Comparison of Different Periodontal Healing of Critical Size Noncontained and Contained Intrabony Defects in Beagles

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Abstract

Background: Regenerative techniques help promote the formation of new attachment and bone filling in periodontal defects. However, the dimensions of intraosseous defects are a key determinant of periodontal regeneration outcomes. In this study, we evaluated the efficacy of use of anorganic bovine bone (ABB) graft in combination with collagen membrane (CM), to facilitate healing of noncontained (1-wall) and contained (3-wall) critical size periodontal defects.

Methods: The study began on March 2013, and was completed on May 2014. One-wall (7 mm × 4 mm) and 3-wall (5 mm × 4 mm) intrabony periodontal defects were surgically created bilaterally in the mandibular third premolars and first molars in eight beagles. The defects were treated with ABB in combination with CM (ABB + CM group) or open flap debridement (OFD group). The animals were euthanized at 8-week postsurgery for histological analysis. Two independent Student's *t*-tests (1-wall [ABB + CM] vs. 1-wall [OFD] and 3-wall [ABB + CM] vs. 3-wall [OFD]) were used to assess between-group differences.

Results: The mean new bone height in both 1- and 3-wall intrabony defects in the ABB + CM group was significantly greater than that in the OFD group (1-wall: 4.99 ± 0.70 mm vs. 3.01 ± 0.37 mm, $P < 0.05$; 3-wall: 3.11 ± 0.59 mm vs. 2.08 ± 0.24 mm, $P < 0.05$). The mean new cementum in 1-wall intrabony defects in the ABB + CM group was significantly greater than that in their counterparts in the OFD group (5.08 ± 0.68 mm vs. 1.16 ± 0.38 mm; $P < 0.05$). Likewise, only the 1-wall intrabony defect model showed a significant difference with respect to junctional epithelium between ABB + CM and OFD groups (0.67 ± 0.23 mm vs. 1.12 ± 0.28 mm, $P < 0.05$).

Conclusions: One-wall intrabony defects treated with ABB and CM did not show less periodontal regeneration than that in 3-wall intrabony defect. The noncontained 1-wall intrabony defect might be a more discriminative defect model for further research into periodontal regeneration.

Key words: Alveolar Bone Loss; Animal Experimentation; Bone Substitute; Guided Tissue Regeneration; Periodontal Disease

Introduction

The ultimate goal of periodontal regenerative therapy is to restore the supporting periodontal tissues (i.e., new periodontal ligament (NPL), new cementum (NC) interspersed with connective tissue fibers, and new alveolar bone) lost due to tooth injury or periodontal disease. In a recent systematic review of preclinical studies, the combined use of graft material and barrier membranes was found to be superior to sole use of barrier membranes or grafts alone, for repair of the so-called noncontained type periodontal defects (i.e., supra-alveolar and 1-wall intrabony with missing buccal and lingual wall).[1]

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One approach to periodontal regeneration involves the filling of the periodontal defects with anorganic bovine

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bone (ABB) and subsequent use of bioresorbable collagen membrane (CM) to cover the graft material and the defects.[2,3] Histological studies in animals and humans have demonstrated the excellent osteoconductive properties of ABB and its ability to integrate into the bone tissue.[3-5] ABB is very well tolerated, and no adverse reactions such as allergies or graft rejection have been reported till date.[5] Controlled clinical studies have shown significantly higher clinical attachment level gains and osseous fill achieved with treatment of intrabony defects with guided tissue regeneration (GTR) + open flap debridement (OFD) + ABB as compared to that achieved with OFD alone or GTR alone.[6-8] Recent studies have shown that the results obtained with ABB + CM can be maintained on a long-term basis (i.e., up to 5 years).[2,5,9]

ABB is derived from cancellous bovine bone, from which all organic components and pathogens are removed. It favors the growth of new tissue and readily integrates with the host tissue, when used for sinus-lifting[10] and ridge-augmentation procedures,[11] as well as in compaction of bone defects adjacent to teeth and implants. The combination of graft material and barrier membrane has been shown to support periodontal regeneration in different acute critical size defects.[12-14] However, the effect on the outcomes of healing and the role of the number of bone walls is still debated. Others have argued that the healing effect depends on the size and angle of periodontal defects, rather than on the number of bone walls. Kim *et al.*[15] evaluated the influence of the number of bone walls on periodontal regeneration following OFD; they found 1- and 3-wall intrabony defects as reproducible models to evaluate candidate technologies for periodontal regeneration. Therefore, the periodontal defect types determine the periodontal healing only treated with OFD, and the type of defects might influence the effect of grafts and membrane on periodontal regeneration. In this study, we evaluated the effect of use of ABB graft in combination with CM, to facilitate healing of noncontained (1-wall) and contained (3-wall) critical size periodontal defects and to select an ideal model for further investigation of novel treatment concepts.

Methods

Study design and animals

The study began on March 2013, and completed on May 2014. Eight male beagles (age: approximately 15 months; weight: 10–15 kg each) were bred exclusively for biomedical research purposes. The animals exhibited intact dentition with a healthy periodontium. Protocol for animal selection and management, surgical protocol, and preparation were approved by the Institutional Animal Care and Use Committee (No. 2014kq-017). The animals had *ad libitum* access to water and a pelleted laboratory diet, with the exception of the first postoperative week when they were fed canned soft dog food diet. Surgery was performed after overnight fasting.

