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ORIGINAL ARTICLE

Wound healing of dehiscence defects following different root conditioning modalities: an experimental study in dogs

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Abstract
Objective The purpose of this study was to investigate the periodontal healing pattern of dehiscence-type defects following different chemical root conditioning modalities.
Materials and methods Buccal osseous dehiscence defects were created on six teeth of seven dogs. After dental plaque accumulation, defects were treated with sterile saline solution (control group) or one chemical conditioning modality: citric acid (CA group), ethylenediaminetetraacetic acid (EDTA group), tetracycline (TTC group), citric acid+tetracycline (CA+TTC group), or tetracycline+citric acid (TTC+CA group). After 3 months of healing, clinical parameters were evaluated, and the animals were killed. Histological sections were processed, and a computer-assisted histometric analysis was used to evaluate the formation of new cementum, new bone, and epithelial apical migration.
Results All treatments yielded significant improvements in terms of probing depth decrease and clinical attachment level gain compared to baseline values; however, without significant differences among the groups ($p>0.05$; one-way ANOVA). The highest amount of new cementum was noted in the EDTA group (3.72±0.83 mm, 77.6 %), while the lowest amount of new bone was observed in the TTC group (0.7±0.94 mm, 14.3 %). However, no statistically significant differences could be observed among the groups regarding epithelial apical migration, new cementum, and alveolar bone formation ($p>0.05$).
Conclusion Chemical root surface conditioning did not promote any significant improvement in periodontal healing pattern of dehiscence-type defects in dogs. Clinical Relevance: Chemical root surface conditioning after surgical debridement did not promote positive or negative effects on periodontal healing pattern of dehiscence-type defects.

Keywords Root surface conditioning · Periodontal wound healing · Regeneration · Cementum · Bone healing

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Introduction

The ultimate goal of periodontal therapy includes not only the arrest of progressive periodontal disease but also the predictable regeneration of periodontium at the site of previous periodontal breakdown, including new cementum, new periodontal ligament, and new alveolar bone formation. However, the wound healing following conventional periodontal therapy has been

frequently characterized by the development of long junctional epithelium and connective tissue adhesion [1, 2].

Several procedures have been proposed to enhance regeneration of periodontal tissues, including root conditioning. During the progression of periodontal disease, root surface becomes exposed to the subgingival and/or oral environment as attachment loss occurs and progresses. The nature of exposed root surface has been identified as one factor that could inhibit predictable periodontal regeneration [3–5]. A number of pathological changes affecting the exposed root surface have been described, such as denudation of fiber attachment, contamination, and alterations in mineral density [6–9]. These changes may have a significant biological influence on the potential of periodontal regeneration. The conventional treatment of pathologically altered root surfaces is based on the mechanical removal of plaque and calculus. However, the smear layer formed during mechanical root instrumentation may act as a physical barrier to the development of a connective tissue attachment to the root surface [10, 11]. Since the root surface serves as a wound margin during regeneration, chemical root conditioning has been directed to create a biologically surface compatible for connective tissue attachment and, consequently, for periodontal regeneration. Citric acid, tetracycline, and ethylenediaminetetraacetic acid (EDTA) are the most widely used substances for this goal.

Although the use of conditioning agents could be considered the oldest and most frequently attempted type of periodontal regeneration, controversies exist concerning the benefits of root surface conditioning as an adjunct to periodontal therapy. In vitro studies have shown that chemical decalcification of root surface enhances adhesion of fibrin clot [12, 13] and results in favorable conditions for connective cells attachment and migration along the affected surface [14, 15]. Animal histological studies have demonstrated an improved healing response with cementum and bone formation, as well as new connective tissue attachment following root conditioning [16–19]. However, other reports suggest that chemical modification of the root surface has no appreciable effect on the course of healing neither in animal nor in human situations [20–24]. Negative effects of etching procedures on wound healing and cementum formation have also been reported [25]. Variations in experimental design, including different methods of conditioner application, inconsistent flap adaptation, inadequate demineralization of periodontitis-affected root surfaces, as well as demineralizing agent effects on the organic matrix of cementum and dentin, may be associated with these inconsistent findings [5, 12]. Since it is difficult to create optimal requirements regarding experimental design and necessary histological evidence in human studies, this study was proposed to evaluate in the same animal model the periodontal healing after root conditioning with different chemical agents. Optimal parameters such as concentration, time, and method of application were employed as previously established. Therefore, the purpose of the present study was to evaluate clinically and histologically the effect of chemical root surface conditioning with citric acid (CA), tetracycline hydrochloride (TTC HCl), or EDTA on periodontal healing pattern of dehiscence-type defects in dogs. The null hypothesis was that periodontal healing pattern of the defects treated or not with chemical root conditioning is not significantly different.

