Characterization of the human root dentin, in the use of EDTA 24%. Intercalated or continuous application?

Anne Karoline Fernandes de Matos[1], Fernanda Aparecida Silva[1], Tomaz Gonçalves Martins Neto[1], Priscila dos Santos Simões[1], Gabriel Gonçalves Amaral[1], Fernando Antônio Mauad de Abreu[1]

[1]Department of Odontology, PUCMG - Pontifical Catholic University of Minas Gerais, Belo Horizonte, MG, Brazil.

Abstract

Aim: Evaluate the action of 24% EDTA at different times application in root samples of human teeth.

Methods: Root samples of human teeth obtained from the university Tooth Bank were divided into control, GI (treated with EDTA 24% for 1 minute), GII (treated with EDTA 24% for 2 minutes intermittently), GIII (treated with EDTA 24% for 2 minutes continuously). Dentin surfaces are assessed and characterized by scanning electron microscopy (SEM) and chemically by energy dispersive spectroscopy (EDS), and distribution is performed by map.

Results: The residual smear layer is removed by the conditioning agent at all times evaluated, but within 2 minutes, there is a partial demineralization of the dentin surface. C, O, N, Ca, Na, P and Mg were observed. A homogeneous distribution over the surface was observed in GI and GII.

Conclusion: The difference in the temporal mode of application changes the morphology of the human dentinal surface; however, the observed chemical constituents suggest a similarity.

Keywords: Ethylenediaminetetraacetic Acid; Dental Root; Scanning Electron Microscopy; Energy Dispersive Spectroscopy, Periodontics.

Introduction

Periodontitis destroys the insertion periodontium, leading to considerable changes in the root (Donos, 2018). The mechanical treatment of periodontal disease produces a layer at the root, known as the smear layer, which contains microorganisms and toxins that interfere with the healing (Lasho *et al*., 1983). The complete decontamination of the root surfaces affected by periodontitis seems complicated when observing the limitations of mechanical therapy because of the various root anatomies. The scraping procedures and root planning provide only a temporary solution for periodontal disease. These methods are less effective in deeper pockets, where dental calculus removal is more complex (Torkzaban & Seyedzadeh Sabounchi, 2016). On the other hand, situations of the healthy periodontium, with gingival recessions, are often associated with root caries, esthetics, and hypersensitivity concerns, which constitute significant therapeutic problems for patients. Exposed root surfaces can sometimes display a hypermineralized layer of cementum and endotoxin contamination; hence, mechanical and chemical preparation of the exposed root was pinpointed to influence the treatment outcome of root coverage procedures (Górski *et al*., 2022).

Root biomodification agents are often used to remove the smear layer, expose collagen fibres and treat their surfaces and make them biologically acceptable for the success of regenerative procedures (Subramanian *et al*., 2017). The literature reports several chemical agents that are used for this purpose, such as citric acid, phosphoric acid, tetracycline hydrochloride (HCl), doxycycline HCl, fibronectin, Ethylenediaminetetraacetic acid (EDTA), minocycline HCl, hydrochloric acid, among others (Nanda *et al*., 2014). EDTA has been

Correspondence to: Fernando A. Mauad de Abreu
E-mail: fmauadabreu@gmail.com

widely studied as a chelating agent for smear layer removal. However, the in vitro biological evidence (Babgi *et al*., 2021; de Vasconcellos *et al*., 2006) and the clinical benefits of using 24% EDTA as an adjunctive treatment to non-surgical periodontal therapy or as a preoperative agent. Root conditioning suggests that there is no benefit in the use of the chemical product. In a recently published randomized clinical trial, the use of 24% EDTA for root conditioning did not improve 12-month outcomes after surgical treatment of gingival recessions (Górski *et al*., 2022a).

