과 김창성 교수님께도 마음 깊이 감사를 전합니다.

대학원 과정을 시작할 수 있도록 격려해 주시고 도전하게 해 주신 이지윤 과장님께 깊은 고마움과 감사의 마음을 전하며 수련 기간동안 치주학에 대한 관심과 가르침을 주시고 부족한 것이 많은 저를 끝까지 지도해 주신 이학철 과장님, 정인원 원장님과 김기태 원장님께 존경과 감사의 마음을 전합니다.

대학원 생활에서도 병원에서도 항상 옆에서 조언과 관심을 가져주시고 학교생활과 병원 생활을 큰 어려움 없이 할 수 있도록 도와주시고 이해해 주신 유정아 과장님과 박필규 부장님께 감사드립니다.

그리고 실험과 수업 및 대학원 생활에서 많은 도움을 주신 치주과 의국 선생님들께 고마운 마음을 전합니다.

늘 곁에서 따뜻이 응원해 주는 사랑하는 시부모님 그리고 친정부모님, 은정, 상우, 은하 언니에게 감사와 사랑의 마음을 전합니다.

마지막으로 올해 놀라움과 신비로움을 느끼게 해준 나의 아들 혁민이에게 엄마의 사랑을 전하며, 나의 신랑 혁이 선생님에게 깊은 사랑의 마음을 전하며 글을 마침니다.

2009년 6월

이 상 민

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ABSTRACT

The histologic evaluation of the biodegradability and tissue response of the absorbable collagen sponge(ACS) following implantation on the 3 wall intrabony defects.

Objective: Absorbable collagen sponge(ACS) has been extensively used as carriers in bone tissue engineering due to its biocompatibility, biodegradability, and low antigenicity. For evaluation of its biocompatibility, this study is compared the 8-weeks histologic results of periodontal healing with open flap curettage implanted buffer/Absorbable collagen sponge or open flap debridement only in the intrabony defects.

Methods: Bilateral 3-wall intrabony periodontal defects were surgically induced in the premolar region in the maxilla and mandible in 8 young adult Korean mongrel dogs. The surgical control groups received a flap operation only, while the collagen groups were treated with phosphate-buffered saline/Absorbable collagen sponge. The subjects were sacrificed 8weeks after the operation, and a comparative histologic evaluation was performed.

Results: Clinical healing was uneventful without adverse effects. No meaningful differences in histologic analysis about regenerated tissues were observed between both groups. Absorbable Collagen Sponge was almost degraded in 8-week histologic specimens. No sites exhibited ankylosis and few sites appeared root resorption.

Conclusions: Absorbable Collagen Sponge has been shown favorable, biocompatible tissue response and relatively rapid biodegradation. So, It may be used a suitable carriers for 3-wall intrabony defects treatment.

Key words: Carriers, Absorbable Collagen Sponge, 3-wall intrabony defects

The histologic evaluation of the biodegradability and tissue response of the absorbable collagen sponge(ACS) following implantation on the 3 wall intrabony defects.

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I. INTRODUCTION

Periodontal therapy is directed at arresting the destruction of periodontium, with the goal of stabilizing the long-term prognosis of the periodontium. More recently, it has been directed towards obtaining a predictable regeneration of the periodontium. Many reports have suggested various modes of therapies, including root debridement with or without flap elevation, the placement of bone grafts or bone substituents, root conditioning, and the use of barrier membranes. But they have limitations about complete regeneration of supporting tissues of periodontium. (Wikesjo et al.1990 ,

Selvig et al.2001)

Recently, advances in the areas of cellular and molecular biology have allowed the elucidation of functions of growth factors and their participation in different phases of periodontal wound healing. Growth factors have been used to treat periodontal defects in human and increased the possibility of periodontal regeneration. (The 6th European workshop on periodontology)

Therapeutic application of growth factors requires a characterized carrier system to ensure safe and effective delivery. Many prerequisites have been suggested for the ideal scaffold material with regard to mechanical properties, biocompatibility, or biodegradability. Nevertheless, none of the carriers evaluated can be considered optimal in all these aspects. The carrier system is divided into synthetic and natural. Synthetic polymers include the polylactides, such as polylactic acid(PLA), polyglycolic acid(PGA), polyethylene oxide(PEO), poly(lactic-co-glycolic)PLGA. Natural materials include type 1 collagen, hyaluronic acid, chitosan, starch. Commonly used carriers for treatment of periodontal defects are collagen in the form of a sponge, membrane or gel and gelatin with varying degrees of cross-linking. Collagen has been extensively described as a beneficial material in tissue engineering due to its biocompatibility, biodegradability, and low antigenicity. But this matrix does not provide space and support the gingival flap in loaded areas. So it might have applicability in cases where mechanical demand is low, such as contained defects.

