

# Influence of concentration, time and method of application of citric acid and sodium citrate in root conditioning

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## ABSTRACT

**O**bjective: The aim of this study was to establish the parameters of concentration, time and mode of application of citric acid and sodium citrate in relation to root conditioning. Material and Methods: A total of 495 samples were obtained and equally distributed among 11 groups (5 for testing different concentrations of citric acid, 5 for testing different concentrations of sodium citrate and 1 control group). After laboratorial processing, the samples were analyzed under scanning electron microscopy. A previously calibrated and blind examiner evaluated micrographs of the samples. Non-parametric statistical analysis was performed to analyze the data obtained. Results: Brushing 25% citric acid for 3 min, promoted greater exposure of collagen fibers in comparison with the brushing of 1% citric acid for 1 minute and its topical application at 1% for 3 min. Sodium citrate exposed collagen fibers in a few number of samples. Conclusion: Despite the lack of statistical significance, better results for collagen exposure were obtained with brushing application of 25% citric acid for 3 min than with other application parameter. Sodium citrate produced a few number of samples with collagen exposure, so it is not indicated for root conditioning.

**Key words:** Periodontics. Dental scaling. Smear layer. Collagen.

## INTRODUCTION

Cementum is a mineralized tissue with primary function to insert the ligament fibers on the root surface and releasing occlusal forces to the surrounding alveolar bone. There is considerable interest in observing the changes that occur on the cementum surface inside periodontal pockets as a result of periodontal disease<sup>14,21</sup>. Periodontitis-affected root surfaces are hypermineralized and contaminated with endotoxins and other biologically active substances<sup>1,20,25</sup>. It has been suggested that endotoxin present in the cement could impair periodontal healing and should be removed to promote a more biologically

acceptable surface than the one obtained only after scaling and root planning<sup>7</sup>.

The most important event in the reattachment of the connective tissue is related to the adhesion of blood elements to the collagen present at the root surface, which retards the apical migration of the sulcular epithelium into the pocket<sup>13,26</sup>. Epithelial downgrowth is exacerbated by the strong adhesion of bacterial products and endotoxins, especially originated from Gram negative bacteria. These bacterial compounds have affinity to mineral structures such as the tooth, promoting a constant aggression on the periodontal tissue, causing loss of tissue support until the loss of the tooth. Endotoxins as bacterial

cell wall lipopolysaccharides remain active even after bacterial death, not being removed by scaling and root planning<sup>1,14</sup>. These bacteria products are found bound to the smear layer produced by the action of curettes, ultrasonic and rotary instruments<sup>1,3</sup>.

In an attempt to remove smear layer and demineralize the contaminated root surface, different approaches have been studied as the use of chemical agents and laser<sup>12,22,24,28</sup>.

Special attention has been focused on the use of chemical agents as a mean to obtain adequate preparation of the root to development of new connective attachment. Certain acids, especially citric acid has been used to clean the root surface by demineralization and, recently, some authors showed that demineralization of exposed dentin can significantly increase the reattachment of connective tissue to the root surface<sup>9,10</sup>.

Previous studies on tissue regeneration have used tetracycline hydrochloride HCl to clean the root surface because its bactericidal and demineralizing effect<sup>9,25</sup>. The parameters for root conditioning with tetracycline HCl were established by Ishi, et al.<sup>12</sup> (2008). However, the search for a less acid substance to avoid tissue necrosis and probably provide better conditions for clot adhesion to the root surface, showed promising results employing ethylenediaminetetraacetic acid (EDTA)<sup>15,29</sup>. However the outcomes were not so favorable in clinical trials. The use of EDTA gel as a root surface conditioning agent negatively affected the outcome of root coverage<sup>3</sup>. EDTA might have inhibited blood element adsorption and adhesion to the dentin surface because of a possible incomplete removal of the gel from the root surface. In addition, EDTA is a calcium chelator; therefore, its residues may have inhibited or retarded coagulation events<sup>17</sup>. Citric acid is a substance such capable of removing smear layer and opening dentinal tubules<sup>18,28</sup>. However, citric acid's low pH may induce cytotoxic effects when in contact with connective tissue<sup>16</sup>.

