J Periodontal Implant Sci. 2022 Feb;52(1):54-64
https://doi.org/10.5051/jpis.2102800140
pISSN 2093-2278·eISSN 2093-2286

JPIS
Journal of Periodontal & Implant Science

Research Article
Periodontal Science

Check for updates

Blood clot stabilization after different mechanical and chemical root treatments: a morphological evaluation using scanning electron microscopy

Martina Stefanini, Edoardo Ceraolo*, Claudia Mazzitelli, Tatjana Maravic, Matteo Sangiorgi, Giovanni Zucchelli, Lorenzo Breschi, Annalisa Mazzoni

Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna - Alma Mater Studiorum, Bologna, Italy

OPEN ACCESS

Received: May 6, 2021
Revised: Jul 14, 2021
Accepted: Sep 2, 2021
Published online: Sep 23, 2021

***Correspondence:**
Edoardo Ceraolo
Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna - Alma Mater Studiorum, Via San Vitale 59, 40125 Bologna, Italy.
Email: ceraoloedoardo@gmail.com
Tel: +39-3336358445
Fax: +39-3336358445

ORCID iDs
Martina Stefanini
https://orcid.org/0000-0002-9154-637X
Edoardo Ceraolo
https://orcid.org/0000-0002-8666-1015
Claudia Mazzitelli
https://orcid.org/0000-0003-2025-4086
Tatjana Maravic
https://orcid.org/0000-0003-1335-2817
Matteo Sangiorgi
https://orcid.org/0000-0003-3778-4243
Giovanni Zucchelli
https://orcid.org/0000-0002-6471-039X
Lorenzo Breschi
https://orcid.org/0000-0001-7621-226X
Annalisa Mazzoni
https://orcid.org/0000-0001-8738-3283

ABSTRACT

Purpose: This *in vitro* study was conducted to evaluate the effects of different debridement techniques and conditioning procedures on root surface morphology and blood clot stabilization.
Methods: Two debridement techniques (curette [CU] vs. high-speed ultrasound [US]) and 2 conditioning procedures (ethylenediaminetetraacetic acid [EDTA] and phosphoric acid [PA]) were used for the study. Seven experimental groups were tested on root surfaces: 1) no treatment (C); 2) CU; 3) US; 4) CU+EDTA; 5) US+EDTA; 6) CU+PA; and 7) US+PA. Three specimens per group were observed under scanning electron microscopy (SEM) for surface characterization. Additional root slices received a blood drop, and clot formation was graded according to the blood element adhesion index by a single operator. Data were statistically analyzed, using a threshold of $P<0.05$ for statistical significance.
Results: The C group displayed the most irregular surface among the tested groups with the complete absence of blood traces. The highest frequency of blood component adhesion was shown in the CU+EDTA group ($P<0.05$), while no differences were detected between the CU, US+EDTA, and CU+PA groups ($P<0.05$), which performed better than the US and US+PA groups ($P<0.05$).
Conclusions: In this SEM analysis, EDTA and conventional manual scaling were the most efficient procedures for enhancing smear layer removal, collagen fiber exposure, and clot stabilization on the root surface. This technique is imperative in periodontal healing and regenerative procedures.

Keywords: Blood clot; EDTA; Fibrin; Periodontal diseases; Phosphoric acid; Smear layer

INTRODUCTION

Obtaining clean, smooth, and decontaminated root surfaces during periodontal surgery is imperative for the organization of new connective tissue re-attachment and enhancement of the tissue healing process [1]. Even though ultrasonic or manual scaling and root planing are the most widely used techniques in clinical settings for the mechanical cleaning of the root surface during periodontal therapy [2,3], they cannot fully decontaminate the dental hard tissue [4]. Moreover, due to mechanical debridement, a compact smear layer is formed in

Author Contributions

Conceptualization: Martina Stefanini, Annalisa Mazzoni; Data curation: Edoardo Ceraolo, Tatjana Maravic; Formal analysis: Claudia Mazzitelli, Tatjana Maravic, Martina Stefanini, Matteo Sangiorgi; Investigation: Edoardo Ceraolo, Tatjana Maravic; Methodology: Tatjana Maravic, Annalisa Mazzoni; Project administration: Martina Stefanini; Supervision: Martina Stefanini, Annalisa Mazzoni, Matteo Sangiorgi; Validation: Giovanni Zucchelli, Lorenzo Breschi; Writing - original draft: Edoardo Ceraolo, Claudia Mazzitelli, Tatjana Maravic; Writing - review & editing: Martina Stefanini, Annalisa Mazzoni, Lorenzo Breschi, Giovanni Zucchelli, Lorenzo Breschi, Matteo Sangiorgi.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

intimate contact with the treated surface, constituted by a mixture of organic and inorganic materials, microbial toxins, and debris [4,5]. From a histological point of view, the presence of a smear layer interposed between the root surface and the adjacent connective tissue could limit the connective tissue re-attachment process [1,4,5].