Materials and methods

Animals

Seven adult mongrel dogs in good systemic health were included in the experiment (average weight of 20 kg). The research protocol of the present study was approved by the local Institutional Ethics Committee of Experimental Research with Animals, São Paulo State University (number 22/2005). The anesthetic procedures used throughout the study included a previous sedation of the animals with intramuscular injection of levomepromazine hydrochloride (Neozine®, Aventis Pharma, São Paulo, Brazil; 0.2 ml/kg) followed by induction of general anesthesia with intravenous injection of sodium thiobarbiturate (Tiopental®, Abbott, São Paulo, Brazil; 25 mg/ml, 0.5 ml/kg). In addition, 2 % lidocaine containing noradrenaline (1:100,000) was infiltrated into the mucosa to control bleeding and to ensure profound anesthesia.

Creation and cronification of dehiscence defects

All surgical procedures were performed by one operator (DLZ). After raising buccal mucoperiosteal flaps, dehiscence defects measuring 3 mm in width and 5 mm in height were surgically created with hand instruments on the mesial roots of the maxillary first molars (M1) and second (P2) and third (P3) premolars. In each defect, a strip of Tofflemire matrix band (Microdont, São Paulo, Brazil) with approximately the defect size was prepared and fixed to the tooth crown using adhesive system and resin. The flaps were replaced to the original position and stabilized with interrupted 4–0 nylon sutures (Ethicon Inc, Johnson & Johnson, São Paulo, Brazil). Sutures were removed after 1 week. Throughout the period of 45 days prior to the surgical treatment procedure, the dogs did not receive any special oral hygiene and were fed only with water-softened food to increase plaque accumulation, which enables the development of chronic inflammatory reaction as well as root contamination.

Defects treatment

After cronification period, band matrices were removed, and clinical measurements were performed (baseline). The following clinical data were obtained from M1, P2, and P3

teeth: presence of visible plaque, presence of marginal gingival bleeding, probing depth (PD), bleeding on probing, marginal gingival level (MGL), and clinical attachment level (CAL). The clinical data were measured by a calibrated examiner (DLZ) in four sites per tooth: mesial site, intermediate site between the mesial and vestibular site (defined as defect site), vestibular site, and distal site. Subsequently, the animals received supragingival scaling and prophylaxis with a rubber cup.

Full thickness flaps were raised, the defects were debrided, and the roots were scaled with periodontal curettes. References notches were placed at the alveolar bone level (apical notch) and at the cemento-enamel junction (coronal notch). Teeth and reflected flaps were isolated with gauze and root surfaces treated by irrigation with 10 ml of sterile saline solution (I, control group) or one of the following conditioning modalities: II, citric acid (CA group); III, EDTA (EDTA group); IV, tetracycline hydrochloride (TTC group); V, citric acid+tetracycline hydrochloride (CA+TTC group); or (VI) tetracycline hydrochloride+citric acid (TTC+CA group). In a previous study of our group [26], citric acid treatment promoted the most organized fibrin network and cells entrapment to the root surface. In order to associate this positive effect of citric acid on clot adhesion with the capacity of root adsorption with slow delivery to the periodontium of tetracycline, these substances were used in combination alternating the order of application. Since EDTA works at neutral pH, this chelating agent was not used in combination with citric acid or tetracycline. The six treatments were rotated among dogs and maxillary M1, P2, and P3 teeth. Thus, each animal received all treatment modalities as well as each tooth received all treatments. The different treatments were applied by one operator (DLZ).

