

# Bone Regeneration in the Extraction Socket Filled with Atelocollagen: Histological and Radiographic Study in Beagle Dogs

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**Purpose:** Alveolar bone develops with tooth eruption and is absorbed following tooth extraction. Various ridge preservation techniques have sought to prevent ridge atrophy, with no superior technique evident. Collagen has a long history as a biocompatible material. Its usefulness and safety have been amply verified. The related compound, atelocollagen, is also safe and displays reduced antigenicity since telopeptides are not present.

**Materials and Methods:** The current study evaluated whether the Rapiderm<sup>®</sup> atelocollagen plug (Dalim Tissen, Seoul, Korea) improves tissue healing of extraction sockets and assessed the sequential pattern of bone regeneration using histology and microcomputed tomography in six beagle dogs. To assess the change of extraction socket, hard tissues were examined 2, 4, 6, and 8 weeks after tooth extraction.

**Result:** The experimental groups showed better bone fill with slow remodeling process compared to the control groups although there was no statistical difference between groups.

**Conclusion:** The atelocollagen seems to have a tendency to slow bone remodeling in the early phase of healing period and maintain remodeling capacity until late phase of remodeling. Also, use of atelocollagen increased the bone-to-tissue ratio compared to healing of untreated extraction socket.

**Key Words:** Atelocollagen; Bone regeneration; Bone remodeling; Tooth extraction

## Introduction

Following tooth loss, the alveolar ridge undergoes

substantial bone modeling that involves marked reduction of ridge dimensions<sup>1</sup>. Ridge contraction from the buccal side is more pronounced than the

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one from the lingual side. Hard tissue modeling and remodeling following tooth extraction have been investigated in dogs<sup>2,3</sup>. In these studies, the extraction socket was initially filled by a coagulum that was replaced with granulation tissue, provisional connective tissue and woven bone. This immature hard tissue was replaced with lamellar bone and bone marrow<sup>2,3</sup>. Morphological changes of the alveolar process following tooth extraction in pre-clinical and clinical studies involve loss of vertical and horizontal ridge dimensions.

As the edentulous ridge shape changes, alveolar bone quality declines<sup>2</sup>. Various ridge preservation techniques have been assessed to prevent ridge atrophy<sup>3,4</sup>. However, the extraction socket grafting materials might interfere with the normal bone healing process<sup>5</sup>. Bio-Oss<sup>®</sup> (Geistlich Pharma AG, Wolhusen, Switzerland) is a common graft material for bony defects. Even with a high degree of contact between the Bio-Oss<sup>®</sup> particles and newly formed bone for 4 months, bone formation was not beneficially enhanced<sup>6</sup>. While ridge preservation using free-dried bone allograft (FDBA) and collagen membrane reportedly improved ridge height and width dimension, FDBA particles were evident histologically after 6 months of ridge preservation<sup>7</sup>.

These results have shifted the focus on the characteristics of rapid resorption in bone graft materials, rather than on the scaffold material itself. Collagen is rapidly absorbed and is easy to use<sup>8</sup>. For the past decade, collagen has been one of the widely used biomaterials in medical applications including resorbable surgical sutures, hemostatic agents, and wound dressings due to its biological and physicochemical properties<sup>9</sup>. Collagen has been studied by a number of authors for the purpose of bone tissue regeneration<sup>9-11</sup>. In the dental fields, collagen membrane has shown favorable regenerative capacities due to their excellent cell affinity and biocompatibility<sup>12</sup>. It is conceivable that the physicochemical properties of collagen might promote wound healing in extraction sockets.

Atelocollagen is prepared by removing telopeptides from collagen. Telopeptides are the main antigenic sites of collagen. This procedure improves the usefulness of collagen as a biomaterial because of the reduced antigenicity and increased safety of the material. Atelocollagen forms fiber-like natural collagen in the body, which persists for a long time without dissolution<sup>13</sup>.

Despite these advantages, little is known of bone regeneration of the extraction socket filled with atelocollagen. The current study evaluated whether the Rapiderm<sup>®</sup> atelocollagen plug (Dalim Tissen, Seoul, Korea) improves bone tissue regeneration of extraction sockets and assessed the sequential pattern of bone regeneration in dogs using histology and microcomputed tomography (microCT) examinations.