Chemical conditioning was, therefore, introduced to support mechanical treatment and detoxify the root surface while dissolving the smear layer [1,6]. The exposure of root surface collagen fibrils using demineralizing solutions activates the physiological cascade of processes involved in periodontal healing, restores the biocompatibility of the root [7], and favors clot stabilization in the earlier stages of periodontal regeneration [8]. The exposure of the dentin matrix allows the interaction between the collagen fibrils of the root surface and the fibrin clot that comes from the periodontal wound, resulting in better regenerative outcomes [4,8].

Several chemical conditioning agents have been proposed to improve biological responses during surgical periodontal therapy. Among these solutions, ethylenediaminetetraacetic acid (EDTA), citric acid, phosphoric acid (PA), and tetracycline hydrochloride are the most widely used demineralizing solutions [9]. However, the lack of standardization of the protocols for the use of these chemical agents in the literature precludes the possibility of precisely indicating the best treatment to promote the healing process in periodontal tissues after surgery.

In particular, EDTA has been commonly used in periodontal practice in different concentrations (12% to 24%) thanks to its neutral pH (up to 7) and its chelating properties towards the mineral structure of the tooth, without a risk of damaging the dentin matrix [9,10]. PA, in contrast, has been extensively used in adhesive dentistry and much less in periodontics, even though it is capable of effectively removing the smear layer and opening up the dentinal tubules, possibly creating a suitable substrate for fibrin clot linkage [11,12].

Thus, the present study aimed to evaluate *in vitro* different debridement techniques (ultrasonic device and manual scaling) and chemical conditioning procedures (EDTA and PA) on root surface morphology and blood stabilization through scanning electron microscopy (SEM) evaluation. The null hypotheses tested were that the type of debridement technique and the conditioning solution would not influence root surface morphology and the adhesion of blood elements to the root surface.

MATERIALS AND METHODS

Specimens' collection

Patients with chronic periodontitis and no history of systemic complications who visited the Department of Periodontology of the University of Bologna (Bologna, Italy) were recruited for this study, in full accordance with ethical principles, including the Declaration of Helsinki. The selected patients were carefully informed about the scope of the study, after which they signed a written informed consent form.

The eligible teeth were required to fulfill the inclusion criteria, such as no history of scaling and root planing in the previous 6 months, an absence of caries or restorations in the proximity of the cementoenamel junction (CEJ), proximal attachment loss of 6 mm or more with bleeding on gentle probing, and grade III mobility that predicted an unfavorable prognosis of the tooth, necessitating extraction therapy.

https://doi.org/10.5051/jpis.2102800140

Three upper premolars were considered suitable for the study, and, after extraction, they were rinsed with saline solution to remove blood, while calculus and other visible debris were hand-removed with curettes (CU). Teeth were then stored in distilled water at 4°C to avoid dehydration until their use, for no longer than 7 days. The protocol was approved by the Ethical Committee of the University of Bologna (Italy; protocol N°: 71/2019/OSS/AUSLBO).

Specimen preparation

Two parallel grooves were made on each tooth using a cylindrical bur mounted on a high-speed handpiece under water irrigation: the first one at the CEJ level and the second one up to 6.5 mm from the first mark, in the apical direction.

The root blocks were longitudinally divided into 2 halves through the root canal, and the pulp tissue was removed with a tweezer, taking care not to touch the walls of the pulp chamber. Each half was randomly assigned to 1 of the following surface mechanical treatments in order to eliminate the contaminated cementum and create a smooth and hard surface: #5-6 Gracey curettes (CU; Hu-Friedy, Chicago, IL, USA) for 1 minute (about 50 apico-cervical traction movements performed) or a high-power ultrasonic device (US; Castellini, Imola, Italy). A single operator performed the debridement procedures. Then, each half specimen was cut lengthwise into several slices (n=6 per half portion, a total of 18 per debridement procedure) (**Figure 1**). Additionally, 2 slices per tooth (n=6) were obtained from the mesiodistal sides of each root (without mechanical treatment) and used as a control group (**Figure 1b**).

Each radicular slice, from either the US or CU group, was randomly allocated into 1 of the following surface conditioning procedure groups: 1) irrigation with saline solution (control group); 2) application of 24% EDTA gel (PrefGel, Straumann, Basilea, Switzerland) for 2

