Effects of EMD liquid (Osteogain) on periodontal healing in class III furcation defects in monkeys

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Abstract

Aim: To evaluate the effect of a novel liquid carrier system of enamel matrix derivative (Osteogain) soaked on an absorbable collagen sponge (ACS) upon periodontal wound healing/regeneration in furcation defects in monkeys.

Materials and Methods: The stability of the conventional enamel matrix derivative (Emdogain) and Osteogain adsorbed onto ACS was evaluated by ELISA. Chronic class III furcation defects were created at teeth 36, 37, 46, 47 in three monkeys (*Macaca fascicularis*). The 12 defects were assigned to one of the following treatments: (1) open flap debridement (OFD) + ACS, (2) OFD+Emdogain/ACS, (3) OFD+Osteogain/ACS, and (4) OFD alone. At 16 weeks following reconstructive surgery, the animals were killed for histological evaluation.

Results: A 20–60% significantly higher amount of total adsorbed amelogenin was found for ACS-loaded Osteogain when compared to Emdogain. The histomorphometric analysis revealed that both approaches (OFD + Emdogain/ACS and OFD + Osteogain/ACS) resulted in higher amounts of connective tissue attachment and bone formation compared to treatment with OFD + ACS and OFD alone. Furthermore, OFD + Osteogain/ACS group showed higher new attachment formation, cementum, and new bone area.

Conclusions: Within their limits, the present data indicate that Osteogain possesses favourable physicochemical properties facilitating adsorption of amelogenin on ACS and may additionally enhance periodontal wound healing/regeneration when compared to Emdogain.

Pre-clinical and clinical studies have provided substantial evidence on the biologic potential of an enamel matrix derivative (EMD) to promote periodontal wound healing/regeneration and improve the clinical outcomes in intra-bony, furcation, and recession-type defects (Miron et al. 2016a). However, due to its fluid

consistency, the use of EMD gel (Emdogain) appears to possess limited space-making potential which, in defects with a more complicated anatomy (e.g. so called non-contained-type defects), may not be able to prevent the collapse of a mucoperiosteal flap thus limiting the available space for regeneration (Mellonig 1999, Lekovic et al. 2000, Cochran et al. 2003, Shirakata et al. 2007). In order to overcome these potential shortcomings, clinicians often combine various types of bone grafting materials with Emdogain, especially when treating non-contained-type defects (Sculean et al. 2011). The combination of Emdogain and different types of grafting materials has been extensively evaluated in pre-clinical and clinical studies (Lekovic et al. 2000, Cochran et al. 2003, Shirakata et al. 2007, Gurinsky et al. 2004, Bokan et al. 2006, Kuru et al. 2006, Yilmaz et al. 2010). Although a recent systematic review reported that the combination of Emdogain with bone grafting materials may lead to statistically significantly higher clinical improvements, the analysis also revealed a high heterogeneity between the clinical outcomes obtained with the various combination approaches (Matarasso et al. 2015). One possible reason for the high clinical variability in the outcomes when using the combination of Emdogain and grafting materials may be related to substantial differences in enamel matrix proteins (EMPs) adsorption and subsequent cell proliferation between the utilized bone grafting materials (Miron et al. 2015). In this respect, very recent data from a series of in vitro studies revealed that the use of EMD without its propylene-glycol-alginate (PGA) carrier markedly improved protein adsorption when compared to conventional EMD (e.g. Emdogain) (Miron et al. 2015). Further advantages of using EMD without its PGA carrier is related to its more fluid consistency and subsequent improvement of surface coating and penetration of EMPs within the bone biomaterials coupled with the capacity for a 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 (Osteogain) specifically designed for mixing it with

different biomaterials including bone grafts and collagen matrices/scaffolds. Very recently, Miron et al. (2016b) reported that pre-coating Osteogain onto a natural bovine mineral (NBM) significantly increased cell adhesion, proliferation, and differentiation of osteoblasts in vitro (Miron et al. 2016b) and improved new bone formation in a rat femur bone defect model (Zhang et al. 2016). However, despite these encouraging data, it is still unclear to what extent Osteogain is effective in promoting periodontal wound healing/regeneration in periodontal defects.

An important aspect that is still controversially discussed in the literature is the need of using bone grafting materials for enhancing periodontal regeneration. Since most findings from histological studies in animals and humans indicate that the healing following the use of bone grafting materials is frequently characterized by persistence of grafting residues embedded either in bone or encapsulated in connective tissue, the biologic rationale of using such materials needs to be questioned (Ivanovic et al. 2014, Sculean et al. 2015). Thus, although clinical consequences of persisting graft residues are still unclear, from a biological point of view, it would be desirable that a biomaterial used as a carrier for biologics (e.g. growth factors or EMD) or to stabilize the wound by supporting the flap, is completely resorbed and replaced by regenerated periodontal tissues (e.g. cementum, periodontal ligament, and bone). During the last decades various types of collagens matrices have been successfully used as carriers for growth factors and Emdogain, pointing to the potential biological advantages of this combination to support wound healing/regeneration (Susin et al. 2015, Stähli et al. 2016).

However, at present, it is unknown whether the combination of EMD and an absorbable collagen sponge (ACS) may represent a potential option for reconstructive surgery in complex non-contained defects such as class III furcation defects. Therefore, the aim of the present pre-clinical study was to evaluate the effects of Osteogain with ACS in chronic class III furcation defects in non-human primates.

Materials and Methods

Emdogain, Osteogain, and ACSs

Emdogain and Osteogain (0.3 ml vials, concentration 30 mg/ml; Straumann AG, Basel, Switzerland) and native type I and III porcine absorbable collagen sponges (ACS: Collacone®; Botiss, Berlin, Germany) were utilized in this study.