## Materials and Methods

### 1. Surgical Procedures

The study was approved by the Institutional Review Board of Kyung Hee University Medical Center, Seoul, Korea (approval number: KHMC-IACUC 13.017). Six beagle dogs aged about 1 year, weighing 10~12 kg, were used. To assess the change of extraction socket, hard tissues were examined 2, 4, 6, and 8 weeks after tooth extraction. Tooth extraction sequence was the left 4th premolar (8 weeks), right 4th premolar (6 weeks), left 3rd premolar (4 weeks), and right 3rd premolar (2 weeks). Each dog was anesthetized with an intravenous injection of Zoletil 50 (0.05 mg/kg; Virbac, Carros, France) and Rompun (0.15 mg/kg; Bayer HealthCare, Leverkusen, Germany). The full thickness flap was elevated to reveal the alveolar crest, and the premolar was hemi-sectioned. Each root was carefully extracted using forceps. To ascertain the healing enhancement of atelocollagen, a Rapiderm<sup>®</sup> plug was inserted in the mesial extraction socket of each dog. The distal socket lacking inserted collagen matrix was the

control. The flaps were replaced and sutured using 3-0 Vicryl (Ethicon, Somerville, NJ, USA). Suture material was removed 1 week after tooth extraction. Gentamicin (0.1 ml/kg) and diclofenac (0.1 mg/kg) were injected intramuscularly for 2 days postoperatively after every tooth extraction. After 2 weeks from the last tooth extraction (right 2nd premolar), the subjects were euthanized with an overdose of Zoletil and saturated KCl and perfused with fixative containing 10% formalin through carotid arteries.

## 2. MicroCT Examination

Each removed mandible was dried and microCT imaged using a Skyscan model 1173 device (Kartuizersweg, Kontich, Belgium) at a voxel resolution of 14.91  $\mu\text{m}$  with a source voltage of 130 kV, source current of 60  $\mu\text{A}$ , and exposure time of 500 ms. To minimize beam hardening artifacts, a 1 mm aluminum X-ray beam filter was used to attenuate soft X-rays at the source. The projections were reconstructed using the built-in software program (NRecon Reconstruction; Kartuizersweg). Each extraction socket was inspected concerning the bucco-lingual aspect of CT images at the lowest point of the socket. Cortical density of lamina dura was considered as old bone. Total tissue volume (TV) and bone volume (BV) were calculated using image analyzing software. Using the software, we could obtain the value of three dimensional volume from microCT. Each section of CT images were reconstructed to three dimensional structure and volume were calculated after boundary was set.

## 3. Histological Evaluation

Specimens for histological analysis were fixed in 10% formalin for 1 week and prepared for sectioning. The specimens were fixed using epoxy resin and sectioned in the buccolingual aspect with a thickness of 5  $\mu\text{m}$ . Histological examinations were performed after staining with H&E and Goldner's trichrome using a light microscope. Histomorphometrical analyses and microscopic observations were performed by one experienced investigator blinded to the specific experimental conditions.

## 4. Statistical Analysis

MicroCT results were compared to each experimental group and the control group concomitantly for the series of extraction sockets. Mann-Whitney U test was conducted to find statistical significance using IBM SPSS Statistics version 22.0 for Windows (IBM Co., Armonk, NY, USA). A P-value <0.05 was considered significant.

## Result

Atelocollagen sponge insertion did not differ from control concerning healing time ( $P=0.240$ ) (Table 1). However, the BV-to-TV ratio (BV/TV) 4 weeks after surgery was increased compared to 2 weeks postoperatively and were statistically significant in both groups ( $P=0.001$ ) (Fig. 1). At 4 weeks after surgery, BV/TV was increased somewhat in both groups, especially with atelocollagen treatment.

### 1. MicroCT

BV/TV increased after surgery in both the

**Table 1.** BV/TV at each time point for the experimental and control groups

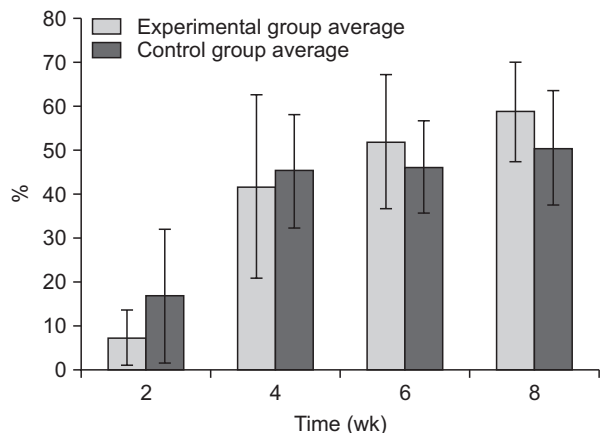
	2 wk (n=6)	4 wk (n=6)	6 wk (n=6)	8 wk (n=6)
Experimental group	7.2±6.4	41.7±20.8	52.0±15.3	58.8±11.3
Control group	16.7±15.3	45.3±12.9	46.2±10.6	50.5±13.1

BV: bone volume, TV: tissue volume.

