DR. YOSHINORI SHIRAKATA (Orcid ID : 0000-0003-0663-0524)

DR. TOSHIAKI NAKAMURA (Orcid ID : 0000-0003-2675-484X)

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Healing of 2-wall intrabony defects treated with a novel EMD-liquid. A preclinical study in monkeys

Yoshinori Shirakata[1]*, Richard J, Miron[2,3], Yukiya Shinohara[1], Toshiaki Nakamura[1], Kotaro Sena[1], Naoto Horai[4], Dieter D. Bosshardt[5], Kazuyuki Noguchi[1], Anton Sculean[6]

[1] Department of Periodontology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

[2] Department of Periodontology, College of Dental Medicine, Nova Southeastern University, Fort Lauderdale, FL, USA

[3] Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA

[4] Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan

[5] Robert K. Schenk Laboratory of Oral Histology, University of Bern, Bern, Switzerland

[6] Department of Periodontology, School of Dental Medicine, University of Bern,

Bern, Switzerland

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Corresponding author:
Yoshinori Shirakata
Department of Periodontology, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan
Tel/Fax:+81-99-275-6202/+81-99-275-6209
E-mail: syoshi@dent.kagoshima-u.ac.jp

Abstract

Aim: To investigate the effect of a novel enamel matrix derivative formulation (EMD-liquid or Osteogain) combined with an absorbable collagen sponge (ACS) on periodontal wound healing in intrabony defects in monkeys.

Materials and Methods: Chronic 2-wall intrabony defects were created at the distal aspect of 8 teeth in 3 monkeys (*Macaca fascicularis*). The 24 defects were randomly assigned to one of the following treatments: (1) Open flap debridement (OFD) + ACS alone, (2) OFD + Emdogain + ACS (Emdogain/ACS), (3) OFD + Osteogain + ACS (Osteogain/ACS), or (4) OFD alone. At 4 months the animals were euthanized for histologic evaluation.

Results: Osteogain/ACS resulted in more consistent formation of cementum, periodontal ligament and bone with limited epithelial proliferation compared to OFD alone, Emdogain/ACS, and OFD + ACS. Among the 4 treatment groups, the Osteogain/ACS group demonstrated the highest amount of regenerated

tissues. However, complete periodontal regeneration was not observed in any of the defects in the 4 groups.

Conclusions: The present findings indicate that in 2-wall intrabony defects, reconstructive surgery with Osteogain/ACS appears to be a promising novel approach for facilitating periodontal wound healing/regeneration, thus warranting further clinical testing.

Clinical Relevance

***Scientific rationale for the study*:** The potential effect of a new liquid formulation of EMD (Osteogain) combined with an absorbable collagen sponge (ACS) on periodontal wound healing/regeneration in 2-wall intrabony defects is at present unknown.

***Principal findings*:** Treatment of intrabony defects with OFD and Osteogain/ACS enhanced periodontal wound healing/regeneration more consistently than treatment with OFD + ACS, OFD + Emdogain/ACS or OFD alone.

***Practical implications*:** The present findings provide for the first time histologic support for the biological potential of Osteogain/ACS to promoting periodontal regeneration in 2-wall intrabony defects.

Introduction

It has been demonstrated that the application of an enamel matrix derivative (EMD) substantially facilitates periodontal wound healing/regeneration including clinical attachment gain and pocket depth reduction for over 20 years (Hammarström et al. 1997, Heijl et al. 1997, Mellonig. 1999, Sculean et al. 2011, Miron et al. 2016a). The viscous consistency of EMD (Emdogain) may limit its space-making potential and, subsequently affect the outcomes in cases where flap collapse due to unfavorable defect anatomy may occur (Tonetti et al. 1993, Mellonig 1999, Lekovic et al. 2000, Cochran et al. 2003). The combination of Emdogain with various grafting materials is one approach to treat non-contained intrabony defects and aims at preventing flap collapse and, at the same time, promoting the regeneration process（Lekovic et al. 2000, Cochran et al. 2003, Sculean et al. 2003, 2007, Shirakata et al. 2007, Yamamoto et al. 2007, Gurinsky et al. 2004, Velasquez-Plata D et al. 2002, Jepsen et al. 2008, Bokan et al. 2006, Kuru et al. 2006, Trombelli et al. 2008, Yilmaz et al. 2010）. A recent systematic review found that the combination of Emdogain with a bone grafting

material led to significantly higher clinical attachment level gains and probing depth reductions compared with Emdogain alone (Matarasso et al. 2015). On the other hand, other studies have failed to reveal adjunctive effects of a combination therapy especially in cases where alloplastic materials were used in combination with Emdogain (Bokan et al. 2006, Jepsen et al. 2008). Despite the fact that the potential influence of the chosen grafting material on the clinical outcomes has been previously discussed, the reasons for this variability are still unclear (Tu et al. 2010, Miron et al. 2014).

