Clin Oral Invest (2006) 10: 29–34
DOI 10.1007/s00784-005-0019-y

ORIGINAL ARTICLE

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Effect of an oily calcium hydroxide suspension (Osteoinductal) on healing of intrabony periodontal defects. A pilot study in dogs

Received: 21 July 2005 / Accepted: 20 October 2005 / Published online: 29 November 2005

Abstract The aim of the present study was to evaluate histologically in dogs the effect of treating intrabony defects with an oily calcium hydroxide suspension (OCHS). Intrabony defects were surgically created bilaterally at the distal aspects of the maxillary first premolars and at the mesial aspects of the third premolars in two mongrel dogs. Subsequently, the defects were randomly treated with (a) access flap surgery followed by the application of an OCHS or (b) access flap surgery alone. After 8 weeks of healing, the animals were killed. Dissected blocks containing the experimental specimens were fixed in formalin, decalcified in EDTA, and embedded in paraffin. The formation of new cementum and bone was assessed histomorphometrically. In the control group, healing was predominantly characterized by the formation of a long junctional epithelium along the root surface and limited periodontal regeneration at the most apical part of the defect. The OCHS-treated defects consistently revealed periodontal regeneration (i.e., new periodontal ligament, new cementum with inserting collagen fibers, and new bone). Within the limits of the present study, it can be concluded that OCHS may favor periodontal regeneration in acute-type intrabony periodontal defects.

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Keywords Intrabony defects · Periodontal regeneration · Oily calcium hydroxide suspension · Access flap surgery · Animal study

Introduction

According to the cause-related concept of periodontal therapy, the main objective of treatment is to control infection and thereby arrest disease progression [7]. Ideally, periodontal therapy does not only include arresting the disease but also regeneration of the periodontal attachment, including cementum, a functionally oriented periodontal ligament, and alveolar bone [2]. Several treatment modalities such as the use of guided tissue regeneration (GTR) alone or in combination with different types of bone grafts, root surface demineralization, enamel matrix derivative (EMD), or the application of growth factors have been employed with varying degrees of success to predictably accomplish this goal [1, 12, 16, 17, 20–22, 24]. Recently, an oily calcium hydroxide suspension (OCHS) has also been supposed to support periodontal regeneration. Calcium hydroxide (CH) is a product of lime slaking from quick lime. The slurry of CH incorporates carbon dioxide from the air and hardens with the formation of calcium carbonate and water. CH is not soluble in organic acids, has a marginal solubility in water, and an improved solubility in glycerine or syrup. The saturated aquaeous solution exhibits an alkalic pH value of 12.4. Several experimental studies have shown that CH may possess antimicrobial [11, 13] and antiinflammatory properties [6]. When applied on the amputated dental pulp or into the root canal close to the apex, CH has been reported to result in the destruction of the vital tissue, leading to the formation of a necrotic layer and, subsequently, the formation of a hard tissue barrier below the exposure site [14, 25]. Additionally, CH also seems to have a positive influence on the healing of periapical lesions [9, 10]. These effects may be mainly due to the alkalic properties of CH, leading to a neutralization of the acidic metabolites of macrophages and osteoclasts [26]. However, the mechanism of CH in promoting the repair of bone tissues may not only

be due to providing rich Ca^{2+} and alkaline environment mineral deposition but also by stimulating calcification enzyme activity of osteoblasts [19]. The oily formulation, available under the name Osteoinductal, contains CH, liquid and solid carbohydrate chains, and fatty acids (myristaleinic, oleic, palmitoleinic, gadoleinic, margaric, pentadecanic, myristic, linolenic, stearic, palmitic, arachidic, lauric, and linolic) esterified with glycerol. The oily parts consist of a natural product of porcine origin, oleum pedum, which was demonstrated to have a low cytotoxic effect on human fibroblasts [18], and vaselinum album.