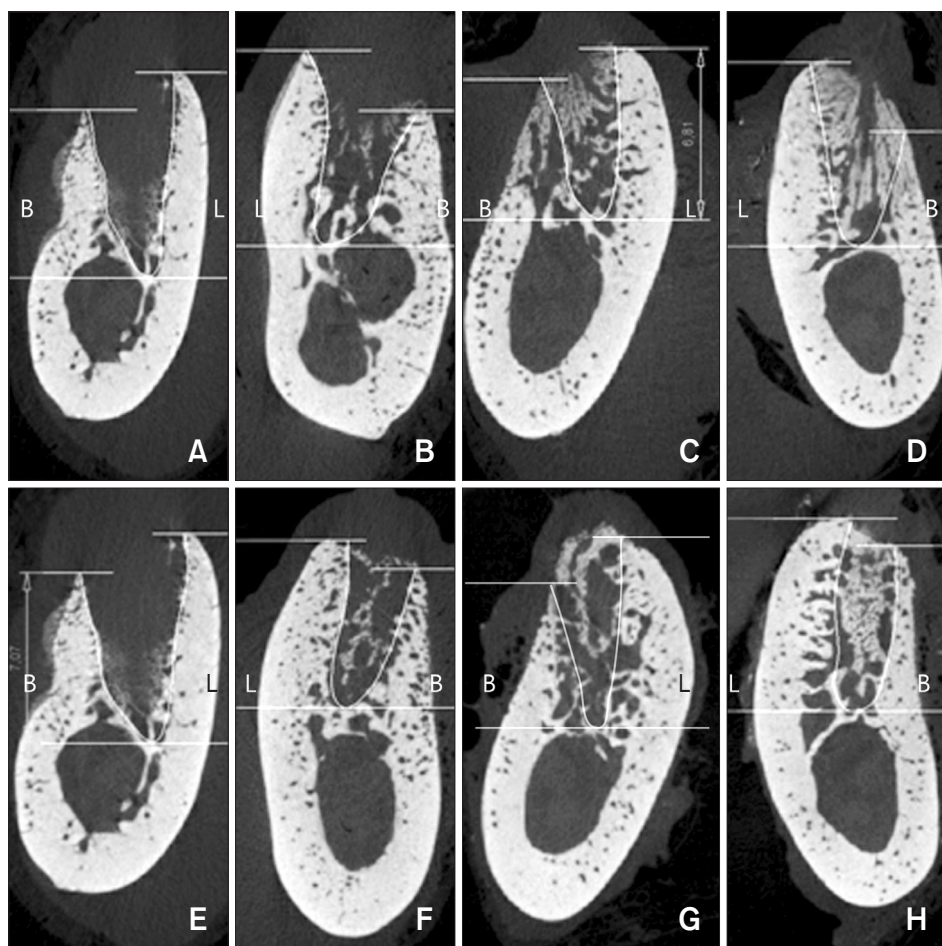
Values are presented as mean±standard deviation.

experimental and control groups. Two weeks after extraction (Fig. 2A, E), hard tissue formation was evident around the apical region in both experimental and control groups. Homogenous

soft tissue filled the middle and coronal third of both sockets. Lingual bone height was slightly less than the buccal height. Four weeks after extraction (Fig. 2B, F), bone formation reached the coronal third. An increased trabecular pattern and higher bone density was observed in the experimental group. The trabecular pattern was more even in the control group, with woven bone bridge evident. Bucco-lingual difference of the alveolar bone height was larger in the experimental group. Six weeks after extraction (Fig. 2C, G), BV/TV of the extraction socket was transposed between the control and experimental groups. BV/TV of the experimental and control group was  $52.0 \pm 15.3$  and  $46.2 \pm 10.6$ , respectively, without significance. The control group displayed a more mature pattern than the experimental group. Eight weeks after extraction (Fig. 2D, H), BV/TV of both groups



**Fig. 1.** Comparison of bone volume/tissue volume (percent bone volume) at different times in each group.



**Fig. 2.** Cross-sectional micro-computed tomography findings at 2 weeks (A, E), 4 weeks (B, F), 6 weeks (C, G), and 8 weeks (D, H). The upper and lower plots display results for the experimental group and control group, respectively. B: buccal, L: lingual.