Recently, in an attempt to characterize protein adsorption of enamel matrix proteins (EMPs) to bone grafts, large variability between commonly utilized bone grafting materials including a bovine-derived natural bone mineral (NBM), demineralized freeze dried bone allograft (DFDBA) and a synthetic calcium phosphate was reported (Miron et al. 2015). More importantly it was found that a liquid formulation of EMD markedly improved protein adsorption when compared to Emdogain (Miron et al. 2015). Further advantages include better surface coating and penetration of EMPs within the bone biomaterials capable of gradual release of EMPs over time (Miron et al. 2015). These prominent findings led to the development of a new liquid carrier system for EMD (EMD-liquid: Osteogain) specifically designed for mixing with bone grafting materials.

In this respect, it has been reported that the fast resorption of residual bone grafts is desirable to avoid the risk for infection and to increase the amount of regenerated tissues in bone/periodontal defects (MacNeil et al. 1999, Shirakata et al. 2002, 2007, Potijanyakul et al. 2010, Yoshinuma et al. 2012). Moreover, when discussing the use of bone grafting materials in regenerative periodontal surgery, it has to be kept in mind that histologically, the healing following bone

grafting is frequently accompanied by persistence of grafting residues surrounded by either bone or connective tissue (Ivanovic et al. 2014, Sculean et al. 2015). Therefore, the biologic rationale for using grafting materials to obtain periodontal regeneration needs to be questioned while, at the same time, the use of completely bioresorbable materials such as collagen matrices/scaffolds may represent a realistic alternative to act both as carriers for biologics and for stabilizing the wound (Susin et al. 2015, Stähli et al. 2016). Due to its high clinical applicability, biocompatibility, and uneventful biodegradation in bone and periodontal surgeries, an absorbable collagen sponge (ACS) has been extensively tested as a putative scaffold or carrier (McPherson. 1992, Cochran et al. 2000, Yamashita et al. 2010, Kim et al. 2013). Practically, ACSs are a relatively low-cost FDA-approved product composed mainly of type I collagen. Type I collagen is also the major organic component of bone extracellular matrix and several studies have reported that collagen application facilitated osteoblastic differentiation, the expression of various osteogenic markers and bone formation (Talley-Ronsholdt et al. 1995, Mizuno et al. 1997, Yamanouchi et al. 2001, Donzelli et al. 2007, Shimoji et al. 2009). Furthermore, collagen products have the ability to adsorb extracellular matrix molecules (e.g. laminin and fibronectin) and growth factors responsible for increasing cell migration and/or proliferation (Itoh et al. 2001, Stähli et al. 2016, Miron et al. 2016c, Shirakata et al. 2017).

A very recent animal study has evaluated the effect of a novel liquid carrier system of enamel matrix derivative (Osteogain) soaked with an ACS (Osteogain/ACS) upon periodontal wound healing/regeneration in class III furcation defects in monkeys (Shirakata et al. 2017). The findings revealed for

the first time that Osteogain/ACS possessed favorable physicochemical properties facilitating adsorption of amelogenin onto ACS and additionally enhanced periodontal wound healing/regeneration of furcation defects when compared to Emdogain/ACS (Shirakata et al. 2017).

However, at present, no data have evaluated the potential effects of Osteogain/ACS in promoting periodontal wound healing/regeneration in intrabony defects. Thus, the aim of the present study was to investigate the potential effects of Osteogain in combination with an ACS on periodontal regeneration in 2-wall intrabony defects in non-human primates.

Materials and Methods

Experimental animals

Three 7-8-year old male monkeys (*Macaca fascicularis*), weighing 6.91 to 7.02 kg, were selected for this study. The animals exhibited intact dentition with healthy periodontium. All procedures during the in life phase for 9 months (from November 6, 2014 to August 3, 2015) were approved by the ethical committee of the Animal Research Center of Kagoshima University, Japan (approval no. D14026).

Enamel matrix derivative and biomaterial

Emdogain (enamel matrix proteins and propylene glycol alginate) and Osteogain (enamel matrix proteins in acetic acid) (0.3 ml vials, working concentration 30 mg/ml) were kindly provided by Straumann AG, Basel, Switzerland. The native type I and III porcine absorbable collagen sponge (ACS: Collacone®, Botiss, Berlin, Germany) was utilized as a candidate material with the ability to efficiently adsorb growth factors due to its 3 dimensional structure.

Preparation of experimental 2-wall intrabony defects

One experienced surgeon (Y.S) performed all surgical procedures under general and local anesthesia using aseptic routines. General anesthesia was achieved with ketamine hydrochloride (0.2ml/kg IM)/medetomidine hydrochloride (0.08ml/kg IM) in combination with maintained spontaneous breathing. Local anesthesia was performed using lidocaine HCl/epinephrine (2%, 1:80,000; Xylocaine).