Quantification of amelogenin adsorption to ACSs

To determine the quantity of Emdogain/Osteogain adsorption to the surface of ACSs, ELISA quantification assay was utilized for amelogenin, the main protein found in EMD encompassing 90–95% of the total protein content. Briefly, 0.3 ml of Emdogain/Osteogain was poured onto cylindrical ACS scaffolds in six-well dishes for 10 min at 37°C. After the coating period incubation, the samples were simply washed with phosphate-buffered saline (PBS), and the remaining PBS solution containing unattached enamel matrix proteins was collected and quantified by a Quantikine Colorimetric Sandwich ELISA (Porcine Amelogenin, X isoform (AMELX) ELISA Kit; MyBioSource Inc, San Diego, CA, USA) according to the manufacturer's protocol. Subtraction of total coated protein from the amount of unadsorbed protein was used to determine the amount of adsorbed material to the surface of ACS as previously described (Miron et al. 2015). Furthermore, in order to determine the quantity of amelogenin protein being released from ACS over time, coated ACSs were soaked in 5 ml of PBS and samples were collected at various time points including 15 min, 1 h, 8 h, 1, 3, and 10 days. All samples were quantified in triplicate and three independent experiments were performed.

Experimental animals

Three 7–8-year old male monkeys (*Macaca fascicularis*), weighing 6.91–7.02 kg, were used. The animals exhibited intact dentition with healthy periodontium. They were kept in individual cages at 23–29°C, relative humidity of 30–70%, and a 12-hour light/dark cycle. Approximately, 108 g of solid food (HF Primate J

12G 5K9J, Purina Mills, LLC, Gray Summit, MO, USA) was provided to each animal daily and water was available ad libitum. All procedures during the in-life phase for about 9 months (from 6 November 2014 to 3 August 2015) were approved by the ethical committee of the Animal Research Center of Kagoshima University, Japan (approval no. D14026) and were performed in accordance with standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals, NIH OACU) of the National Institutes of Health Policy on Human Care and Use of Laboratory Animals.

Preparation of experimental defects

One surgeon (Y. S) performed all surgical procedures under general and local anaesthesia using aseptic routines. Before the operation, buprenorphine hydrochloride (Lepetan injection 0.2 mg; Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan, 0.1 ml/Kg), to ameliorate pain, and an antibiotic (Mycilinzol Meiji, 0.05 ml/kg; Meiji Seika Pharma Co., Ltd, Tokyo, Japan), to prevent infection, were administered intramuscularly. General anaesthesia was achieved with a ketamine hydrochloride (0.2 ml/kg IM; Supriya Lifescience Ltd, Mumbai, India)/medetomidine hydrochloride (Domitor, 0.08 ml/kg IM; Orion Corporation, Espoo, Finland) combination maintaining spontaneous breathing. Local anaesthesia was performed using lidocaine HCl/epinephrine (2%, 1:80,000; Xylocaine, Fujisawa Inc., Osaka, Japan). After the operation, atipamezole hydrochloride (Antisedan, 0.08 ml/kg, Orion Corporation) was administered intramuscularly. Ketoprofen (Capisten IM 50 mg, 2 mg/kg, 0.1 ml/kg; Kissei Pharmaceutical Co., Ltd, Matsumoto, Japan), to ameliorate pain, and an antibiotic (Mycilinzol Meiji, 0.05 ml/kg; Meiji Seika Pharma Co., Ltd), to prevent infection, were intramuscularly administered to all animals for 2 days after operation.

As a pre-treatment, the second premolars and third molars in the mandible were extracted for flap management to prevent material exposure and obtain primary closure as previously described (Donos et al.2003, Gkranias et al. 2012). After a healing period of 2 months, the mucoperiosteal flaps were raised and class III furcation defects were surgically created at the first and the second mandibular molars with the use of bone chisels and slowly rotating diamond burs (12 defects in total). The dimensions of the exposed furcation defects were 5 mm wide and 5 mm high. In order to prevent spontaneous healing and induce plaque accumulation, the defects were filled with impression materials (EXAFINE PUTTY TYPE, GC Corporation, Tokyo, Japan) (Fig. 1a). Subsequently, the flaps were repositioned and stabilized with 4-0 silk sutures (Mersilk™; Ethicon Ltd, Edinburgh, UK). Sutures were removed at 10 days following surgery. During 8 weeks following the first surgery, no oral hygiene measures were performed and the animals were fed a soft diet. After 8 weeks, the impression material was removed from the defects and a plaque control regimen, consisting of oral cavity flushing with a chlorhexidine gluconate solution (5% HIBITANE®, 25 ml of a 2% solution; Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan) was performed for a period of 4 weeks.

Reconstructive surgery

At 16 weeks following creation of the defects (Fig. 1b), intra-sulcular incisions were performed and full-thickness buccal and lingual flaps were elevated in order to expose the furcation defects. All granulation tissue (Fig. 1c) was removed and the exposed root surface was carefully scaled and planed. Cementum was removed using Gracey curettes and a chisel (Fig. 1d). Reference notches were made using a #1 round bur on the root surface at the base of the defects for histometric analysis. Class III furcation defects received 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. The experimental conditions were rotated between defect sites in subsequent animals. Emdogain/ACS and Osteogain/ACS were not placed in the same unilateral side in the same animal. In the ACS group, ACS was mixed with sterile saline before being applied to the defect. Root surfaces that received Emdogain or Osteogain were conditioned with a 24% EDTA gel (PrefGel®; Straumann AG) for 2 min and then, along with the adjacent mucoperiosteal flaps, thoroughly rinsed with sterile saline. 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 min (Fig. 1e). The constructs were then filled in the defect with moderate pressure (Fig. 1f). Maximum care was taken during surgery to prevent mixing of Emdogain or Osteogain to the other site in the same side of the mandible.

A periosteal releasing incision was made to allow coronal displacement 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). After reconstructive surgery, the animals received similar treatments of the antibiotics and analgesics which were used during preparation of experimental defects. Sutures were removed after 14 days of healing and postoperative plaque control was maintained as previously described. Then, 4 months after the reconstructive surgery (Fig. 1h) the animals were anesthetized by an intravenous injection of sodium pentobarbital (64.8 mg/ml, 0.4 ml/kg; Tokyo Chemical Industry Co., Ltd, Tokyo, Japan) and killed by exsanguination.