Among this diversity of biomodifying agents, 24% of EDTA has been widely used and has gained popularity for being within the Enamel Matrix Derived (EMD) use protocol. After the working protocol and its combination with periodontal regenerative therapies that use proteins from the EMD. Its application on the root must be treated with EMD for two minutes (Heden *et al*., 1999). In a clinical study, the modified technique of coronally advanced tunnel combined with subepithelial connective tissue graft with or without EMD in the treatment of gingival recession types 1 and 2. EDTA 24% was used seed in test sites (use of EMD). The results were similar. However, the authors concluded that even though the treatment modalities were equally effective in treating with similar improvements in clinical parameters, the application of EMD as an adjunct resulted in less postoperative pain and better professionally evaluated aesthetic results (Górski B *et al*., 2020). In another recently published study, the authors investigated the factors influencing 12-month outcomes after treatment of multiple gingival recessions with modified coronally advanced tunnel and subepithelial connective tissue graft with enamel matrix derivative (EMD) (tests) or without. (controls). Again, EDTA 24% was used seed in the test sites (use of EMD) for 2 minutes. The results concluded that the additional use of EMD significantly increases the chances of obtaining better results 12 months after the technique was employed (Górski *et al*., 2022b).

EDTA 24% is used as a tooth root conditioning agent before using EMD. However, no is in the literature on the differences that can occur on the dental surface if this application is made in a continuous time of two minutes followed by washing with saline solution, or if it is intercalated, that is, one minute, washing with saline solution, added another minute and another wash with saline solution, completing the two minutes. In this context, the present work evaluated and characterized through Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS) the action of the root biomodifier composed of 24% EDTA, in different application times, on the root surface of human teeth.

Materials and Methods

Sampling and preparation

The Research Ethics Committee approved this research under number CAAE 23001119.1.0000.5137. The study was carried out in triplicate, using nine upper human central incisor teeth from the Dental Bank of the Dentistry Department of PUC Minas. The same operator treated the teeth. Being carefully shaved with a specific instrument, washed under filtered running water and then stored, for 24 hours, in a glass vial containing 10% neutral buffered formalin solution. Subsequently, the samples were rewashed under running filtered water and stored in a glass bottle under 70% alcohol until they were sectioned. The action of the fixing agents was carried out to keep the smear layer produced by the root planning process.

The teeth were sectioned, separating the dental crown from the root portion, the root portion being sectioned into its apical, middle and cervical thirds, using the middle root third. The cervical and apical thirds were discarded, and the middle third was divided into four parts in the direction of its long axis, allowing the integrity of the root surface for the proposed treatments (Figure 1). An abrasive carborundum disc, a diameter of 22.2 mm and thickness of 0.6mm (ref. 223, Dentorium products, USA), was used for the dental section. It presents a double, fast cut and produces little heating, being mounted on a straight piece and dental micromotor (Marathon 3 Champion) and under constant irrigation by sterile injection water.

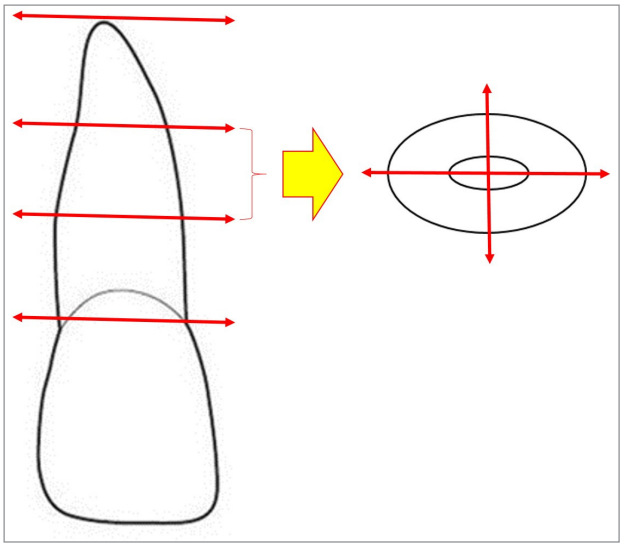

Figure 1. Schematic drawing of the model for obtaining the area used for research trials.

Study design

The study was divided into four groups. The control group received a rapid bath of saline and tests conditioned with EDTA 24%, commercially available 24% EDTA gel of pH 7.3 (PrefGel, Biora, Malmo, Sweden). Group I was conditioned for 1 minute, followed by washing with saline; group II was conditioned for 1 minute, followed by washing with saline, plus 1 minute of conditioning and a new wash with saline and group III was conditioned for 2 minutes interrupted, followed by washing with saline (Table 1). Each part was stored in Eppendorf to prepare the samples for analysis by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS).