The objectives of this study is to evaluate the biocompatibility and biodegradability of collagen carrier system in the 3-wall intrabony defects in the dog.

II. MATERIALS AND METHODS

1. Animals

Four 2-year-old Korean mongrel dogs were used. The animals had intact dentition and healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved by the Instittutional Animal Care and Use committee, Yonsei Medical Center, Seoul, Korea.

2. Surgical Protocol

The surgical procedure was performed under general anesthesia induced by an intravenous injection of atropine(0.04mg/kg: Kwangmyung Pharmaceutical Ind. Co., LTD, Seoul, Korea), and the intramuscular induction with a compound of xylazin (Rompun, Bayer Korea Co.,Seoul, Korea) and ketamin (Ketara, Yuhan Co., Seoul, Korea) followed inhalation. Local infiltration with 2% lidocaine hydrochloride with 1/80,000 epinephrine was given for hemostasis and to reduce postoperative pain.

The maxillary second and mandibular third premolars had been extracted prior to the experimental surgeries, and the extraction sites had been allowed to heal for 8weeks. The remaining dentition received oral prophylaxis in conjugation with the extraction procedures.

At reconstructive surgery, buccal and lingual mucoperiosteal flaps were elevated and 4*4*4mm^3 3-wall intrabony defects were created at the distal aspect of maxillary first and mandibular second premolars. Following root planing, a reference notch was made with a 1/4round bur on the root surface at the base of the defect.

The bilateral maxillary and mandibular intrabony defects each received alternately : **buffer/ACS**(experimental groups) , **sham surgery** (control groups). The defects of buffer groups were treated with ACS, cut into 25*25mm, then soaked in a sufficient amount of PBS for 30min and again cut into 8*8mm sections and folded twice for implantation. . The defects of sham surgery groups were performed open flap curettage only and no filler or barrier was placed before suturing. Primary ,tension-free wound closure was accomplished. Sutures were removed after 7 to 10 days. Postsurgery management included IM administration of antibiotics, soft diet, and daily topical application of a 2% chlorhexidine solution throughout the healing sequence. After 8weeks, the aninmals were euthanized

3. Clinical Procedures and Histologic Procedure

The aninmals were killed after 8weeks by an intravenous injection of concentrated sodium pentobarbital(Entobar® Hanmim Pharmaceuticals Co., Seoul, Korea) Tissue blocks, which included teeth, bone, and tissue were removed, rinsed in saline, then fixed in 10% buffered formalin for 10days. Subsequently, the bolck sections were decalified in 5% nitric acid for 7-8 days and embedded in paraffin. Serial

sections(5um thick) were made in the mesiodistal direction at intervals of 80um. They were stained with hematoxylin/eosin for examination by light microscopy.

4. Histologic Analysis

Histologic evaluation of both groups was performed as follows:

Infiltration of inflammatory cells

Junctional epithelium migration

Connective tissue adhesion

The formation of new bone and new cementum

The absorption of the ACS

Arrangement of the PDL fibers and fibroblasts

Root resorption and Ankylosis

III. RESULTS

1. Clinical observations

Primary closure was successfully maintained in all defects. Clinical wound healing was uneventful and no serious adverse events were reported.

2. Histologic observations

Sham group

The junctional epithelium(JE) extended apically to various degrees and composed of thick cell layers thinning in a apical direction. Inflammatory cells infiltration was minimal in all defect sites. (Fig.1-1)

At the point of junctional epithelium termination, a thin cementum layer was observed with connective tissue fiber adhesion showing a parallel orientation.(Fig.1-2)

Newly formed bone was more or less observed on the coronal root surface notch. The coronal thinning of new bone was apparent and many blood vessels are developed at the periodontal ligament space around bone crest. (Fig.1-3)

Newly formed cementum with inserting collagen fibers was observed on the instrumented root portions (Fig.2). It was in continuity with the original