Histological processing

All the defects, including the experimental and control sites, were then dissected with the surrounding soft and hard tissues. The tissue blocks were fixed in 10% buffered formalin, trimmed, and rinsed in PBS. The samples were decalcified in Kalkitox™ solution (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 3 weeks, dehydrated, and embedded in paraffin. Step serial sections of 6 μm thickness were then prepared along the mesio-distal plane, stained with haematoxylin/eosin or with Azan–Mallory at intervals of 90 μm.

Histometric analysis

All the specimens were analysed histometrically under a light microscope (Eclipse E800; Nikon Inc., Tokyo,
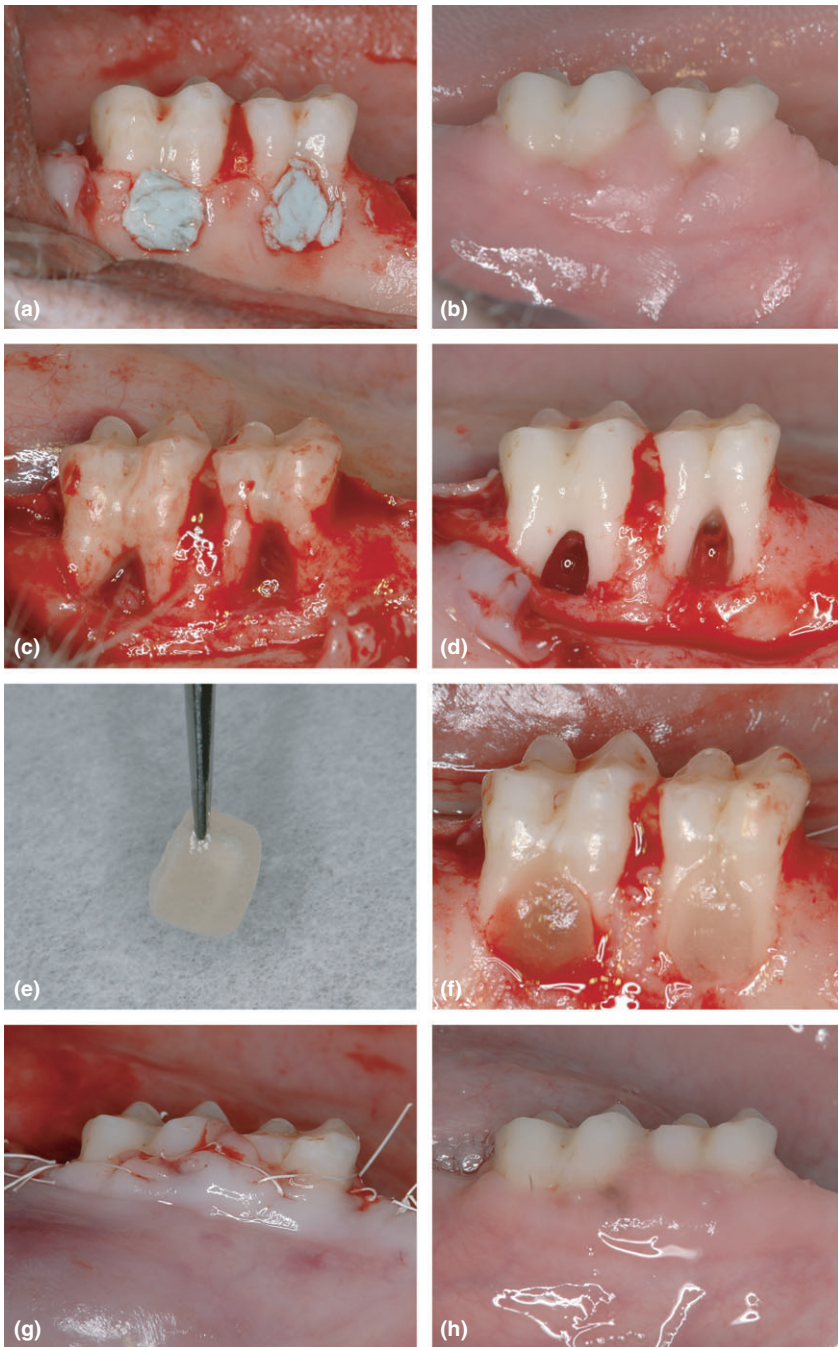
Fig. 1. Clinical appearance of the mandibular buccal aspect of *Macaca fascicularis*. (a) Induction of chronic inflammation. After fabrication of Class III furcation defects, impression materials were placed to encourage growth of oral microflora along the exposed root surfaces. (b) Prior to reconstructive surgery. (c) Immediately after flap reflection. Note the excessive granulation tissue in the chronic defects. (d) Defects were exposed and debrided again at the time of reconstructive surgery. (e) Osteogain/absorbable collagen sponge (ACS) construct before surgical implantation. (f) left (second molar): ACS alone, right (first molar): placement of Osteogain/ACS. (g) Flaps were coronally repositioned and sutured. (h) 16 weeks after reconstructive surgery.

notches defined the apical limit of the defect and the following parameters were measured by the same experienced and masked examiner (T. N.). The mean value of each histometric parameter was then calculated for each site.

Area measurements (in mm² and %)

1 Bone defect area (BDA): area limited by the apical line and the root surface in the furcation region;
2 Non-filled area (NFA): portion of the BDA not filled with any tissue, partially filled with plaque deposited on the root surface;
3 Epithelium tissue area (ETA): portion of the BDA filled with epithelium tissue;
4 Connective tissue area (CTA): portion of the BDA filled with connective tissue;
5 New bone area (NBA): portion of the BDA filled with new bone

Area measurements, except for BDA, were calculated as the percentage of the BDA within each defect.

Linear measurements (in mm and %)

1 Length of the root surface (LRS): length of the root surface from the mesial notch to the distal notch;
2 Tissue-free defect length (TFL): portion of the LRS with the absence of any new tissue formation;
3 Junctional epithelial migration (JE): total linear extensions of the root surface covered by epithelial tissue;
4 Connective tissue adhesion (CT): total linear extensions of the root surface covered by connective tissue without cementum;
5 New cementum formation (NC): total linear extensions of the root surface coved by new cementum:
6 New attachment formation (NA): total linear extensions of the root surface covered by NC adjacent to newly formed bone, with functionally oriented collagen fibres

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

Examiner calibration

Thirty-six sections from all sites were read by the examiner without calibration before the measurements.