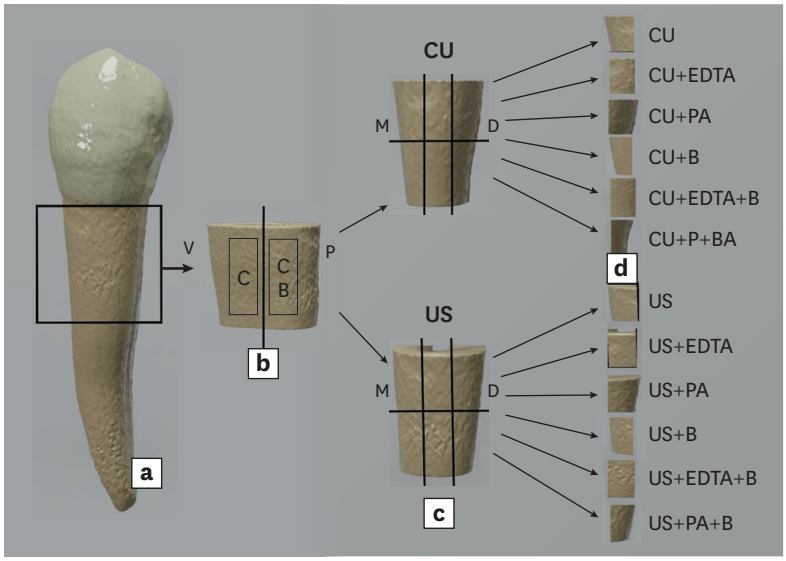

Figure 1. Schematic representation of specimen preparation. Root dentin blocks were obtained from 3 upper premolars (a). Each block was sectioned lengthwise in the mesiodistal direction into 2 halves (b). For each block, 2 slices were obtained from the mesiodistal side and used as CB or C. The 2 halves (c) were allocated to the following surface mechanical treatments: CU or US. Vertical and horizontal cuts were made to obtain 6 slices treated with the tested chemical surface treatments (no treatment, EDTA and PA), with (B) or without blood coating (d).
CB: control with blood application, C: without blood application, CU: curette, US: ultrasound, EDTA: ethylenediaminetetraacetic acid, PA: phosphoric acid, B: with blood coating, V: vestibular, P: palatal, M: mesial, D: distal.

https://doi.org/10.5051/jpis.2102800140

minutes, renewing the solution each 30 seconds (EDTA); 3) application of 35% PA gel (Ultra-Etch, Ultradent, South Jordan, UT, USA) for 15 seconds (PA). The final experimental groups were: 1) no treatment (C); 2) CU; 3) US; 4) CU+EDTA; 5) US+EDTA; 6) CU+PA; and 7) US+PA.

The solutions were brushed onto the entire surface of the radicular slices with a clean cotton pellet and the procedures were repeated thrice. After surface conditioning procedures, the specimens were abundantly rinsed with saline solution (0.9% NaCl) 3 times for 5 minutes in a multi-well dish, with a gentle swirling motion using a rotating tabletop shaker at low speed.

One slice per group was set aside for morphological evaluation. Further, to evaluate the influence of surface conditioning on blood adhesion, 1 drop of fresh human blood taken from a healthy volunteer donor was placed on the top of the remaining pre-treated slices. The blood was allowed to clot over the specimens for 20 minutes in a humidified chamber at 37°C. The specimens were finally rinsed in phosphate-buffered saline (3 times×5 minutes each) in multi-well dishes with gentle rotating movements [4]. After rinsing, the specimens were fixed with 2.5% glutaraldehyde in 0.15 M cacodylate buffer solution (pH 7.2) for 4 hours, rinsed in 0.15 M cacodylate buffer, and dehydrated in ascending ethanol series solutions (50%, 70%, 90%, 95%, 100%; 5 minutes each concentration) [13]. The dehydration process was concluded in a carbon dioxide (CO_2) critical point drier (CPD 030, BalTec, Pfäffikon, Switzerland).

SEM evaluation

Specimens were mounted on aluminum stubs with colloidal graphite, gold-sputtered, and examined using SEM (Nova Nanosem, 450, FEI Europe, Eindhoven, Netherlands) at a voltage of 15 kV. Images of each sample were obtained at ×1,000, ×4,000, and ×10,000 magnification from the most representative areas. Micrographs of the samples that received blood drops were scored using the blood elements adhesion index (BEAI) score [14] as follows:

- Score 0: absence of fibrin network and blood cells;
- Score 1: scarcely distributed fibrin network and/or blood cells;
- Score 2: moderate number of blood cells and thin fibrin network with poor interlacing;
- Score 3: dense fibrin network with rich interlacing and the presence of blood cells.

A single, independent investigator carried out the imaging and scoring of the SEM microphotographs. Data were collected and statistically analyzed. Since data were not normally distributed (Kolmogorov-Smirnov test, $P>0.05$) the Kruskal Wallis non-parametric test was run to analyze the groups' BEAI score values. The Mann-Whitney test was used for pairwise comparisons (IBM SPSS version 26, IBM Corp., Armonk, NY, USA). The level of significance was set at $P<0.05$.