However, it is currently unknown to what extent OCHS may influence healing when used in conjunction with periodontal surgery. Therefore, the aim of the present study was to histologically investigate the healing of artificially created intrabony periodontal defects in dogs following treatment with access flap surgery and the application of OCHS to access flap surgery alone.

Material and methods

Animals

Two 3-year-old male mongrel dogs (approximate weight 25 kg) were used in the study. The animals exhibited a fully erupted, healthy, permanent dentition. During the experiment, the dogs were fed ad libitum with soft-food diet and water to minimize mechanical trauma to the flaps. Animal selection, management, and surgery protocol were approved by the Animal Care and Use Committee of Victor Babes University of Medicine and Pharmacy of Timisoara, Romania. The experimental segment of the study started after an adaption period of 4 weeks.

Study design

The study was performed in two surgical phases. In the first phase, extraction of the maxillary second premolars was performed bilaterally. The remaining dentition received oral prophylaxis in conjunction with the extraction procedures. After 10 weeks of healing, three-wall intrabony defects were surgically created bilaterally at the distal aspects of the maxillary first premolars and at the mesial aspects of the third premolars. In both dogs, the intrabony periodontal defects were randomly treated either with access flap surgery and the application of an OCHS or access flap surgery alone according to a split-mouth design (Table 1). Throughout the study period, oral hygiene procedures were performed three times a week, including tooth brushing. The animals were killed after a healing period of 8 weeks.

Surgical procedure for both phases

All surgical procedures were performed by one experienced operator. The dogs were sedated with 0.5–1 mg kg^{-1} diazepam (Terapia SA, Cluj-Napoca, Romania) and anesthetized with 10–15 mg kg^{-1} Calypsol 5% (Gedeon Richter, Budapest, Hungary), and 6.5 mg kg^{-1} and 1 mg kg^{-1} propofol 1% (Fresenius Kabi, Bad Homburg, Germany). To maintain hydration, both animals received a constant rate infusion of lactated Ringer's solution while anesthesized. Prophylactic antibiotic medication was administrated intraoperatively with cephalexin (Insporin, Industrial Veterinaria SA Invesa, Barcelona, Spain) 1 ml 18 kg^{-1} day^{-1} and maintained for 3–4 days postsurgically. In the first surgery, second maxillary premolars were carefully removed following a reflection of full-thickness mucoperiosteal flaps and tooth separation. After wound closure by means of matress sutures, the sites were allowed to heal for 10 weeks. In the second surgery, after raising mucoperiosteal flaps, three-wall intrabony defects (4×4× 4 mm) were surgically created with burs on the distal aspect of maxillary first premolars and the mesial aspect of the third premolar in either right- or left-jaw quadrants. Using a round bur, reference notches indicating the bottom of the defect were prepared on the respective root surfaces. Thus, any periodontal ligament tissue which may later develop coronally to the notch in the root surface will be de novo formed and clearly distinguishable in the histological sections. The intrabony defects were randomly treated with (a) access flap surgery followed by the application of an OCHS according to the instructions given by the manufacturer (Osteoinductal, Osteoinductal GmbH, Munich, Germany) (n=4 defects) or (b) access flap surgery alone (n=4 defects). In both groups, mucoperiosteal flaps were repositioned, and primary wound closure was achieved using resorbable 5.0 Polyester mattress sutures (Resorba, Nürenberg, Germany).

Table 1 Individual mean values (mm±SD) of the histomorphometric analysis of new cementum and new bone in both groups

Specimen	Dog	Group	New cementum	New bone
1	1	OCHS	3.1±0.5	4.4±0.4
2	1		3.9±0.1	3.6±0.2
3	2		5.5±0.1	4.8±0.1
4	2		2.9±1.2	2.6±0.2
Mean±SD			3.9±1.0	3.8±0.8
1	1	C	0.2±0.1	0.4±0.1
2	1		1.6±0.3	1.7±0.2
3	2		0.4±0.1	0.2±0.1
4	2		0.5±0.2	0.1±0.1
Mean±SD			0.7±0.5	0.6±0.7