In an attempt to find a substance that could efficiently remove smear layer and expose collagen fibers, Leite, et al.<sup>18</sup> (2010) tested the sodium citrate in the clot stabilization. However, this substance has not been tested before and the application parameters are not established yet.

In order to establish the application parameters for sodium citrate and ascertain that this substance is capable of smear layer removal, we suggested conducting this study. In addition, as citric acid presented better results than EDTA for clot stabilization<sup>18</sup> and as citric acid combined with platelet-derived growth factor-BB showed better results than EDTA and tetracycline hydrochloride on attachment of periodontal ligament cells on

root surfaces<sup>2</sup>, we proposed to test if a lower concentration of citric acid applied by a shorter time is capable of removing smear layer and exposing collagen fiber. The aim of this study was to establish concentrations, times and modes of ideal applications of citric acid and sodium citrate in removing smear layer and exposure of collagen fibers.

## MATERIAL AND METHODS

A total of 124 periodontally involved human teeth were obtained at the Oral and Maxillofacial Surgery and Periodontics clinics at the Araraquara School of Dentistry, UNESP – Univ. Estadual Paulista, Brazil. This study was approved by the institutional Human Ethics Committee.

### Sample preparation

The samples were prepared according to previous published data<sup>12</sup> and briefly described as follows. For sample preparation the buccal and lingual root surfaces of each tooth were used. Two parallel grooves with approximately 0.8 mm deep were made using a high speed cylindrical bur (#3099 - Ø 1.6 mm) (KG Sorensen, Medical Burs, Cotia, SP, Brazil) under copious irrigation. One groove was made at the cemento-enamel junction and another one approximately 3 mm distant from the first, in the apical direction. The same bur was used to remove the surface layer of the root between the two grooves.

In order to create a smear layer, 50 apical to cervical strokes were performed using a sharp #5-6 Gracey curette (Hu-Friedy, Hu-Friedy, Chicago, IL, USA). A total of 495 samples measuring about 2x3 mm were obtained cutting teeth with a flexible double faced diamond disc (#7020 - Ø 0.22 mm - thickness: 0.15 mm) (KG Sorensen, Medical Burs, Cotia, SP, Brazil) at low speed. The samples were equally distributed among the groups.

The citric acid was applied in five concentrations 0.5, 1, 2, 15 and 25% (five groups n=45 samples each). The sodium citrate was also divided into five groups with the following concentrations 3, 10, 20, 30 and 40% (n=45 each). The control group was represented by conditioning with saline solution (n=45).

Five different concentrations were tested in order to determine if lower concentrations, and consequently less aggressive substances, could be effective in removing smear layer and exposing collagen fibers. In the same way, different modes and times of application were evaluated in an attempt to reduce the contact of the substances with the periodontal cells.

The groups were divided into subgroups

according to the mode and time of application solutions. Three modes of application were used (n=15 each): 1) simple positioning of a small cotton pellet embedded in solution (passive application); 2) brushing application with a soft brush (Disposable Brush Tips Ø2, 3M ESPE, Seefeld, Germany); and 3) burnishing application (friction) with a small cotton pellet. Each of these three subgroups was further divided into the three application periods of 1, 2, or 3 min (n=5 each).

Samples were dehydrated in an increasingly graded series of ethanol: 30, 50, 70, 80, 95 and 100%. Then, the samples were dried overnight in a dehydration jar (Corning, Corning Life Sciences, São Paulo, SP, Brazil), mounted on metallic stubs (Senai, São Paulo, SP, Brazil) and sputter-coated with a thin 25 nm layer of 99.99% pure gold (Balt-Tec SCD-050, Balt-Tec, Gnathole Farm, Kettleshulme, High Peak, Cheshire, UK).