Table 1. Study design.

	Treatment	Temporal model
Control	Saline solution	-
Group I	EDTA 24% for 1 minute	interrupted treatment
Group II	EDTA 24% for 1 + 1 minute	interleaved treatment
Group III	EDTA 24% for 2 minutes	interrupted treatment

Morphological and chemical characterisation and distribution

SEM performed the morphological characterisation. EDS evaluated the chemical characterisation. The MAPA technique observed the distribution of chemical components on the root surface. For the analysis of SEM and EDS, the samples underwent the standard treatment. That is, the samples, after dehydration, were treated with a gold-coated sputter. The evaluations were performed using the JSM-6400 Electron Microscope (JEOL), equipped with NORAN 6 X-ray Microanalysis System and Semafore Digitizer, from the Metallurgical Engineering department of PUC Minas. SEM performed the morphological characterisation. EDS evaluated the chemical characterisation. For the analysis of SEM and EDS, the samples underwent the standard treatment. That is, the samples, after dehydration, were treated with a gold-coated sputter. The evaluations were performed using the JSM-6400 Electron Microscope (JEOL), equipped with NORAN 6 X-ray Microanalysis System and Semafore Digitizer, from the Metallurgical Engineering department of PUC Minas. Random fields were selected in each sample, in increments of 500x, 1,000x, 1500x, 2000x and 3000x, for the descriptive morphological analysis of the root surface and at a 100x magnification to analyse the chemical composition and MAPA. The results obtained for the EDS were measured in weight percentage (wt%). All chemical elements identified were described and presented in a graphic, which acts in the periodontal healing process. The wt% of an element is the weight measured in the sample divided by the weight of all elements multiplied by 100. If the results are normalised, the wt% tells about the relative concentration of the element in the sample.

Statistical evaluations

A two-way ANOVA test analyzed the EDS data. Values less than 0.05 were considered statistically significant. Means and standard deviation (SD) are reported in charts (GraphPad Prism version 6 for Windows, GraphPad Software Inc., San Diego, CA, USA). All sample was analyzed in triplicate (n=3).

Results

Root surface morphology

Samples of the human root surface were obtained by SEM and described (Figure 2). The smear layer with an irregular root surface in the control group is observed. In more significant increases, it was possible to observe the overlap of material layers that line the root surface. In test group 1, translated with EDTA 24% for 1 minute, the images still show an irregular surface, with the opening of numerous pores, suggesting that there was a removal of the smear layer surface, and the most significant increases confirmed its residue. In group I, EDTA 24% was applied intermittently, applied for 1 minute, washed with saline, treated for another minute, and washed again. A less irregular surface was observed compared to the control and group I. However, compared to group III, the minimum surface irregularities are more evident, suggesting that washing with saline solution in the application interval influenced the agent biomodifier. Finally, in Group III, the biomodifying agent was also left for 2 minutes, but in an interrupted manner and, in the end, washed with saline solution. The images suggest that an excellent removal of the smear layer occurred in the group where the agent was used interrupted. A less irregular surface was observed and confirmed in the most significant increases.