Two months prior to the start of the experiment, the mandibular and maxillary 2nd premolars and lateral incisors were extracted to provide enough space for defect creation. Following elevation of mucoperiosteal flaps, 2-wall intrabony defects with a depth of 5 mm measured from the bone crest were produced by means of a slowly rotating round and fissure burs with a sterile saline coolant at the distal surfaces of both maxillary and mandibular first premolars and central incisors (i.e., 24 defects in total) according to a previously described protocol (Sculean et al. 2000a). In order to prevent spontaneous healing and induce plaque accumulation, ligature wires were placed into the intrabony defects (**Fig. 1a**). The flaps were repositioned and stabilized with 4-0 silk sutures. 10 days following surgery, the sutures were removed. For the first 2 months following the 1st surgery, no oral hygiene measures were performed and the animals were fed a soft diet. After removal of the ligature wires, a plaque control program was resumed including routine flushing (three times a week) of the oral cavity with a chlorhexidine solution (chlorhexidine gluconate; 25ml of a 2 % solution) for 4 weeks.

Reconstructive surgery

At 12 weeks following the defect creation (**Fig. 1b**), intrasulcular incisions were performed and full-thickness buccal and lingual mucoperiosteal flaps were

elevated in order to expose the intrabony defects (**Fig. 1c**). All granulation tissue was removed and the exposed root surface was carefully scaled and planed (**Fig. 1d**). Cementum was removed using Gracey curettes and a chisel. Reference notches were made using a #1 round bur on the root surface at the base of the defects, and at the cemento-enamel junction (CEJ) for histometric analysis, and on the crown surface to indicate the precise center plane of the two-wall intrabony defects and to aid in optimal histologic processing. Two-wall intrabony defects were randomly assigned to one of the following treatments: ACS alone, Emdogain with ACS (Emdogain/ACS), Osteogain with ACS (Osteogain/ACS), and open flap debridement (OFD) as a surgical control. In the ACS group, ACS was mixed with sterile saline before being applied to the defect. The root surfaces at the experimental defects that received Emdogain or Osteogain were conditioned with a 24% EDTA gel (PrefGel®, Straumann AG, Basel, Switzerland) for 2 minutes and then, along with the adjacent mucoperiosteal flaps, thoroughly rinsed with sterile saline to remove EDTA residues. Prior to the placement of Emdogain/ACS or Osteogain/ACS, the ACS was fully saturated with Emdogain or Osteogain and the constructs were allowed to rest for 10 minutes. The constructs were then filled in the defect close to the residual bone crest (**Fig. 1e, Fig. 1f**). A periosteal releasing incision was made to allow tension-free coronal repositioning of the flap, followed by suturing (Gore-Tex CV-6 Suture, W. L. Gore & Associates Inc., Flagstaff, AZ, USA) slightly coronal to the CEJ (**Fig. 1g**).

Postsurgical protocol

After the operation, the animals received a single dose of intramuscularly administered antibiotics. The sutures were removed after 14 days of healing and postoperative plaque control was maintained by routine flushing (three times a

week) of the oral cavity with a chlorhexidine solution (chlorhexidine gluconate; 25ml of a 2% solution). Then, 4 months after the reconstructive surgery (**Fig. 1h**), the animals were euthanized by an overdose injection of sodium thiopental.

Histological processing

All the defects, including the experimental and control sites, were then dissected free along with the surrounding soft and hard tissues. The tissue blocks were fixed in 10% buffered formalin, trimmed, and rinsed in phosphate buffered saline. These samples were then analyzed using a micro-computed tomography system (Scan Xmate-E080®, Comscantecno Corporatioin., Kanagawa, Japan) and software (TRI/3D VIEWER, Ratoc system engineering Co., Ltd, Tokyo, Japan) for observing bucco-lingual appearances (including defect margin and root location in the alveolar bone) and for discerning the central portion of the defects to obtain the optimal histological sections. The samples were decalcified, dehydrated, and embedded in paraffin. Step serial sections of 6 µm thickness were then prepared along the mesio-distal plane, stained with hematoxylin/eosin or with azan-mallory at intervals of 90 µm.

Histometric analysis

All the specimens were analyzed histometrically under a light microscope (BX51, Olympus Corp., Tokyo, Japan) equipped with a computerized image system (cellSens, Olympus Corp., Tokyo, Japan). For histometric analysis, 3 sections approximately 90 µm apart were selected from the most central area of each 2-wall defect, identified by the length of the root canal and the reference notches. The mean value of each histometric parameter was then calculated for each site. The following parameters were measured by the same experienced and masked examiner (T. N). Intra-examiner reproducibility was ensured by reading twenty

four sections from all sites by the examiner and repeating the same procedure forty-eight hours later. Calibration was accepted at the 90% level.

