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The evaluation of root surface modifications after different subgingival mechanical instrumentation techniques – an *in vitro* study

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ABSTRACT

Objectives. The aim of this *in vitro* study was to observe through scanning electron microscopy (SEM) the surface morphology of root samples treated with different mechanical instrumentation methods and the additional application of a nanocolloidal silver-based antiseptic solution.
Material and methods. Root samples were prepared from extracted molars and divided in four groups: group 1 of samples was instrumented with Gracey curette (7/8), group 2 of samples was instrumented with ultrasonic tip 1S, group 3 of samples was instrumented with ultrasonic tips 1S plus H4R/L, group 4 of samples was treated with ultrasonic tips 1S plus H4R/L and application of the silver-based antiseptic solution. All samples were then subjected to SEM examination.
Outcomes. Group 1 of samples presented a smoother surface compared to the samples in the other groups. Group 2, 3 and 4 displayed superficial grooves parallel to the direction of action of the ultrasonic tip. In group 3 and 4, root planning with tip H4R/L did not ameliorate the smoothness of the surface. The smear layer was present, regardless the instrumentation method. In group 4, the additional application of the antiseptic solution did not influence the surface morphology or the amount of smear layer.
Conclusions. Gracey curettes created a smoother radicular surface compared to ultrasonic tips. Smear layer was obvious on the radicular surfaces, no matter the instrumentation method. The application of the silver-based antiseptic solution had no additional impact on surface morphology.

Keywords: subgingival mechanical instrumentation, root surface, antiseptic, silver nanoparticles, scanning electron microscopy

INTRODUCTION

The subgingival mechanical instrumentation is an efficient method to remove the calculus and bacterial biofilm from the root surfaces and the subgingival regions of periodontally affected teeth, without surgical reflection of the soft tissues around the teeth [1]. However, numerous publications indicate that none of the mechanical instrumentation techniques can eliminate completely the deposits [2]. By comparing various methods used in nonsurgical periodontal therapy, no major clinical differences were found between these approaches [3].

Following the reduction of the subgingival bacterial load, the host tissues can better manage residual microorganisms, leading to improvements in clinical parameters such as gingival bleeding, periodontal pocket depth and clinical attachment level,

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changes dependent on several factors: baseline probing depth, tooth type (mono- or pluriradicular) and behavioral factors (oral hygiene, smoking) [4,5].

The insufficient cleaning of subgingival areas difficult to access (deep pockets, furcation lesions), even after a thorough subgingival mechanical instrumentation, supports the use of adjunctive local therapies. Recent therapeutic guidelines state that local antiseptic products with prolonged release (i.e. chlorhexidine-based – *PerioChip*®) can be considered as adjuvants in non-surgical periodontal therapy, but no significant improvements have been observed following the use of subgingival irrigation solutions [1].

The root surfaces treated by subgingival mechanical instrumentation were also subjected to microscopic analysis. Scanning electron microscopy (SEM) has highlighted variable degrees of damage of the root surfaces following instrumentation, dependent on the nature of the instruments, the applied force, and the number of strokes over the root surface [5]. Surface alterations were described in terms of roughness (irregularities, the arrangement and depth of grooves created in the root surface), the amount of tooth structure removed, or the presence of the smear layer [6]. The clinical aim of the subgingival instrumentation is the obtention of a smooth and clean root surface, which is less prone to bacterial colonization, delays the formation of new biofilm and supports the healing of periodontal tissues [7,8].

The present *in vitro* study aims to evaluate through SEM the modifications of root surfaces of extracted teeth treated with manual and mechanical instrumentation and the adjuvant application of a less-studied nanocolloidal silver-based local antiseptic product.

MATERIAL AND METHODS

Study design

The study protocol was approved by the Ethics Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca (No. 472/19.12.2018). Before tooth collection and after receiving details of the study, the patients signed an informed consent.