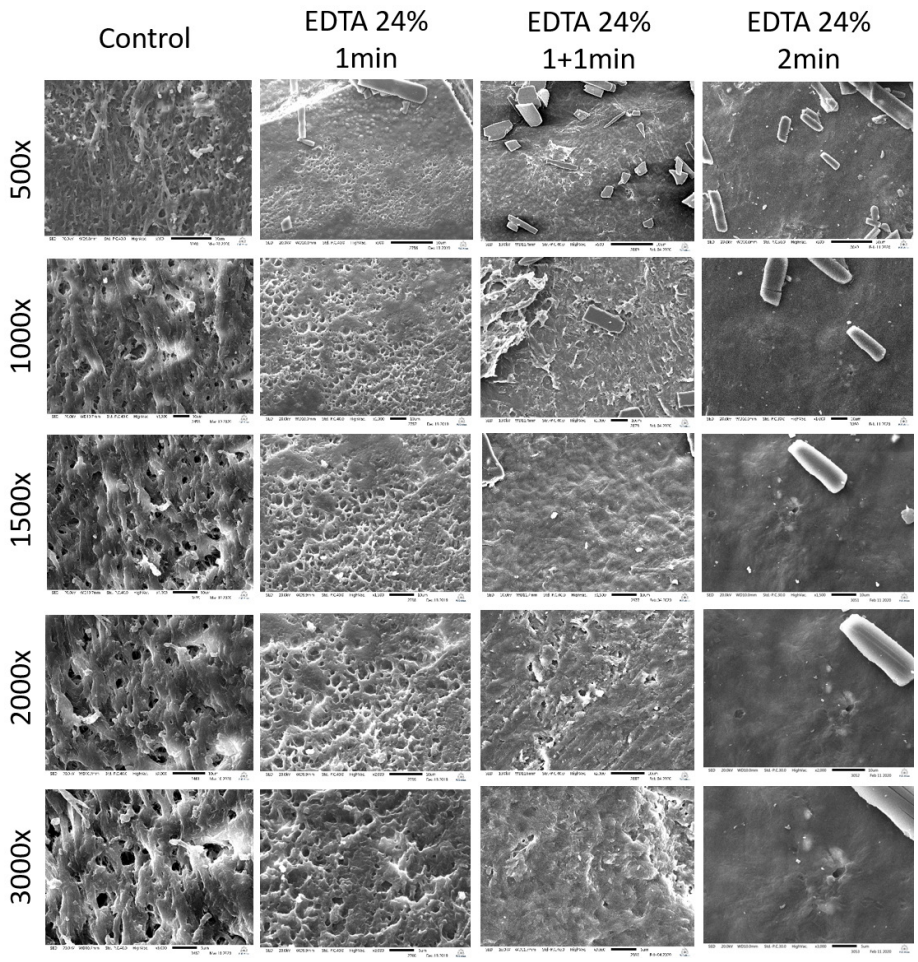

Figure 2. SEM images of human dentin treated by 24% EDTA.

Characterization and chemical distribution on the root surface

EDS performed chemical analysis. The human root surface samples were treated with 24% EDTA in different time forms. In all treatments performed, there was a similarity in the exposure of chemical constituents, with carbon (C), calcium (Ca), phosphorus (P), nitrogen (N), oxygen (O), and sodium (Na) being observed and in some samples the magnesium (Mg). The tooth root conditioning agent aims to remove the smear layer and expose organic molecules that can promote regeneration, such as collagen protein. Organic molecules present in the smear layer or the demineralized dentin matrix were also identified by C. The primary constituents of mineralized tissues are Ca and P. Only the principal molecules (C, Ca, P and O) were presented in the wt% table (Table 2).

Table 2. Data were obtained from the EDS on the percentage of chemical elements in the area. Carbon (C), oxygen (O), calcium (Ca), phosphorus (P) and magnesium (Mg) were evaluated. The table shows the mean and standard deviation of the analyzed samples.

	Carbon (C)	Oxygen (O)	Calcium (Ca)	Phosphor (P)	Magnesium (Mg)
Control	40.90% ± 13.8	26.46% ± 5.92	18.70% ± 3.64	8.63% ± 2.14	0.35% ± 0.21
EDTA 24% - 1 minute	39.93% ± 2.00	29.66% ± 5.08	17.06% ± 0.56	7.16% ± 1.25	ND
EDTA 24% - 1+1 minute	37.66% ± 9.93	28.26% ± 2.25	16.40% ± 7.54	6.76% ± 3.10	ND
EDTA 24% - 2 minutes	44.63% ± 4.91	33.70% ± 4.12	5.60% ± 2.91	1.73% ± 1.50	0.03% ± 0.05

In the control group (Figure 3), the evaluated C showed a high variation in the standard deviation, suggesting that the smear layer is variable, even after mechanical instrumentation standardized by specific periodontal curettes before applying the conditioning agent. The same occurred in test group II, where the action of root hydration by saline solution suggests interfering with the action of 24% EDTA when applied intermittently. This situation can be explained by retaining a saline solution on the root surface after washing between times. Even though we are careful to dry it, the hydration of this surface suggests interfering with the action of a new application of the conditioning agent.

