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Histologic study of absorbable atelocollagen sponge on healing of a fresh extraction socket in the mongrel dog

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치의학과

문 상 식

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흡수성 atelocollagen sponge가 변견의 신선 발치와의
치유에 끼치는 영향에 관한 조직학적 연구

2008년 8월 일

조 선 대 학 교 대 학 원

치 의 학 과

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지도교수 김 병 옥

이 논문을 치의학 박사학위신청 논문으로 제출함.

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치 의 학 과

문 상 식

문상식의 박사학위논문을 인준함

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문상식
(지도교수: 김 병 옥)
조선대학교 대학원 치의학과

치아가 발거된 발치와는 시간이 경과됨에 따라 흡수가 일어나게 된다. 현재 이러한 무치악부위를 임플란트로 수복하기 전에 발치와 함께 발치와를 보존시키려는 술식이 보고되고 있다.

이 연구는 흡수성 atelocollagen sponge가 변견의 신선 발치와의 치유에 끼치는 영향을 평가하기 위해서 시행되었다.

이 실험을 위해서 총 8마리의 개가 이용되었다. 2개의 하악 소구치를 절단한 후 조심스럽게 발치하였다. 실험부위는 임의로 선택되었으며 2군으로 나누었다. 1군; 협측골결손부가 있으며 atelocollagen sponge를 사용한 군, 2군; 협측골결손부가 있으며 atelocollagen sponge를 사용하지 않은 군. 발치와의 협측골은 발치와 총깊이의 2/3까지 삭제하였다. 1주, 4주, 6주, 그리고 10주 후에 개를 희생하였고, 조직학적으로 평가하였다.

1군과 2군 모두 술후 4주후부터 발치와에서 신생골 형성이 관찰되었다. 이 기간에 1군의 근단부위에서 신생골이 더 성숙되어 있음이 관찰되었다. 형성된 신생골의 양은 1군이 2군에 비해 많았다. 또한 1군은 발치와와 주위 골과의 구별이 힘들었던 반면에 2군은 주변골과의 구분이 쉬웠다. 또한, 술후 6주에서도 신생골의 형성은 계속 진행되었으며 그 양은 1군이 2군보다 더 많았다. 술후 10주째에는 골의 재개조과정이 관찰되었으며 이는 1군과 2군 모두에서 나타났다. 이 기간에는 1군과 2군에서 발치와의 형태적인 차이가 발견되었는데, 1군은 '둥근천장'으로 치유되었으나, 2군은 신생골로 채워진 형태로 치유되었다.

이 제한된 연구에서, 흡수성 atelocollagen sponge를 신선 발치와에 위치시키는 것은 신생골 형성을 촉진하고 특히 변연골을 재건할 수 있어 발치와의 치유에 적합한 재료가 될 수 있음을 알 수 있었다. 향후, 신생골 형성양의 향상을 평가하기 위해 조직계측학적 연구가 필요할 것으로 생각된다.

I. Introduction

Following tooth extraction, the edentulous site of the alveolar process will undergo both quantitative and qualitative changes.[1-4] An average of 40% to 60% of original height and width is expected to be lost after tooth extraction, with the greatest loss occurring within the first year.[5]

Especially, in the anterior maxilla, the buccal plate often is thin and friable, consistent bone resorption is found after extraction.[6] Araújo and Lindhe[7] demonstrated that the resorption of the buccal/lingual walls of the extraction site occurred in two overlapping phases; phase 1 (resoprtion of bundle bone and replacement with woven bone) and phase 2 (resorption of the outer surfaces of both bone walls. Araújo et al[8] studied ridge alterations following implant placement in fresh extraction sockets in an experimental study. They concluded that vertical bone loss was more pronounced at the buccal than at the lingual aspect of the ridge and the resorption of the socket walls that occurs following tooth removal must be considered in conjunction with implant placement in fresh extraction sockets.