Molars extracted for reasons unrelated to this study were used. After removing the soft tissues, the teeth were preserved in chloramine T 4% and used within a month from extraction as current protocols recommend [9].

The molars were treated with four treatment approaches. Then root samples were made. They were processed and examined by SEM describing the qualitative changes.

Preparation of the root samples

Root instrumentation was performed using three mechanical instrumentation techniques, on 10 molars: a) Gracey 7/8 curette (Hu-Friedy, Chicago, IL, USA), b) tip 1S (Satelec Acteon, Acteon Group, Mount Laurel, NJ, USA), and c) tip 1S plus tip H4R/L (Satelec Acteon, Acteon Group) used in the green zone. The Acteon Satelec P5 Booster ultrasonic scaler (Acteon Group) was used for scaling. Some roots instrumented with the third technique were also treated with a commercial nanocolloidal silver-based local antiseptic solution *Perioflush*® (Dental Life Sciences, Niemce, Poland).

The root samples were prepared with high-speed handpiece flame red-ring diamond burs (no. 8852.314.012, Komet Dental, Lemgo, Germany), under continuous cooling. External root areas of 2 mm thickness were obtained and used to generate the experimental samples with uniform dimensions (5x5x2 mm).

Four root sample groups were obtained depending on the treatment approach applied to molars' roots: group 1 (9 samples) – root samples instrumented by technique 1 (Gracey curette); group 2 (9 samples) – root samples instrumented by technique 2 (ultrasonic tip 1S); group 3 (9 samples) – root samples instrumented by technique 3 (ultrasonic tips 1S and H4R/L); group 4 (9 samples) – root samples instrumented by technique 3 and treated with a silver-based local antiseptic solution.

All treatments were performed by a single operator (IS) in the presence of a senior periodontist.

Scanning electron microscopy analysis

The root samples were thoroughly rinsed with water spray and dried with air spray, then left to dry at room temperature for 30 minutes. Samples were fixed with conductive carbon double-adhesive discs and coated with a 7 nm layer of platinum/palladium using an argon evaporator from Agar Scientific (Essex, UK). Images were obtained with a Jeol JSM 5510LV scanning electron microscope (JEOL, Tokyo, Japan), and the cell surface morphology was descriptively analyzed.

RESULTS

This *in vitro* study evaluated, through SEM analysis, the changes induced by root instrumentation alone or associated with the application of an antiseptic preparation, at the level of four groups of root samples obtained from extracted teeth.

SEM examination revealed, in general, the presence of an abundant smear layer at the level of all samples, regardless the root instrumentation approaches. Even in the absence of obvious linear traces, all surfaces had a generally rough appearance provided by the presence of randomly distrib-