All surgical extractions and experimental procedures were performed under general anesthesia induced by an intravenous injection of 3% pentobarbital sodium solution (pentobarbital sodium salt, 0.4 ml/kg; Guoan Biotechnology Co., Ltd., Shaanxi, China) and an intramuscular injection of xylazine (0.05 ml/kg; Huamu Animal Health Products Co., Ltd., Jilin, China). Routine dental infiltration anesthesia (Xylocaine 2%, 1:80,000 dilution; Harvest Co., Ltd., Shanghai, China) was used at the surgical sites. The study included two surgeries. In the first stage of surgery, the mandibular second and fourth premolars of eight beagle dogs were extracted. Eight-week later, acute critical size intrabony defects were created in a second-stage surgery [Figure 1].

Thirty-two bilateral 1- and 3-wall intrabony defects were created around the mandible first molar (M1) and the mandible third premolar (PM3) in eight dogs.

One-wall intrabony defects were created mesial to the M1 (width: 4 mm; depth: 7 mm). Three-wall intrabony defects were created distal to the PM3 (width 4 mm; depth: 5 mm).

Following root planing, a reference notch was made into the root surface at the base of the defects using a small round bur. Thus, any periodontal ligament tissue which later might develop coronally to the notch in the root surface will be formed de novo and clearly distinguishable in the histological sections.

ABB + CM group (1-wall intrabony defect [n = 10]) and 3-wall intrabony defect [n = 10]): The defects were filled with ABB (Bio-Oss Geistlich AG, Wolhusen, Switzerland) and covered by a CM (Bio-Gide Geistlich AG, Wolhusen, Switzerland).

OFD group (1-wall intrabony defect [n = 6]) and 3-wall intrabony defect [n = 6]): After OFD and planing of the root, control defects were left empty and allowed to heal spontaneously.

In all cases, the mucogingival flaps were repositioned and sutured in a coronally displaced position with vertical and horizontal mattress sutures.

Postsurgery procedures

After the operation, the animals received a single dose of antibiotic (Cefazolin Sodium 20 mg/kg intramuscular; North China Pharmaceuticals Co., Ltd., Hebei, China). Sutures were removed after 14 days. Hygiene procedures were performed daily including brushing of teeth and topical application of 0.2% chlorhexidine (Shenzhen South Pharmaceutical Co., Ltd., Guangdong, China) until suture removal. For the rest of the experiment, oral hygiene measures were performed twice weekly as described above. Two months after the last surgical procedure, the animals were sacrificed with an overdose of pentobarbital sodium.

Micro computed tomography analysis

All samples in the mandible were examined using a micro computed tomography (CT) scanner (Siemens Inveon, Munich, Germany) at 80 kV, 500 μA and an isotropic voxel size of 27 μm. Images of the specimens were reconstructed in three dimensions (3D) using Inveon Research Workplace

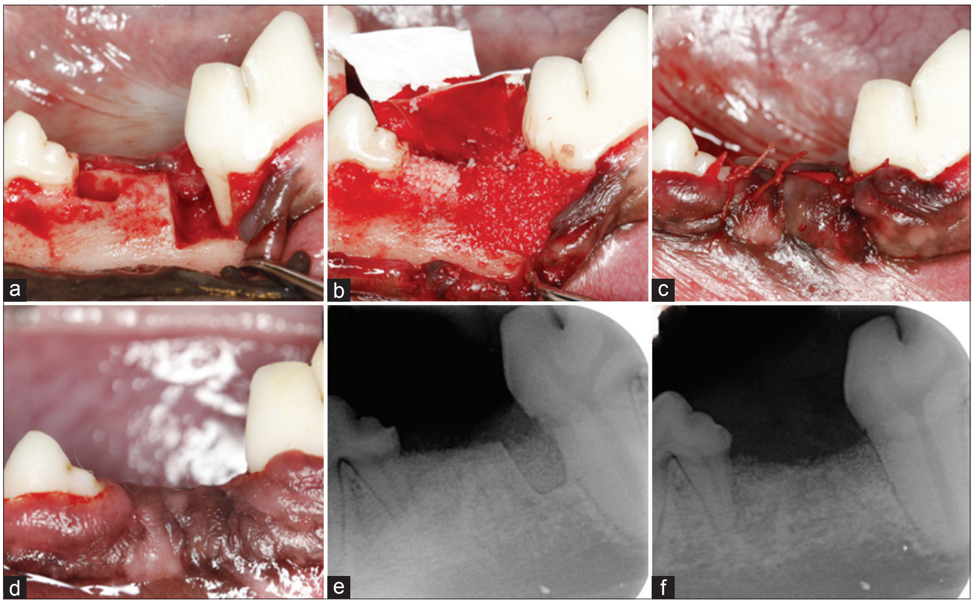

Figure 1: Clinical photographs showing the surgically created critical size 1- and 3-wall intrabony defects. (a) Defects at the distal aspect of the mandibular third premolar and the mesial aspect of the mandibular first molar, (b) defects were treated with ABB + CM; (c) a tension-free primary wound closure was achieved at all defect sites, (d) healing at 8-week postsurgery, (e) radiograph obtained at treatment with ABB + CM, (f) radiographs obtained 8-week postsurgery. ABB: Anorganic bovine bone; CM: Collagen membrane.

