Ridge preservation of a novel extraction socket applying Bio-Oss® collagen: An experimental study in dogs

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Abstract  Background: Bio-Oss® collagen (BC) has been used in clinical applications for years but the ridge preservation property of BC remains controversial. There is no animal model accurately simulates the extraction socket in people. The aim of this study was to assess the ridge preservation of a novel extraction sockets with a thin buccal plate using BC. Materials and methods: Two beagle dogs were used to assess the characterization of the novel extraction socket. The width and height of the socket were measured and biopsies of the socket were collected for histologic examination. Four beagle dogs were used to assess the ridge preservation property of BC. BC was placed in the socket and socket left untreated was set as control group (CT). Cone-beam computed tomography analysis, histological examination, and micro-CT analysis were used to evaluate the ridge preservation. Results: The novel extraction socket had obvious larger volume with a markedly narrow buccal wall than mandible extraction sockets. At 12 weeks, the width of the crest of the alveolar ridge preservation ratios was 34% for the CT and 82% for the BC. BC group had larger socket volume compare to CT group. BC group had a significant higher bone density in the middle and apical areas of the alveolar bone. Socket placed with BC showed significantly less vertical bone loss compared with CT group. Conclusion: Extraction site with a significantly larger dimension and a very thin buccal plate was established. Extraction sockets filled with BC exhibit excellent maintenance of alveolar bone volume.
Introduction

Extensive experimental evidence from both animal and human studies suggested that it is unavoidable for a severe alveolar bone resorption to occur after tooth extraction.1-4 Significant loss of the alveolar ridge occurs within six months after tooth extraction, averaging 3.8 mm in width and 1.24 mm in height.5 The resorption of the alveolar bone is not conducive for future implant treatment and may result in devastating esthetical consequences.6,7 Ridge preservation is a procedure aiming to control alveolar bone resorption following tooth extractions.8 Ridge preservation involves the application of different bone graft materials to fill the extraction socket.9 Numerous studies demonstrated that ridge preservation could preserve more bone volume when compared with tooth extraction alone.10-12 However, there is no report showing that the bone volume of the extraction socket could be completely preserved and ridge preservation technique does not completely meet the clinical demand.13 Various bone graft materials have been used for ridge preservation.14 Bone grafts such as hydroxyapatite, bioactive glass, deproteinized bovine bone mineral (DBBM), human demineralized bone matrix and others are effective for maintaining the bone volume of extraction socket.15-17 However, there is no evidence to suggest the superiority of one bone graft over others.18 Bio-Oss® collagen (BC, Geistlich, Wolhusen, Switzerland) is a kind of xenogenic bone substitute material consists of 90% DBBM extracted from cattle and 10% highly purified porcine collagen matrix.8 BC possesses osteoconductive and biocompatibility properties and has been currently used in the clinical applications.19 However, there are concerns over the residence of DBBM particles that may influence the new bone formation.18,20 More studies are needed to evaluate the ridge preservation property of BC.

Most animal studies so far evaluate extraction socket on mandible (with very thick buccal wall), which does not reflect clinical circumstances where majority ridge preservation are needed on maxilla and at least parts of the buccal wall is missing or will resorb during socket remodeling.18,19 Today, there is no animal model that accurately simulates the extraction socket in people.

Based on these concerns, the aim of the present study was to assess the ridge preservation result of a novel extraction socket on maxilla with a thin buccal plate using BC. In this study, the characterization of the novel extraction socket and the ridge preservation property of BC were the main focus.

Material and methods

The research protocol was approved by the Ethics Committee of School and Hospital of Stomatology, Wuhan University, China. Animal experiments followed the National Institutes of Health guide for the Care and Use of Laboratory Animals. Six female beagle dogs about 12 months old and weighing about 10 kg were used for the study. The dogs were anesthetized with intramuscular administered with ketamine (20 mg/kg) and xylazine hydrochloride (1 mg/kg). In addition, the dogs received local anesthesia with articaine with adrenaline (1:100,000).

Characterization of the novel extraction socket

The novel extraction socket was located on the distal roots of the 4th maxillary premolar (4P4). 4P4 of two beagle dogs were sectioned into two parts with fissure burs. The distal roots of 4P4 were extracted atraumatically while the mesial portion was retained. The width and height of the sockets were measured with a periodontal probe and dogs were killed by injecting an overdose of xylazine hydrochloride. Block biopsies of the sockets were collected for histologic examination. Extraction socket of the distal roots of 3rd mandibular premolars (3P3), the most commonly studied mandible extraction sockets, were used as control.

Ridge preservation surgical protocol

In four dogs, 4P4 were sectioned hemisected and the distal roots were atraumatically removed. Sockets were allocated to the following two experimental groups: (1) no treatment (CT, control group) (2) BC group. In BC groups, BC were placed to fill the socket while in CT group, blood clot was killed by injecting an overdose of xylazine hydrochloride. Block biopsies of the sockets were collected for histologic examination. Extraction socket of the distal roots of 3rd mandibular premolars (3P3), the most commonly studied mandible extraction sockets, were used as control.