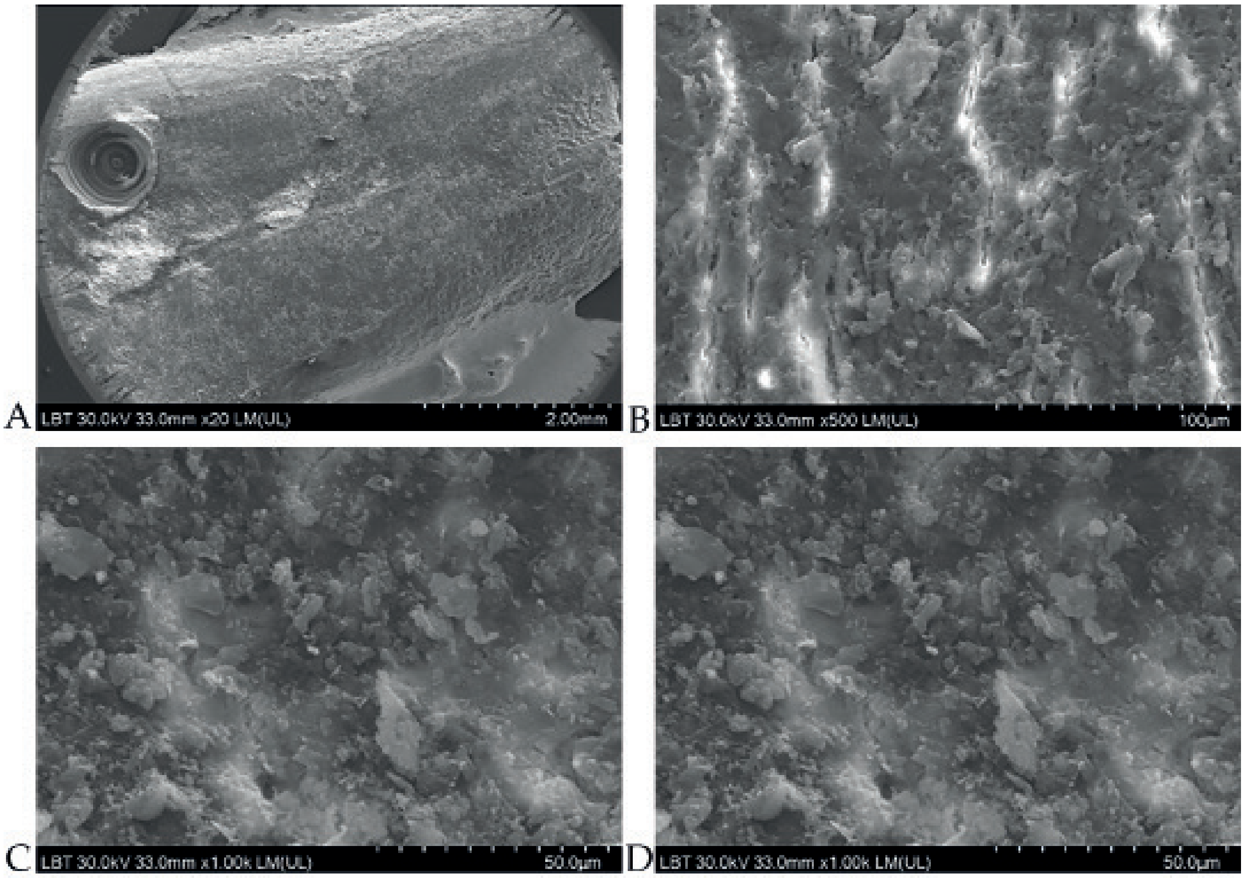

FIGURE 1. The microscopic aspect of the root surfaces after mechanical instrumentation with Gracey 7/8 curette, at different magnification: A. x20; B. x500; C. x1000; D. x1000