1. Defect height (DH): the distance between the apical extent of root planing and CEJ.
2. Apical extension of the junctional epithelium (JE): distance between apical extension of JE and CEJ.
3. Connective tissue adhesion (CT; without cementum): distance between apical extent of JE and coronal extent of newly formed cementum.
4. New bone formation (NB): distance between apical extent of root planing and coronal extent of newly formed alveolar bone along the root surface.
5. New cementum formation (NC): distance between apical extent of root planing and coronal extent of newly formed cementum on denuded root surface.
6. New attachment formation (NA): total linear length of the root surface covered by NC adjacent to newly formed bone, with functionally oriented collagen fibres;

Linear measurements, except for DH, were also expressed as the percentage of the DH within each defect.

Results

Clinical observations

All surgical treatments were well tolerated by the animals, and clinical healing was uneventful at all 24 sites with limited signs of inflammation and limited gingival recession. No adverse reactions including material exposure, increased tooth mobility, infection and suppuration were observed throughout the entire experimental period.

Histologic observations

OFD group

The healing pattern in the OFD group was characterized by extensive collapse of the flap, resorption of the parent bone crest and limited periodontal regeneration **(Fig. 2a)**. Considerable apical migration of the junctional epithelium was observed. Thick new cellular cementum with or without collagen fibers obliquely to the root surface was detected in the lower portion of the defect **(Fig. 2b)**. New bone formation with narrow bone growth parallel to the root surface occurred to a varying extent. In one defect, a slight ankylosis was found in the apical area of the notch. Connective tissue fibers were observed aligned parallel to or detached from the denuded root surface **(Fig. 2c)**.

ACS group

In the ACS-applied sites, apical extension of the junctional epithelium was more suppressed than in the OFD group. Newly formed bone was observed along and around the root surface **(Fig. 3a)**. Thick new cellular cementum with or without inserting collagen fibers was detected in the middle portion of the defect **(Fig. 3b)**. The collagen fibers apperared to be sparser than those observed in the Emdogain/ACS and Osteogain/ACS groups **(Fig. 3c)**.

Emdogain/ACS group

Three of six sites in the Emdogain/ACS group healed well with robust periodontal regeneration **(Fig. 4a)**. A continuous layer of new mixed acellular/cellular cementum was observed on the previously denuded root surface. Dense collagen fibers were seen inserting into the newly formed cementum, obliquely oriented to the root surface **(Fig. 4b)**. Bone formation was noted extending from the parent bone crest toward the coronal region of the defect **(Fig. 4a)**. However, in three samples, periodontal regeneration was

modest or limited to the apical half of the defects **(Fig. 4c)**. The area of connective tissue adjacent to the root surface without cementum formation was broad and varied more in the Emdogain/ACS-treated sites than in the other groups **(Fig. 4c, Fig. 4d)**.

Osteogain/ACS group

In this group, new cementum with perpendicularly inserting collagen fibers was consistently observed. Cellular intrinsic fiber cementum was mostly found at the apical portion and tended to change to acellular extrinsic fiber cementum towards the coronal portion of the denuded root surface **(Fig. 5b, Fig. 6b, Fig. 6c)**. Bone formation was noted extending from the parent bone crest toward the coronal region of the defect **(Fig. 5a, Fig 6a)**. The newly formed periodontal ligament was highly vascularized and tightly confined between the newly formed cementum and bone, maintaining its width up to the coronal portion **(Fig. 5, Fig. 6)**.

ACS appeared to be completely resorbed after 16 weeks of healing in the ACS, Emdogain/ACS and Osteogain/ACS groups. There was no extensive root resorption or ankylosis in these treatment groups.

Histometric analysis

The results of the histometric analysis is summarized in **Table 1**.

The length of JE observed in the Osteogain/ACS (3.18±1.34mm) group was shorter than those in the OFD (4.57±0.98mm), ACS (3.63±1.13mm) and Emdogain/ACS (3.76±1.58mm) groups. The amount of CT (without cementum) in the Emdogain/ACS (1.00±1.33mm) group was greater than in the OFD (0.45±0.42mm), ACS (0.80±0.67mm) and Osteogain/ACS (0.56±0.78mm) groups. The Osteogain/ACS group showed the greatest amount of NB among

the groups examined. The amounts of NC and NA formation in the Osteogain/ACS (4.00±1.49mm, 3.59±1.65mm) group were greater compared to the OFD (3.21±1.60mm, 1.49±0.90mm), ACS (3.60±1.22mm, 2.59±1.57mm) and Emgoain/ACS (3.58±1.58mm, 2.98±1.69mm) groups.