Japan) equipped with a computerized imaging system (Image-pro Plus Media Cybernetics, Silver Spring, MD, USA). For the histometric analysis, three sections approximately 90 μm apart were selected from the most central area of each class III furcation defect, identified by the length of the root canal and the reference notches. A line connecting both

Forty-eight hours later, the same examiner read all 36 sections again to evaluate intra-examiner reproducibility. Inter-calibration of the examiner was accepted at the 90% level. Because of low sample size due to the selected model chosen, the statistical analysis was restricted to descriptive statistics.

Statistical analysis

For the amelogenin ELISA quantification experiments, means and standard errors (SE) were calculated. Statistically significant differences were examined by multiple *t*-tests between both groups. Statistical significance was defined as a *p*-value of 0.05 corrected using the Bonferroni–Dunn method utilizing GraphPad Prism software (La Jolla, CA, USA).

Results

Ability to adsorb and release Emdogain and Osteogain over time

ELISA was utilized to investigate the amount of adsorbed amelogenin when Emdogain or Osteogain were loaded onto ACS (Fig. 2). While it was first found that both carriers efficiently loaded amelogenin onto the collagen sponges at time point 0, a simple saline rinse with PBS significantly removed over 20% more (from >90% to 70%) of the total amelogenin content from Emdogain when compared to Osteogain where the total protein content remained <90% of the initial concentrations (Fig. 2). At each of the remaining time points thereafter, a 20–60% significantly higher amount of total adsorbed amelogenin was found for collagen sponges loaded with Osteogain when compared to Emdogain

(Fig. 2). After a 10-day period, nearly 60% of the initial amelogenin protein content found in Osteogain remained present within the collagen sponges, whereas in the Emdogain samples, no remaining amelogenin could be quantified as values approached 0% (Fig. 2).

Clinical observations

All surgical treatments were well tolerated by the animals, and clinical healing was uneventful at all 12 sites. No visible adverse reactions including material exposure, infection, and suppuration were observed throughout the experimental period.

Histological observations

OFD group

In the OFD group, apical migration of the junctional epithelium considerably occurred (Fig. 3a,e,i) with varying degrees of new attachment, and new bone formation was observed. In one defect, considerable new cementum formation and moderate new attachment formation occurred (Fig. 3a). Artefacts (separations between the new cementum and the root surface) were consistently detected in all three defects. Thick new cellular cementum with or without collagen fibres obliquely oriented to the root surface was seen in the lower portion of the defect (Fig. 4a,b). The collagen fibres appeared to be sparser than those observed in the Emdogain/ACS and Osteogain/ACS groups (Figs 3e,i and 4b).

ACS group

The healing pattern in the ACS group was characterized by limited periodontal regeneration (Fig. 3b, f

and j). Junctional epithelium migrated to the most coronal extension of new cementum with less connective tissue adhesion (Fig. 4c,d). A small amount of new bone formed in the lower portion of the defect (Fig. 3b,f,j). New cementum formation and new attachment formation were minimal in one defect, and restricted to the mid portion of the rest of two defects. Moderate thick and thin cellular cementum with or without collagen fibres obliquely oriented to the root surface was observed (Fig. 4c,d).

Emdogain/ACS group

In the Emdogain/ACS *group*, apical extension of the junctional epithelium was more restrained than in the OFD and ACS groups. A greater amount of new cementum was observed in the EMD group than in the control and ACS groups. New bone formation was noted extending from the apical notch towards the coronal region of the defect (Fig. 3g, k). Thin acellular cementum and thick cellular cementum, with collagen fibres obliquely oriented to the root surfaces, were observed (Fig. 4e,f). Furthermore, the collagen fibres appeared to be denser than those observed in the OFD and ACS groups. Many blood vessels were observed within the newly formed periodontal ligament (Fig. 4e,f).

Osteogain/ACS group

In general, the healing pattern in the Osteogain/ACS group was similar to the Emdogain/ACS group. However, migration of junctional epithelium was more restrained in the Osteogain/ACS group than in the OFD, ACS, and Emdogain/ACS groups. Furthermore, new attachment and new bone formation were consistently noted extending from the apical notches towards the coronal region of the defect in all defects (Fig. 3d,h, l). Moderately thick new cellular and thin acellular cementum, with dense collagen fibres obliquely or perpendicular oriented to the denuded root surface was more consistently observed (Fig. 4g, h). Highly vascularized new periodontal ligament-like tissue, tightly confined to between the new cementum and new bone, maintained its width up to the coronal portion (Fig. 4g,h).
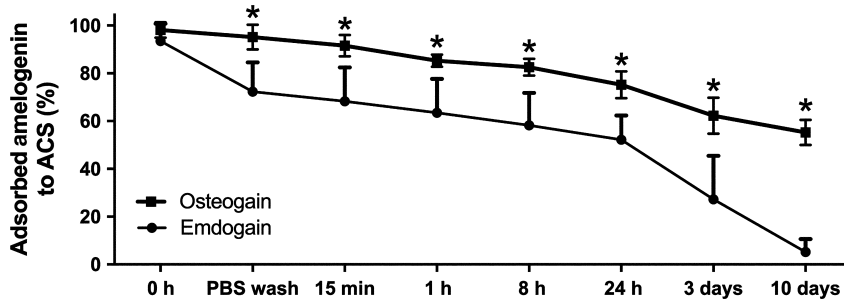