Killing of animal and retrieval of specimens

The animals were killed (overdose of sodium pentobarbital, 200 mg kg^{-1}, i.v.) after 8 weeks. The jaws were dissected, and blocks containing the experimental specimens were obtained. All specimens were fixed in 10% neutral-buffered formalin solution for 4–7 days. Specimens were decalcified in EDTA under radiographic control of the decalcification

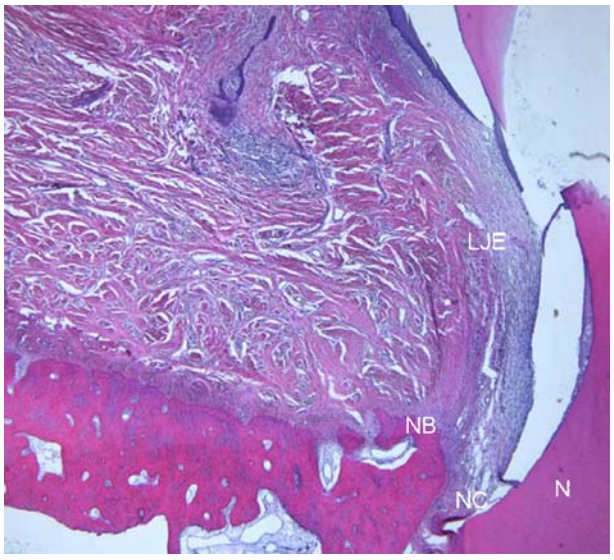

Fig. 1 Histological view of specimen 1 of the control group. Healing was predominantly characterized by the formation of a long junctional epithelium along the root surface. A minute amount of new bone formation was observed only occasionally and was limited to the most apical part of the defect (magnification ×4)

process, dehydrated, and fixed in paraffin. Mesiodistal serial sections were cut parallel to the long axis of the teeth, with the micrometer set at 5 μm. Sections representing the central part of the defect were stained with hematoxylin–eosin and selected for histomorphometric analysis.

Histological and histomorphometric analysis

Histomorphometric analyses and microscopic observations were performed by one experienced investigator masked to the specific experimental conditions. For histomorphometric measurements, images were obtained using a light microscope (BX50, Olympus, Hamburg, Germany) at a magnification of ×100, associated with a video camera (SIS Color View3, Soft imaging System GmbH, Münster, Germany). Digital images were evaluated using a software program (SIS analySIS Auto Software 3.2, Soft imaging System). The cementoenamel junction (CEJ) and the apical extension of the notch were used as the reference points. The following parameters were measured: (a) cementum regeneration—distance from the apical extension of the reference notch to the coronal extension of newly formed cementum on the root surface and (b) bone regeneration—distance from the apical extension of the reference notch to the coronal extension of newly formed bone along the root surface.

Results

Clinical observation

The postoperative healing was uneventful in all cases. No complications such as abscesses or infections were observed throughout the study period.

Histological and histomorphometric analysis

Individual values of the histomorphometric analysis of new cementum and new bone in both groups are presented in Table 1. In particular, specimens treated with OCHS exhibited significantly higher amounts of both newly formed bone and cementum than control specimens. The histological evaluation of the control group revealed that healing