Citric acid conditioning was carried out by the apposition of sterilized cotton pellets soaked with a saturated aqueous solution (25%, pH 1.3, Pharma Nostra, Campinas, Brazil) to the root surface during 3 min [27, 28]. EDTA conditioning was performed by a 3-min continuous application of 24 % EDTA gel (pH 7.0, Pref Gel, Biora AB, Malmö, Sweden) using sterilized soft brush according to previous studies [26, 29]. Tetracycline conditioning was performed by burnishing sterilized cotton pellets soaked with fresh aqueous solution of tetracycline hydrochloride (50 mg/ml) during 3 min [30, 31]. The saturated solution was prepared by adding tetracycline hydrochloride powder from capsules (Valde Química, São Paulo, Brazil) to distilled water at room temperature. An effort was made to avoid the contact of the substances with the surrounding tissues by suctioning. Chemical agents were renewed every 30 s. Following etching procedures, root surfaces were rinsed with 10 ml of sterile saline solution. Subsequently, the flaps were sutured tightly at the cemento-enamel junction. All flaps were secured in position with vertical or horizontal mattress sutures (4–0 nylon, Ethicon Inc, Johnson & Johnson, São Paulo, Brazil). Chemical plaque control regimen was instituted with topical application of 0.2 % chlorhexidine digluconate five times per week until the end of the study. The sutures were removed 1 week later.

After a healing period of 3 months, clinical parameters were evaluated (final), and the dogs were sedated and killed with overdose of sodium thiobarbiturate. The maxillary M1, P2, and P3 were removed in blocks.

Histological and histometric analysis

The maxilla samples were fixed in 10% neutral buffered formalin for 1 week and decalcified in Morse solution (1:1 of formic acid 50 % and sodium citrate 20 %) for approximately 4 months. Histological sections, 5 μm thick, were cut in a bucco-lingual direction through the entire mesio-distal plane of the tooth (Jung Supercut 2065 Leica, Chicago, IL, USA). Five sections were selected for each tooth: the first and the last section where the reference notches could be noted on the root surface, and three other sections that were equally spaced between the first and last sections. Hematoxylin and eosin (HE) and Masson trichrome staining were used.

Histological analysis was carried out by a blinded, trained, and experienced pathologist using a microscope set for light microscopy. Type and quality of the tissues were evaluated as well as the presence of inflammatory process and fibrous tissue, ankylosis, and root resorption. Histometric measurements were performed on digitally captured images using an image analysis system (UTHSCSA ImageTool, University of Texas Health Science Center, San Antonio, TX, USA). All measurements were recorded by a blinded, trained, and calibrated examiner (FRML). After a training period, 10% of the measurements were performed twice (7 days apart), and intraexaminer reproducibility was checked by means of the paired *t* test. No statistical significant differences were found between the two lots of measurements ($p>0.05$). The following measurements were obtained: (1) total defect length (DL), total length of the root surface between coronal and apical notches; (2) gingival margin (GM), linear measurement from the gingival margin to the coronal notch; (3) epithelial migration (EP), linear measurement from the coronal notch to the apical extension of the junctional epithelium, or linear measurement from the gingival margin to the apical border of the junctional epithelium if gingival recession was present; (4) new formed cementum (CE), linear measurement from the apical notch to the most coronal part of the new cementum; (5) new formed bone (B), linear measurement from the apical notch to the most coronal part of the bone; and (6) new bone area (BA), area of newly formed bone coronal to the apical notch.

Three sections per site representing the central portion of the defect were used to obtain the mean value for each histometric parameter. The histometric linear parameters EP, CE, and B were also expressed as a percentage of the linear DL.

Statistical analysis

One-way ANOVA and paired Student's *t* tests were used to detect respectively inter- and intragroup differences in PD and CAL. Since MGL clinical parameter did not present normal distribution, nonparametric tests were used: Friedman and Wilcoxon. Descriptive statistics for the histological parameters were expressed as mean±SD. One-way ANOVA test was used to detect differences among the groups regarding the linear measurements DL, GM, EP, CE, and B. Nonparametric Friedman test was used to detect differences among the groups regarding the area measurement BA. One-way ANOVA test was also used to compare the parameters EP, CE, and B in terms of percentage of the defect length. In case of statistically significance, the Tukey–Kramer (parametric variables) or Dunn (nonparametric variables) tests were used for multiple comparisons. A significance level of 0.05 was used.