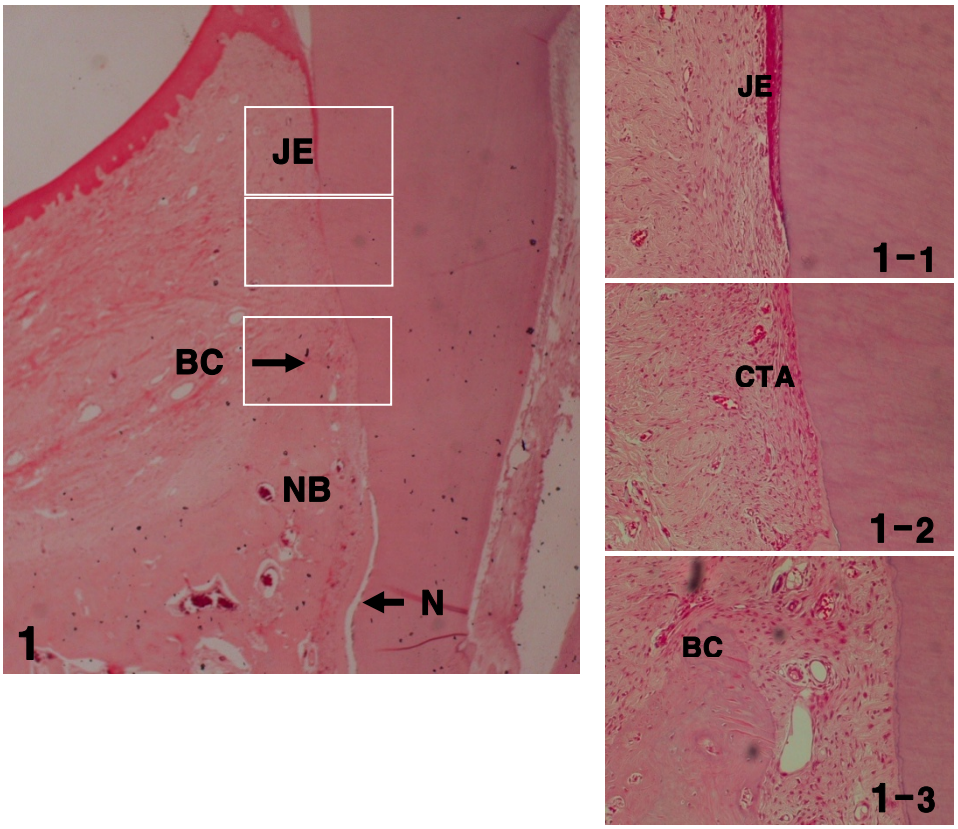

Fig.1 Section of sham surgery site showing apical migration of the junctional epithelium(JE), connective tissue attachment of root surface and newly formed cementum and bone above reference notch(N). No signs of inflammatory reactions are visible. (H-E: original magnification ×20) (BC: Bone Crest, NB: New Bone)

Fig.1-1 The apical migration of the junctional epithelium(JE) (H-E:original magnification ×200)

Fig.1-2 The connective tissue fibers were apposed directly to the root planed dentin or newly formed cementum surface.(H-E: original magnification ×200) (CTA:Connective Tissue Attachment)

Fig.1-3 Bone and cementum regeneration were seen on the bone crest. Irregularly oriented periodontal ligament fibers and many blood vessels were seen. (H-E: original magnification ×200)

cementum layer apical to the instrumented part of the root and was thickest in the apical portions (Fig.1) The new regenerated cementum also have a tendency for the portions to separate from the underlying cementum during histologic processing. (Fig.3) The periodontal ligament area had collagen fibers running perpendicularly or obliquely from the root surface.(Fig.2)

The finding of ankylosis was not seen, root resorption area was observed in only one animal. In areas of root resorption, multinucleated cells were seen along the dentin surface.