The distribution of chemical molecules was irregular in the control and group III groups. In the control group, due to partial removal of the smear layer (Figure 3). In group III, due to partial demineralization of the root surface after removing the smear layer (Figure 6). The conditioning agent removes mineralized particles from dentin and exposes collagen. Interestingly, in treatment groups I and II, a more homogeneous distribution of chemical constituents over the root surface (Figures 4-5). Again, it is demonstrated that the application of saline solution between applications of 24% EDTA suggests reducing demineralization, allowing the removal of the smear layer and limiting its action on the root surface.

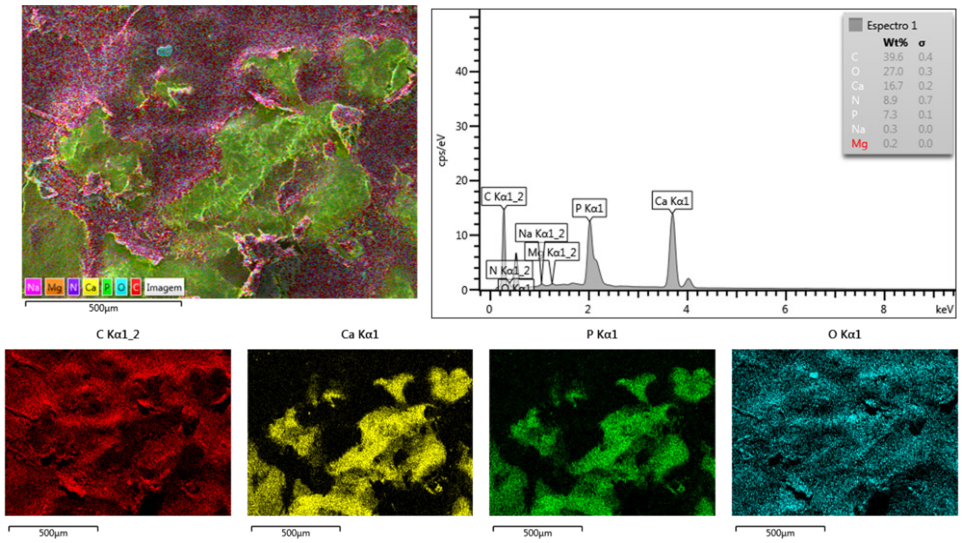

Figure 3. Distribution map of chemical elements from the control group, 100x magnification. After mechanical treatment, the presence of the smear layer in most of the dentin surface is confirmed by carbon. Calcium and phosphorus exposure suggests areas where root scaling removed the smear layer—carbon in red, calcium in yellow, phosphorus in green and oxygen in ocean green.

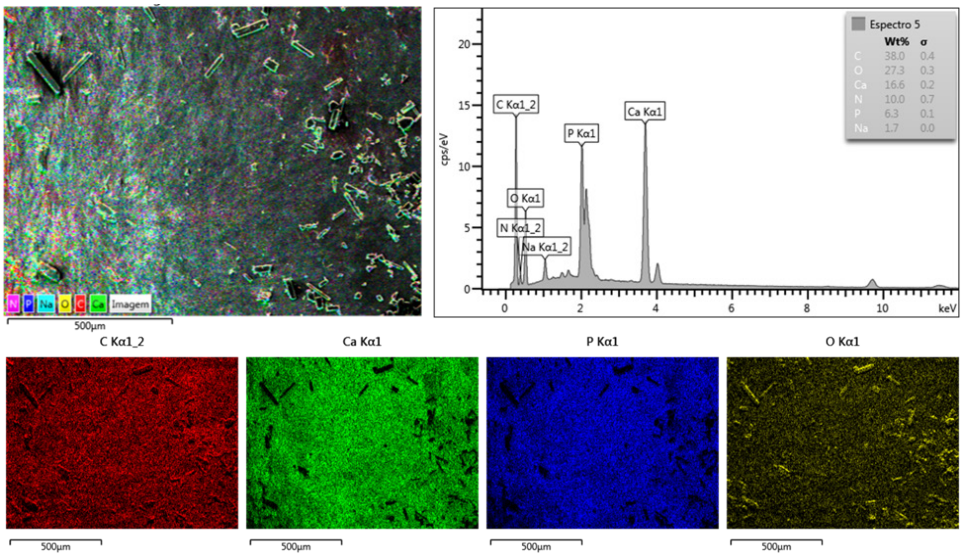

Figure 4. Distribution map of chemical elements of the group treated with 24% EDTA for 1 minute at 100x magnification. Carbon in red, Calcium in green, phosphorus in blue and oxygen in yellow.