The supporting bone can be preserved at the time of tooth extraction, or augmented at the time of implantation, using a variety of regenerative materials. Nevins et al[9] concluded that a patient has a significant benefit from receiving grafting materials at the time of extraction in a study of the fate of the buccal wall of extraction sockets of teeth with prominent roots. To prevent bone resorption after extraction, many kinds of techniques and materials have been evaluated, including various graft materials and/or barrier membranes.

Artzi et al[10] reported that the resorbability of porous bovine bone mineral in healing of human extraction sockets could not be recognized in a 9-month period. Froum et al[11] reported that bioglass had a positive effect on socket healing at 6 to 8 months postextraction.

Especially, a deproteinized bovine cancellous bone mineral (Bio-Oss, Geistlich, Switzland) has recently been widely used in the treatment of defects such as

sinus floor elevation procedures, and extraction sockets. Botticelli et al[12] concluded that a high degree of contact was established between the Bio-Oss particles and the newly formed bone at 4 months, and they did not enhance the process of bone formation and defect closure. In some of these human and animal studies, socket preservation techniques apparently were successful, whereas in other researches the benefits of such grafting therapy were less clear. Therefore there is a need to modify the established bone graft techniques that could be quickly replaced by the host bone and easily performed by surgeons.

Collagen is a highly versatile material, and is capable of being prepared into cross-linked compacted solids or into lattice-like gels, and its resorbable forms have been used to dress oral wounds, for closure of graft and extraction sites, and to promote healing.[13]

The purpose of this study was to evaluate the influence of absorbable atelocollagen sponge (TERUPLUG™, TERUMO, JAPAN) on soft and hard tissue healing of a fresh extraction socket in mongrel dogs.

Ⅱ. Materials and Methods

1. Materials

1 to 2-year old mongrel dogs (total 8 dogs) which had been grown in same condition were used. 2 dogs were applied per a cycle.

In this study, absorable atelcollagen sponge (TERUPLUG™, JAPAN) was used to evaluate the healing of a fresh extraction socket in the mongrel dogs

2. Methods

Anesthetic injection was done with Zoletil 50® (0.05 ㎎/㎏, Verbac Lab, France) and Xylazine-HCl (Rompun® 0.15 ㎖/㎏, Bayer, Korea) per each dog. Custom tray impression taking was done by next. After that, flap was elevated and 2nd, 4th premolar were extracted carefully with forcep after hemisection (Fig. 1-2). After measuring the socket depth, buccal bone at the right mandibular socket was trimmed as much as 2/3 its length (Fig. 3-4). The experimental sites (group 1) were 2nd premolar: buccal defect with atelocollagen sponge, and the control site (group 2) were 4th premolar: buccal bone defect without ateolcollagen sponge (Fig. 5a, and 5b). Primary suture was done and stitch-out was done 1 week later (Fig. 6). After surgery, Gentamicin (0.1 ㎖/㎏) was injected as an antibiotics to the all experimental subjects.

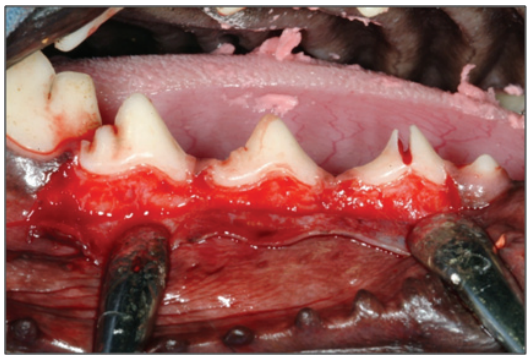


Fig. 1. Flap was raised and hemisected.

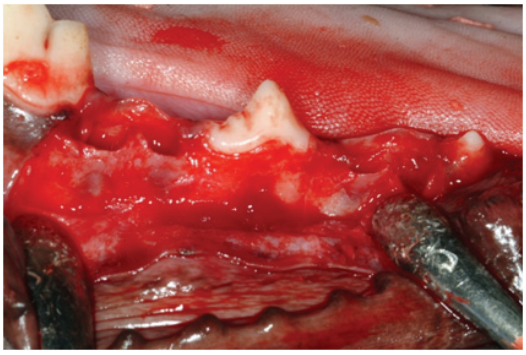


Fig. 2. View of the socket extracted with forcep.