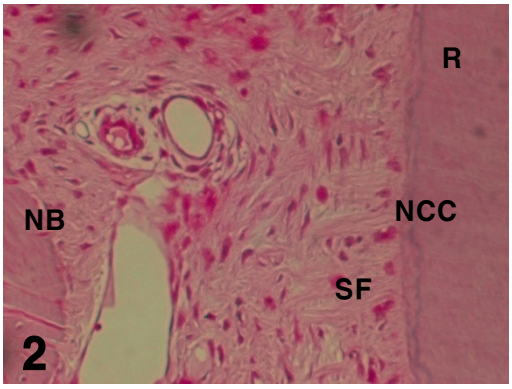

Fig2. Section of the sham site showing irregular and perpendicular insertion of the periodontal ligament fibers in the new bone(NB) and new cellular cementum(NCC) (H-E: original magnification ×400)
(R: root,SF: Sharpey`s Fiber)

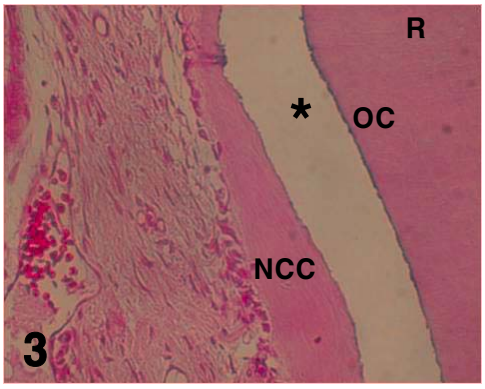

Fig.3 Section of the sham site showing a tendency to separate(*****) of the regenerated cementum from the underlying cementum during histologic processing (H-E: original magnification ×400)
(NCC: newly cellular cementum, OC: old cementum, R: root)

ACS/buffer group

The apical extension of the junctional epithelium appeared. It was composed thick cell layers and extended long and tapered apically. (Fig.4-1) Inflammatory cells infiltration was minimal.

At the terminal point of junctional epithelium, connective tissue attachment was in varying dimension along the root surface. Connective tissue fibers arranged parallel to the newly formed cementum or instrumented dentin.(Fig.4-2)

Above the apical notch, more or less new bone and cementum was formed along the root surface (Fig.4). In a section, a row of osteoblasts surrounded alveolar bone crest were seen.(Fig.5) The newly formed cementum appeared thicker in the notch area and thinner more coronally. In the cementum layer below the bone crest, collagen fibers were found inserted perpendicularly. Above the bone crest, cementum formed a thin strip along root surface and fibers showed an oblique or parallel arrangement.

Periodontal ligament fibers exhibited irregular or perpendicular alignment with the root surface and many blood vessels were observed around the periodontal ligament space at the bone crest. Some Sharpey`s fibers were embedded into the newly formed cementum and bone. (Fig.4-3, Fig.6)

No sites exhibited evidence of ankylosis. Root resorptions were observed in some sections. In only a section, there were seen osteoclasts around remnant ACS in the new bone. (Fig.7)