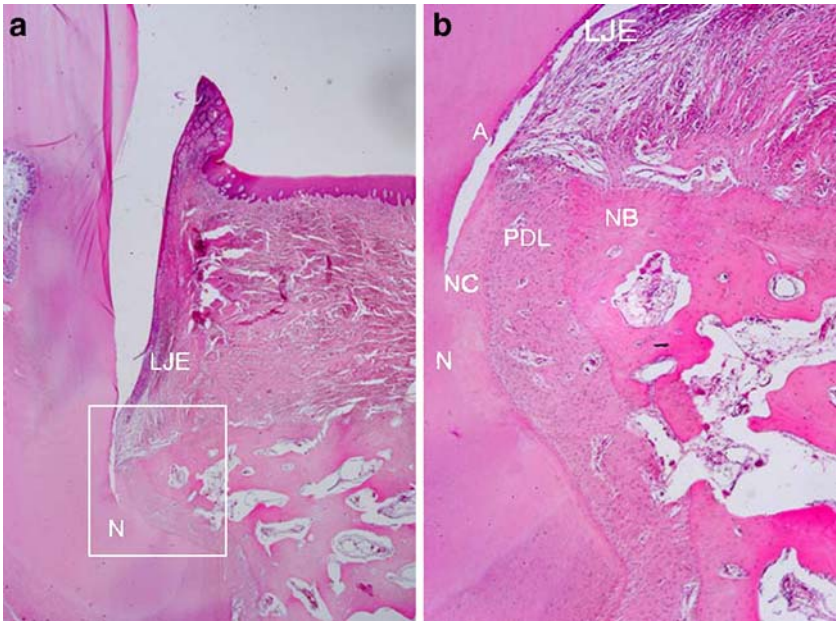

Fig. 2 **a** One specimen (2) of the control group also revealed signs of regeneration (magnification ×1.25). **b** Higher magnification of the regenerated area shown in **a** demonstrated formation of cementum, periodontal ligament, and alveolar bone (magnification ×4)

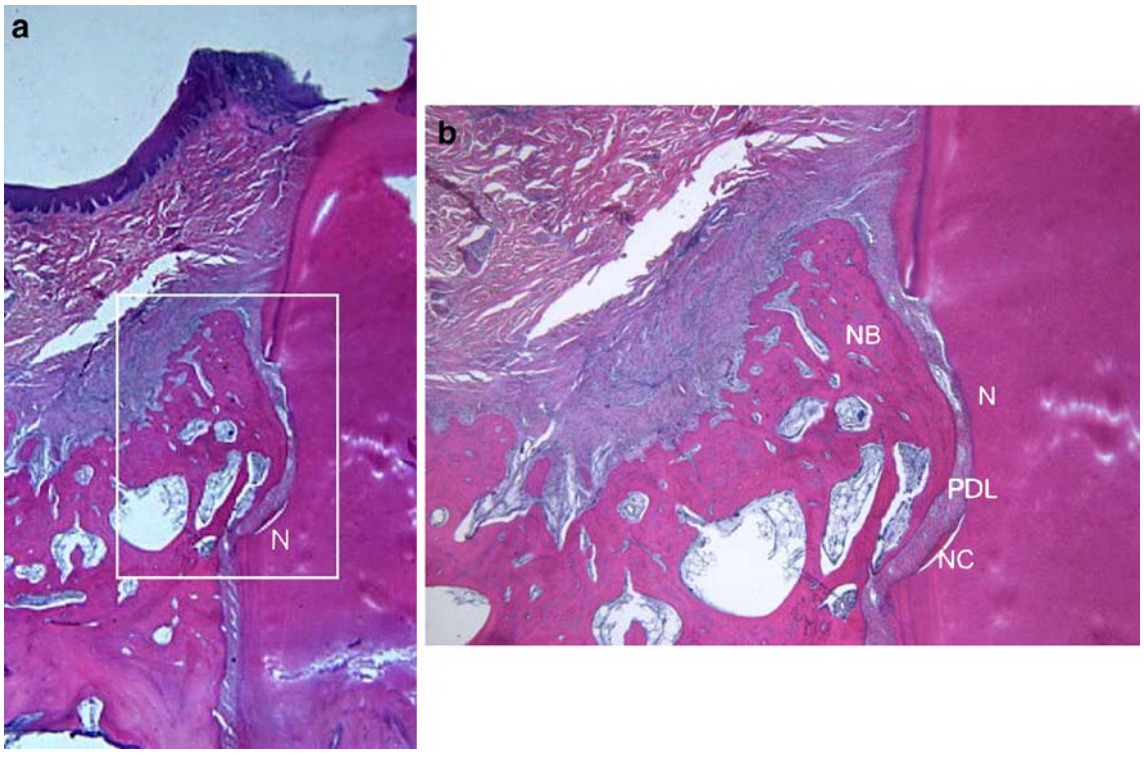

Fig. 3 **a** Histological view of specimen 1 following application of OHCS showing periodontal regeneration (magnification ×1.25). **b** Higher magnification of the regenerated area shown in **a** demonstrated formation of cementum, periodontal ligament, and alveolar bone (magnification ×4)