### SEM evaluation

Two micrographs were obtained from the center area of each sample with 1,500x and 3,500x magnifications, using a scanning electron microscope operated at an accelerating voltage of 20 kV (Jeol T330 A; Jeol Ltd., Peabody, MA, USA). The micrographs were evaluated according to a root surface modification index adapted for this study. A previously calibrated (kappa score=0.93) and experienced examiner<sup>18</sup> evaluated three times each image, with an interval of 15 days between each evaluation. The score attributed to each sample was the most prevalent score in the three evaluations. The adapted index used for this study

consisted of eight scores as shown in Figure 1.

### Statistical analysis

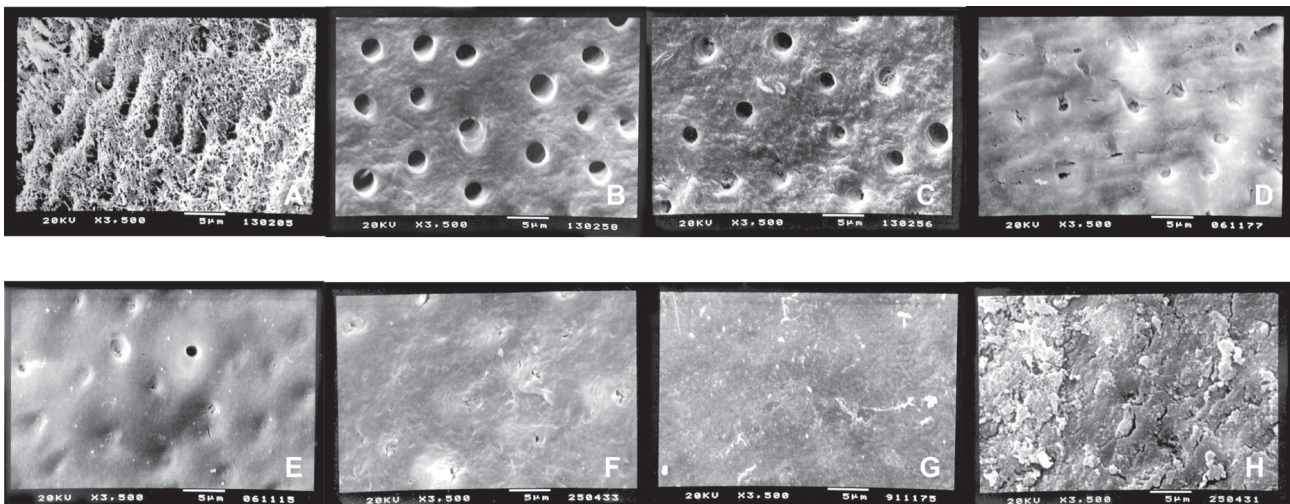
The non-parametric analysis of variance (Kruskal-Wallis test) was applied to independently evaluate the effect of the three dependent variables: solution concentration, mode of application and period. The test was performed separated for both the substances evaluated. The level of significance adopted was 5%. If  $p \leq 0.05$ , Dunn's Multiple Comparison *post hoc* test was applied to detect statistically significant differences among groups. Statistical analysis was made with a computer software (GraphPad Prism 5.00; GraphPad Software Inc., San Diego, CA, USA).

## RESULTS

### Citric acid

Citric acid was evaluated at concentrations of 0.5, 1, 2, 15 and 25%. All concentrations were used for 1, 2 and 3 min under the passive, brushing or burnishing forms.

Statistically significant differences were found between control group and the other concentrations used (Table 1). Even without statistical significance, the concentration of 25% and 15% showed less variation in score values (Figure 2), the same way that the concentration of 25% presented more samples with score 1 (Table 1). Score 1 represents complete smear layer removal with dentin collagen fibers exposure and complete opened dentin tubules, without



**Figure 1-** Root Modification Index. A) Score 1. Complete smear layer removal with dentin collagen fiber exposure. Completely open dentinal tubules, without smear layer on root surface. B) Score 2. Complete smear layer removal. No collagen fiber exposure. Completely open dentin tubules. C) Score 3. Traces of smear layer remaining in the openings of dentinal tubules. D) Score 4. Partial opening of dentinal tubules. E) Score 5. Smear layer covering the surface, formed by chemical dissolution of dentinal surface. F) Score 6. A uniform smear layer covering dentin surface with some signs of tubule openings. G) Score 7. Dentin surface covered by uniform a smear layer, with no signs of dentinal tubule opening. H) Score 8. Rough smear layer covering dentin surface

smear layer on root surface. This is the aim of the chemical root conditioning (Figure 1).