Results

Clinical analysis

The healing response was favorable in all groups. No suppuration, abscess formation, or other clinical complication could be observed. No significant differences were detected within and among the groups regarding the clinical parameters MGL, PD, and CAL in mesial, vestibular, and distal sites ($p>0.05$). In the defect site, all treatments groups yielded significant improvements in terms of PD decrease and CAL gain compared to baseline values ($p<0.05$), however, without significant differences among the experimental groups ($p>0.05$; Fig 1).

Histological and histometric analysis

Defects treated with or without chemical root conditioning showed similar periodontal healing patterns (Figs. 2 and 3). New cementum was observed in all specimens, and cementoblasts could be observed along the surface of the newly formed cementum (Figs. 4 and 5). A continuous layer of new cementum was deposited coronally to the apical radicular notches up to the epithelial tissue (Figs. 2 and 3). The apical migration of epithelium was limited to the coronal third of the defect in all treatment groups. The dentin surface underneath the newly formed cementum presented irregularities or surface resorption. A fibrous connective tissue with collagenous fibers in a regular disposition was present in all groups, and inflammatory infiltrate was found in two teeth of the CA group. New bone was observed coronally to the apical notches in most of the specimens, but usually limited to the apical third of the defect. Periodontal ligament was present between new cementum and new bone (Figs. 4 and 5) in all groups. No area of active resorption and dentoalveolar ankylosis was present.

The descriptive statistics as well as the statistical analysis of linear (mm) and area measurements (mm^2) are summarized in Table 1. No statistically significant differences were detected among the groups in regard to the linear (DL, GM, EP, CE, and B) and area (BA) measurements ($p>0.05$). The parameters EP, CE, and B were also expressed as percentage of the linear defect length; however, no significant differences were observed among the groups ($p>0.05$).

Discussion

The present study was designed to evaluate the effect of different modalities of root conditioning on periodontal healing pattern of dehiscence-type defects in the same animal model. The establishment of a new connective tissue attachment to the periodontitis-affected root surface following periodontal surgery has not been considered a predictable biological event. Various approaches to therapy have been proposed in an attempt to achieve regeneration of the lost supporting tissues. Root conditioning has been used to create a more biocompatible surface for periodontal healing. This conditioning is intended to decontaminate and to demineralize the root surface, removing the smear layer and exposing some components of the extracellular matrix of dentin and cementum, such as type I collagen, proteoglycan, fibronectin, and growth factors [5, 10, 32].

For the purpose of the study, 42 chronic dehiscence defects were treated with or without root conditioning in combination with flap surgery. The optimum concentration, method, and time of each chemical agent application were defined based on the results of previous in vitro investigations [26–31]. Despite the use of established parameters, no significant improvement could be observed in periodontal healing of dehiscence defects treated with root conditioning when compared to the control group that did not receive etching procedures. Therefore, the null hypothesis of the present study could not be rejected.

Clinically, a significant PD decrease and CAL gain compared to baseline values could be observed in the defect sites of all treatment groups, however, without significant differences among the groups (Fig 1). Similarly, root conditioning with citric acid in combination with modified Widman flap did not yield significant improvements in terms of PD

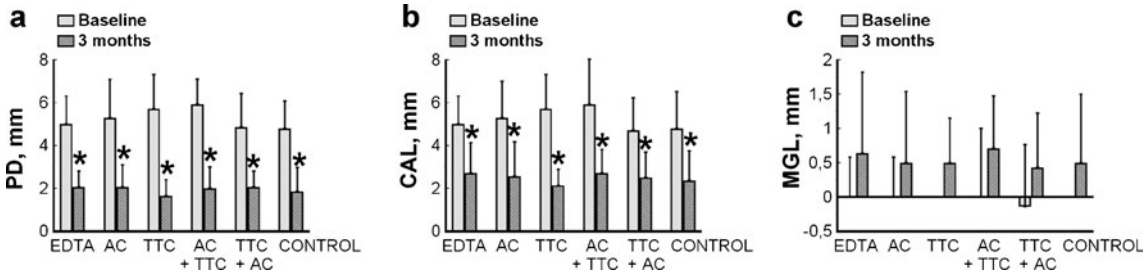

Fig. 1 Clinical parameters for each group at baseline and 3 months after treatment. **a** Probing depth (*PD*). **b** Clinical attachment level (*CAL*). **c** Marginal gingival level (*MGL*). *$p<0.05$, significant difference between the baseline and 3-months post treatment (Student's paired *t* test). No significant differences were found among groups at each period of clinical evaluation ($p>0.05$; ANOVA one-way test)