Fig. 2. In vitro release profiles of Emdogain and Osteogain from absorbable collagen sponge. (*, *p* values < 0.05 was considered significant).
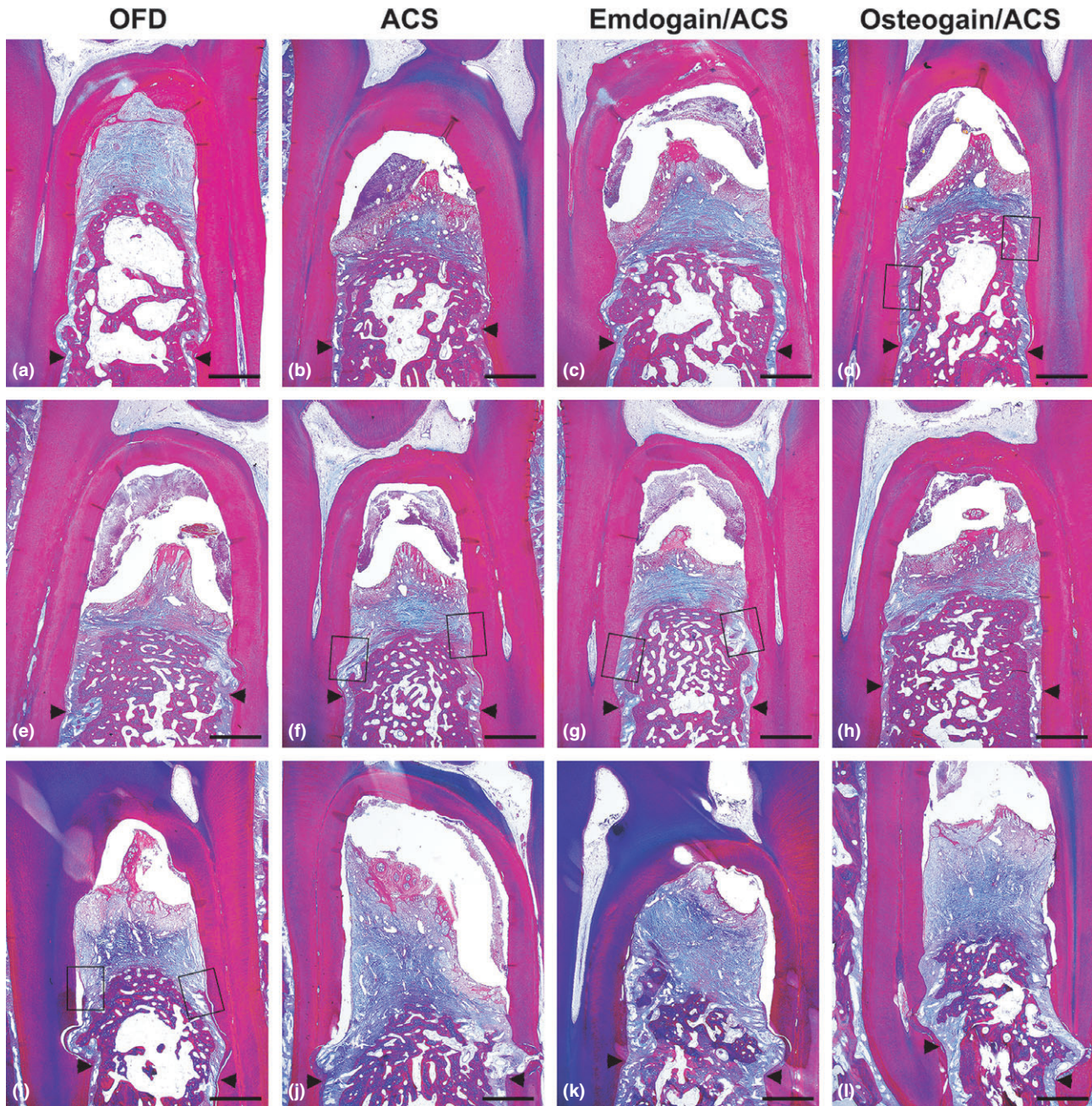
Fig. 3. Overview photomicrographs of all Class III furcation defects in different groups (Azan-Mallory staining). (a), (e), and (i): Open flap debridement (OFD) group Overview. (scale bar: 1 mm). (b), (f), and (j): Absorbable collagen sponge (ACS) group Overview. (scale bar: 1 mm). (c), (g), and (k): Emdogain/ACS group Overview. (scale bar: 1 mm). (d), (h), and (l): Osteogain/ACS group Overview. (scale bar: 1 mm). Arrowhead: notch (apical extent of root planing).

ACS appeared to be completely resorbed after 16 weeks of healing in the ACS, Emdogain/ACS, and Osteogain/ACS groups. Complete defect resolution of furcation defects was not achieved in any of defects in all four treatment groups. There was neither extensive root resorption nor ankylosis, irrespective of the experimental group.