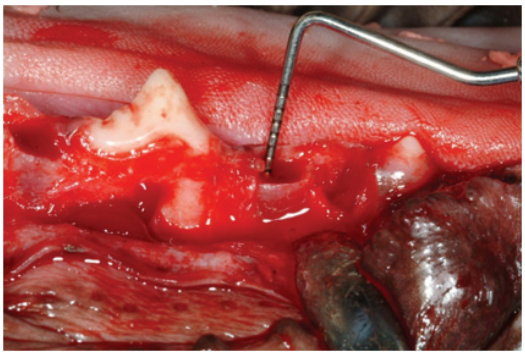


Fig. 3. Measurement of full length of extracted socket in the buccal wall.

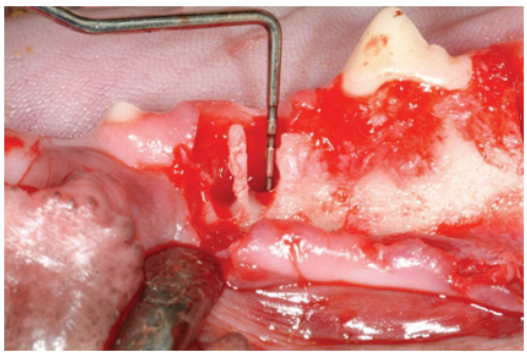
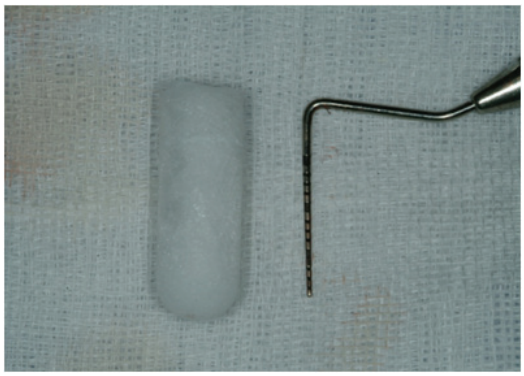


Fig. 4. View of buccal wall trimmed as much as 2/3 extracted socket.



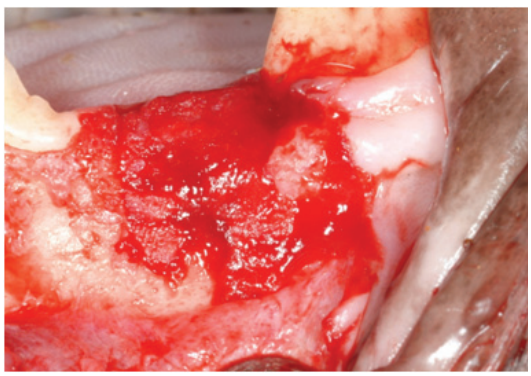


Fig. 5b.

Fig. 5. View of absorbable atelocollagen sponge in this study used.
a: TERUPLUG™. b; View of extraction socket with TERUPLUG™.

Fig. 6. Primary closure was achieved.

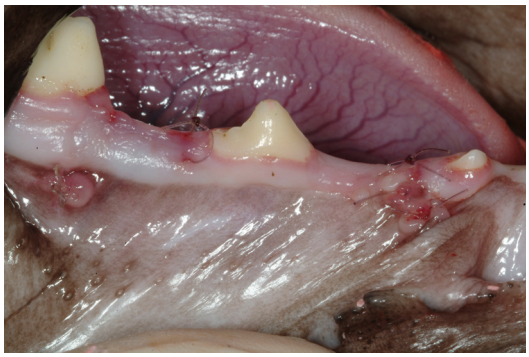


Fig. 7. View of healed site.