Discussion

The present study has for the first time evaluated the effect of Osteogain/ACS on periodontal wound healing/regeneration in 2-wall intrabony defects in non-human primates. The use of Osteogain/ACS in conjunction with reconstructive surgery resulted in higher amounts of newly formed cementum, periodontal ligament and bone compared to the use of Emdogain/ACS, ACS or OFD alone. In both EMD-treated groups (Emdogain/ACS and Osteogain/ACS), the newly formed periodontal ligament was well vascularized, while the collagen fibers inserting in the new cementum appeared to be denser compared to those observed in the OFD and ACS groups, providing additional evidence for the positive effect of EMD in promoting periodontal wound healing. The present results obtained with Emdogain/ACS are comparable to those reported previously following the use of Emdogain demonstrating periodontal regeneration in both animal and human intrabony defects (Sculean et al. 1999, 2000a, 2000b, 2015, Shirakata et al. 2007, 2010, Ivanovic et al. 2014).

In the present study, the application of Osteogain/ACS appeared to induce periodontal regeneration more consistently than Emdogain/ACS. This finding is in agreement with our very recent report evaluating the effects of the same treatments on chronic class III furcation defects in monkeys (Shirakata et al. 2017). Despite the fact that in the mentioned study, none of the treatments achieved complete regeneration in class III furcations, the application of

Osteogain/ACS yielded consistently more cementum, periodontal ligament and bone compared to the other 3 treatments (e.g. Emdogain/ACS, ACS and OFD). One explanation for the discrepancy in the amount of the regenerated tissues beween Osteogain/ACS and Emdogain/ACS may be related to the adsorption of amelogenins in Osteogain/ACS which was 20-60 % higher compared to that in Emdogain/ACS as revealed by an ELISA assay. Furthermore, the ACS loaded with Emdogain began to degrade in PBS by 3 days whereas those pre-coated with Osteogain showed more stable properties suggesting that in this novel formulation, EMD may be present for a longer time in the wound area, which in turn may influence the healing process (Shirakata et al. 2017). These findings appear to suggest that Osteogain/ACS not only maintains a higher concentration of remained and sustained release of amelogenins, but may also provide a more favorable environment for periodontal regeneration compared to Emdogain/ACS. Furthermore, they are also in line with findings from previous *in vitro* experiments which have demonstrated that Osteogain significantly increased cell adhesion, proliferation and differentiation of osteoblasts when combined with bone grafting particles or ACS (Miron et al. 2016b, 2016c) and significantly upregulated the expression of genes encoding BMP2 and TGF-ß1 while decreasing expression of IL-1ß (Miron et al. 2016d). Moreover, the differentiation potential of both osteoblasts and PDL cells was further retained following the use of Osteogain as demonstrated by the increased collagen and osteocalcin gene expression and significantly higher alizarin red staining (Miron et al. 2016d).

When interpreting the results, it has to be kept in mind that none of the defects in the 4 treatment groups demonstrated complete resolution of the intrabony component without substantial differences. These results may, on the one hand,

be explained by the limited number of included animals/teeth not allowing for appropriate statististical comparisons and, on the other hand, by the chronic type of defects. In this study, non-human primates were chosen since the anatomical, microbiological and immunological features including turnover rate of bone remodeling have been shown to be quite similar to those of humans (Giannobile et al. 1994, Pellegrini et al. 2009, Oz et al. 2011), despite the fact that the expenses, demanding maintenance and ethical issues restrict their broad use. The rationale to create chronic periodontal defects with minimal potential for spontaneous repair was based on previous reports indicating that such defects provide a clinically valuable model for evaluating new treatment strategies for periodontal regenerative therapy (Caton et al. 1994, Giannobile et al. 1994, Sculean et al. 2000a).

The rationale for using ACS as a carrier for EMD instead of bone grafting materials was based on the fact that ACS is easily applied to the variously shaped bone/periodontal defects with moderate elasticity (McPherson. 1992, Cochran et al. 2000, Yamashita et al. 2010, Kim et al. 2013). Another reason to consider the use of an ACS carrier was due to outcomes reported in animals and humans demonstrating that following grafting, the healing is frequently characterized by persistence of grafting particles either encapsulated in connective tissue or surrounded by a bone-like tissue (Sculean et al. 2003, 2015, Schwarz et al. 2007, Shirakata et al. 2010, Yamashita et al. 2010, Yoshinuma et al. 2012, Kim et al. 2013, Ivanovic et al. 2014). These findings indicate that complete regeneration of the tooth's supporting tissues is rather insonsistent despite the observed clinical improvements (Ivanovic et al. 2014, Matarasso et al. 2015, Sculean et al. 2015). The choice of ACS as a potential carrier for

biologics was additionally supported by very recent data from *in vitro* studies which have shown that the adsorption rate of amelogenin to ACS was higher than those observed on NBM, FDBA or a synthetic calcium phosphate material (Miron et al. 2015, 2016c, Stähli et al. 2016). Thus, from a biological point of view, the use of collagen constructs aiming at stabilizing the blood clot and serving as carriers for biologics such as EMD, growth factors etc., appears to represent a potential novel approach to enhance periodontal wound healing/regeneration. However, it cannot be excluded that in 2-wall intrabony defects, the used ACS did not possess the mechanical properties needed to ensure sufficient wound stability and space provision for periodontal regeneration (Yamashita et al. 2010, Kim et al. 2013, Susin et al. 2015, Shirakata et al. 2017).