Histometric analysis

The results of histometric analysis are summarized in Tables 1 and 2. The ETA/BDA in the Emdogain/ACS ($14.6 \pm 2.7\%$) and Osteogain/ACS ($14.3 \pm 1.5\%$) groups were lower than those in the OFD ($19.2 \pm 3.9\%$) and ACS ($21.7 \pm 3.2\%$) groups. Osteogain/ACS group showed the greatest amount of newly formed bone (NBA/BDA) among the groups examined. The length of junctional epithelium migration observed in the Osteogain/ACS group was shorter than those in the OFD, ACS, and Emdogain/ACS groups. The Emdogain/ACS and Osteogain/ACS groups showed greater cementum formation than the ACS group. The amount of
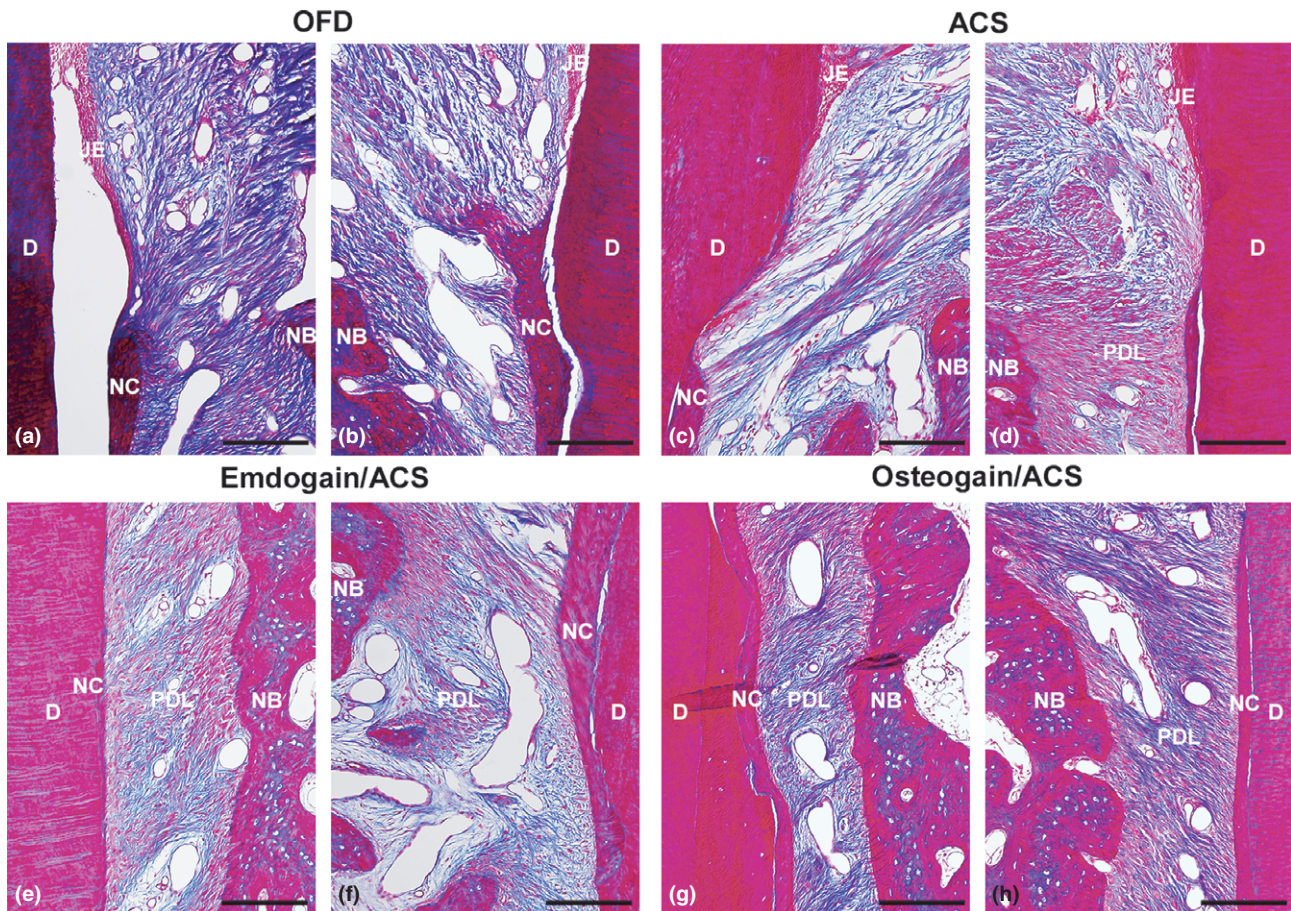
OFD

ACS

Emdogain/ACS

Osteogain/ACS

Fig. 4. Representative photomicrographs of Class III furcation defects in open flap debridement (OFD) group (Azan-Mallory staining). (a) Higher magnification of the framed area (left) in Fig. 3i (scale bar: 200 μm), (b) Higher magnification of the framed area (right) in Fig. 3i (scale bar: 200 μm), in ACS group (c) Higher magnification of the framed area (left) in Fig. 3f (scale bar: 200 μm), (d) Higher magnification of the framed area (right) in Fig. 3f (scale bar: 200 μm), in Emdogain/ACS group (e) Higher magnification of the apical framed area (left) in Fig. 3g, (f) Higher magnification of the framed area (right) in Fig. 3g (scale bar: 200 μm), and in Osteogain/ACS group (g) Higher magnification of the framed area (left) in Fig. 3d (scale bar: 200 μm), (h) Higher magnification of the framed area (right) in Fig. 3d (scale bar: 200 μm). JE: junctional epithelium, NB: new bone, D: root dentin, NC: new cementum, PDL: periodontal ligament.

new cementum in the Osteogain/ACS group (40.5 ± 7.2%) was twice as great as in the ACS group (19.4 ± 7.5%). Moreover, new attachment formation was most extensive in the Osteogain/ACS (37.4 ± 4.6%) group when compared to the OFD (19.4 ± 6.9%), ACS (14.1 ± 9.6%), and Emdogain/ACS (25.0 ± 1.8%) groups.

Discussion

To the best of our knowledge, this is the first report evaluating the potential effects on periodontal wound healing/regeneration of a new liquid carrier system for EMD (Osteogain) in chronic class III furcation defects in non-human primates. Many investigators have

become interested in the plausible reasons for the high clinical variability for studies reporting the combination of EMD with bone grafting materials (Tu et al. 2010, Miron et al. 2014). Previous data have shown that the use of Emdogain, although ideal for root surface adsorption, displayed drastically increased thickness of coating to the bone grating surface which was easily dissolved following a simple PBS rinse. Contrarily, the use of Osteogain (EMD dissolved in acetic acid solution) exhibited more favourable surface coating by demonstrating an increase and more complete surface loading of porous graft materials and tighter and more stable surface coatings with enamel matrix proteins (Miron et al. 2015).

In this study, we used ACS as a putative carrier for EMD since ACS has been extensively used as an appropriate carrier including high clinical applicability, biocompatibility, and uneventful biodegradation in bone and periodontal surgeries (McPherson 1992, Cochran et al. 2000, Yamashita et al. 2010, Kim et al. 2013). In addition, it has been reported that the fast resorption of a residual material is desirable to avoid the risk for infection and to increase the amount of regenerated tissues in bone/periodontal defects (MacNeill et al. 1999, Shirakata et al. 2002, 2007, Potijanyakul et al. 2010, Yoshinuma et al. 2012). Histological findings demonstrated that ACS was completely resorbed after 16 weeks of healing in the ACS, Emdogain/ACS,

Table 1. Histomorphometric area measurement in each group (mean $\pm$ SD in mm^2 and %; n = 3 animals, n = 12 sites)