RESULTS

The morphology of the root dentin surfaces after the conditioning treatments, observed on SEM, seemed to be relatively consistent and was characterized by smear layer-covered surfaces with no underlying tubule exposure (**Figure 2**). Specifically, the surface textures of the C group had a rough appearance, with sparsely distributed traces of plaque and microorganisms (**Figure 2A**). Similar morphological structures were found in the CU, US, CU+EDTA, and US+EDTA groups, with the presence of sparsely distributed honeycomb filamentous formations (**Figure 2B-E**). The specimens decontaminated with PA presented a foamy surface (**Figure 2F and G**).

https://doi.org/10.5051/jpis.2102800140

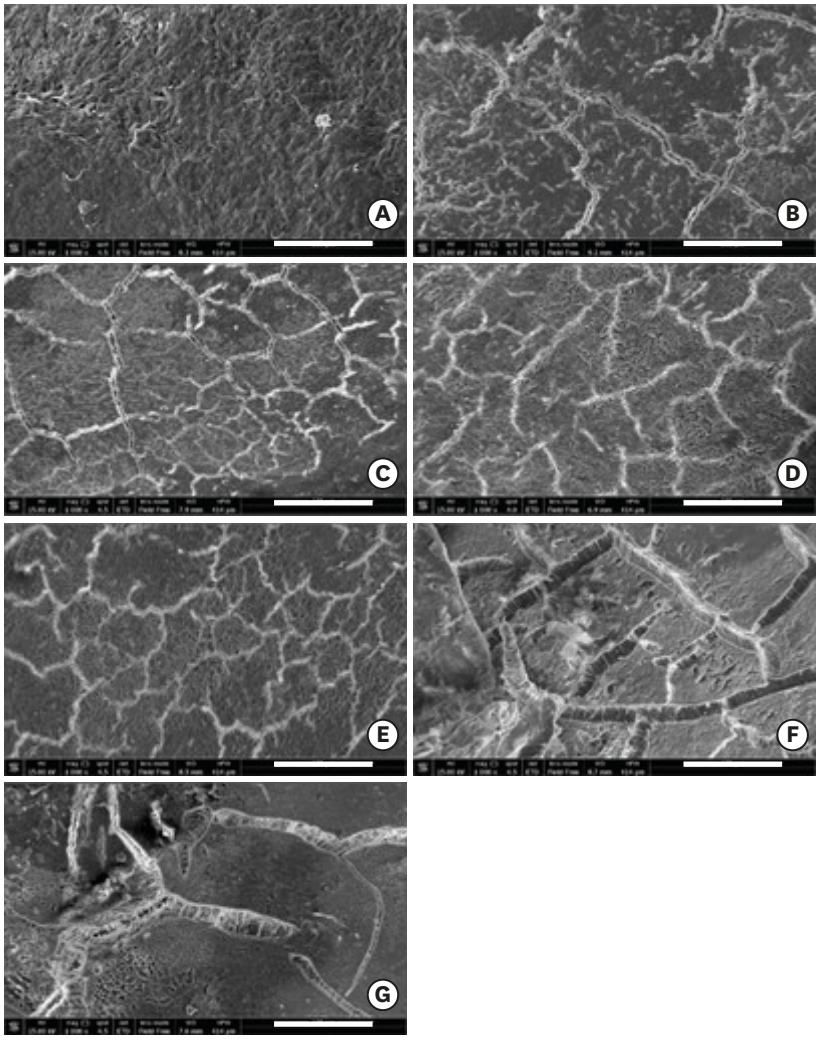

Figure 2. A panel of representative root surface beams observed under SEM (Scale bar: 100 μm). The experimental groups mainly differed from the control groups by having less irregular surfaces. Less rough surfaces were observed in the US and CU groups than in the control group (B and C, respectively). (A) Control; (B) CU; (C) US; (D) CU+EDTA; (E) US+EDTA; (F) CU+PA; (G) US+PA.
SEM: scanning electron microscopy, US: ultrasound, CU: curette, EDTA: ethylenediaminetetraacetic acid, PA: phosphoric acid.

Representative SEM images used for BEAI grading and the score distribution among the tested groups are presented in **Figures 3** and **4**, respectively. SEM images of representative radicular slices that received blood application were evaluated by the BEAI and are shown in **Figure 5**.

Differences in blood cell attachment were observed among the tested groups on SEM microphotographs. The C group showed a surface completely devoid of fibrin network linkage and blood cell adhesion (**Figure 5A**). These differences translated to BEAI scores as follows. Among the experimental groups, the CU+EDTA group revealed the highest distribution of a fibrin network, with a statistically significant difference from other groups (100% of score 3, $P<0.05$). According to pairwise comparisons, the following results were obtained: CU+EDTA ($P<0.05$) > CU = US+EDTA = CU+PA ($P<0.05$) > US = US+PA ($P<0.05$) > C ($P<0.05$). The C group showed a total absence of blood elements on the root surface and it was the only one to present a score of 0 (32%) among the tested groups (**Figure 4**).