was predominantly characterized by the formation of a long junctional epithelium along the root surface. Minute amounts of reparative cementum were observed only occasionally and were limited to the most apical part of the defect (Fig. 1). However, one specimen in the control group also revealed the formation of new connective tissue attachment (i.e., cementum with inserting collagen fibers) coronally to the notch on the root surface. In this specimen, the new attachment was also accompanied by new bone. The newly formed cementum seemed to be connected to the newly formed bone (Fig. 2a,b). In contrast, periodontal healing associated with OCHS was consistently characterized by new bone formation above the notch area extending to the level of the newly formed cementum. The newly formed cementum exhibited a well-organized periodontal ligament with perpendicularly oriented collagen fibers

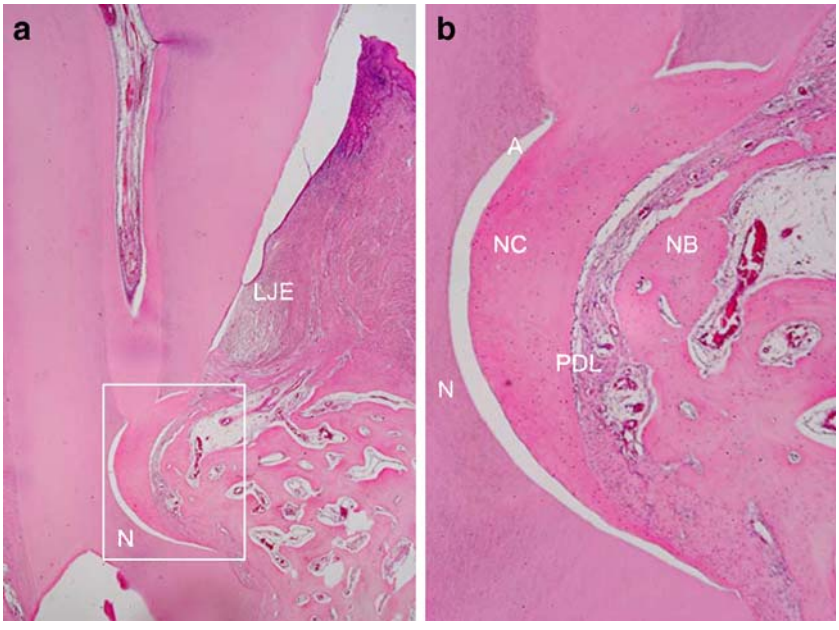

Fig. 4 **a** Specimen exhibiting a thick layer of new cementum in the notch area, whereas a thinner new cementum layer was observed more coronally (OHCS, specimen 2, magnification × 1.25). **b** Higher magnification of the regenerated area shown in **a** (magnification ×4). *A*, artifact; *N*, notch; *NB*, new bone; *NC*, new cementum; and *PDL*, new periodontal ligament (hematoxylin–eosin stain)

(Fig. 3a,b). Most specimens exhibited a thick layer of new cementum in the notch area, whereas a thinner new cementum layer was observed more coronally (Fig. 4a,b). Histologically, there were no identifiable residues of OCHS noted in all four specimens. Furthermore, no signs of root resorption or ankylosis were observed in both groups. However, artifacts [splits between long junctional epithelium (LJE) or regenerated cementum and the root surface] were observed occasionally in all specimens (Figs. 1, 2, 3, and 4).