Histometric parameter	Animal No	Experimental condition			
		Control	ACS	Emdogain/ACS	Osteogain/ACS
BDA (mm^2)	1	12.3	11	16.2	12.7
	2	13.1	10.2	11.6	14.5
	3	12.1	18	11.6	17.5
	Mean $\pm$ SD	12.5 $\pm$ 0.5	13.1 $\pm$ 4.3	13.1 $\pm$ 2.7	15.0 $\pm$ 2.4
NFA in mm^2 and (%)	1	0 (0.4)	2.7 (25.0)	4.9 (30.3)	3.2 (25.6)
	2	5.9 (45.4)	3.1 (30.5)	3.4 (28.8)	2.6 (17.7)
	3	1.5 (12.4)	5.2 (28.6)	1.1 (9.5)	4.2 (24.3)
	Mean $\pm$ SD	2.5 $\pm$ 3.1 (19.4 $\pm$ 23.4)	3.7 $\pm$ 1.3 (28.0 $\pm$ 2.8)	3.1 $\pm$ 1.9 (22.9 $\pm$ 11.6)	3.4 $\pm$ 0.8 (22.5 $\pm$ 4.3)
ETA in mm^2 and (%)	1	2.5 (20.5)	2.6 (23.8)	2.4 (14.7)	1.6 (13.0)
	2	1.9 (14.8)	1.8 (17.9)	1.4 (11.9)	2.1 (14.1)
	3	2.7 (22.4)	4.2 (23.3)	2.0 (17.2)	2.8 (15.9)
	Mean $\pm$ SD	2.4 $\pm$ 0.4 (19.2 $\pm$ 3.9)	2.9 $\pm$ 1.2 (21.7 $\pm$ 3.2)	1.9 $\pm$ 0.5 (14.6 $\pm$ 2.7)	2.2 $\pm$ 0.6 (14.3 $\pm$ 1.5)
CTA in mm^2 and (%)	1	2.0 (16.7)	1.4 (13.1)	2.4 (14.7)	1.2 (9.5)
	2	1.3 (9.6)	1.9 (18.4)	1.4 (11.9)	1.8 (12.1)
	3	2.4 (20.1)	3.8 (21.0)	2.9 (25.3)	3.2 (18.2)
	Mean $\pm$ SD	1.9 $\pm$ 0.6 (15.4 $\pm$ 5.4)	2.4 $\pm$ 1.2 (17.5 $\pm$ 4.0)	2.2 $\pm$ 0.8 (17.3 $\pm$ 7.1)	2.1 $\pm$ 1.0 (13.3 $\pm$ 4.5)
NBA in mm^2 and (%)	1	3.2 (26.3)	3.8 (34.5)	5.4 (33.4)	5.4 (42.4)
	2	3.2 (24.1)	2.8 (27.1)	4.3 (37.0)	6.9 (47.2)
	3	4.1 (34.3)	3.1 (17.2)	3.6 (30.9)	5.3 (30.2)
	Mean $\pm$ SD	3.5 $\pm$ 0.6 (28.3 $\pm$ 5.3)	3.2 $\pm$ 0.5 (26.3 $\pm$ 8.7)	4.4 $\pm$ 0.9 (33.8 $\pm$ 3.0)	5.8 $\pm$ 0.9 (39.9 $\pm$ 8.8)

ACS, absorbable collagen sponge; BDA, bone defect area; NFA, non-filled area; ETA; epithelium tissue area; CTA, connective tissue area; NBA, new bone area.

Table 2. Histomorphometric linear measurement in each group (mean $\pm$ SD in mm and %; n = 3 animals, n = 12 sites)

Histometric parameter	Animal No	Experimental condition			
		Control	ACS	Emdogain/ACS	Osteogain/ACS
LRS (mm)	1	12.3	10.1	13.1	12
	2	11.3	10.7	11.7	12.3
	3	12.2	14.5	10.9	16.5
	Mean $\pm$ SD	11.9 $\pm$ 0.5	11.8 $\pm$ 2.4	11.9 $\pm$ 1.1	13.6 $\pm$ 2.5
TFL in mm and (%)	1	1.4 (11.6)	5.8 (57.2)	7.0 (53.8)	5.4 (44.6)
	2	7.9 (69.8)	5.5 (51.7)	5.7 (48.6)	5.5 (45.1)
	3	3.8 (31.0)	7.3 (50.5)	4.6 (42.2)	7.3 (44.3)
	Mean $\pm$ SD	4.4 $\pm$ 3.3 (37.5 $\pm$ 29.6)	6.2 $\pm$ 1.0 (53.1 $\pm$ 3.6)	5.8 $\pm$ 1.2 (48.2 $\pm$ 5.8)	6.1 $\pm$ 1.1 (44.7 $\pm$ 0.4)
JE in mm and (%)	1	6.2 (50.7)	2.0 (19.9)	1.6 (11.9)	1.5 (12.5)
	2	0.9 (7.6)	2.7 (25.4)	1.6 (14.1)	1.5 (12.5)
	3	5.0 (40.6)	4.9 (33.9)	3.7 (33.9)	3.6 (22.0)
	Mean $\pm$ SD	4.0 $\pm$ 2.8 (33.0 $\pm$ 22.5)	3.2 $\pm$ 1.5 (26.4 $\pm$ 7.0)	2.3 $\pm$ 1.2 (19.9 $\pm$ 12.1)	2.2 $\pm$ 1.2 (15.7 $\pm$ 5.5)
CT in mm and (%)	1	0.4 (2.9)	0 (0)	0.2 (1.1)	0.1 (1.1)
	2	0.1 (0.4)	0.3 (2.5)	0.6 (5.5)	0.2 (1.6)
	3	0.1 (1.2)	0.1 (0.7)	0.3 (2.8)	0.4 (2.4)
	Mean $\pm$ SD	0.2 $\pm$ 0.2 (1.5 $\pm$ 1.3)	0.1 $\pm$ 0.1 (1.1 $\pm$ 1.3)	0.4 $\pm$ 0.3 (3.1 $\pm$ 2.2)	0.2 $\pm$ 0.1 (1.7 $\pm$ 0.7)
NC in mm and (%)	1	5.1 (41.6)	2.8 (27.3)	3.9 (29.9)	5.0 (41.3)
	2	2.6 (23.1)	2.0 (18.5)	3.8 (32.6)	5.8 (47.2)
	3	3.3 (26.9)	1.8 (12.4)	4.3 (39.7)	5.4 (32.9)
	Mean $\pm$ SD	3.7 $\pm$ 1.3 (30.5 $\pm$ 9.8)	2.2 $\pm$ 0.5 (19.4 $\pm$ 7.5)	4.0 $\pm$ 0.3 (34.1 $\pm$ 5.1)	5.4 $\pm$ 0.4 (40.5 $\pm$ 7.2)
NA in mm and (%)	1	3.3 (26.9)	2.4 (23.4)	3.0 (23.1)	4.9 (40.8)
	2	2.1 (18.1)	1.6 (14.7)	3.1 (26.6)	4.8 (39.2)
	3	1.6 (13.2)	0.6 (4.2)	2.8 (25.4)	5.3 (32.3)
	Mean $\pm$ SD	2.3 $\pm$ 0.9 (19.4 $\pm$ 6.9)	1.5 $\pm$ 0.9 (14.1 $\pm$ 9.6)	3.0 $\pm$ 0.2 (25.0 $\pm$ 1.8)	5.0 $\pm$ 0.3 (37.4 $\pm$ 4.6)