The comparison of the different periods of application of citric acid, showed no differences among the groups (Figure 3). Furthermore, Table

1 shows a slight tendency of the 3-min application to be more effective in exposing collagen fibers (score 1).

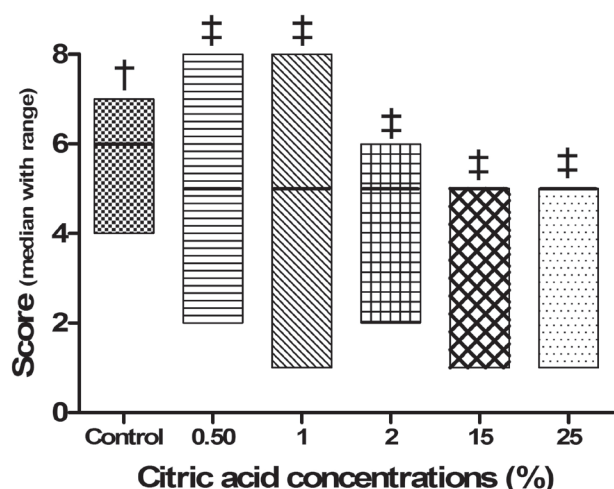
The same way, the evaluation of the different modes of application showed no significant

**Table 1-** Sample distribution for the citric acid concentrations, periods and modes of application

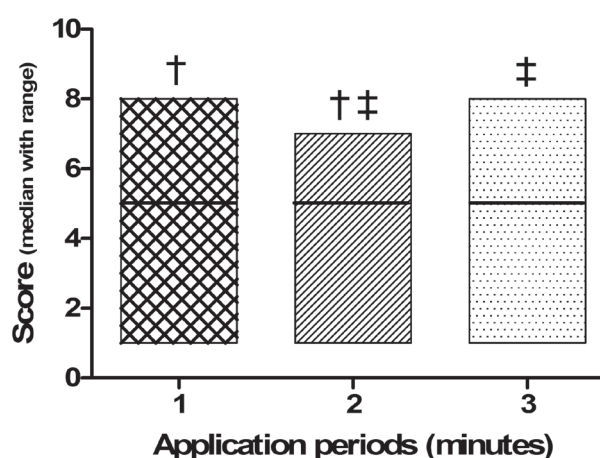
Citric acid concentration						
	Control	0.5%	1%	2%	15%	25%
n	45	45	45	45	45	45
Median/Mean (SD)	6.00/6.00 (0.78)	5.00/5.00 (1.20)	5.00/4.80 (1.40)	5.00/4.60 (0.75)	5.00/4.50 (1.30)	5.00/3.80 (1.80)
p Value	A	B	B	B	B	B
Number of samples with score 1 (%)	0	0	3 (6.67)	0	4 (8.89)	12 (26.67)
Periods of application						
	1 minute	2 minutes	3 minutes			
n	90	90	90			
Median/Mean (SD)	5.00/5.00 (1.60)	5.00/4.80 (1.00)	5.00/4.60 (1.60)			
p Value	NS	NS	NS			
Number of samples with score 1 (%)	7 (7.78)	4 (4.44)	8 (8.89)			
Modes of application						
	Passive	Brushing	Burnishing			
n	90	90	90			
Median/Mean (SD)	5.00/4.90 (1.50)	5.00/4.50 (1.70)	5.00/5.00 (0.79)			
p Value	NS	NS	NS			
Number of samples with score 1 (%)	4 (4.44)	14 (15.55)	1 (1.11)			

Kruskal-Wallis test and Dunn’s Multiple Comparison post hoc test. p=0.05

\* Within the same category, scores with the same letter are not statistically different. SD= standard deviation