2.2 (Siemens Inveon, Munich, Germany). The images were used to visualize the samples; new bone volume (NBV) (%), which represented the percentage of the volume of the newly formed bone in the defect, was measured on 3D images. All specimens were analyzed and the average value for each group was obtained at 8-week.

Histological processing

The experimental tissues were fixed by perfusion with 10% buffered formalin (Biosharp Biotechnology Co., Ltd., Anhui, China) administered through the carotid arteries. Following this procedure, the block sections including defect sites and surrounding alveolar bone and soft tissues were collected and placed in 10% buffered formalin. The specimens were rinsed in sterile saline and decalcified in 10% EDTA (Biosharp Biotechnology Co., Ltd., Anhui, China) for 3 months, dehydrated and embedded in paraffin. Mesial-distal serial sections (6 μm) were cut parallel to the long axis of the teeth with a microtome. The sections were stained with hematoxylin/eosin and Masson's trichrome staining.

Histological and histometric analysis

For histometric measurements, three sections 100 μm apart, which represented the central part of the defect, were used. The following histological measurements were performed by a calibrated and masked investigator [Figure 2].

- Defect height (DH, mm): Distance between the apical notch and the cementoenamel junction
- Junctional epithelium (JE, mm): Distance coronal to apical extent of a JE along the root surface
- NC (mm): Distance between the apical notch and the coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root
- Connective tissue attachment (CTA, mm): Distance from the end of the JE to the coronal extension of the NC

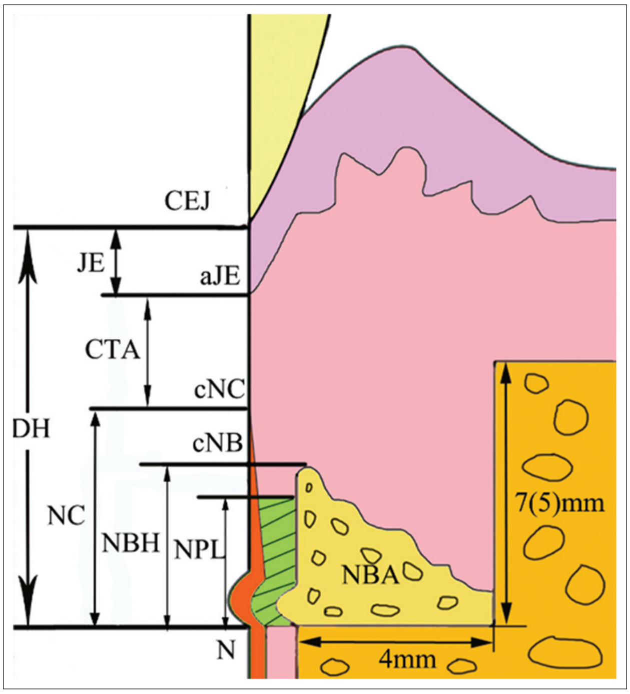

Figure 2: Landmarks/parameters used in the histometric analysis. The yellow template served as a proxy for the defect site for the estimation of bone regeneration area. DH: Defect height; NC: New cementum; CTA: Connective tissue attachment; cNC: Coronal extension of new cementum; cNB: Coronal extension of newly formed bone; CEJ: Cementoenamel junction; JE: Junctional epithelium; aJE: Apical extension of junctional epithelium; NBH: New bone height; NBA: New bone area; NPL: New periodontal ligament; N: Notch; 7(5): The depth of intrabony defect was 7 mm (1-wall) or 5 mm (3-wall).

- NPL (mm): Length of functional periodontal ligament between the newly formed cementum-like tissue and new bone

- New bone height (NBH, mm): Distance between the apical notch and the coronal extension of regenerated alveolar bone
- NBH (%): Ratio of regenerated bone height within DH
- New bone area (NBA, mm²): Area represented by new alveolar bone within a standardized area of interest (AoI)
- NBA (%): Ratio of regenerated bone/marrow spaces within AoI
- Root resorption (RR, mm): Combined linear heights of distinct resorption lacunae on the planed root
- Ankylosis (mm): Combined linear heights of ankylotic union between the regenerated alveolar bone and the planed root
- Histological signs of inflammation:
    1. Foreign body reaction adjoining biomaterial particles
    2. Multinuclear osteoclastic cells within regenerated bone

    The safety assessment of the stained biopsy specimens has to yield each in a six step scale: 0: Not present; +: Minimal; ++: Slight; +++: Moderate; ++++: Marked; and +++++: Severe
- Quality and maturity of newly formed bone.

  The quality assessment of the stained biopsy specimens has to yield each in a six step scale: 0: No bone; +: Few immature trabeculae with minimal contact to calcium phosphate; ++: Immature trabeculae with active osteoblasts and broad osteoid, biomaterial present; +++: Mature trabeculae, narrow osteoid, biomaterial present; ++++: Few areas of haversian bone, biomaterial grossly degraded; and +++++: Completely remodeled, no biomaterial.

  The frequency of RR and ankylosis was also documented in each group.