3. Tissue processing

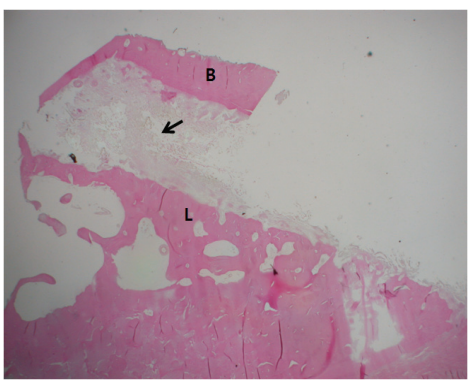
1-, 4-, 6-, and 10-week after surgery, 2 number of dogs were sacrificed at each cycle with using phentobarbital (100 ㎎/㎏) injection in vein. After that, impression taking with custom tray was done. Histologic biopsy sample was taken from 1st premolar distal to 1st molar mesial at both sides. Fixation of dog`s mandible was done with 4% paraformaldehyde and decalcification during 7 months was done with 10% EDTA. The sample was embedded in paraffin and sliced in 6 ㎛ thickness. The sliced section was observed with light microscope after H& E staining.

Ⅲ. Results

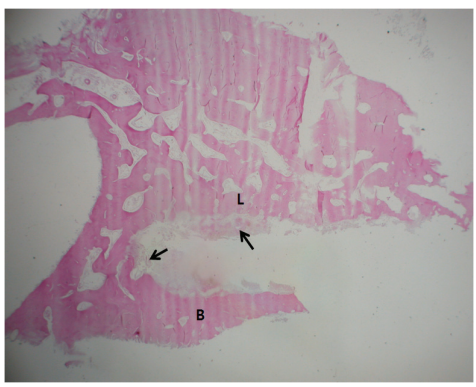
Group 1 showed better result in new bone formation than group 2. These results were same in all cycle groups.

① 1 week after surgery

Group 1 (with TERUPLUG^{TM}) showed lots of inflammatory cells with richly fibroblast-like cells which were held in collagen fiber through entire extraction socket. (Fig. 8a). But Group 2 (without TERUPLUG^{TM}) showed less inflammatory cells and fibroblast-like cells around extraction socket walls. (Fig. 8b)



a



b

Fig. 8. Histologic view of group 1(a) and group 2(b) in 1 week after surgery (X 12). Arrow: granulation tissue with richly fibroblast-like cells held in collagen fibers. B: buccal wall of extraction socket. L: lingual wall of extraction socket.

② 4 weeks after surgery

Group 1 showed new bone formation in the extraction socket. Extraction socket was filled with woven bone. The new bone were more matured in apical regions at this time interval (Fig. 9a). Group 2 also showed new bone formation (Fig. 9b), but the amount of new bone formed was lower than that of group 1. Extraction socket of group 1 was hardly recognized, on the other hand, extraction socket of group 2 was recognized more easily.

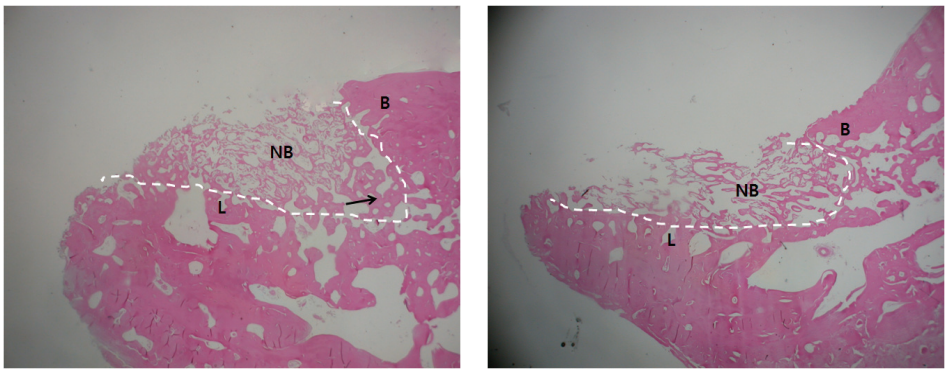
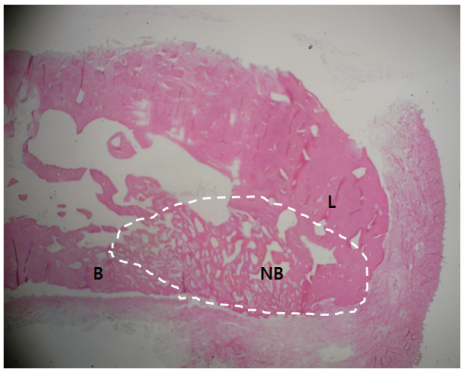