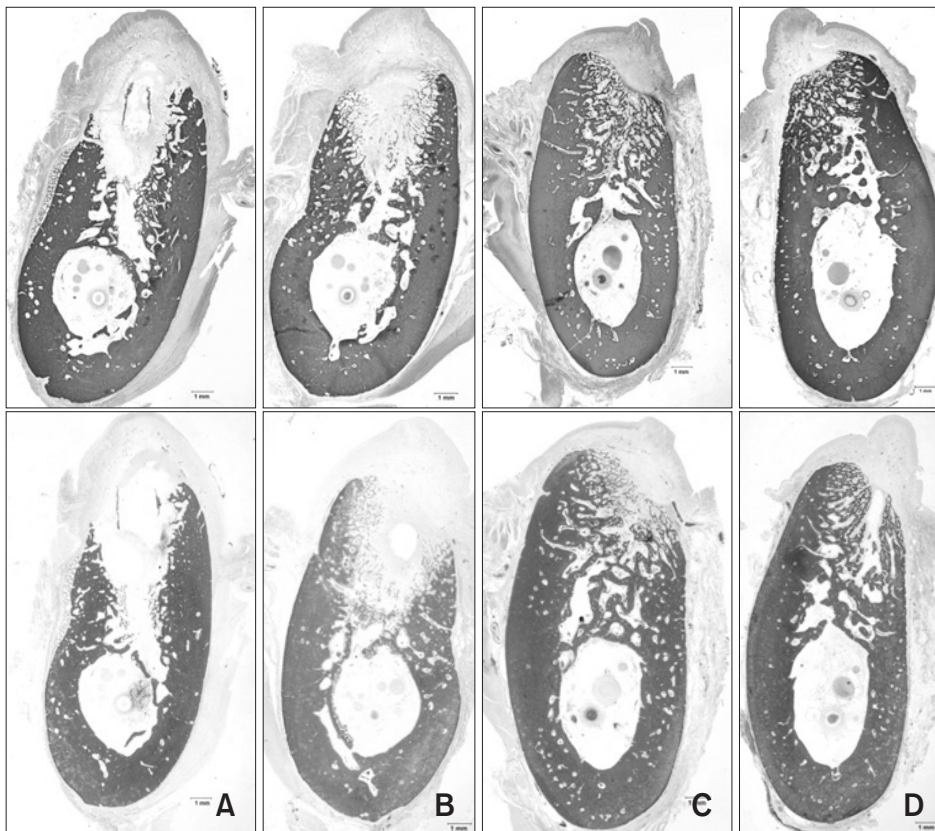
increased compared to BV/TV at 6 weeks. The trabecular pattern of the control group was nearly indistinguishable from old bone. Otherwise, the patterns of experimental group demonstrated immature woven bone.

## 2. Histological Examination

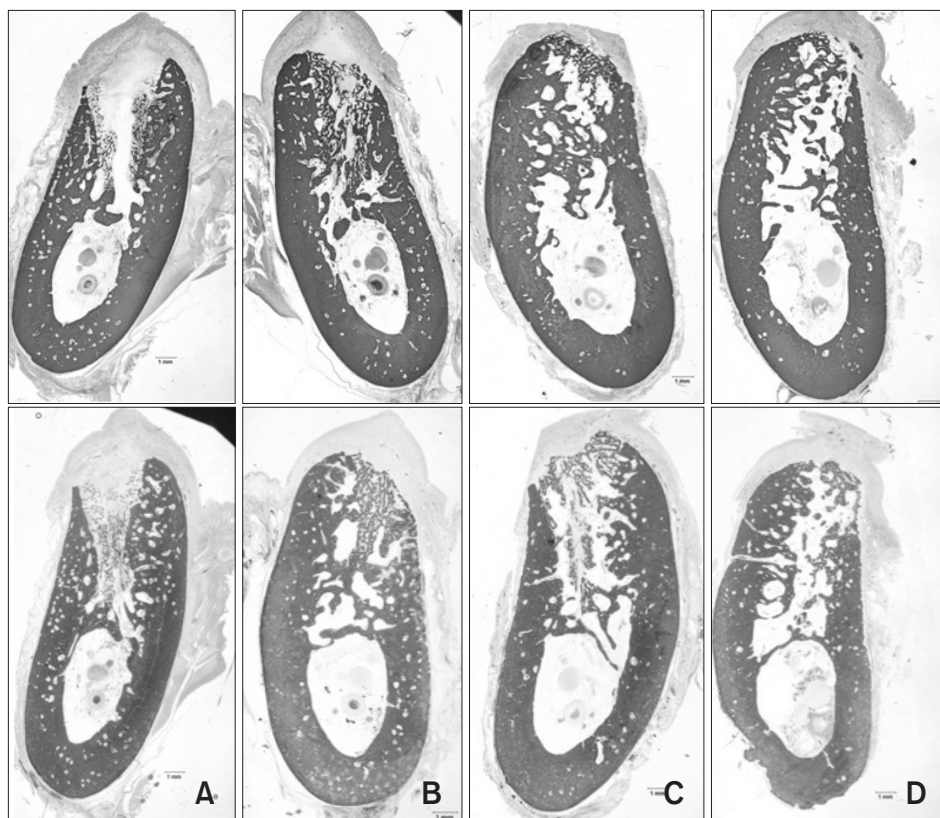
The experimental group showed less *de novo* bone formation than the control group at 2 and 4 weeks after extraction (Fig. 3). After 6 weeks, the control group showed more mature bone portion. In contrast, the experimental group consisted of woven bone rather than lamellar bone (Fig. 4C). After 8 weeks from surgery, woven bone of the control group was almost replaced by lamellar bone and it was difficult to distinguish old bone and new bone (Fig. 4D). On the other hand, experimental group was almost entirely composed of woven bone.