Statistical analysis

Based on previous experiences, a total of thirty-two sites in eight animals (four sites per animal) were used for histological examination. Statistical analyses were performed using the GraphPad Prism (version 5.0, GraphPad Inc., CA, USA). Data are expressed as mean ± standard deviation (SD). Two independent Student's *t*-tests (1-wall [ABB + CM] vs. 1-wall [OFD] and 3-wall [ABB + CM] vs. 3-wall [OFD]) were used to compare experimental groups and control groups. A value of $P < 0.05$ was considered statistically significant.

Results

Clinical observations

Postoperative healing was uneventful in all eight beagles [Figure 1]. Gingival recession was found in the 1-wall OFD group and in the 3-wall OFD group.

Micro computed tomography analysis

Figure 3 showed 3D reconstructed images and cross-sectional images of the defect sites by treatment groups. All mineralized tissues in the defect area of groups (1-wall [ABB + CM], 1-wall [OFD], 3-wall [ABB + CM], and 3-wall [OFD]) were newly formed bone [Figure 3a-3d]. The height and volume of the mineralized tissue in the defect area (1-wall [OFD] and 3-wall [OFD]) was smaller than that of the newly formed bone at the experimental sites (1-wall [ABB + CM] and 3-wall [ABB + CM]). In groups of 1-wall (ABB + CM) and 3-wall (ABB + CM), the mineralized tissue with scattered graft particles was connected to the pristine bone over the notch level [Figure 3a and 3b].

The experimental groups (1-wall [ABB + CM] and 3-wall [ABB + CM]) showed significantly higher NBV values [Figure 3e] as compared to that in the OFD groups (1-wall [OFD] and 3-wall [OFD]) ($P < 0.05$), respectively. However, no statistically significant between-group difference with respect to NBV was found between 1-wall (ABB + CM) and 3-wall (ABB + CM) groups ($P > 0.05$).

Histological observations

All experimental sites showed new bone and cementum formation along the planed root surface [Figures 4–6].

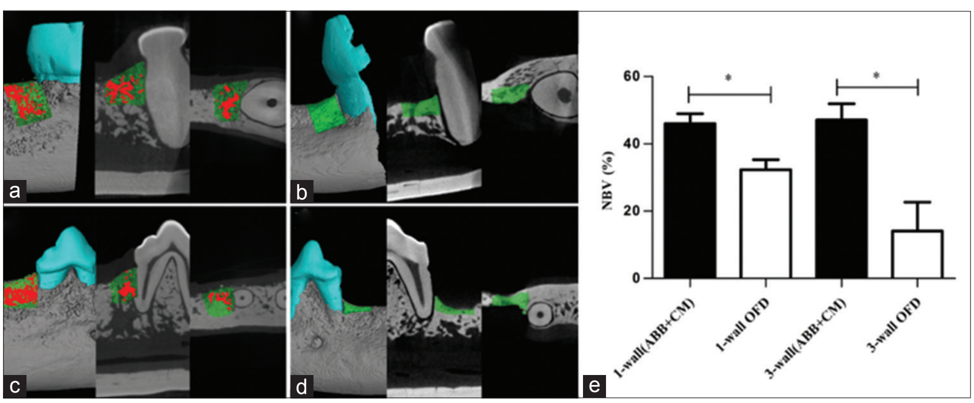

Figure 3: Representative micro-CT images of the defects and the quantification of bone healing at the defects. Three-dimensional reconstructed and sagittal and transverse sectioned views of the ABB + CM (a and c) and OFD group (b and d) in 1-wall intrabony defects (a and b) and 3-wall intrabony defects (c and d). Colored area indicated new bone and graft particles in the region of interest. Red region indicated the residual ABB; green region indicated the new bone (original magnification ×100). (e) NBV (%) from three-dimensional micro-CT analysis. *Indicated $P < 0.05$. NBV (%): New bone volume in the defect/volume of the defect; ABB: Anorganic bovine bone; CM: Collagen membrane; OFD: Open flap debridement.