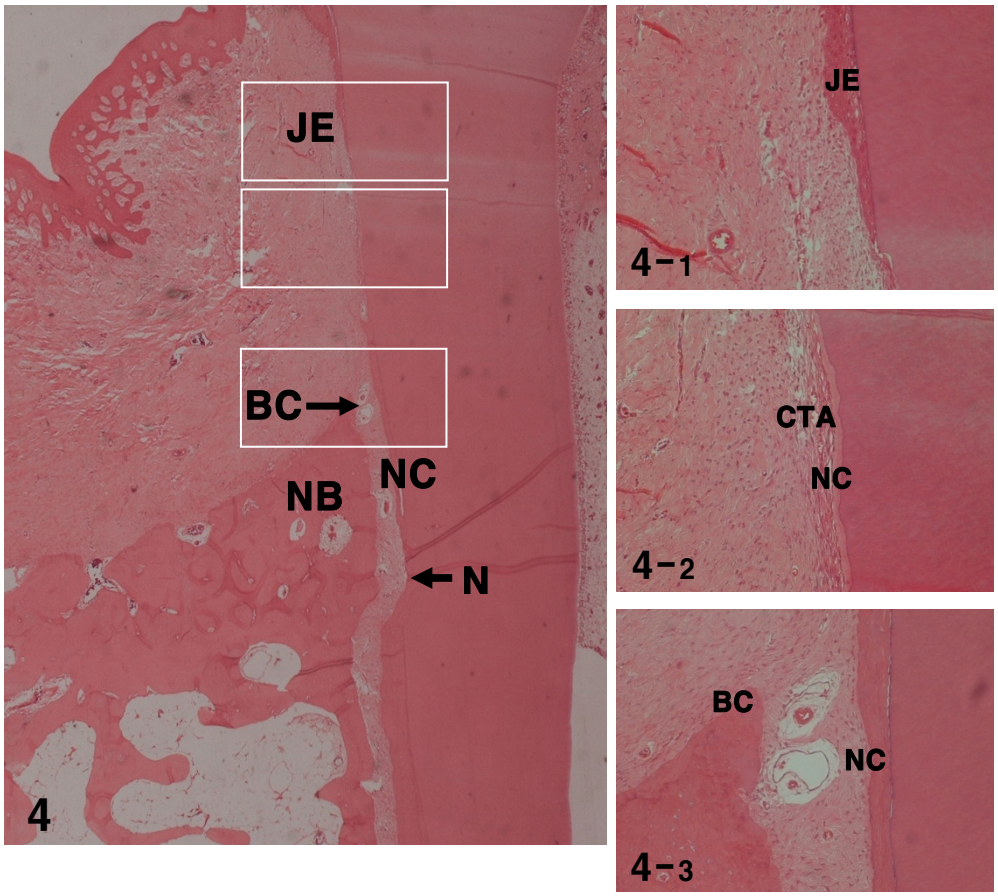

Fig.4 Section of ACS/buffer site showing long junctional epithelium(JE), newly formed cementum(NC) and bone(NB). The new cementum is continuity with the original cementum layer and becomes gradually thinner in the coronal direction. (H-E : original magnification ×20)(N: notch, BC: bone crest)

Fig.4-1 The long junctional epithelium adhered root surface. (H-E: original magnification ×200)

Fig.4-2 Connective tissue contact to the root surface with a cementum formation. Collagen fibers are aligned parallel along the root surface. (H-E: original magnification ×200)

Fig.4-3 Newly formed cellular cementum, alveolar bone formation may be observed, and many blood vessels are developed. (H-E: original magnification ×200)

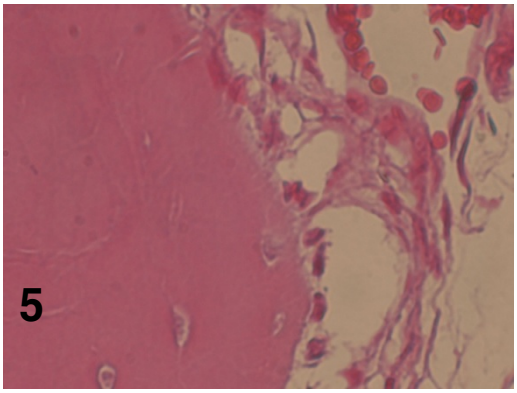

Fig5. Section of the ACS/buffer site showing a row of osteoblasts and a few osteocytes at the bone crest. (H-E: original magnification×400)

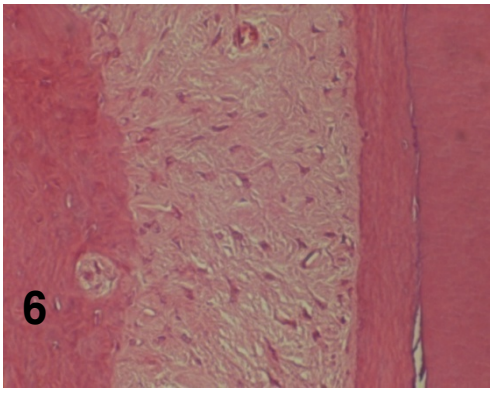

Fig6. Section of the ACS/buffer site showing periodontal ligament fibers running irregular or perpendicular (H-E: original magnification ×400)

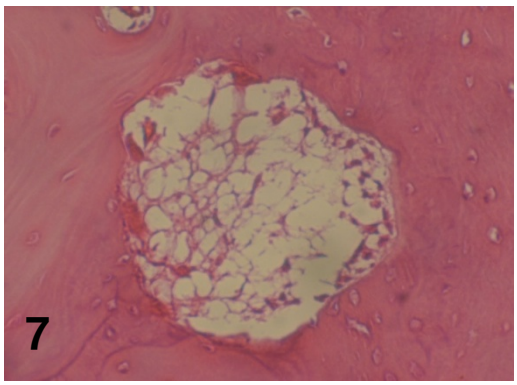

Fig7. Remnant ACS and osteoclasts were observed. (H-E: original magnification ×400)

IV. Discussion

The objective of this study was to evaluate the biocompatibility of absorbable collagen sponge (ACS) in the 3 wall intrabony defects in the dogs. Some 4*4*4mm^3 3 wall intrabony defects in the dogs implanted buffer/ACS and others received sham surgery (open flap debridement). Clinical and histologic evaluations following 8 week healing period showed no meaningful differences in regeneration of attachment apparatus between defects receiving the buffer/ACS and defects receiving sham surgery only.