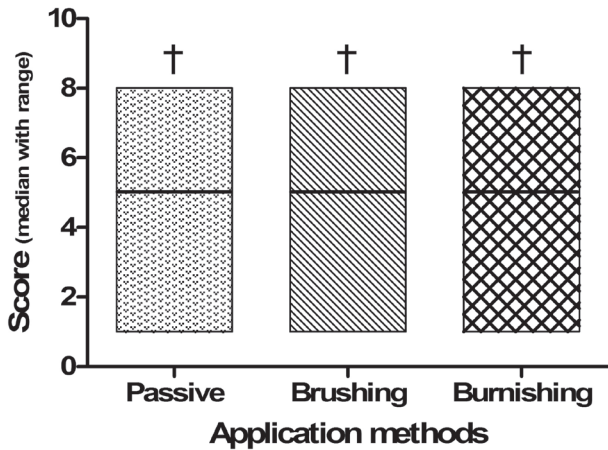


**Figure 2-** Median with range of the scores obtained for the five concentrations of citric acid and the control group. Concentrations with the same symbol are not statistically different

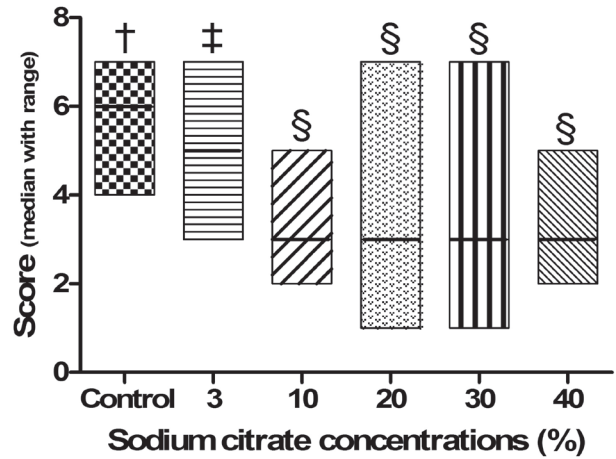


**Figure 3-** Median with range of the scores obtained for the three application periods of the citric acid. Application periods with the same symbol are not statistically different

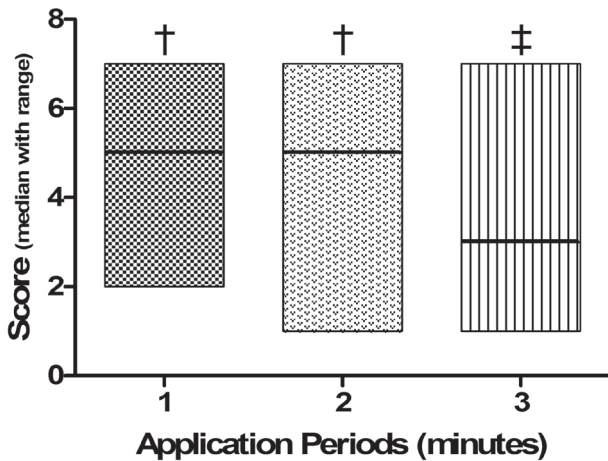




**Figure 4-** Median with range of the scores obtained for the three application methods of the citric acid. Application methods with the same symbol are not statistically different



**Figure 5-** Median with range of the scores obtained for the five concentrations of sodium citrate and the control group. Concentrations with the same symbol are not statistically different



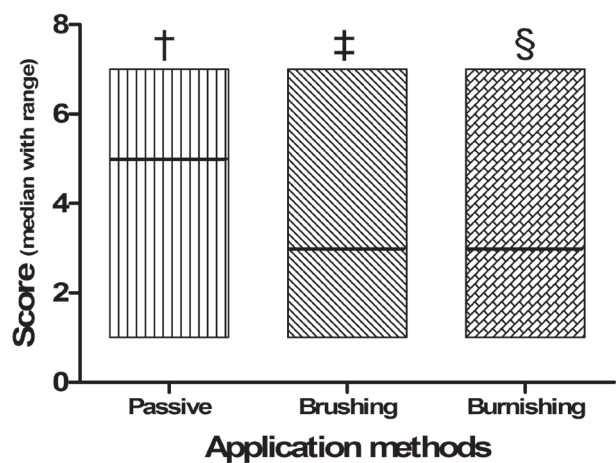
**Figure 6-** Median with range of the scores obtained for the three application periods of the sodium citrate. Application periods with the same symbol are not statistically different

difference between groups (Figure 4). However, application by brushing seems to have favored the production of samples with more exposure of collagen fibers as seen in Table 1.