https://doi.org/10.5051/jpis.2102800140

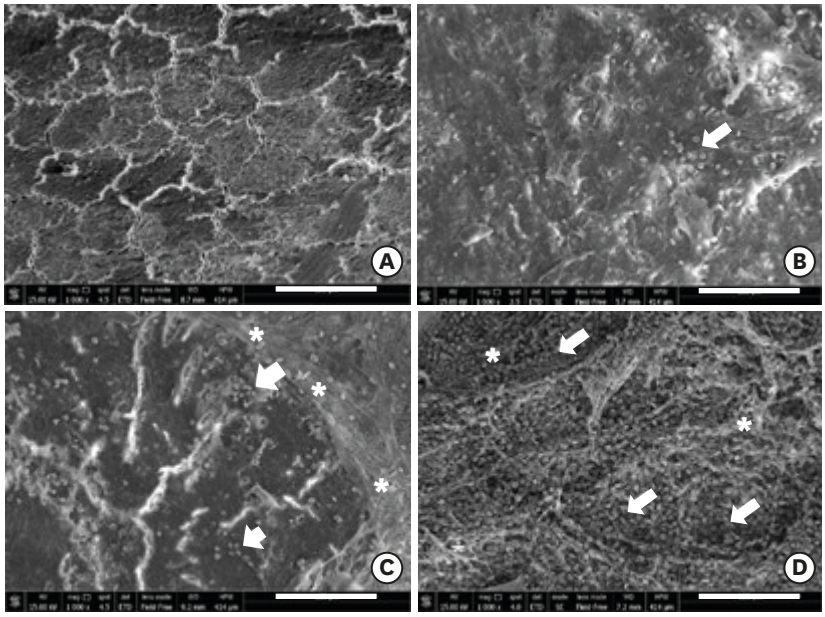

Figure 3. BEAI of representative beams observed under SEM (Scale bar: 100 μm) used for the blood adhesion score (0–4). (A) Absence of fibrin network and blood cells; (B) Scarcely distributed fibrin network (white asterisks) and/or blood cells (white arrows); (C) A moderate number of blood cells (white arrows) and thin fibrin network with poor interlacing (white asterisks); (D) Dense fibrin network with rich interlacing (white asterisks) and presence of blood cells (white arrows).
BEAI: blood elements adhesion index, SEM: scanning electron microscopy.

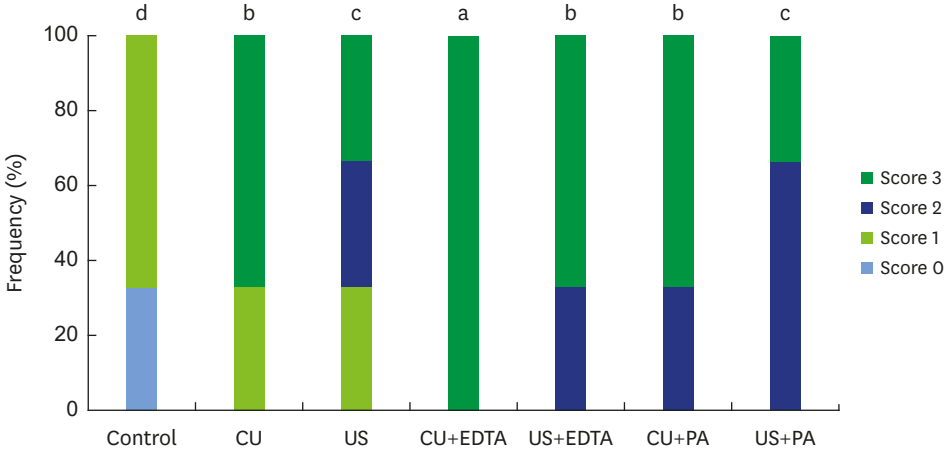

Figure 4. Score distribution according to the BEAI in the specimens of the control; high-speed US; CU; US+EDTA (US+24% EDTA); CU+EDTA (CU+24% EDTA); US+PA (US+35% PA); CU+PA (CU+35% PA). Different letters indicate statistically significant differences among the groups ($P<0.05$).
BEAI: blood elements adhesion index, US: ultrasound, CU: curette, EDTA: ethylenediaminetetraacetic acid, PA: phosphoric acid

Conventional manual scaling and root planing resulted in a higher frequency of fibrin and blood interlacing than the ultrasonic instrumentation ($P<0.05$).