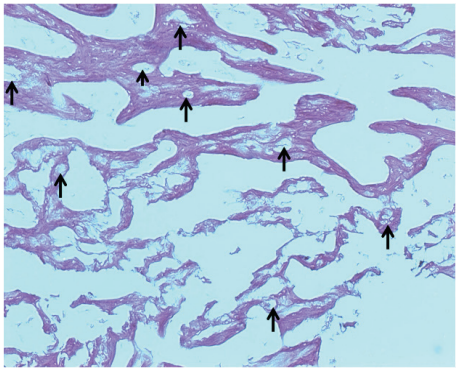
Fig. 9. Histologic view of group 1(a) and group 2(b) in 4 weeks after surgery (X 12). a: arrow: more matured bone in apical region. White line: outline of the extraction socket. B: buccal wall of extraction socket. L: lingual wall of extraction socket. NB: new bone.

③ 6 weeks after surgery

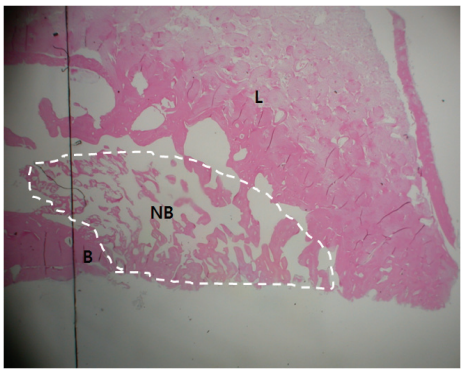
Group 1 showed a lot of new bone which consisted of woven bone and parallel-fibered bone. This specimen exhibited signs of modeling that indicated by the presence of woven bone with its primary osteons in view of high magnification (Fig. 10a and b).

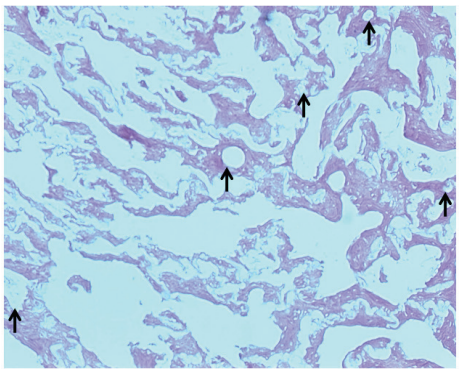
Group 2 also showed complete filling of extraction socket with new bone. But amount of new bone was lower than group 1. New bone layer closed extraction socket, but the socket were recognizable yet. View of high magnification exhibited signs of modeling as well. But primary osteons was not much showing as group 1 (Fig. 10c and d).





10a 10b





10c. 10d.

Fig. 10. Histologic view of group 1 (a and c) and group 2 (b and d) in 6 weeks after surgery. a and c (x 12); white line: outline of the extraction socket. B: buccal wall of extraction socket. L: lingual wall of extraction socket. NB: new bone. b and d (x 100); arrow: primary osteon with woven bone.

④ 10 weeks after surgery

Group 1 showed highly matured, and remodeled bone. A 'dome-shaped' portion of newly formed mineralized bone placed the defect entrance. The shape of new bone was dome-shape which was filled in bone defect completely. The extraction socket was not recognizable and in harmony with old bone. The woven bone has been replaced with bone marrow through osteoclast activity and subsequent bone marrow formation. In high magnification view, secondary osteon within the bone trabeculae was seen with large portion of bone marrow space (Fig. 11a and b).