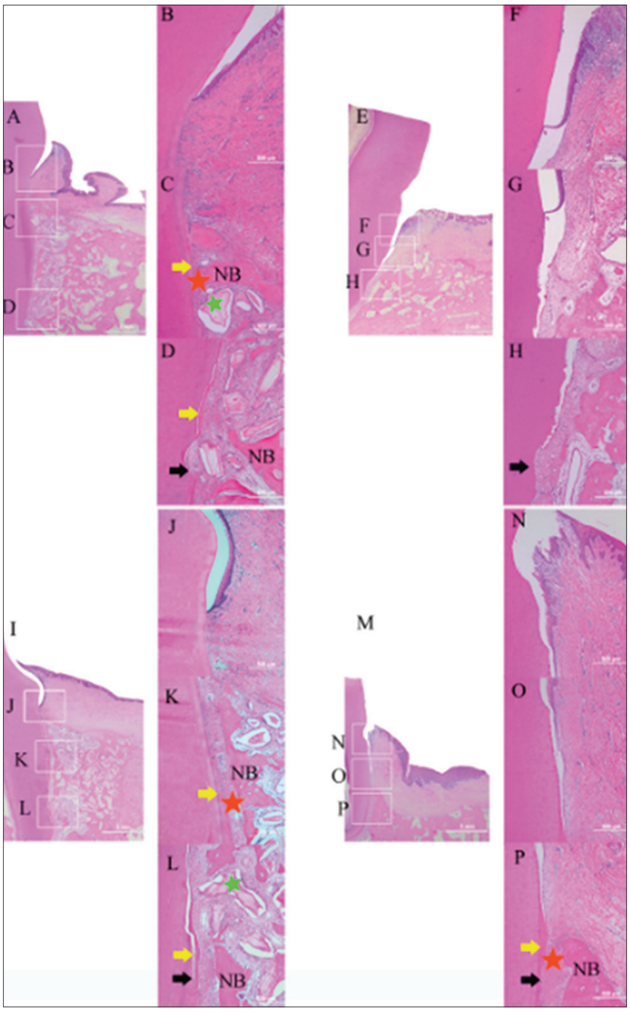

Figure 4: Photomicrographs of 1-wall (A–H) and 3-wall (I–P) intrabony defects implanted with ABB + CM (A–D, I–L) or treated by OFD (E–H, M–P) (hematoxylin/eosin, original magnification ×8 and ×40). Coronal inserts showed the apical extent of the junctional epithelium; mid-root inserts showed the coronal extension of newly formed alveolar bone; and apical inserts showed the apical extension (notch: Black arrows) of the defect. Residual ABB particles (green stars) were seen surrounded by new bone. Red stars indicated new periodontal ligament. Yellow arrowheads indicated new cementum. ABB: Anorganic bovine bone; CM: Collagen membrane; OFD: Open flap debridement; NB: New bone.

Figure 5: Photomicrographs of 1-wall (A–H) and 3-wall (I–P) intrabony defect sites implanted with ABB + CM (A–D, I–L) or treated by OFD (E–H, M–P) (Masson's trichrome stain, original magnification ×8 and ×40). Sites which received ABB + CM showed more lamellar bone, while OFD sites showed mostly woven bone with primary osteons. Obliquely or perpendicularly oriented blue collagen fibers (red stars) inserting into newly formed predominantly cellular cementum (yellow arrowheads) and new bone. Black arrows indicated the notch level. Green stars indicated residual ABB particles. ABB: Anorganic bovine bone; CM: Collagen membrane; OFD: Open flap debridement; NB: New bone.

One-wall intrabony defect: A significantly large amount of new cementum, new periodontal ligament, and new bone was observed in the ABB + CM sites as compared to that in the OFD sites [Figures 4 and 5]. Almost all ABB + CM-treated defects were occupied by newly formed bone and scattered residual graft particles. The crestal bone height adjacent to the defect was maintained without soft tissue invagination. The sites which received ABB + CM showed more lamellar bone whereas OFD sites included mostly woven bone with primary osteons. A part of newly-formed periodontal fibers were observed extending into the newly formed cementum, oriented obliquely to the root surface [Figure 6]. In the OFD group, minimal bone regeneration and new cementum formation were observed; soft tissue was collapsed into the defect or the collagen fibers were oriented in parallel to the root surface. The JE migrated apically along the root surfaces and the gingival margin receded.

Three-wall intrabony defect: All sites in the ABB + CM group exhibited a new attachment such as new cementum, new periodontal ligament, and new bone, formed within the boxing defect [Figures 4–6]. The intrabony defects were mostly occupied by lamellar bone and woven bone, and the height of newly formed bone was more than that in the OFD group. ABB + CM group showed osteoblasts enclosing residual ABB granules [Figure 7], which suggested a gradual biodegradation. Although there were still some new cementum and periodontal ligament in OFD group, most of defects were occupied by minimal mature new bone and provisional connective tissue.