decrease and CAL gain in teeth presenting residual pockets with probing depth >5 mm [33]. The mean CAL gain on the surfaces treated with citric acid was 2.1 mm in comparison with 1.5 mm on the surfaces not conditioned [33]. In the present study, the CAL gain was 2.72 mm in the CA group and 2.43 mm in the control group (Fig 1). On the other groups that received root conditioning, the CAL gain ranged from 2.21 to 3.57 mm. TTC group showed the highest improvement in CAL gain (Fig 1); however, this difference did not reach a level of statistical significance.

Histological evidence indicated that new connective tissue could not attach to a denuded root, and apical migration of epithelium along the root surface was observed [16]. On the other hand, demineralization of the denuded root surface with citric acid inhibited the apical migration of epithelium and resulted in new connective tissue attachment [16–18]. In contrast to these findings, our results revealed similar epithelium apical migration, new cementum, and bone formation in defects treated or not with root conditioning (Table 1). In agreement with our data, root conditioning with citric acid, tetracycline, or EDTA provided no benefit or clinical significance to regeneration in patients with chronic periodontitis [34].

The results of the histometric analysis demonstrated that new cementum formation was greater than apical epithelium migration in all treatment groups, without significant

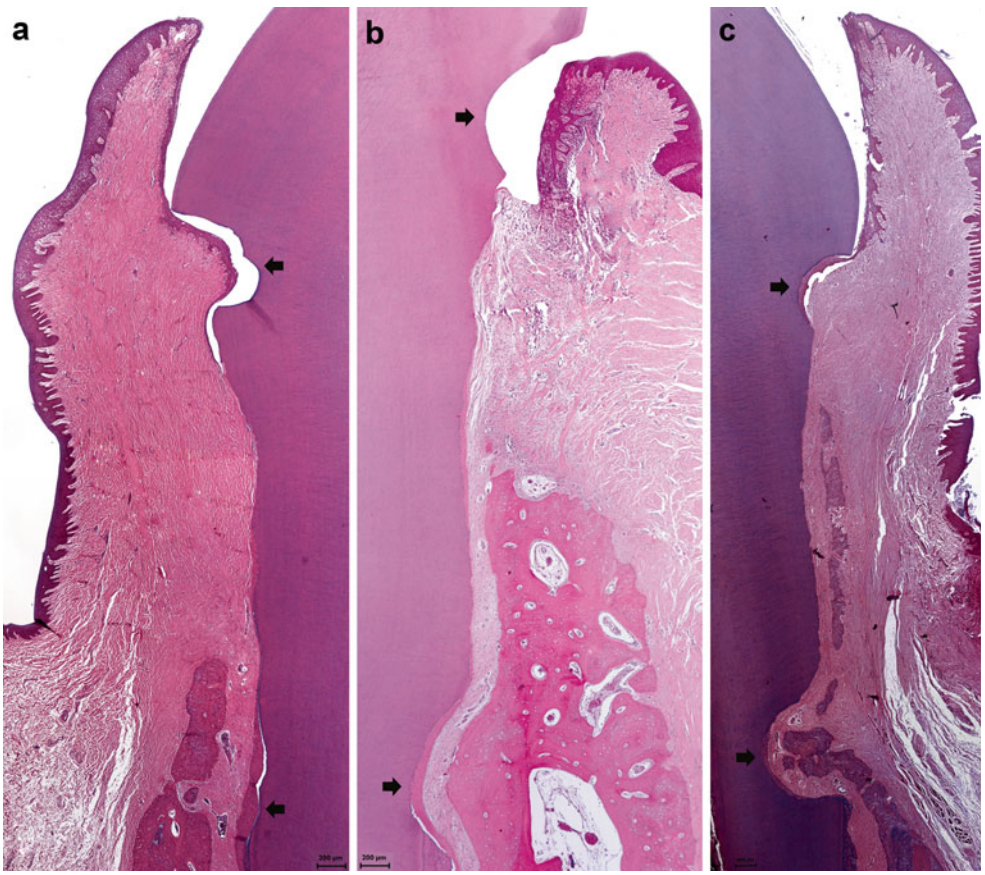

Fig. 2 Panoramic view of the defects with the radicular notches (*arrows*). New bone and new cementum are observed extending coronally from the apical notch after 3 months of treatment. **a** Control group. **b** CA group. **c** EDTA group (HE, bar=200 μm, original magnification ×5)