Thus, within their limits, the present findings indicate that in 2-wall intrabony defects, reconstructive surgery with Osteogain/ACS may represent a potential novel approach for facilitating periodontal wound healing/regeneration. Obviously, prior to the clinical use in humans, additional studies aiming at further investigating the effects and the mechanisms of action of Osteogain combined with ACS or with various types of bone grafting materials on periodontal regeneration are needed.

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Table 1. **Histomorphometric linear measurment in each group (mean± SD in mm and (%) ; n=3 animals, n=24 sites; 6 sites/group)**

DH: defect height **JE:** junctional epithelium migration **CT:** connective tissue attachment (without cementum) **NB:** new bone **NC:** new cementum **NA**: new attachment formation

Histometric parameters	Treatment modality			
	OFD	ACS	Emdogain/ACS	Osteogain/ACS
DH (mm)	8.08±2.09	8.09±1.93	8.31±2.30	7.76±2.47
JE in mm and (%)	4.57±0.98 (58.56±15.94)	3.63±1.13 (45.76±14.95)	3.76±1.58 (47.68±18.44)	3.18±1.34 (41.05±15.19)
CT in mm and (%)	0.45±0.42 (5.47 ± 5.02)	0.80±0.67 (9.07±6.28)	1.00±1.33 (10.20±11.62)	0.56±0.78 (5.67±6.53)
NB in mm and (%)	2.64±1.30 (31.90±11.18)	3.08±1.62 (37.79±20.40)	3.63±2.25 (42.28±18.44)	3.89±1.47 (51.61±16.99)
NC in mm and (%)	3.21±1.60 (38.31±13.07)	3.60±1.22 (44.30±10.97)	3.58±1.58 (42.53±14.03)	4.00±1.49 (52.31±15.01)
NA in mm and (%)	1.49±0.90 (19.66±12.04)	2.59 ± 1.57 (31.49±19.56)	2.98±1.69 (34.40±15.78)	3.59±1.65 (46.52±15.17)

Figure legends

Figure 1. Clinical appearance of the maxillary buccal aspect of Macaca fascicularis. **(a)** Induction of chronic inflammation. After fabrication of the 2-wall intrabony defects, plaque-retentive ligatures were placed to encourage growth of oral microflora along the exposed root surfaces. **(b)** Prior to reconstructive surgery. **(c)** Immediately after flap reflection. Note the excessive bone resorption

in the chronic defects. **(d)** Defects were exposed and debrided again at the time of reconstructive surgery. **(e)** Osteogain/ACS construct was placed into the defect **(f)** Emdogain/ACS construct was placed into the defect **(g)** Flaps were coronally repositioned and sutured. **(h)** 16 weeks after reconstructive surgery.

Figure 2.

Representative photomicrographs of a 2-wall intrabony defect treated with open flap debridement (OFD). **(a)** Overview. (Bar: 1 mm; Azan-Mallory staining) **(b)** Higher magnification of the apical framed area in (a) (Bar: 200 µm; Azan-Mallory staining). **(c)** Higher magnification of the coronal framed area in (a) (Bar: 200 µm; Azan-Mallory staining). NB: new bone, NC: new cementum, PDL: periodontal ligament, JE: junctional epithelium, CT: connective tissue, N: notch (apical extent of root planing), D: root dentin.