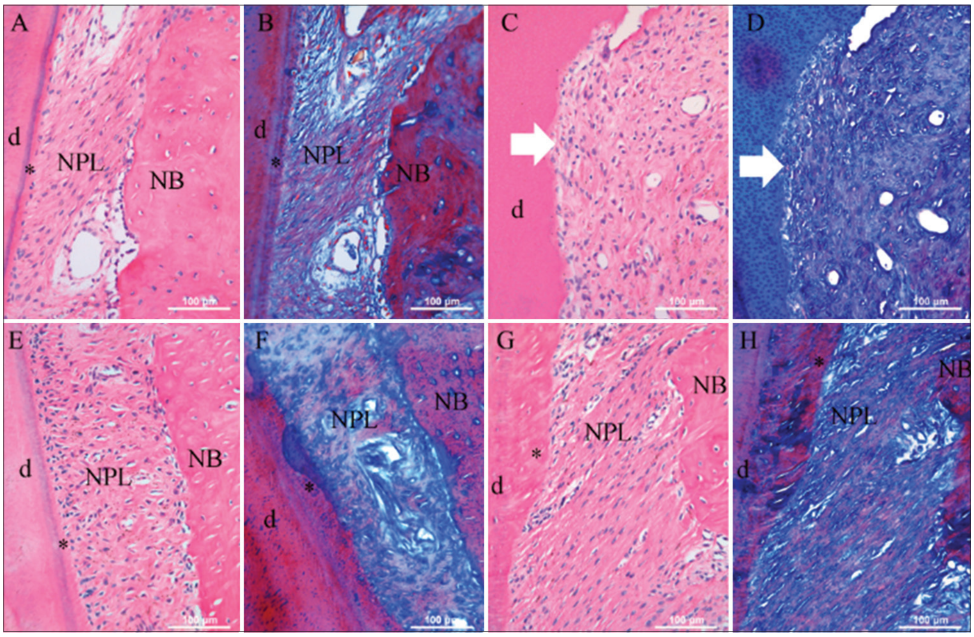

Figure 6: Photomicrographs of 1-wall (A–D) and 3-wall (E–H) intrabony defect sites (hematoxylin/eosin; Masson's trichrome stain, original magnification ×200). The sites treated by ABB + CM (A, B and E, F) showed dense new periodontal ligament tissue; the periodontal ligament fibers were embedded perpendicularly and obliquely into newly formed cementum (asterisks) and alveolar bone. OFD (C, D and G, H) showed fewer periodontal ligament fibers. Root resorption (white arrows) and some cementum formation (asterisks) were observed in OFD group. d: Denuded dentin; ABB: Anorganic bovine bone; CM: Collagen membrane; OFD: Open flap debridement; NB: New bone; NPL: New periodontal ligament.

Histometric analysis

The results of the histomorphometric evaluation were shown in Table 1. The mean NC for the 1- and 3-wall intrabony defects in the ABB + CM groups was 5.08 ± 0.68 mm and 3.01 ± 0.50 mm, respectively; the corresponding values in the OFD group were 1.16 ± 0.38 mm and 2.61 ± 0.56 mm, respectively. The 1-wall intrabony defect in the ABB + CM group showed significantly greater NC and NPL as compared to their correspondent OFD groups ($P < 0.05$). However, no statistically significant difference was observed with respect to NC and NPL for 3-wall intrabony defects between the ABB + CM and OFD groups.

The 1- and 3-wall intrabony defects (4.99 ± 0.70 mm and 3.11 ± 0.59 mm) in the ABB + CM groups exhibited significantly greater NBH compared with their correspondent OFDs (3.01 ± 0.37 mm and 2.08 ± 0.24 mm) ($P < 0.05$). Moreover, NBA and the NBA (%) were significantly enhanced for the 1- and 3-wall intrabony defects in ABB + CM groups as compared to that in their correspondent OFD groups ($P < 0.05$).

The 1-wall OFD group exhibited a significantly extended JE as compared to that in the 1-wall ABB + CM group ($P < 0.05$). However, no significant difference with respect to JE in 3-wall intrabony defect was observed between the ABB + CM and OFD groups.

In addition, there were no significant differences in the histological signs of inflammation, quality and maturity of newly formed bone in the 1-wall intrabony defects between the ABB + CM and OFD groups [Table 2]. However, the 3-wall intrabony defects in ABB + CM groups showed lower quality and maturity of newly formed bone as compared to that in their correspondent OFD and there was no obvious difference with respect to the histological signs of inflammation. The frequency of abnormal healing, including RR and ankylosis, was shown in Table 2. RR was evident in six 1-wall intrabony defect sites in the ABB + CM and OFD groups, whereas the RR only occurred in three 3-wall intrabony defect sites in the ABB + CM group and in the OFD group. The 1- or 3-wall intrabony defect demonstrated

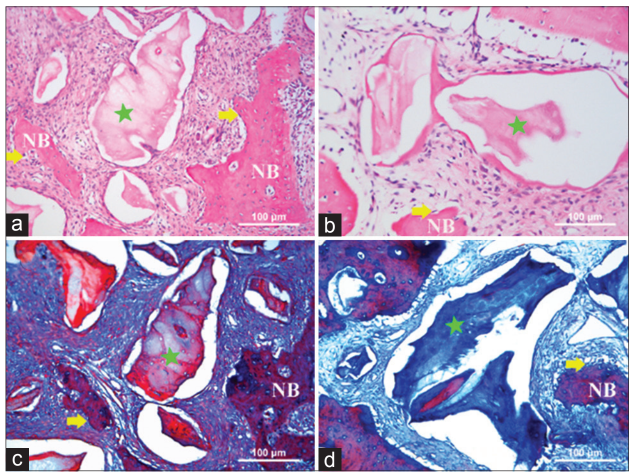

Figure 7: Photomicrographs of the two types of defect sites implanted with ABB + CM (a-d). Residual ABB (green stars) in remodeling and incorporation phase in both 1-wall group (a and c) and 3-wall group (b and d). Numerous osteoblasts (yellow arrows) were seen around new bone. (hematoxylin/eosin; Masson's trichrome stain; original magnification ×200). ABB: Anorganic bovine bone; CM: Collagen membrane; NB: New bone.

slight ankylosis, regardless of the treatment in only one site in the 1-wall ABB + CM group.

Discussion

Evaluation of periodontal regenerative techniques requires an analysis of all components of the periodontal attachment. Previous publications have mainly reported on intrabony periodontal defects that have been treated with GTR, GTR, and bone substitute materials or the combination of cells or growth factors. These approaches lead to a more predictable periodontal regeneration as compared to periodontal OFD only. Some studies[15,16] have suggested that the healing potential of intrabony defects was primarily influenced by the number of residual bone walls after periodontal regenerative therapy. Kim *et al.*[15] reported that an increase in the number of bone walls generally correlated with a significant increase in periodontal ligament and cementum and a decrease in formation of JE in self-healing condition after OFD. However, in this study, we compared the possible healing difference in the ABB + CM following acute surgically created 1- and 3-wall intrabony defects in the same dogs. Apparently, the healing of intrabony periodontal defects may not only be influenced by the number of bone walls.