https://doi.org/10.5051/jpis.2102800140

JPIS
Journal of Periodontal & Implant Science

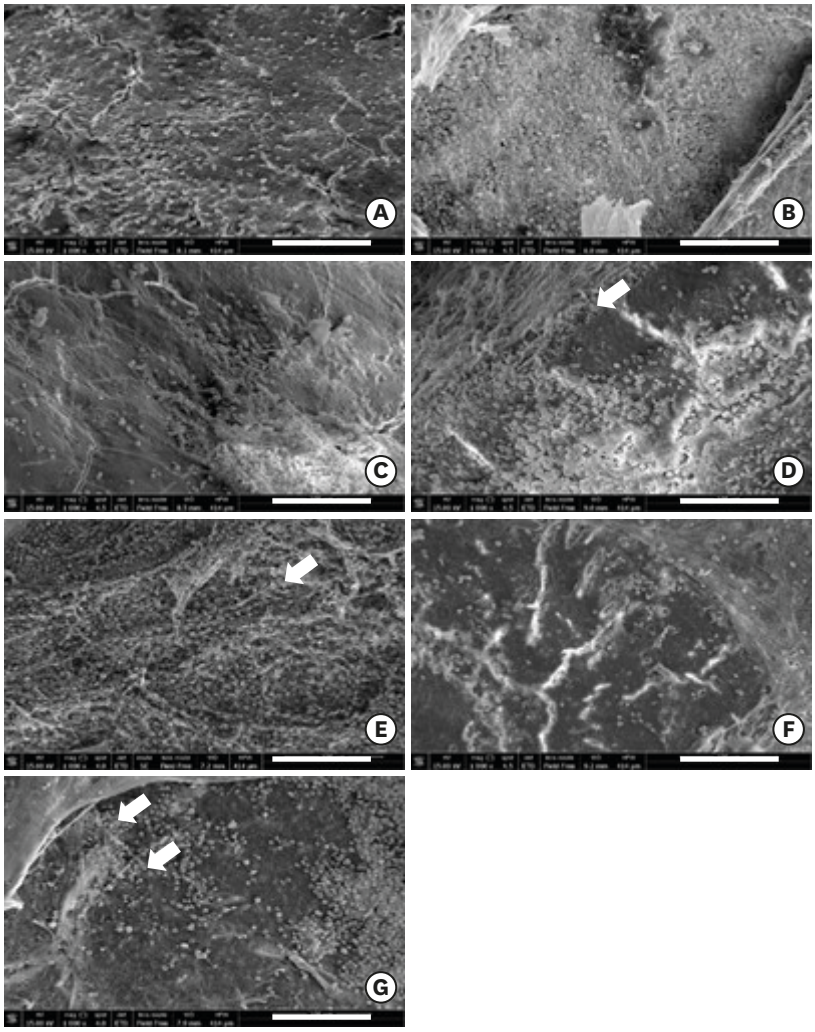

Figure 5. Panel of representative SEM microphotographs of the tested groups after blood application (scale bar: 100 μm). Differences in blood cell attachment were observed, with CU+EDTA (E) and US+EDTA (D) revealing more prominent formation of fibrin ligaments adhering to the treated surfaces (white arrows). Moderate blood cell adhesion and fibrin networking (white arrows) characterized the US+PA group (G). Conversely, when PA was used to decontaminate the surface after US debridement, the surface was still foamy with sparsely distributed fibrin filaments and rare clot adhesion (F). (A) Control; (B) CU; (C) US; (D) CU+EDTA; (E) US+EDTA; (F) CU+PA; (G) US+PA. SEM: scanning electron microscopy, US: ultrasound; CU: curette, EDTA: ethylenediaminetetraacetic acid, PA: phosphoric acid.

DISCUSSION

The results of the present study demonstrated that different debridement techniques and conditioning of the root surface influenced its morphology and improved the adhesion of blood elements to the root surface, especially conventional manual scaling combined with EDTA. Hence, the null hypothesis could be rejected.

Root preparation during periodontal surgical therapy aims to decontaminate the infected root surface to make it smoother, harder, more biocompatible, and suitable for the healing process [1,2].

https://doi.org/10.5051/jpis.2102800140

Several studies have shown that the smear layer formed after root surface debridement may prevent the migration and the attachment of the blood elements from the periodontal wound [8,14]. The adhesion and stability of the blood clot between the gingival flap and the root surface are crucial for the healing process, especially in regenerative and mucogingival procedures for the formation of new connective tissue attachment. Thus, chemical demineralizing agents have been introduced to remove the smear layer and promote the exposure of dentin collagen fibrils, which represents the substrate for the linkage with the fibrin clot [10,15]. However, despite the promising results of several *in vitro* studies, other histological and clinical studies have reported inconsistent results regarding those agents' positive or negative effects [15-18]. The lack of evidence about real clinical effectiveness also stems from differences in the techniques and experimental procedures [15].

To the best of our knowledge, this is the first study to evaluate the effects of the combination of 2 different techniques of scaling and root planing and 2 different modalities of chemical conditioning. Our findings show that contaminated root surfaces do not represent a proper substrate for the healing process since they are covered by residual tissue, plaque, and calculus that do not promote the adhesion of the blood elements, corroborating previous results [4,19-21]. The samples from the control group showed the worst results according to the BEAI (**Figure 4**), with only a few blood cell traces without any organization, mixed with fibrous structures and debris (**Figures 2A** and **5A**). Therefore, root debridement is strongly recommended as a crucial procedure for proper periodontal healing.

Root surface instrumentation for 1 minute has shown promising results even without conditioning, although, in accordance with previous studies, scaling and root planing could not fully decontaminate the root surface, further producing a variable amount of debris and smear layer [2,4,8,15,21]. The 2 tested scaling and root planing techniques (manual vs. US) did not lead to relevant morphological differences of the treated surfaces [22,23]. However, in the present study, it was noteworthy that CU attained statistically significant higher BEAI index than US only. Nevertheless, it should be pointed out that the results of 1-minute-long instrumentation of the root can be strongly affected by the operator's manual skill, especially for CU use [3].