ACS, absorbable collagen sponge; LRS, length of the root surface; TFL, tissue-free defect length, JE, junctional epithelial migration; CT, connective tissue adhesion; NC, new cementum; NA, new attachment formation.

and Osteogain/ACS groups. Complete resolution of all furcation defects was not achieved in any of the specimens. On the one hand, the incomplete regeneration may be explained by the large chronic-type, furcation defects exhibiting a lower healing potential and by the difficulties to ensure a plaque-free healing environment in this animal model (Caton et al. 1994, Donos et al. 2003). It has been extensively demonstrated that periodontal wound healing and the outcomes following conventional and regenerative periodontal surgery are negatively influenced by plaque accumulation of the wound area (Rosling et al. 1976,

Nyman et al. 1977, Lindhe et al. 1995, Tonetti et al. 1996, Rossa et al. 2000, Gkranias et al. 2012). On the other hand, it cannot be excluded that the used ACS did not possess the optimal mechanical characteristics to ensure sufficient stability of the wound, subsequently resulting in a collapse of the mucoperiosteal flap and more limited space for regeneration (Susin et al. 2015). Although the expenses and demanding maintenance may restrict the broad use of non-human primates, the microbiological, immunological, and morphological features are quite similar to those of humans (Pellegrini et al. 2009). Furthermore, chronic periodontal defects with minimal spontaneous repair are valuable for evaluating new medical formulations or drugs as a putative periodontal regenerative therapy prior to clinical application in humans (Caton et al. 1994, Giannobile et al. 1994). The amount of new tissue formation obtained in the Emdogain/ACS and Osteogain/ACS groups was greater than those in the OFD and ACS groups in the present animal model. Furthermore, the migration of the junctional epithelium was more restricted in the Emdogain/ACS and Osteogain/ACS groups compared to the OFD and ACS groups. For the newly formed cementum, no distinct qualitative differences were observed between the Emdogain/ACS and the Osteogain/ACS group, they were composed of mixed cellular/acellular cementum (Araujo & Lindhe 1998, Donos et al. 2003, Shirakata et al. 2007, Gkranias et al. 2012). Functionally oriented collagen fibres with many blood vessels in some parts along the denuded root surface were observed, and they appeared to be denser than those observed in the control and ACS groups. These findings are comparable to those of previous studies reporting that EMD histologically presented positive regeneration results in animals and human biopsies (Hammarström et al. 1997, Heijl et al. 1997, Mellonig 1999, Donos et al. 2003, Hovey et al. 2006, Shirakata et al. 2007, Gkranias et al. 2012, Ivanovic et al. 2014, Sculean et al. 2015).

Interestingly, the amount of the regenerated tissue (i.e. NBA, NC, and NA) was the greatest in the Osteogain/ACS group, superior to that obtained in the Emdogain/ACS group. This may be due to the fact that the ELISA assay demonstrated that a 20–60% significantly higher amount of total adsorbed amelogenin was found for the Osteogain/ACS group when compared to Emdogain/ACS. Furthermore, the ACS loaded with Emdogain started to degrade in PBS by 3 days, whereas those pre-coated with Osteogain showed more stable properties. These findings suggest that Osteogain-adsorbed ACS not only maintains the sustained release of amelogenin but also provides an environment conducive to accelerating periodontal regeneration. Furthermore, the positive effects of Osteogain on periodontal regeneration may be explained by previous findings indicating that Osteogain significantly increased cell adhesion, proliferation, and differentiation of osteoblasts in vitro (Miron et al. 2016b), as well as promoted the upregulation of genes encoding BMP-2, TGF-β1, collagen 1 and osteocalcin (Miron et al. 2016c).

Within their limits, the present data indicate that Osteogain possesses favourable physicochemical properties facilitating adsorption of amelogenin on ACS and may additionally enhance periodontal wound healing/regeneration when compared to Emdogain. None of the treatments achieved complete regeneration, that is, class III furcation still persisted after treatment. Further pre-clinical and clinical studies are thus warranted to evaluate the biologic and clinical value of this novel EMD formulation on periodontal wound healing/regeneration.

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Clinical Relevance

Scientific rationale for the study: A new liquid carrier system for enamel matrix derivative (EMD) (Osteogain) has recently been developed to facilitate bone biomaterial mixing. This study evaluated the effect of Osteogain with absorbable collagen sponges (ACS) in chronic class III furcation defects in monkeys.

Principal findings: Statistically significantly higher amount of total adsorbed amelogenin was detected for Osteogain/ACS when compared to Emdogain, which yielded higher amounts of new connective tissue attachment and bone formation in furcation defects.

Practical implications: Osteogain/ACS promoted periodontal wound healing/regeneration in class III furcation defects, thus warranting further evaluation in preclinical and clinical settings.