In this study, the 1-wall intrabony defect model was evaluated in beagles and the histological observations are consistent with most findings reported elsewhere.[9,17] In most reports, the formation of new bone and cementum formation did not exceed 30–35% of the height of 1-wall intrabony defects in dogs with OFD group over an 8-week healing interval.[15,18] However, in the study, alveolar bone and cementum formation almost reached 64–79% of the height of defect and 33–41% bone area formation (not including the residual ABB granules) in the ABB + CM groups. Although the 1-wall intrabony defect size (7 mm × 4 mm) was found healing within an 8-week period in this study, which indicated greater challenge to spontaneous healing than the generally reported 1-wall intrabony defects (4 mm × 5 mm or 3 mm × 5 mm) within an 8- or 12-week,[19,20] ABB + CM group exhibited more bone regeneration (71.38% on average in height) than those reported only with grafts (36.5–60.0% in newly formed bone height)[19,21,22] and even than that using grafts combined with cells or growth factors (31–71% in newly formed bone height).[23,24] In addition, treatment with ABB + CM did not show less periodontal regeneration in 1-wall intrabony defects than that in the 3-wall intrabony defect within the limits of this study.

Table 1: Histometric analysis of 1- and 3-wall intrabony defects treated with ABB + CM and OFD

Parameters	1-wall (ABB + CM), *n* = 10	1-wall (OFD), *n* = 6	*t*	*P*	3-wall (ABB + CM), *n* = 10	3-wall (OFD), *n* = 6	*t*	*P*
DH (mm)	6.97 ± 0.46	6.94 ± 0.26	0.14	0.890	4.89 ± 0.34	4.89 ± 0.17	−0.62	0.540
JE (mm)	0.67 ± 0.23*	1.12 ± 0.28	−3.55	0.003	0.70 ± 0.32	0.74 ± 0.41	−0.22	0.830
CTA (mm)	1.22 ± 0.45	0.86 ± 0.32	1.70	0.110	1.13 ± 0.44	1.33 ± 0.28	−1.02	0.320
NC (mm)	5.08 ± 0.68*	1.16 ± 0.38	12.85	<0.001	3.01 ± 0.50	2.61 ± 0.56	1.50	0.160
NPL (mm)	3.19 ± 0.63*	0.69 ± 0.32	8.99	<0.001	2.43 ± 0.53	1.95 ± 0.20	2.10	0.060
NBH (mm)	4.99 ± 0.70*	3.01 ± 0.37	6.31	<0.001	3.11 ± 0.59†	2.08 ± 0.24	4.08	0.001
NBH (%)	71.38 ± 7.67*	43.45 ± 6.43	7.17	<0.001	63.51 ± 10.08†	41.67 ± 4.46	4.97	<0.001
NBA (mm^2)	10.41 ± 1.84*	5.90 ± 0.81	5.64	<0.001	6.57 ± 0.62†	3.19 ± 0.46	11.52	<0.001
NBA (%)	37.17 ± 3.93*	21.05 ± 2.06	9.23	<0.001	32.85 ± 3.03†	16.31 ± 2.17	11.62	<0.001
RR (mm)	1.03 ± 1.26	0.86 ± 0.25	–	–	0.08 ± 0.16	0.20 ± 0.29	–	–
Ankylosis (mm)	0.08 ± 0.24	0	–	–	0	0	–	–

Data shown as mean ± standard deviation. *Statistically significant difference between ABB + CM and OFD in the 1-wall group; †Statistically significant difference between ABB + CM and OFD in the 3-wall group. DH: Defect height; JE: Junctional epithelium; CTA: Connective tissue attachment; NC: New cementum; NPL: New periodontal ligament; NBA: New bone area; RR: Root resorption; ABB: Anorganic bovine bone; CM: Collagen membrane; OFD: Open flap debridement; –: Not applicable; SD: Standard deviation; NBH: New bone height.

Table 2: Frequency of main qualitative histological observations in the 1- and 3-wall intrabony defects treated with ABB + CM and OFD

Groups	Histological signs of inflammation						Quality and maturity of newly formed bone						RR	Ankylosis
	0	+	++	+++	++++	+++++	0	+	++	+++	++++	+++++		
1-wall (ABB + CM)	0/10	2/10	3/10	3/10	2/10	0/10	0/10	2/10	4/10	2/10	2/10	0/10	6/10	1/10
1-wall (OFD)	0/6	1/6	2/6	2/6	1/6	0/6	0/6	1/6	3/6	1/6	1/6	0/6	6/6	0/6
3-wall (ABB + CM)	0/10	2/10	5/10	2/10	1/10	0/10	0/10	2/10	3/10	4/10	1/10	0/10	3/10	0/10
3-wall (OFD)	0/6	1/6	3/6	1/6	1/6	0/6	0/6	0/6	0/6	2/6	3/6	1/6	3/6	0/6

The data were shown as X/Y. X/Y: The number of positive sites (X) occurred in ABB + CM group (Y = 10) or OFD group (Y = 6). Histological signs of inflammation (sites): 0: Not present; +: Minimal; ++: Slight; +++: Moderate; ++++: Marked; +++++: Severe. Quality and maturity of newly formed bone (sites): 0: No bone; +: Few immature trabeculae with minimal contact to calcium phosphate; ++: Immature trabeculae with active osteoblasts and broad osteoid, biomaterial present; +++: Mature trabeculae, narrow osteoid, biomaterial present; ++++: Few areas of Haversian bone, biomaterial grossly degraded; +++++: Completely remodeled, no biomaterial. RR: Root resorption; ABB: Anorganic bovine bone; CM: Collagen membrane; OFD: Open flap debridement.