Furthermore, in the present study, chemical conditioning increased the biocompatibility of the treated surfaces, leading to better adhesion and organization of the blood clot. The blood clot could adhere to the root surface even without any chemical treatment, but in the samples treated with EDTA or PA, greater interaction between the collagen structure of the dentin and the fibrin clot weave was recorded. The reason could be related to the etching procedures that effectively removed the smear layer and exposed the underlying dentin substrate, promoting links between the fibrils and the fibrin. Thus, we can speculate that the healing process may be more predictable when the mechanical debridement is associated with etching of the root surface, which seems to promote better adhesion of blood cells.

EDTA is one of the most frequently used solutions for this purpose in periodontics at different concentrations and with various application times. It has been previously reported to remove the smear layer while preserving the dentin collagen matrix and avoiding excessive acidification of the surrounding tissues [4,8,9,19,21,22,24]. Blomlöf et al. [25] showed that a 24% EDTA solution was significantly more effective for the removal of the smear layer than lower concentrations. Moreover, gel formulations, such as the one used in the present study, allow better control of etching properties [26]. Nevertheless, the real efficacy

https://doi.org/10.5051/jpis.2102800140

of EDTA in terms of promoting blood cells' adhesion to the root remains controversial [4]. If not properly removed from the root surface, EDTA could have a negative effect on blood clot adhesion. Indeed, EDTA is a calcium chelator, and its residues could slow or inhibit coagulation processes [19,26]. However, the results of the present study demonstrated that these potential drawbacks can be prevented if EDTA is thoroughly rinsed after application. Moreover, the most effective application time remains unclear. Although Gamal et al. reported a positive correlation between the application of EDTA for 4 minutes and the adhesion of the periodontal ligament cells to the root [16], it is reasonable to prefer an application time that is clinically effective, but simultaneously compatible with the surgical timing. The 2-minute application time used in the present study seemed to effectively remove the smear layer and promote blood clot adhesion, while remaining within a clinically acceptable time frame.

PA is widely used in adhesive dentistry to remove the smear layer and demineralize peritubular and intertubular dentin during etch-and-rinse or selective enamel etching bonding procedures [11,12]. Some investigators have proposed also using PA in the periodontal setting, thanks to its ability to remove the radicular smear layer and expose the dentinal tubules, allowing interactions between the collagen fibrils and the fibrin clot in the periodontal wound [11,24]. Both EDTA and PA led to good blood clot stability. Nevertheless, some samples etched with PA showed a greater exposure of dentin collagen fibrils, probably due to its lower pH and, as a consequence, greater etching properties. Our choice to use it for 15 seconds, a common application time in adhesive dentistry, comes from evidence that prolonged contact with the root surface can lead to dentin matrix degradation, as reported after the application of acid substances on the dentin substrate [7,15]. The high etching potential of PA could have interesting clinical implications since it seems to guarantee the exposure of the dentin collagen components in only 15 seconds, thereby saving time during the surgical procedure. However, the effect on the surrounding periodontal tissues remains unclear, and if the gingival flap does not completely cover some areas at the end of surgery, this could lead to dentinal hypersensitivity [24].

In conclusion, the results of this preliminary study confirm the crucial role of scaling and root planing in creating reliable adhesion of blood cells to the root as the first step of the healing process. The additional use of chemical decontaminants, such as EDTA or PA, enhances the interaction between dentinal collagen fibrils and the fibrin clot, suggesting a potential clinical benefit in periodontal reconstructive surgical procedures. Additional *in vitro* investigations with an increased number of specimens, as well as clinical studies, are needed to validate the procedure further.

ACKNOWLEDGEMENTS

We would like to thank the Centro Interdipartimentale Grandi Strumenti (CIGS) of the University of Modena and Reggio Emilia for assistance in scanning electron microscopy (SEM) analysis.