Group 2 also showed highly matured and remodeled bone. The entrance of the socket was 'closed' by a bridge of mineralized bone that connected the crest of buccal defect and lingual wall. And there was less bone marrow space. In high magnification view, secondary osteon within the bone trabeculae was seen as well (Fig. 11c and d).

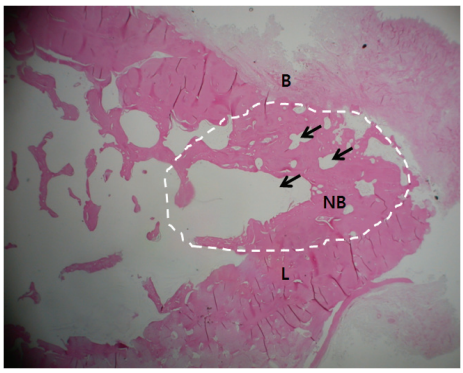


Fig. 11a.

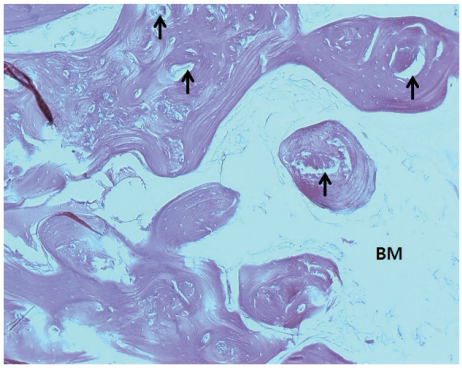


Fig. 11b.

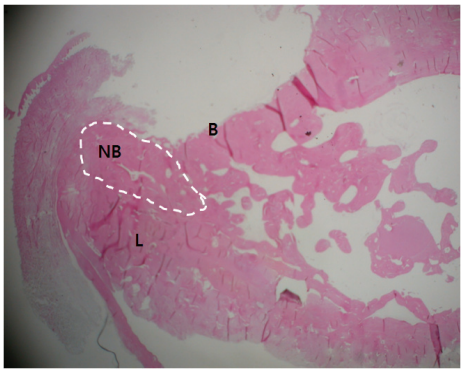


Fig. 11c.

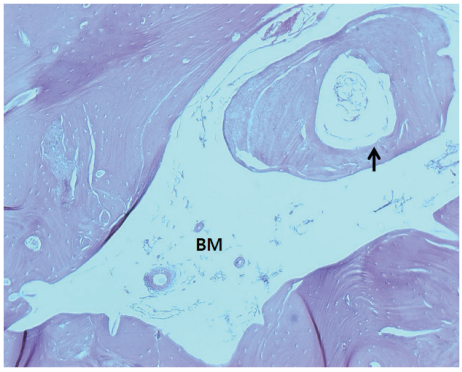


Fig. 11d.

Fig. 11. Histologic view of group 1 (a and c) and group 2 (b and d) in 10 weeks after surgery. a and c (x 12); white line: outline of the extraction socket. B: buccal wall of extraction socket. L: lingual wall of extraction socket. b and d (x 100); arrow: primary osteon with woven bone. BM: bone marrow space.

Ⅳ. Discussion

The rationale for augmentation of the residual alveolar socket at the time of tooth removal such as socket reservation, socket augmentation and ridge preservation depends on the knowledge that alveolar ridge resorption is an unavoidable sequela of tooth loss.[14)]

TERUPLUG™ is made by 85 to 95% collagen type I and 1 to 5% collagen type III. The basic material from which it is fabricated is obtained from American bovine skin dermis. It is primarily made of atelocollagen, to minimize antigenicity, which is cross-linked by heat treatment for biocompatibility, in a sponge block configuration, is shaped for easy placement in the extraction wound, and consists of fibrillar and heat-denatured collagen.[15,16)]