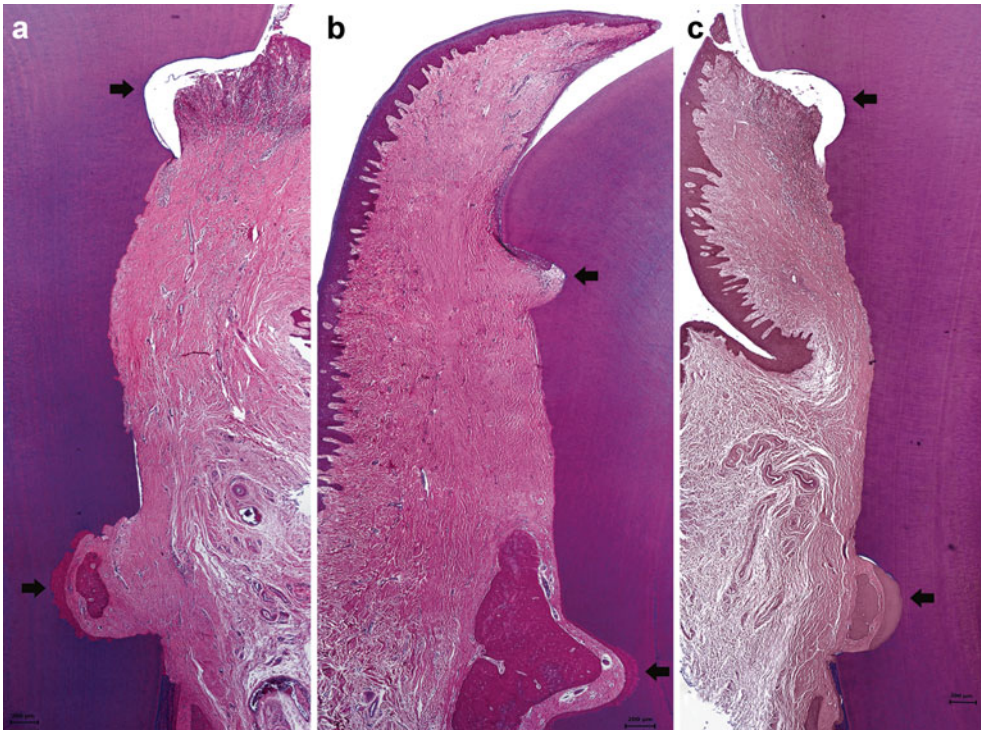

Fig. 3 Panoramic view of the defects with the radicular notches (*arrows*). New bone and new cementum are observed extending coronally from the apical notch after 3 months of treatment. **a** TTC group. **b** CA+TTC group. **c** TTC+CA group (HE; bar=200 μm; original magnification ×5)

differences among them (Table 1). The mean percentage of the defect covered with epithelium ranged from 20.36 to 35.73 %, while the mean percentage of newly formed cementum ranged from 60.98 to 77.6 %. In addition, most of the specimens presented newly formed bone coronally to the apical radicular notch (Figs. 2 and 3). The linear (B) and area (BA) measurements indicated that the amount of new bone formation was greater in CA and CA+TTC groups when compared to the other groups, however, without statistically significant difference (Table 1).