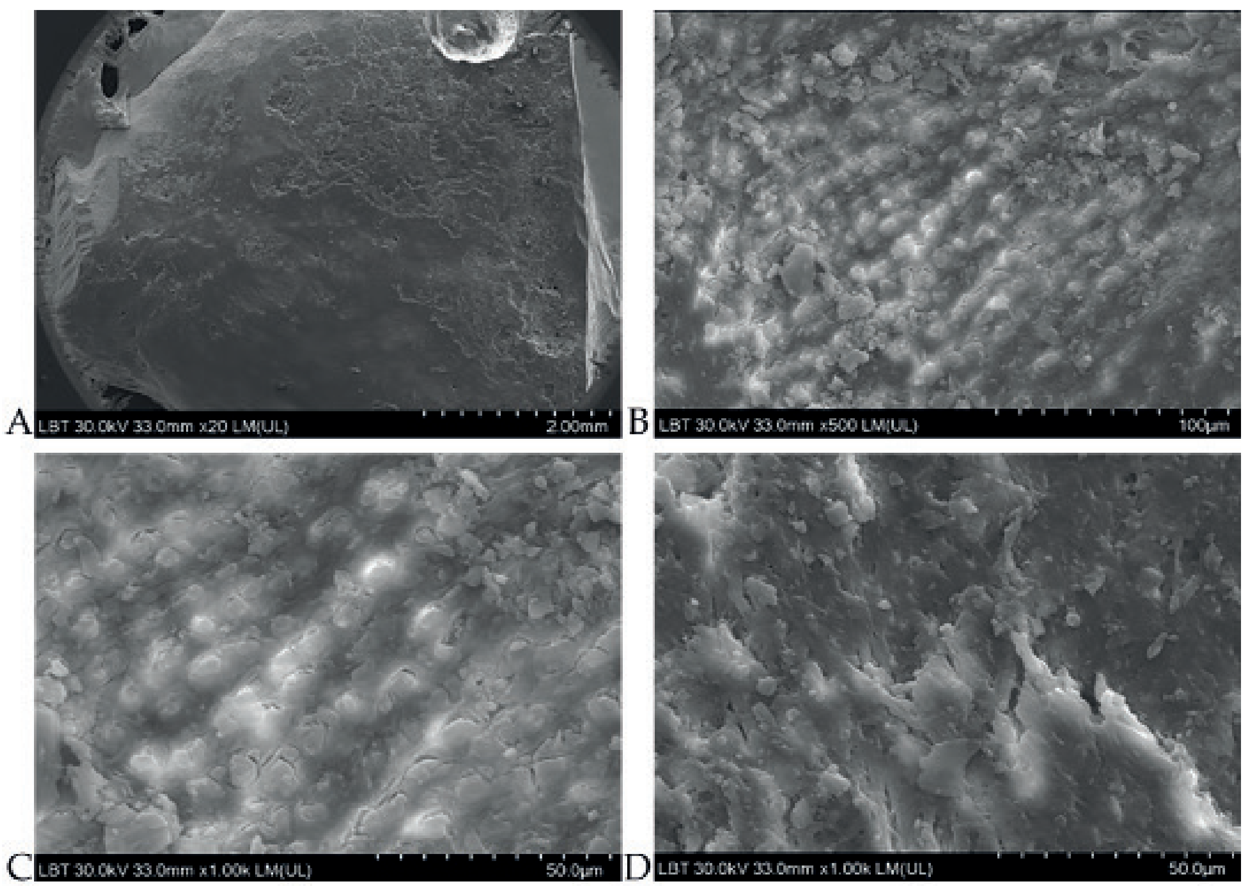

FIGURE 2. The microscopic aspect of the root surfaces after mechanical instrumentation with ultrasonic 1S tip, at different magnification: A. x20; B. x500; C. x1000; D. x1000