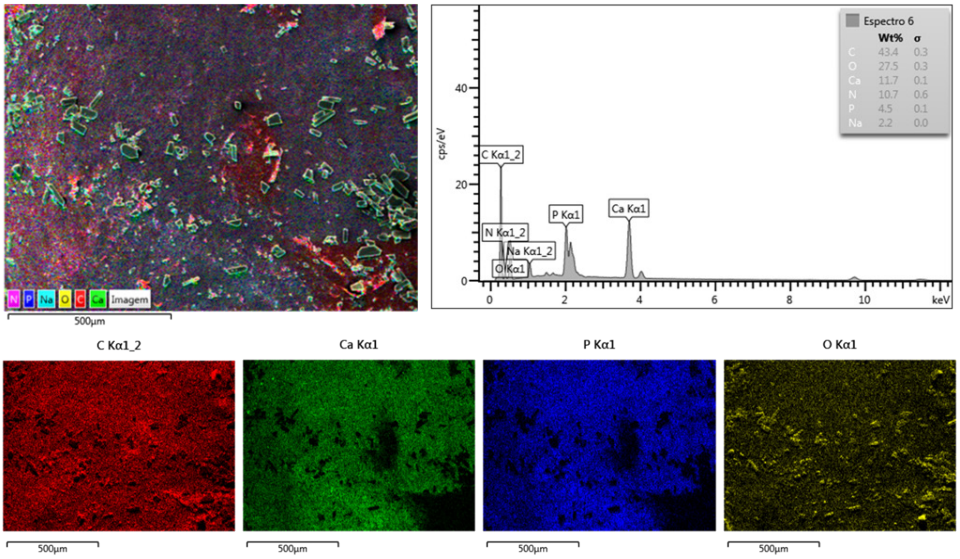

Figure 5. Distribution map of chemical elements of the group treated with 24% EDTA for 1 + 1 minute at 100x magnification. Carbon in red, Calcium in green, phosphorus in blue and oxygen in yellow.

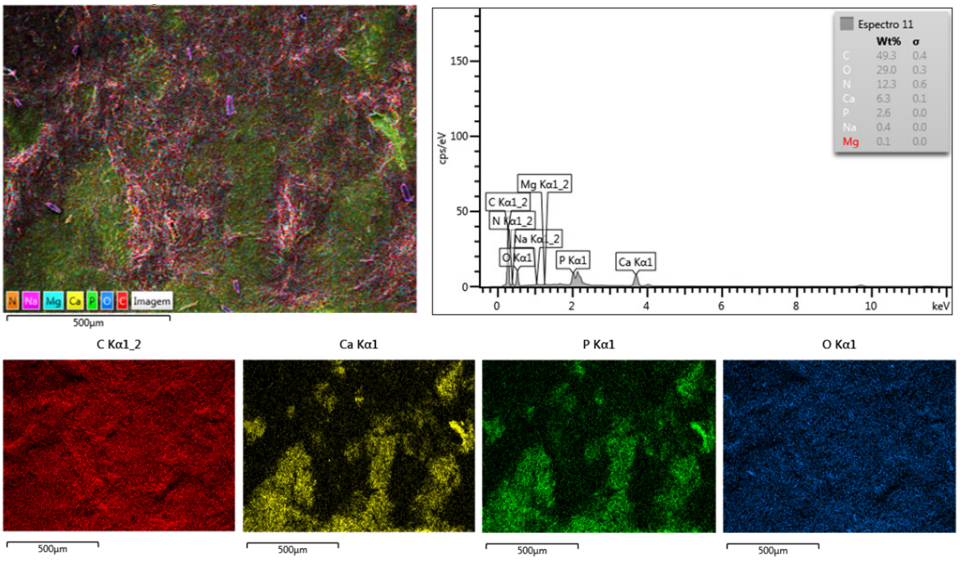

Figure 6. Distribution map of chemical elements of the group treated with 24% EDTA for 2 minutes at 100x magnification. Carbon in red, Calcium in yellow, phosphorus in green and oxygen in blue.