In the study of Claffey et al. [35], the connective tissue attachment gain after application of TTC HCl was similar to that obtained with citric acid. The root surface conditioned with TTC HCl showed connective tissue attachment extending to the cemento-enamel junction in most of the specimens. The extent of alveolar bone regeneration was approximately 62% of the root planed surface length. However, only two animals were used in this study, and no control group was included in the analysis. In contrast, the lowest amount of new bone formation was noted in the TTC group (0.7±0.94 mm, 14.3 % of the defect length; Table 1). This amount increased when the TTC HCl conditioning was preceded or followed by the application of citric acid (1.51± 1.32 mm, 27.91 % of the defect length, and 1.07±1.28 mm,

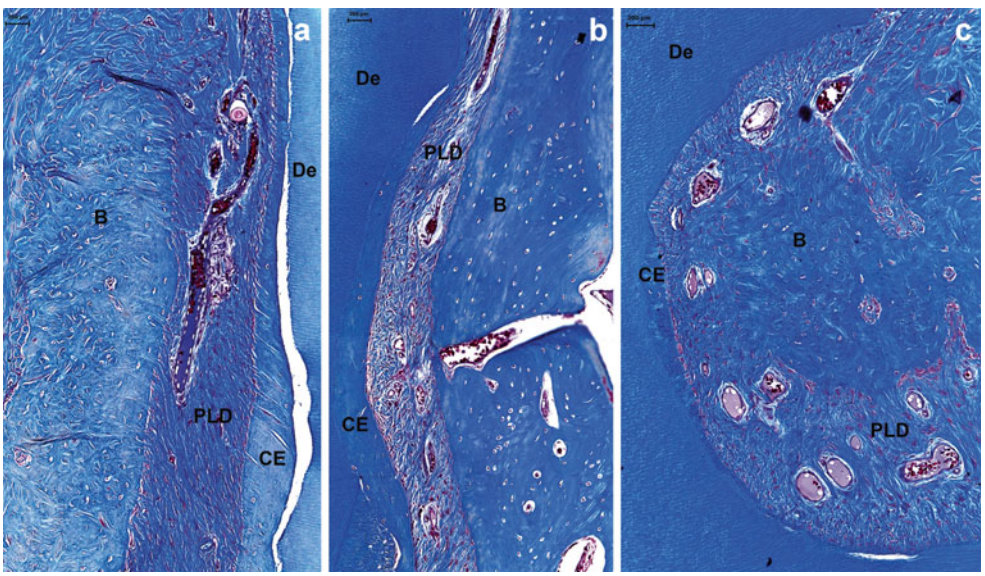

Fig. 4 Higher magnification of the radicular notch area in Fig. 2 showing new cementum (*CE*), periodontal ligament (*PLD*), and alveolar bone (*B*) formation. Dentin (*De*). **a** Control group. **b** CA group. **c** EDTA group (Masson trichrome; bar=200 μm; original magnification ×20)

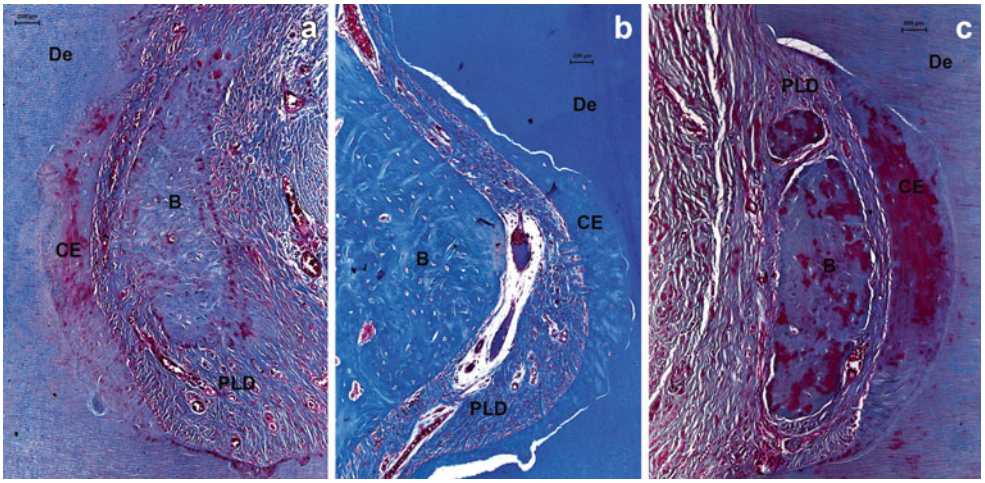

Fig. 5 Higher magnification of the radicular notch area in Fig. 3 showing new cementum (*CE*), periodontal ligament (*PLD*), and alveolar bone (*B*) formation. Dentin (*De*). **a** TTC group. **b** CA+TTC group. **c** TTC+CA group (Masson trichrome; bar=200 μm; original magnification×20)

20.69 % of the defect length, respectively), but this difference was not statistically different (Table 1). Nagata et al. [21] observed that the amount of new attachment as well as bone and cementum formation was similar in the groups conditioned or not with TTC HCl. Accordingly, the demineralization with TTC HCl did not produce any additional effect on periodontal regeneration of dehiscence defects surgically created in monkeys.