Cone-beam computed tomography (CBCT) analysis

The CBCT images were taken with a CBCT machine (NewTom, Verona, Italy). After general anesthesia, beagle dogs underwent a low-dose CBCT imaging with voxel size 0.300 mm, tube voltage of 110 KV, current of 0.56 mA, and exposure time of 3.6 s. A comparison was made between the 2 weeks postoperative CBCT radiographs and the 12 weeks postoperative CBCT radiographs. The alveolar bone width (B-P, buccal—palatal width) was measured at the top of the center alveolar ridge.

Histological examination

The biopsies were processed according to the methods described by Araujo.19 They were dehydrated in increasing concentrations of ethanol and embedded in resin. Serial
sections were cut in the B–P plane. The microtome was set at 10 µm. For each biopsy, three sections stood for the central part of the extraction socket, about 30 µm apart, were selected for histological examination. The sections were stained with Goldner trichrome staining, allowing the distinct classification of mineralized bone (green), non-mineralized osteoid (red-orange). Total socket area and the area occupied by mineralized bone (MB), non-mineralized osteoid (NMO), and bone marrow (BM) in the socket were measured. In addition, Bio-Oss® like particles (BP) in BC group were also measured.

Micro-CT analysis

The socket samples were scanned with micro-CT (Bruker Skyscan-1176, Kontich, Belgium) at 82 kV, with 18 µm pixel size. The projection image data were reconstructed using Skyscan NRecon software (Bruker, Kontich, Belgium) to create 3D images and analyzed using the Mimics 21.0 (Materialise, Leuven, Belgium). Bone density were measured as described in other study. A line between mesial and distal wall was drawn and the socket was divided into three parts at the apical, middle and coronal area with the same vertical height. Bone density of these areas were evaluated through variations of grayscale varied from 0 (transparent) to 255 (opaque) using the Mimics 21.0 software. Vertical bone loss was measured according to the methods described before. Briefly, a horizontal base line was drawn on the basis of bone height of the flanking teeth. The vertical bone loss was then measured at the center positions of the socket (B–P plane) with respect to the horizontal base line.

Results

Result of the characterization of the extraction socket

Compared to \(3P_3(A)\), the extraction socket of \(4P_4(B)\) had obvious larger volume. The buccal bone wall of \(4P_4\) was markedly narrow than \(3P_3\) counterpart (Fig. 2). The mean socket width and socked depth of \(4P_4\) was \(5.2 \pm 0.2\) mm and \(11.8 \pm 0.6\) mm. The corresponding value for \(3P_3\) was \(3.2 \pm 0.1\) mm and \(8.5 \pm 0.2\) mm. There was a significant difference \((P < 0.05)\) between the two groups (Fig. 2, C).

CBCT analysis result

The CBCT results showed that BC group result in greater bone volume compared with CT group. The structure of alveolar bone in CT group was clear high-density edges and internal low-density shadow (12 weeks). The original buccal bone wall disappeared at 12 weeks in two groups (Fig. 3). The width of the crest of the alveolar ridge (WD) was presented in Table 1.

Data analysis

Statistical analyses were performed using the SPSS version 25 software (IBM Software, New York, USA). The values for the parameters were measured and presented as the means ± standard deviation. Two sample t test was used to analyze experimental data.
Histological examination result

Because this study was performed in a novel socket with very thin buccal wall, the original buccal bone wall in all groups got lost at three months after tooth extraction (Fig. 4). Unmineralized matrix could be found on the region of bone marrow (Fig. 5). Tissue proportions of all groups were reported in Fig. 6.

The socket of CT group was composed of mineralized bone (MB) (55.6% ± 12%) in the peripheral and bone marrow (BM) (41.4% ± 12.3%) in the central (Figs. 4 and 6). The socket in BC group was filled by MB (51.1% ± 2.5%), bone marrow (BM) (30.4% ± 3.1%), and remnants of the BP (14.7% ± 2.8%) (Fig. 6). The majority of the spaces between BP were the newly formed bone (Figs. 4 and 5).

BC group had obvious larger socket volume compared with the CT group. No significant difference of MB% could be found between two groups. The values of BM% in BC group had a significant difference compared with the CT group ($P < 0.05$).

Micro CT analysis result

The original buccal wall in two groups disappeared 12 weeks after tooth extraction. The bone density of CT group was $88.9 ± 10.9$ (coronal area), $32.3 ± 14.9$ (middle area) and $34.4 ± 27.6$ (apical area). The bone density for BC group was $71.4 ± 7.0$ (coronal area), $69.4 ± 12.8$ (middle area) and $78.6 ± 12.4$ (apical area). The vertical bone loss of CT group was $2.2 ± 0.7$ mm. The corresponding value for BC was $1.1 ± 0.4$ mm (see Fig. 7).