Discussion

After the periodontal mechanical instrumentation, the residual cement and the residues caused by the root scraping form a structure known as a "smear layer", which is the term used to describe the micro fragments and microdetrites left on the dentin after the mechanical instrumentation; this smear layer may also be contaminated by microorganisms, which suggest a harmful action to the periodontal healing process in regenerative procedures (Nanda *et al*., 2014). In the present study, human dentin samples were scraped, and SEM images were obtained. The smear layer was evident in the control group, where it did not undergo any chemical treatment, only the mechanical treatment of root scraping.

Several chemical agents known as biomodifiers or conditioners are employed to remove the smear layer. Among them is EDTA. In vitro studies present different times for the application of EDTA. Amaral *et al*. (2011) compared four conditioning agents to remove the smear layer and open dentin tubules. They used teeth lost due to advanced periodontal disease. Among the agents studied, they used EDTA 24% for 3 minutes. Preeja *et al*. (2013) evaluated and compared the degree of adhesion of the fibrin clot to the root surfaces treated with two types of root conditioning agents. For EDTA 24%, the human dentin blocks were conditioned for 3 minutes and then rinsed three times for 5 min in 10 mL of PBS. Torkzaban *et al*. (2016) compared the surface characteristics of human teeth extracted after exposure to four root conditioners in different periods. 24% of EDTA was used in periods of 1,2,3 and 4 minutes. The EDTA used was a commercially available 24% EDTA gel of pH 7.3 (PrefGel, Biora, Malmo, Sweden). In this study, we used the time of 2 minutes for evaluation criteria, considering the importance of clinical applicability and following the reference to the protocol used for EMD.

Clinical studies have also assessed whether the use of EDTA could improve clinical response. Barootchi *et al*. (2018) conducted a systematic literature review and meta-analysis on the effect of root conditioning with EDTA on advanced coronary flaps with a connective tissue graft. The authors concluded that there is limited evidence available when assessing the effectiveness of root conditioning with EDTA with CAF + CTG. However, the adjuvant application of EDTA with CAF + CTG appears beneficial. The concentration of EDTA and the time used in its application on the tooth root do not report. In another study, 145 periodontal infra-bone defects were evaluated after regenerative therapy using EDTA 24% for 2 minutes associated with the application of the EMD protein, with defects of 1, 2 and 3 walls. The authors observed a reduction in the pocket depth and again in the insertion level (Heden *et al*., 1999). In therapies involving EMD, EDTA 24% for 2 minutes is part of their protocol; in this context, we cannot say that EDTA helped in this response since EMD has antimicrobial and regenerative properties (Miron *et al*., 2016). The present study results show a change in the root surface treated with EDTA 24% that varies according to the time and the way it was applied. This change in root morphology, with the proper elimination of the smear layer, suggests influencing the results of tissue repositioning on the root surface. The inclusion of the application time and concentration of the conditioning agent should be evaluated in critical analysis studies on the clinical responses reported in the literature. On the other hand, chemical analysis shows a similarity in the chemical constituents exposed on the root surface after different application modes.

Although the sample was tripled, its size can be considered a limitation of the present study. From a clinical point of view, root biomodifiers are an exciting complement to regenerative periodontal therapy with DME. Its use can favourably influence the substantivity of EMD on the tooth root's surface, indirectly helping the healing of periodontal wounds. However, further studies with larger sample sizes and comparisons with other biomodifying agents and cell cultures on these treated surfaces are necessary to confirm the effect on dental roots and the benefit for clinical use in the search for stability and healing of the periodontal wound.

Conclusions

Given the limitations of this study, the observed data demonstrate smear layer and changes in the morphology of the human dentin surface within two continuous minutes of 24% EDTA action. The chemical constituents observed suggest a similarity.

References

Donos N. The periodontal pocket. Periodontol 2000. 2018;**76**(1):7-15.