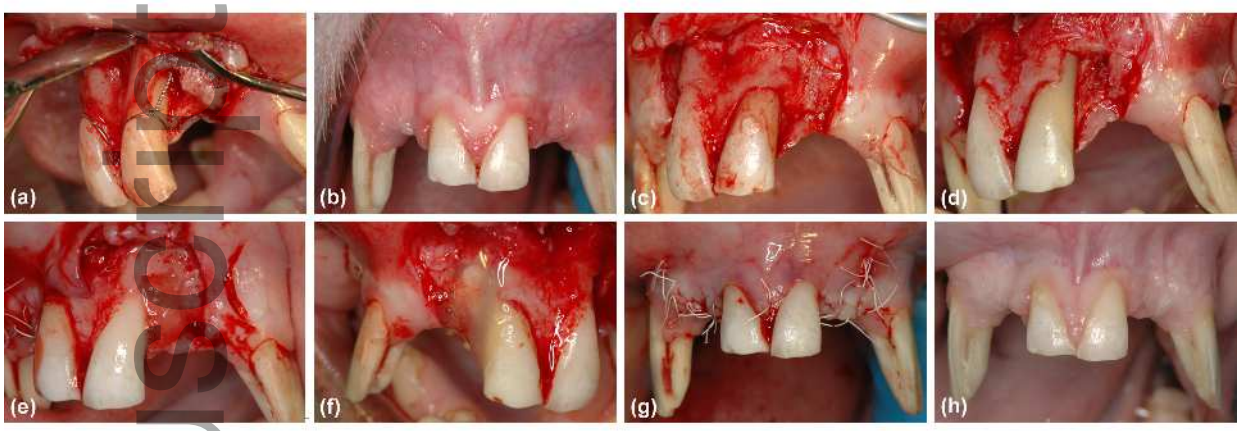
Figure 3. Representative photomicrographs of a 2-wall intrabony defect treated with ACS. **(a)** Overview. (Bar: 1 mm; Azan-Mallory staining) **(b)** Higher magnification of the apical framed area in (a). **(c)** Higher magnification of the coronal framed area in (a). (Bar: 100 µm; Azan-Mallory staining). NB: new bone, NC: new cementum, PDL: periodontal ligament, N: notch (apical extent of root planing), D: root dentin.

Figure 4. Photomicrographs of 2-wall intrabony defects treated with Emdogain/ACS. **(a)** A well-healed 2-wall intrabony defect in the incisor site. Overview. (Bar: 1 mm; Azan-Mallory staining) **(b)** Higher magnification of the coronal framed area in (a). (Bar: 100 µm; Azan-Mallory staining). **(c)** A poorly-healed 2-wall intrabony defect in the premolar site. Overview. (Bar: 1 mm; Azan-Mallory staining) **(d)** Higher magnification of the coronal framed area in (**c**). (Bar: 100 µm; Azan-Mallory staining). NB: new bone, NC: new cementum, PDL:

periodontal ligament, JE: junctional epithelium, CT: connective tissue, N: notch (apical extent of root planing), D: root dentin.

Figure 5. Representative photomicrographs of a 2-wall intrabony defect (incisor site) treated with Osteogain/ACS. **(a)** Overview. (Bar: 1 mm; Azan-Mallory staining) **(b)** Higher magnification of the coronal framed area in (a). (Bar: 100 µm; Azan-Mallory staining). NB: new bone, NC: new cementum, PDL: periodontal ligament, N: notch (apical extent of root planing), D: root dentin.

Figure 6. Representative photomicrographs of a 2-wall intrabony defect (premolar site) treated with Osteogain/ACS**. (a)** Overview. (Bar: 1 mm; Azan-Mallory staining) **(b)** Higher magnification of the apical framed area in (a). (Bar: 100 µm; Azan-Mallory staining) **(c)** Higher magnification of the coronal framed area in (a). (Bar: 100 µm; Azan-Mallory staining). NB: new bone, NC: new cementum, PDL: periodontal ligament, N: notch (apical extent of root planing), D: root dentin**.**



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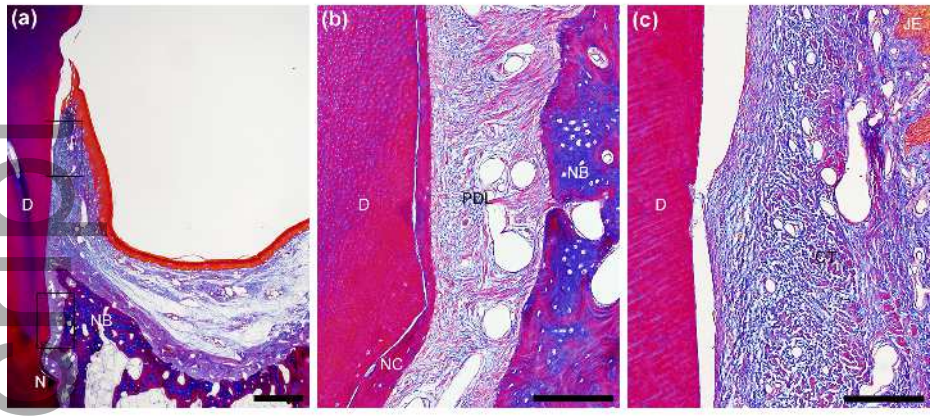