**Sodium citrate**

The effect of sodium citrate in smear layer removal was evaluated in five different concentrations and in a control group. Among the concentrations evaluated, the 3% showed less effective results than the others and significant statistical difference was observed. The concentrations of 10, 20, 30 and 40% showed no statistically significant differences among them (Figure 5). Table 2 shows the median values and standard deviation (SD) for the concentrations, periods of applications and modes of application.

The analysis of the application periods showed significant differences among the groups.



**Figure 7-** Median with range of the scores obtained for the three application methods of the sodium citrate. Application methods with the same symbol are not statistically different

Even though, the results for 2 and 3 min were statistically the same, the 3-min application seems to have been slightly better than others (Figure 6).

Regarding the mode of application, more favorable results were obtained with application by brushing or by burnishing than the passive application (Figure 7). A statistically significant difference was observed among the modes of application and a slight tendency of best results can be seen with the application by burnishing (Table 2).

**Table 2-** Sample distribution for the sodium citrate concentrations, periods and modes of application

Sodium citrate concentration						
	Control	3%	10%	20%	30%	40%
n	45	45	45	45	45	45
Median/Mean (SD)	6.00/6.00 (0.78)	5.00/5.00 (1.70)	3.00/3.80 (1.10)	3.00/3.20 (1.50)	3.00/3.20 (1.40)	3.00/3.60 (0.96)
p Value	A	B	C	C	C	C
Number of samples with score 1 (%)	0	0	0	3 (6.67)	4 (8.89)	0
Periods of application						
	1 minute	2 minutes		3 minutes		
n	90	90		90		
Median/Mean (SD)	5.00/4.70 (1.40)	5.00/4.20 (1.70)		3.00/3.40 (1.40)		
p Value	A	A		B		
Number of samples with score 1 (%)	0	3 (3.33)		4 (4.45)		
Modes of application						
	Passive	Brushing		Burnishing		
n	90	90		90		
Median/Mean (SD)	5.00/4.80 (1.60)	3.00/4.10 (1.50)		3.00/3.40 (1.60)		
p Value	A	B		C		
Number of samples with score 1 (%)	1 (1.11)	2 (2.22)		4 (4.45)		

Kruskal-Wallis test and Dunn's Multiple Comparison *post hoc* test.  $p=0.05$

\* Within the same category, scores with the same letter are not statistically different. SD= standard deviation

## DISCUSSION

With the methodology used in this study it is possible to evaluate the morphological aspect of the conditioned root surface. The micrographs taken at 1,500 and 3,500x has excellent definition and quality for observation of the root surface. This methodology has been successfully used for this kind of evaluation<sup>12,17,18,24</sup>.

The SEM micrographs should represent the evaluated sample, but it is not possible to take a micrograph of the entire sample. To solve this difficulty, the visualization was directed to the center of the sample and all the micrographs were taken from the center of the sample.

Larger samples take longer to be sputter-coated and are difficult to be obtained. The samples used in this study were about 2x3 mm. These dimensions were obtained after sample reduction from the coronal root third and comprise approximately the measure between the cemento-enamel junction and the furcation entrance in molars.

Despite the limitations presented, the methodology in this study has been extensively used before and is accepted as a method to

evaluate root conditioning and blood cell adhesion with *in vitro* studies<sup>12,17,18,22,24</sup>.

The use of different concentrations, application modes and times was efficient in determine and standardize the parameters of application of a chemical substances and allows the use of the obtained parameters in other studies such as studies to evaluate blood cell adhesion and periodontal ligament cell attachment to the root surface<sup>2,18</sup>.