In both groups, apical extension of junctional epithelium was observed. This finding is similar to the previous histometric studies. (Choi et al.2002, Park et al.2003)

No marked differences in connective tissue adhesion and fibers arrangement between the buffer/ACS groups and control groups are observed. At the termination of junctional epithelium, gingival connective tissue contacts to the planed dentin or cementum tightly and connective tissue fibers orient parallel along the root surface in both groups.

Bone and cementum regeneration extending from above notch to coronal were observed and no significant differences were found in both groups. Newly formed bone was generally lined with osteoblast-like cells and osteoid, suggesting continued bone remodeling after 8-week period. (Sigurdson et al. 1994) The regenerated cementum appeared most cellular, usually covered with cementoblast-like cells and cementoid materials. The cementum layer extended from notch to coronal of the

newly formed alveolar bone and was gradually thinner in the coronal direction. Irregular or perpendicular arrangement of the periodontal ligament fibers in newly formed bone and cementum was observed in both groups.

The evidence of root resorption was observed in both groups. In many previous studies, it was explained that root resorption was more pronounced when the connective tissue was directly opposed to dentin rather than when the root surface was covered by cementum. (Wikesjo et al. 1994, Schroeder et al. 1992) Ankylosis was not observed in any of the intrabony defects.

Carrier architecture is designed to used inherent regenerative capability of osseous tissue by allowing osteoprogenitor cells to populate and mineralize in the carrier. And it is to minimize local tissue response and allow replacement by newly formed bone and allow for space maintenance in bony defect. Furthermore, the mechanical, chemical, and biological properties of the scaffold should be suitable for the specific application.

In this study, comparing the histologic observation of new attachment apparatus formation in buffer/ACS groups and sham surgery groups, biocompatibility of collagen as carrier system was evaluated. Histologic healing appearances of the both groups are similar and the amount of regenerated tissues were not significantly different by previous studies. (Park et al.2003, Choi et al.1993, Kim et al.1998) Remnants of the collagen and signs of inflammation could not almost be seen at 8 week period.

Collagen may be resorbed without beneficial or harmful effect on the periodontal

healing. This property may make absorbable collagen sponge a suitable carrier for study to evaluate osteogenic effect of a variety of growth factors.

V. CONCLUSION

Absorbable collagen sponge (ACS) has been shown favorable, biocompatible tissue response and relatively rapid biodegradation. So, it may be used a suitable carrier to study the effect of growth factors on the periodontal regeneration of the contained defects.

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국문요약

삼벽성 골내낭에서 흡수성 콜라겐 스폰지의 흡수정도와 조직반응에 대한 조직학적 연구

<지도교수 **김 종 관**>

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목적: 흡수선 콜라겐 스폰지는 생체 적합성, 생체 흡수성, 낮은 알러지 반응으로 인해 조직 공학에서 carriers로서 널리 이용되고 있다. 그것의 생체 적합성을 평가하기 위해 이 연구는 3벽성 골내낭에서 흡수성 콜라겐을 매식한 부위와 외과적 처치만을 한 부분의 치주조직 치유에 대한 8주 조직학적 결과를 비교해 보고자 한다.

방법: 8마리의 성견에서 양측성으로 상하악 소구치 부위에 삼벽성 골내낭을 형성했다. 대조군으로는 외과적 처치만을 시행했으며 실험군으로는 콜라겐 스폰지를 외과적 처치와 함께 매식하였다. 8주 후 성견을 희생하여 조직학적 비교를 시행했다.

결과: 두 그룹간의 조직학적 차이는 거의 없었다. 임상적으로 부작용 없이 치유가 일어났으며 8주 조직시편에서 흡수성 콜라겐 스폰지는 거의 흡수가 일어났다. 유착이 일어난 곳은 없었고 치근 흡수를 보이는 시편도 거의 없었다.

결론: 흡수성 콜라겐 스폰지는 양호하고 조직 친화적 반응을 가지며 비교적 빠른 흡수가 일어난다. 따라서 3벽성 골내낭 처치시 안전한 carriers로서 이용될 수 있을 것이다.

핵심이 되는 말: 운반체, 흡수성 콜라겐 스폰지, 삼벽성 골내낭