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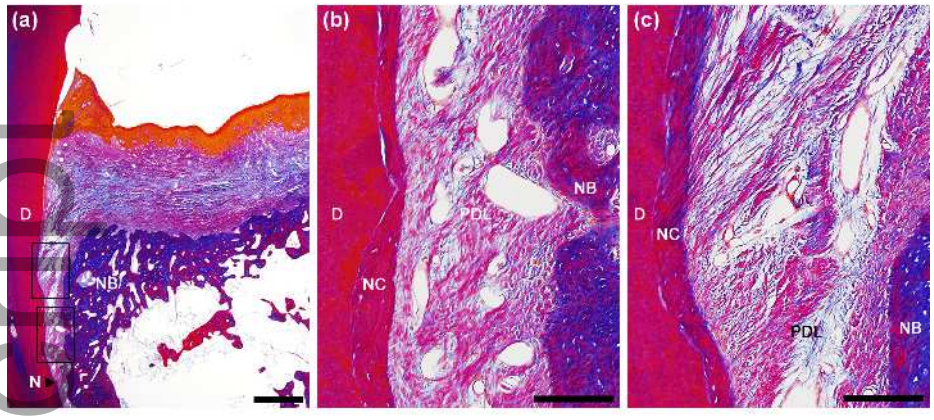

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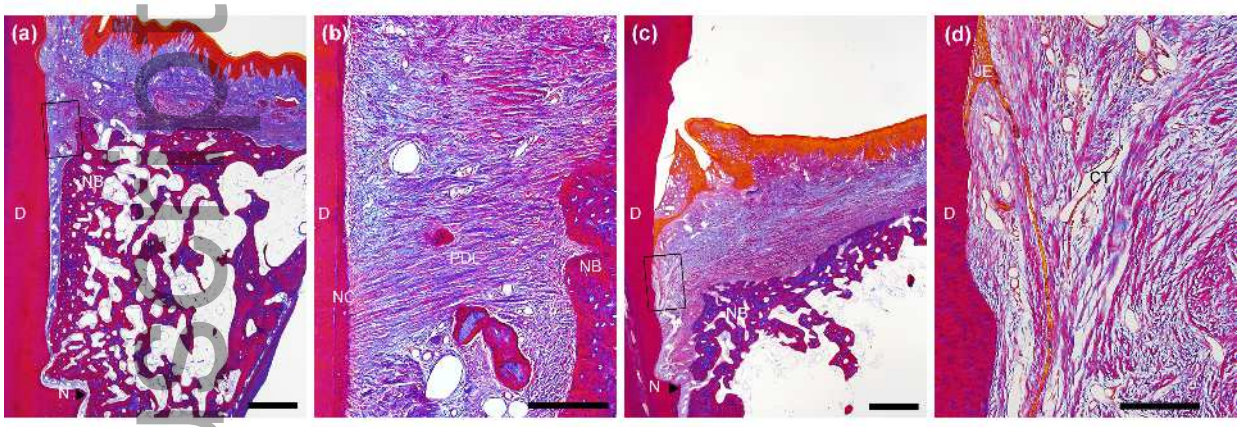

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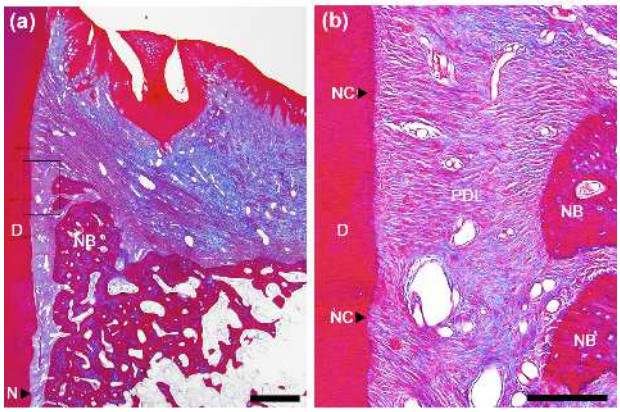

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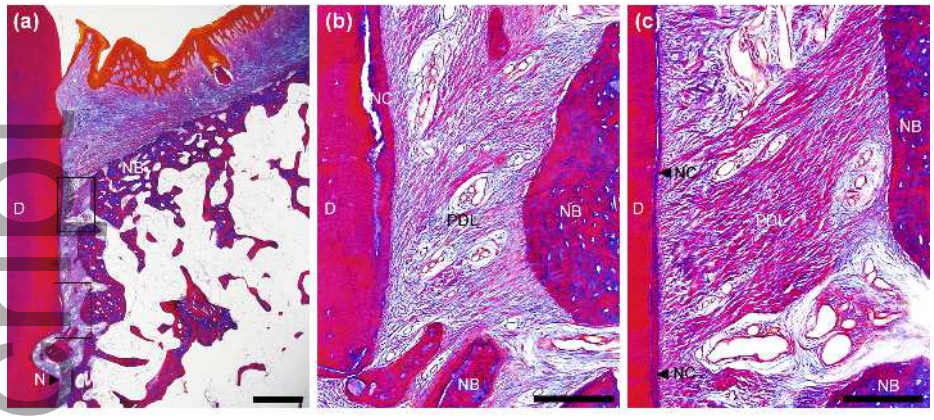

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