Discussion

The results of the present study have indicated that treatment of intrabony periodontal defects with OCHS leads more predictably to periodontal regeneration when compared to coronally repositioned flap surgery alone. Furthermore, it was observed that OCHS neither led to postoperative complications nor to impaired clinical wound healing. The present histological evaluation has shown that in the control group, healing was predominantly characterized by the formation of an LJE along the root surface. The formation of a minute amount of cementum with inserting collagen fibers and of alveolar bone was found only occasionally and was limited to the most apical part of the defects. These observations are in agreement with findings from previous histological studies, which have shown that healing following conventional access flap surgery results mainly in the formation of an LJE to the bottom of the defect and no predictable de novo formation of connective tissue attachment and regrowth of alveolar bone [3, 4, 23]. In particular, in a study on monkeys, Sculean et al. [23] reported that the amount of new attachment varied from 0.0 to 2.78 mm, whereas the amount of new bone varied from 0.0 to 2.25 mm. In comparison to the control group, all four specimens treated with OCHS generally exhibited new bone formation above the notch area extended to the level of the newly formed cementum. Both tissues were connected by a newly formed periodontal ligament, identifiable as an oblique or perpendicular collagen fiber arrangement. In this context, it needs to be pointed out that these specimens are the first data evaluating the use of OCHS for the treatment of intrabony periodontal defects. The positive effect of OCHS on periodontal wound healing noted in the present study may be explained by a stimulation of the calcification enzyme activity of osteoblasts [19]. However, further experimental studies are needed to examine the effects of OCHS on the differentiation and proliferation of regenerative potential cells. Until now, periodontal regeneration has been histologically observed only after a combination of surgical access and various additional procedures such as barrier membranes, alone or in combination with different types of bone grafts, root surface demineralization, EMD, or the application of growth factors [1, 12, 16, 17, 20–22, 24]. However, the histomorphometric results assessed in the OCHS group seemed to be within the range of new cementum and bone formation following GTR procedures or the application of EMD [8, 23]. In particular, Sculean et al. [23] evaluated histologically in monkeys the effect of treating intrabony defects with EMD, GTR, or combined EMD and GTR. After 5 months of healing, the amount of newly formed attachment varied from 0.40 to 3.85 mm in the EMD group, from 2.95 to 3.61 mm in the GTR group, and from 2.31 to 3.68 mm in the EMD + GTR group. The amount of newly formed bone varied from 0.58 to 2.88 mm in the EMD group, from" 2.25 to 3.45 mm in the GTR group, and from 2.77 to 3.67 mm in the EMD + GTR group [23]. Similar results were also reported by Cochran et al. [8] following application of EMD in intrabony defects of baboons. After 5 months of healing, EMD-treated 4-mm-wide sites revealed a new cementum height of 2.38 mm and a new bone height of 1.22 mm [8]. When interpreting these results, however, it has also to be queried whether periodontal wound healing in monkeys may be transferable to dogs. Furthermore, in these studies, the defects were of a chronic type (i.e., they were surgically created and plaque-infected). In contrast, the acute-type defects involved in the present study might not necessarily represent the real situation encountered in a chronic, plaque-infected periodontal defect [5]. Indeed, histological studies in nonhuman primates have shown that in acute-defect models, approximately 50–70% spontaneous regeneration can be expected, which in turn may lead to difficulties in interpreting the results [5]. On the other hand, in a study on monkeys, Isidor et al. [15] have demonstrated that no histological differences in the result of healing were discernible between the specimens of previously periodontitis-affected roots and roots with surgically created defects. New cementum with inserting collagen fibers had formed in the apical part of the instrumented surface in both groups of teeth. Similar results were also reported by Wikesjö et al. [27], since regeneration of alveolar bone and cementum was comparable for both acute and chronic defect conditions. All these data, taken together with the results from the present study, may justify controlled clinical trials to evaluate the effectiveness of OCHS for the treatment of intrabony periodontal defects.

Within the limits of the present study, it can be concluded that OCHS may favor periodontal regeneration in acute-type intrabony periodontal defects.

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