## Discussion

This study was designed to evaluate the serial change of extraction socket with/without atelocollagen insertion using histologic and microCT analyses. We had expected that the treatment would produce a significant beneficial effect on alveolar bone repair because of the accumulation of growth factors and cells derived from the adjuvant bone marrow. Extraction sockets filled with collagen matrix showed better bone fill with slow remodeling process compared to the unfilled control extraction sockets after 6 and 8 weeks of healing. Collagen are the most abundant elements in the extracellular matrix of bone and may act as a reservoir of osteogenic cells and many local factors<sup>9</sup>. Atelocollagen used in the study may have served as a reservoir for osteogenic cells in the extraction sockets. However, bone regeneration of the experimental groups was not significantly higher than the control groups.



**Fig. 3.** Histologic findings from experimental groups at 2 weeks (A), 4 weeks (B), 6 weeks (C), and 8 weeks (D). The upper and lower plots display results for Goldner's trichrome stained samples ( $\times 10$ ) and H&E stained samples ( $\times 10$ ), respectively.



**Fig. 4.** Histologic findings from control groups at 2 weeks (A), 4 weeks (B), 6 weeks (C), and 8 weeks (D). The upper and lower plots display results for Goldner's trichrome stained samples ( $\times 10$ ) and H&E stained samples ( $\times 10$ ), respectively.

Better bone healing using an atelocollagen matrix has been reported previously<sup>14-17</sup>. Presently, BV/TV increased significantly between 2 and 4 weeks in both the experimental and control groups. In both groups, BV/TV gradually increased thereafter until 8 weeks from extraction. The pattern differed, however. In the experimental group, BV/TV increased steadily, while in the control group BV/TV increased rapidly until 4 weeks and nearly plateaued until 8 weeks. This period represents the remodeling phase (biologic process) of the extraction socket. Although the two groups showed a similar healing pattern, insertion of atelocollagen seemed to slow osteoclastic activity and delay remodeling. Hamanishi et al.<sup>18</sup> reported that atelocollagen membrane coverage inhibited cartilage repair. They postulated that the cause of the inhibition was blockage of the entry of cells and factors derived from the synovium by the atelocollagen plug, which markedly increased pressure in the chondral defect. This prevented

efflux of cells and growth factors from the bone marrow. Alternately, the high pressure may interfere with migration of cells from the bone marrow, which would have delayed remodeling.

Presently, extraction sites with atelocollagen graft showed pronounced resorption of the buccal bone wall. After 8 weeks of healing following tooth extraction, the loss of alveolar ridge dimension was similar between the treatment and control groups. Socket preservation with atelocollagen alone does not seem to be effective enough to prevent dimensional ridge alterations, although the number of sample sites was small. This insufficiency may reflect the fast resorption of collagen compared to other biomaterials.

The benefit gained by inserting atelocollagen in the extraction socket may require gentle insertion, to allow infiltration of cells derived from adjuvant bone marrow. The mechanism of atelocollagen remains unclear and will require a long-term study.

Atelocollagen can be used as a carrier for the

delivery of various proteins and genes<sup>9)</sup>. Applications of atelocollagen as a carrier for tissue engineering including the effect of atelocollagen combined with various proteins, such as bone morphogenic protein, on ridge preservation are underway or anticipated.

Limitations of the present study include small sample size and relatively short follow-up period.

## Conclusion

The atelocollagen seems to have a tendency to slow bone remodeling in the early phase of healing period and maintain remodeling capacity until late phase of remodeling. However, there was no statistical significance between the control and the experimental group. Use of atelocollagen increased the bone-to-tissue ratio compared to healing of untreated extraction socket. The use of atelocollagen as a carrier of various bioactive proteins including bone morphogenic proteins for socket preservation should be studied further.

## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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