The dimensions of the intraosseous defects determine periodontal regeneration outcomes. Considering the healing factors including the difference in wound stability between different intrabony defects, the 3-wall intrabony defects were more favorable for spontaneous healing than 1- or 2-wall intrabony defects and could provide high predictable outcomes in regenerative procedures. Therefore, the use of 3-wall intrabony defects for testing the regeneration effect has evoked much interest among researchers. In this study, wound healing in the 3-wall intrabony defects showed a significant difference from that in the OFD group [Table 1]. However, ideal 3-wall intrabony defects are rarely encountered in clinical settings. Selvig *et al.*[25] reported that the percentage of 3-wall intrabony defect was less than 30% and a combination of 1- and 2-wall intrabony defects was mainly observed due to the most common occlusal component.

In the present study, the 1-wall intrabony defects in the OFD group exhibited a large ratio of JE downgrowth on root surface as compared to their counterparts in the OFD group, which suggests that the number of bone walls might have inherently compromised wound stability due to the defect morphology. In addition, regardless of the sites treated by ABB + CM or OFD, the 1-wall group frequently showed RR, which suggests that periodontal healing in the 1-wall intrabony defects was unpredictable as compared to that in the 3-wall group. Periodontal wound healing in 1-wall intrabony defect was limited by the lack of buccal and lingual periodontal tissue component in this type of defect. Therefore, the spontaneous regenerative potential of 2- and 3-wall intrabony defects might be generally higher than that of 1-wall intrabony defect.[16,26] Furthermore, only a few publications have reported on the ideal dimensions of the 2-wall intrabony defects, which make it a difficult and less reproducible model.[27,28] In general, the presence of lateral bone walls in 2- and 3-wall intrabony defects might provide a better support for the soft tissue flap and the blood clot at the tooth surface, thus providing a more stable milieu for periodontal regeneration. The 1-wall intrabony defect provides less favorable support to the flap and the blood clot; the other two defect models might be less discriminative than the 1-wall intrabony defects to investigate new treatments. Therefore, it would be a more challenging and more clinically relevant experimental model.

Bone graft materials provide an environment for host cells that stimulates regeneration.[29,30] Resorption is one of the vital factors that affect the osseous penetration of the bone graft material. Most researchers thought that ABB was a slow resorption[31,32] or no resorption[33,34] of the material. In addition, they claimed that unresorbed granules within the newly formed bone were undesirable because it interfered with new bone growth and compromised the properties of the regenerated tissue, especially the osteointegration capacity. Furthermore, various studies have demonstrated that a limited amount of bone slowly replaces ABB.[35,36] In this study, residual ABB granules were observed in all ABB + CM groups at 8-week. The quality and maturity of newly formed bone in the 3-wall FD group was greater than that in the other groups although there was no significant difference between the 1- and 3-wall intrabony defects in the ABB + CM group with respect to the quality and maturity of the newly formed bone. It is possible that the residual ABB granules may not influence the quality and maturity of the newly formed bone. Wiltfang *et al.*[36] reported that autologous grafts in combination with nonresorbable ABB particles were used as a protection against graft resorption and to achieve long-term bone preservation. In the study, in terms of histological examination, ABB with its osteoconductive properties acted as a scaffold and stimulated the regeneration of new cementum and bone, by virtue of the close contact between this material and the newly formed bone.

The limitations of the present study should be mentioned. There was no enough evidence to prove that the completely new periodontal ligament, new cementum, and new bone were regenerated by the implanted biomaterial. The sample size was also relatively small. However, ABB is a natural bone mineral with excellent osteoconductivity.[32] The 1- and 3-wall OFD groups also showed little new periodontal tissue, which further confirmed that the periodontal regeneration from experiment group was formed by ABB. Further studies incorporating the use of the tracer technique and immunohistochemistry should be carried out to verify the new periodontal tissue formed from the bone graft. In addition, larger studies are required to provide more robust evidence.

In conclusion, in this study, the use of ABB, which has good osteoconductive properties, in combination with CM enhances comparable periodontal regeneration in 1- and 3-wall intrabony defects. One-wall intrabony defect showed more significant difference in the parameters between the ABB + CM and OFD group as compared to that in the 3-wall intrabony defect model. The 1-wall intrabony defect (7 mm × 4 mm) might act as a more discriminative and clinically relevant defect model for further evaluation of new regeneration procedures in periodontal treatment, and even in implant surgery or surgery for jaw bone defects.

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Conflicts of interest

There are no conflicts of interest.

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