BC group had a significant higher bone density in middle and apical area of the alveolar bone compare with CT group. The vertical bone loss of BC group was significant lower ($P < 0.05$).
The objective of this study was to evaluate the ridge preservation result of a novel extraction sockets with a very thin buccal plate using BC. A number of studies performed on socket with thick buccal plate suggest positive outcome of ridge preservation. However, sites with a thick buccal plate (more than 1 mm) usually receive immediate implant placement. Those sockets with very thin buccal plate or partial missing buccal wall will need ridge preservation. In our study, we successfully established a novel extraction socket. The sockets have a significantly larger dimension and thinner buccal plate compared with the most commonly studied mandible extraction sockets. The buccal bone wall of this novel extraction socket resorbed three month later which is highly consistent with clinical findings. The novel extraction socket left untreated lead to severe horizontal and vertical bone resorption which is very similar to the situation in humans. These results suggest that this novel extraction socket highly reflects clinical circumstances and is suitable for ridge preservation research.

An adequate alveolar ridge maintenance to accommodate the implant is a prerequisite for the successful dental implantation. In our study, BC is able to maintain 82% of alveolar bone width while CT group is only able to maintain 37%. These results are like the results of other research that sockets treated with BC demonstrated a loss of less than 20% of the alveolar bone width in 79% of test sites, whereas in untreated sockets 79% of extract sites showed a loss of more than 20% of the alveolar bone width. In the cases with considerable narrow alveolar ridge, bone increment operations may be required for implantation.

Our histology study showed that the socket of no treat control group at the 12-week was composed of new formed mineralized bone in the peripheral and bone marrow in the central. This finding is highly consistent with other studies. This phenomenon is referred to as "corticalization" of the socket which means after a series of reconstruction events the cortical bone wall was eventually developed. Socket filled with BC led to obviously increased bone dimension when compared to the no treatment group. This is also in agreement with the histology study of other researches. Some studies showed the adhesion of osteoblasts to the BC surface which demonstrated that BC could serve as a scaffold for osteogenic cells. However, there are concerns over the residence of DBBM particles that may influence the new bone formation. In our study, there is no significant

### Table 1

|       | CT        | BC        |
|-------|-----------|-----------|
|       | 2 weeks   | 12 weeks  |
|       | 2 weeks   | 12 weeks  |
|       | 2 weeks   | 12 weeks  |
|       | 2 weeks   | 12 weeks  |
| 2 weeks | 4.8 ± 0.2 | 5.0 ± 0.2 |
| 12 weeks | 1.7 ± 0.1 | 4.1 ± 0.3* |
| Ratio  | 0.3 ± 0.04| 0.8 ± 0.1* |

*P < 0.05.

a Control.
b Bio-Oss® collagen.

**Discussion**

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Figure 4 Result of histological examination (A: control group, B: Bio-Oss® collagen group). The black arrows indicate the contours of the alveolar ridge. Original magnification: 1×.

Figure 5 Highly magnified of the buccal side of the alveolar ridge (A: control group, B: Bio-Oss® collagen group). The arrows indicate the non-mineralized osteoid (red-orange). BM: bone marrow, BP: Bio-Oss® like particles. Original magnification: 20×. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
difference of MB% could be found between BC (51.1%) and CT (55.6%). This suggests that the amount of new bone formation is not influenced by the residence of DBBM particles in this novel extraction socket.

Micro CT result suggested that socket filled with BC had a significantly higher bone density in the middle and apical area of the socket compared with control group. The higher bone density is not only because of BC but also the new formed bone in the area. Implant placed in this socket may have a better stability since a high bone density is contributory to the primary stability of implantation. Our study showed that socket left untreated showed significantly more vertical bone loss compared with BC. It is consistent with other studies. Severe vertical bone loss can pose serious problems such as increased crown-to-implant ratio and higher aesthetics concerns.

Space maintenance for bone regeneration is one of the key factors for bone regeneration. BC showed a good ability to maintain alveolar bone volume. This is partly because BC offer stability for the coagulum can avoid volume reduction and BC particles act as a scaffold for new bone formation. On the contrary, blood clot in CT could not maintain the space after buccal wall absorption. This may partially explain the severe alveolar bone resorption that occur in CT. Present study showed that 14.7% of the

![Figure 6](image1.png)

**Figure 6** Compositions (%) of the different tissues. CT: control group, BC: Bio-Oss® collagen group, BP: Bio-Oss® like particles, BM: bone marrow, MB: mineralized bone, NMO: non-mineralized osteoid.

![Figure 7](image2.png)

**Figure 7** The result of micro-CT analysis. A: B–P plane of CT group, B: B–P plane of BC group, C: the result of the bone density, D: the result of the vertical bone loss. CT: control group, BC: Bio-Oss® collagen group. *P < 0.05.
socket volume are composed of remnants of the BP after 3 months. The fate of these remnants and long-term effects of these remnants on implant loading requires a further study.

In conclusion, a novel extraction socket with a significantly larger dimension and a very thin buccal plate was established for the study. BC filled in this novel extraction sockets exhibited excellent maintenance of alveolar bone volume.

Ethics statement

The present study is a part of the project that approved by the Ethics Committee of School and Hospital of Stomatolog, Wuhan University (Ethical approval code: 2020A39). All animal care and use were performed in accordance with the ethical guidelines of the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Authorship

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Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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