REFERENCES

1. Baker PJ, Rotch HA, Trombelli L, Wikesjö UM. An *in vitro* screening model to evaluate root conditioning protocols for periodontal regenerative procedures. J Periodontol 2000;71:1139-43.
PUBMED | CROSSREF
2. Adriaens PA, Adriaens LM. Effects of nonsurgical periodontal therapy on hard and soft tissue. Periodontol 2000 2004;36:121-45.
PUBMED | CROSSREF
3. Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: How many options for one disease? Periodontol 2000 2017;75:152-88.
PUBMED | CROSSREF
4. Nascimento GG, Leite AA, Leite ER, Sampaio JE, Leite FR. Blood clot stabilization on root dentin conditioned by the combination of tetracycline and EDTA. Braz J Oral Sci 2014;13:83-8.
CROSSREF
5. Fontanari LA, Pinto SC, Cavassim R, Spin-Neto R, Ishi EP, Sampaio JE. Influence of dental exposure to oral environment on smear layer removal and collagen exhibition after using different conditioning agents. Braz Dent J 2011;22:479-85.
PUBMED | CROSSREF
6. Baker DL, Stanley Pavlow SA, Wikesjö UM. Fibrin clot adhesion to dentin conditioned with protein constructs: an *in vitro* proof-of-principle study. J Clin Periodontol 2005;32:561-6.
PUBMED | CROSSREF
7. Zandim DL, Leite FR, da Silva VC, Lopes BM, Spolidorio LC, Sampaio JE. Wound healing of dehiscence defects following different root conditioning modalities: an experimental study in dogs. Clin Oral Investig 2013;17:1585-93.
PUBMED | CROSSREF
8. Leite FR, Sampaio JE, Zandim DL, Dantas AA, Leite ER, Leite AA. Influence of root-surface conditioning with acid and chelating agents on clot stabilization. Quintessence Int 2010;41:341-9.
PUBMED
9. Manzolli Leite FR, Nascimento GG, Manzolli Leite ER, Leite AA, Cezar Sampaio JE. Effect of the association between citric acid and EDTA on root surface etching. J Contemp Dent Pract 2013;14:796-800.
PUBMED | CROSSREF
10. Ruggeri A Jr, Prati C, Mazzoni A, Nucci C, Di Lenarda R, Mazzotti G, et al. Effects of citric acid and EDTA conditioning on exposed root dentin: an immunohistochemical analysis of collagen and proteoglycans. Arch Oral Biol 2007;52:1-8.
PUBMED | CROSSREF
11. Héritier M. Ultrastructural study of new connective tissue attachment following phosphoric acid application on human root dentin. J Periodontol 1983;54:515-21.
PUBMED | CROSSREF
12. Héritier M. Effects of phosphoric acid on root dentin surface. A scanning and transmission electron microscopic study. J Periodontal Res 1984;19:168-76.
PUBMED | CROSSREF
13. Josic U, Maravic T, Bossù M, Cadenaro M, Comba A, Ierardo G, et al. Morphological characterization of deciduous enamel and dentin in patients affected by osteogenesis imperfecta. Appl Sci (Basel) 2020;10:7835.
CROSSREF
14. Dantas AA, Fontanari LA, Ishi EP, Leite FR, Zandim DL, Rached RS, et al. Blood cells attachment after root conditioning and PRP application: an *in vitro* study. J Contemp Dent Pract 2012;13:332-8.
PUBMED | CROSSREF
15. Amaral NG, Rezende ML, Hirata F, Rodrigues MG, Sant'ana AC, Greghi SL, et al. Comparison among four commonly used demineralizing agents for root conditioning: a scanning electron microscopy. J Appl Oral Sci 2011;19:469-75.
PUBMED | CROSSREF
16. Gamal AY, Mailhot JM. The effects of EDTA gel conditioning exposure time on periodontitis-affected human root surfaces: surface topography and PDL cell adhesion. J Int Acad Periodontol 2003;5:11-22.
PUBMED
17. 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. Clin Oral Investig 2018;22:2727-41.
PUBMED | CROSSREF

18. Caffesse RG, De LaRosa M, Garza M, Munne-Travers A, Mondragon JC, Weltman R. Citric acid demineralization and subepithelial connective tissue grafts. J Periodontol 2000;71:568-72.
PUBMED | CROSSREF

19. Leite FR, Moreira CS, Theodoro LH, Sampaio JE. Blood cell attachment to root surfaces treated with EDTA gel. Braz Oral Res 2005;19:88-92.
PUBMED | CROSSREF

20. Subramanian S, Appukuttan D, Tadepalli A, Gnana PP, Athmarao RT. Root conditioning with citric acid and ethylenediaminetetraacetic acid and their effect on fibrin clot adhesion to dentin-a scanning electron microscopic study. J Clin Diagn Res 2017;11:ZC82-5.
PUBMED | CROSSREF

21. Silva AC, Moura CC, Ferreira JA, Magalhães D, Dechichi P, Soares PB. Biological effects of a root conditioning treatment on periodontally affected teeth--an *in vitro* analysis. Braz Dent J 2016;27:160-8.
PUBMED | CROSSREF

22. Singh S, Uppoor A, Nayak D. A comparative evaluation of the efficacy of manual, magnetostrictive and piezoelectric ultrasonic instruments--an *in vitro* profilometric and SEM study. J Appl Oral Sci 2012;20:21-6.
PUBMED | CROSSREF

23. Babay N. Attachment of human gingival fibroblasts to periodontally involved root surface following scaling and/or etching procedures: a scanning electron microscopy study. Braz Dent J 2001;12:17-21.
PUBMED

24. Soares PB, Castro CG, Branco CA, Magalhaes D, Neto AJ, Soares CJ. Mechanical and acid root treatment on periodontally affected human teeth – a scanning electron microscopy. Braz J Oral Sci 2010;2:128-32.

25. Blomlöf J, Blomlöf L, Lindskog S. Effect of different concentrations of EDTA on smear removal and collagen exposure in periodontitis-affected root surfaces. J Clin Periodontol 1997;24:534-7.
PUBMED | CROSSREF

26. Blomlöf JP, Blomlöf LB, Lindskog SF. Smear removal and collagen exposure after non-surgical root planing followed by etching with an EDTA gel preparation. J Periodontol 1996;67:841-5.
PUBMED | CROSSREF