Many studies for socket augmentation have been reported.[17-27)] Sclar[28)] proposed the Bio-Col technique. In his technique, bleeding socket with intact bony walls is grafted to the level of the alveolar crest with Bio-Oss natural bone mineral, and CollaPlug absorbable collagen dressing is condensed over the grafted bone mineral, and then a horizontal mattress suture is loosely secured to prevent its coronal displacement. Recently, Wang and Tsao[29)] published the mineralized bone allograft-plug socket augmentation, and reported promising results. Ajure et al[30)] demonstrated that the placement of Bio-Oss Collagen in fresh extraction sockets promoted hard tissue formation in the crest region, that the elimination of Bio-Oss is a slow process that may require many years, and that Bio-Oss collagen may promote additional hard tissue formation and further enhance the dimension of the crest due to residual Bio-Oss particles. Nevins et al[31)] studied that the fate of the buccal wall of extraction sockets of teeth with prominent roots, and reported that 15 of 19 Bio-Oss treated sockets experienced a reduction of less than 20% of the buccal plate, while as many as 12 of 17 control sites suffered a reduction of more than 20%.

Although there are difference in materials which were used in studies, The

results of this study could are similar as previous studies. In this present study, both group 1 and group 2 showed new bone formation in the extraction socket. Especially, the placement of absorbable atelocollagen in fresh extraction sockets enhanced hard tissue formation in the defected area at 4-week after surgery. At this period, the new bone were more matured in apical regions in group 1. The amount of new bone formed of group 2 was lower than that of group 1. And extraction socket of group 1 was hardly recognized, on the other hand, extraction socket of group 2 was recognized more easily. And Further histometric study may be required to determine difference in quantity of new bone formation.

Lindhe et al[32] described bone tissue formation in tooth extraction sites in dogs. They examined healing of fresh extraction socket without any graft or defect by histologic sections. At 7 days healing, a richly vascularized granulation tissue with large numbers of inflammatory cells. At 14 days healing, the formation of woven bone and provisional connective tissue rich in fibroblast - like cells is formed. At 30 days of healing, the socket is filled with woven bone which contains a large number of cells and primary osteons. At 60 days of healing, woven bone has been replaced with bone marrow through osteoclastic activity and subsequent bone marrow formation. In this study, similar result are revealed. Histologic view at 7 days of this study, granulation tissue with richly fibroblast - like cells are shown. In a group 1, richly collagen fibers was shown due to atelocollagen sponge. At 28 days and 42 days, There are woven bone and parallel-fibered bone with primary osteon like 30 days in literature. And at 70 days after surgery, remodeling process were seen like 60 days.

Previous studies shows graft of Bio-Oss may delay process of remodeling with respect to tissue composition.[19),33-36)] But, there is no difference in this study compared with description of Lindhe et al. So, author could find that atelocollagen sponge did not interfere the remodeling process unlike Bio-Oss. Araujo et al demonstrated that the placement of Bio-Oss Collagen in fresh

extraction sockets promoted hard tissue formation in the crest region. they described that a 'dome-shaped' portion of newly formed mineralized bone occupied the socket entrance in grafted sites, whereas the entrance of the socket was 'closed' by a bridge of mineralized bone that connected the buccal and lingual crests. Like this study, a 'dome-shape entrance in group with atelocollagen sponge and 'closed' entrance in group without atelocollagen sponge. Atelocollagen sponge can hold the blood clots and prevent the collapse of soft tissue above extraction socket like grafted Bio-Oss.

Ⅴ. Conclusion

Within this limited study, the placement of a absorbable atelocollagen sponge in an extraction socket may promote new bone formation and compensate for marginal ridge reconstruction. It suggests that new bone might be formed from the adjacent remaining bone and the periosteum while the atelocollagen sponge maintain the shape of the extraction socket which had buccal bone defect. And it is probably a suitable material for healing of a fresh extraction socket, especially maintenance of it shape. Further histomorphometric research will be needed to observe the enhancement of the dimension of the new bone formation during the healing period.

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논문제목	한글 : 흡수성 atelocollagen sponge가 변견의 신선 발치와의 치유에 끼치는 영향에 관한 조직학적 연구 영어 : Influence of absorbable atelo-collagen sponge on healing of a fresh extraction socket in the mongrel dog				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2008년 6월 일

저작자 : 문 상 식 (서명 또는 인)

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