Lasho DJ, O'Leary TJ, Kafrawy AH. A scanning electron microscope study of the effects of various agents on instrumented periodontally involved root surfaces. *Journal of Periodontology*. 1983; **54**:210–20.

Torkzaban P, Seyedzadeh Sabounchi S. The Effect of Four Commonly Used Root Conditioner Agents in Different Time Periods Applied on Periodontally Diseased and Healthy Teeth. *Journal of Dental Biomaterial*, 2016; **3**(2):241-247.

Górski B, Szerszeń M, Kaczyński T. Effect of 24% EDTA root conditioning on the outcome of modified coronally advanced tunnel technique with subepithelial connective tissue graft for the treatment of multiple gingival recessions: a randomized clinical trial. *Clinical Oral Investigations* 2022;**26**(2):1761-1772.

Subramanian S, Appukuttan D, Tadepalli A, Gnana PPS, Athmarao RT. Root Conditioning with Citric Acid and Ethylenediaminetetraacetic Acid and their Effect on Fibrin Clot Adhesion to Dentin-A Scanning Electron Microscopic Study. *Journal of Clinical and Diagnostic Research*. 2017; **11**(8):ZC82-ZC85.

Nanda T, Jain S, Kaur H, Kapoor D, Nanda S, Jain R. Root conditioning in periodontology - *Revisited*. *Journal of Natural Science, Biology and Medicine*. 2014; **5**(2):356-358.

Babgi W, Alhajaji M, Al-Mehmadi L, Elbaqli R, Khayat N, Aldahlawi S, Youssef AR. Effect of root conditioning agents hyaluronic acid, EDTA and chlorhexidine on the attachment of human gingival fibroblasts to healthy root surface. *Saudi Dental Journal* 2021; **33**(6): 342-347.

de Vasconcellos LM, Ricardo LH, Balducci I, de Vasconcellos LG, Carvalho YR. Histological analysis of effects of 24% EDTA gel for nonsurgical treatment of periodontal tissues. *Journal of Oral Science* 2006; **48**(4): 207-14.

Heden G, Wennström J, Lindhe J: Periodontal tissue alterations following Emdogain. A treatment of periodontal sites with angular bone defects. A series of case reports. *Journal of Clinical Periodontology* 1999; **26**: 855–860.

Górski B, Górska R, Wysokińska-Miszczuk J, Kaczyński T. Tunnel technique with enamel matrix derivative in addition to subepithelial connective tissue graft compared with connective tissue graft alone for the treatment of multiple gingival recessions: a randomized clinical trial. *Clinical Oral Investigations* 2020; **24**(12): 4475-4486.

Górski B, Górska R, Szerszeń M, Kaczyński T. Modified coronally advanced tunnel technique with enamel matrix derivative in addition to subepithelial connective tissue graft compared with connective tissue graft alone for the treatment of multiple gingival recessions: prognostic parameters for clinical treatment outcomes. Clinical Oral Investigations. 2022;26(1):673-688.

Amaral NG, Rezende ML, Hirata F, Rodrigues MG, Sant'ana AC, Greghi SL, Passanezi E. Comparison among four commonly used demineralizing agents for root conditioning: a scanning electron microscopy. Journal of Applied Oral Science. 2011; 19(5):469-475.

Preeja C, Janam P, Nayar BR. Fibrin clot adhesion to root surface treated with tetracycline hydrochloride and ethylenediaminetetraacetic acid: A scanning electron microscopic study. Journal of Dental Research. 2013; 10(3):382-388.

Barootchi S, Tavelli L, Ravidà A, Wang CW, Wang HL. Effect of EDTA root conditioning on the outcome of coronally advanced flap with connective tissue graft: a systematic review and meta-analysis. Clinical Oral Investigations. 2018; 22(8):2727-2741.

Miron RJ, Sculean A, Cochran DL, Froum S, Zucchelli G, Nemcovsky C, Donos N, Lyngstadaas SP, Deschner J, Dard M, Stavropoulos A, Zhang Y, Trombelli L, Kasaj A, Shirakata Y, Cortellini P, Tonetti M, Rasperini G, Jepsen S, Bosshardt DD. Twenty years of enamel matrix derivative: the past, the present and the future. Journal of Clinical Periodontology. 2016; 43(8):668-83.