The rationale for this study is that the evident contamination of the cementum and dentin with bacterial toxins could impair periodontal healing<sup>1</sup>. These products may be responsible for a marked progressive and irreversible destruction of the periodontal structures of support. Scaling and root planning can be accomplished by manual instruments, rotary instruments, sonic and ultrasonic instruments. Such methods often do not appear to be totally effective in removing mineralized debris of the root surface. Furthermore, root instrumentation causes grooves and results in a root surface covered with smear layer, which contains remnants of dental calculus, contaminated cementum, bacterial endotoxin and subgingival plaque<sup>5,7,19</sup>.

Over the past decade a number of alternatives have been presented as compensation for the limitations inherent to the mechanical therapy. *In vitro*<sup>11</sup> and *in vivo* studies<sup>9,10</sup> have emphasized the conditioning of the root surface using different methods as an adjuvant treatment to scaling and root planning in regenerative procedures.

Several chemical agents have been proposed including citric acid<sup>10,23,27-29</sup>, EDTA<sup>4-8,17,23,24</sup>, tetracycline hydrochloride<sup>12,13,15</sup> among others. An important factor that should be considered and is often neglected is the mode of application, time, composition and concentration of the conditioning agent in addition to the pH of the substance used. Root surfaces exposed to biofilm are hypermineralized<sup>20,25</sup> and therefore more difficult to be both mechanically and chemically decontaminated, and thus, low pH substances were proposed to aid smear layer removal.

With respect to pH, it was possible to demonstrate that citric acid which possesses a low pH, causes cell death when in contact with periodontal ligament (PDL) cells<sup>16</sup> and thus could delay cell proliferation and repair of the area. Because of this necrotizing effect, the use of 24% EDTA at neutral pH was proposed as an alternative for low pH substances<sup>6</sup>. This way, studies were carried out to compare different formulations, concentrations, modes and length/duration of the application. Most studies have shown that EDTA is a substance that provides smear layer removal and exposure of collagen fibers<sup>4,5,7,8,24</sup>. However, researches on clot adherence to root surface bioengineered with EDTA obtained worse results when compared to citric acid and tetracycline hydrochloride<sup>18</sup>. This may be because EDTA is an anticoagulant or even because it removes the calcium ions from the surface of root dentin that are important in blood clotting cascade<sup>18</sup>.

Considering these results, a new substance was proposed in the present study - sodium citrate - which has the same principle of EDTA, but is more biocompatible and is also currently used in blood collection bags. It was also evaluated the citric acid different concentrations in order to ascertain the optimal parameters for its use. Actually, the problem of this substance is its low pH and its high power to demineralization. This way, in the present study lower concentrations with consequently higher pH were evaluated.

This high demineralization power can be observed after analysis of the SEM micrographs, in which we found that almost all groups of citric acid concentrations of 15, 20, 25 and 30% can cause chemical dissolution of tooth surface (score 5) with collagen destruction. These results disagrees with the majority of studies published<sup>10,15,27,28</sup>, not being compatible with the principle of root conditioning,

which requires the exposure of dentin collagen fibers for adhesion of the clot on the tooth surface. Thus, these results suggest that the concentration should be decreased so that the smear layer could be effectively removed but without destroying the collagen fibers network. This way, the citric acid concentration was reduced to 0.5, 1.0 and 2.0% to try to solve the problem, but no difference was observed. Citric acid even at lower concentrations produced excessive demineralization not having a standard of performance (Table 1).

Despite being more biocompatible, sodium citrate was not able to expose collagen fibers of the root surface (Table 1) even in the group with best results, which was vigorous application for 3 min (Graphs 5 and 6).

## CONCLUSION

Within the limitations of the methodology, it can be concluded that, despite the lack of statistical significance, the best results for collagen exposure using citric acid were obtained with brushing application at 25% for 3 min. Sodium citrate was not able to adequately remove smear layer an expose collagen fibers, so it is not indicated for root conditioning.

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