Root conditioning with a neutral pH agent such as EDTA appeared to produce in a short-term perspective a more biocompatible surface compared to conditioning at low pH. Etching at low pH induces an immediate necrosis, while etching at neutral pH preserves the integrity of exposed collagen fibers and the vitality of the adjacent tissues [36, 37]. According to Sammons et al. [38], root conditioning with citric acid and tetracycline may temporarily inhibit the migration and proliferation of periodontal ligament fibroblasts and delay early wound healing. However, no statistically significant differences between the groups conditioned with low or neutral pH agents concerning clinical (Fig 1) and histological parameters (Table 1) could be observed after 3 months of healing. The application of CA and TTC HCl did not jeopardize the periodontal repair compared to EDTA and control groups. Contradictorily, Blomlöf et al. [39] demonstrated significantly improved healing with respect to histological attachment, less gingival recession, and probing depth in EDTA etched group compared to control and citric acid groups.

In general, the results of the present investigation demonstrated that groups treated with root surface conditioning did not show significant clinical or histological improvements on periodontal healing pattern of dehiscence defects compared to the control group that did not receive any chemical conditioning. Although no advantage could be

Table 1 Histometric parameters (mean±SD) for the control and conditioned groups

Histometric Parameter	Group						*p* value
	Control (*n*=7)	CA (*n*=7)	EDTA (*n*=7)	TTC (*n*=7)	AC+TTC (*n*=7)	TTC+AC (*n*=7)	
DL[a] (mm)	4.53±0.55	4.74±0.61	4.79±0.62	4.96±0.74	4.93±1.02	5.18±0.61	0.65
GM[a] (mm)	0.85±0.42	1.05±0.80	0.75±0.69	0.82±0.63	0.92±0.64	0.73±0.72	0.94
EP[a] (mm)	1.52±0.95	1.27±1.02	0.98±0.58	1.75±1.94	1.80±1.27	1.31±0.86	0.78
CE[a] (mm)	2.89±1.41	3.01±1.73	3.72±0.83	2.87±1.36	2.77±1.18	3.65±1.09	0.59
B[a] (mm)	1.10±0.90	1.53±1.29	1.16±0.96	0.70±0.94	1.51±1.32	1.07±1.28	0.75
BA[b] (mm^2)	279.87±307.51	518.03±619.76	343.42±364.50	229.42±380.81	588.92±545.66	184.85±209.16	0.61
%EP[a]	35.73±24.75	27.15±20.51	20.36±13.28	32.90±33.72	35.51±28.30	24.88±16.59	0.74
%CE[a]	61.80±26.06	63.41±32.69	77.60±14.39	60.98±32.30	58.07±28.83	71.43±21.48	0.79
%B[a]	24.49±20.54	33.21±26.53	25.06±19.99	14.3±19.97	27.91±22.88	20.69±23.73	0.72

Linear parameters: *DL* total defect length, *GM* gingival margin, *EP* epithelial migration, *CE* new formed cementum, *B* new formed bone. Area parameter: *BA* new bone area. Linear parameters expressed as a percentage of the defect length: %EP, %CE, and %B

*p value refers to statistical significance differences among groups ($p<0.05$)

[a] One-way ANOVA test

[b] Friedman test

observed with root surface conditioning alone, the exposure of collagenous matrix of dentin or cementum could be useful to retain biologically active substances such as growth factors in regenerative procedures.

Within the limits of the present study, it could be concluded that root surface conditioning with citric acid, tetracycline, and EDTA did not promote any significant improvement in periodontal healing pattern of dehiscence defects surgically created in dogs.

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Conflict of interest The authors declare that they have no conflict of interest.

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