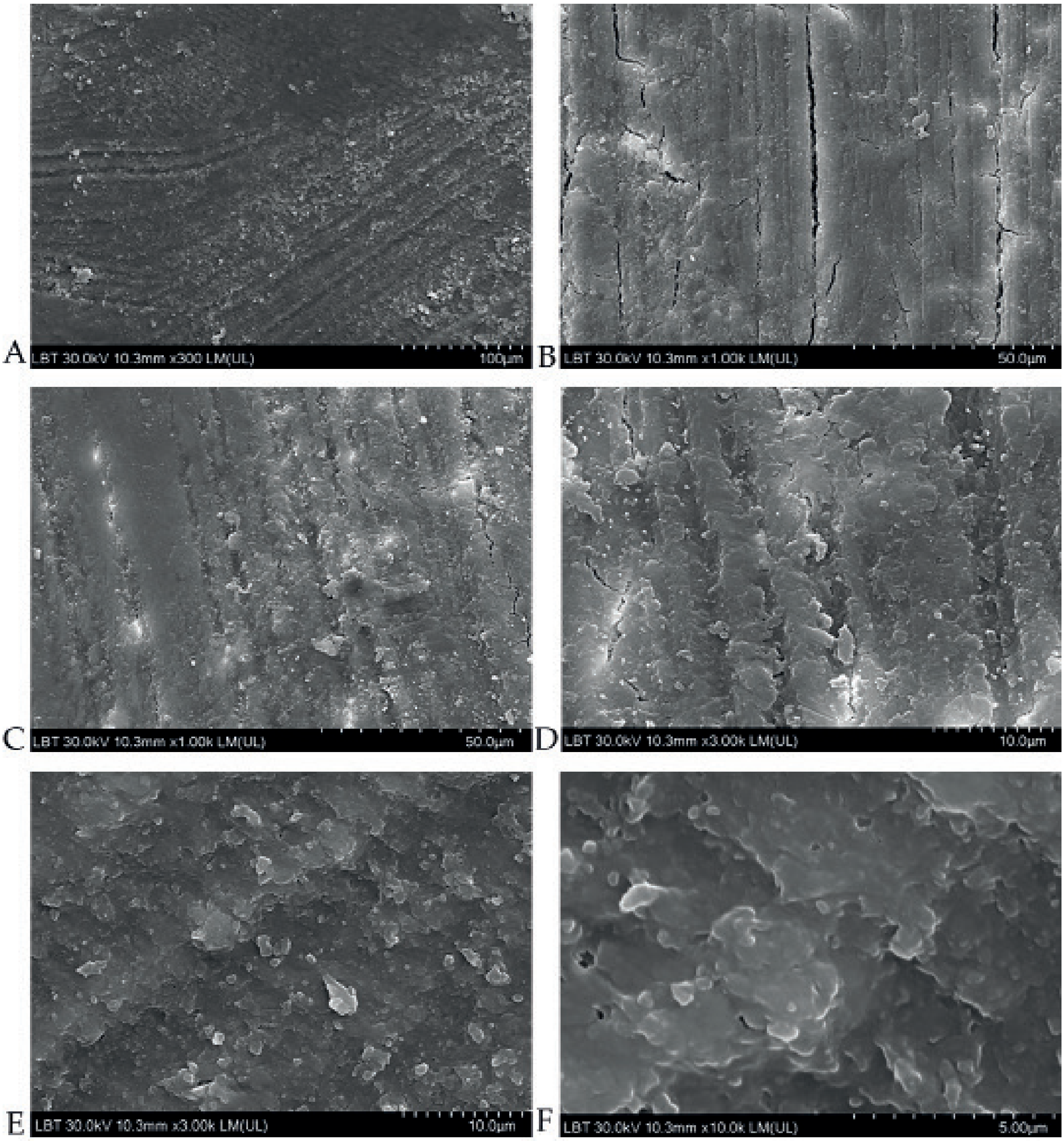

FIGURE 3. The microscopic aspect of the root surfaces after double ultrasonic mechanical instrumentation (1S tip and H4R/L tip), at different magnification: x300; B. x1000; C. x1000; D. x3000; E. x3000; F. x10000

uted detritus. Detritus deposits and surface roughness were even more evident at high magnifications of microscopic observations.

In group 1 of samples (Figures 1A, B), the appearance of the surface randomly covered with smear layer was observed. At higher magnifications (Figures 1B, C), most of the samples in this category were covered by a more obvious and randomly arranged smear layer deposits.

In group 2 of samples, at lower magnifications, the root surfaces displayed a relatively uniform distribution of the smear layer (Figure 2A). Higher magnifications (Figures 2B-D) highlighted relatively shallow grooves traced by the tip 1S, with a linear disposition, covered by abundant smear layer. The linear traced aspect of the surface is evident.

In group 3 of samples, the lower and higher magnifications (Figures 3A, B) showed alteration of the root surfaces, with a linear aspect, with obvious traces that followed the direction of action of the ultrasonic tips 1S and H4R/L. The smear layer was evident for these samples as well (Figures 3C, D), its arrangement following the linear disposition of the grooves resulting from instrumentation.

In group 4 of samples, the alterations of the root surfaces were maintained even after Perioflush®

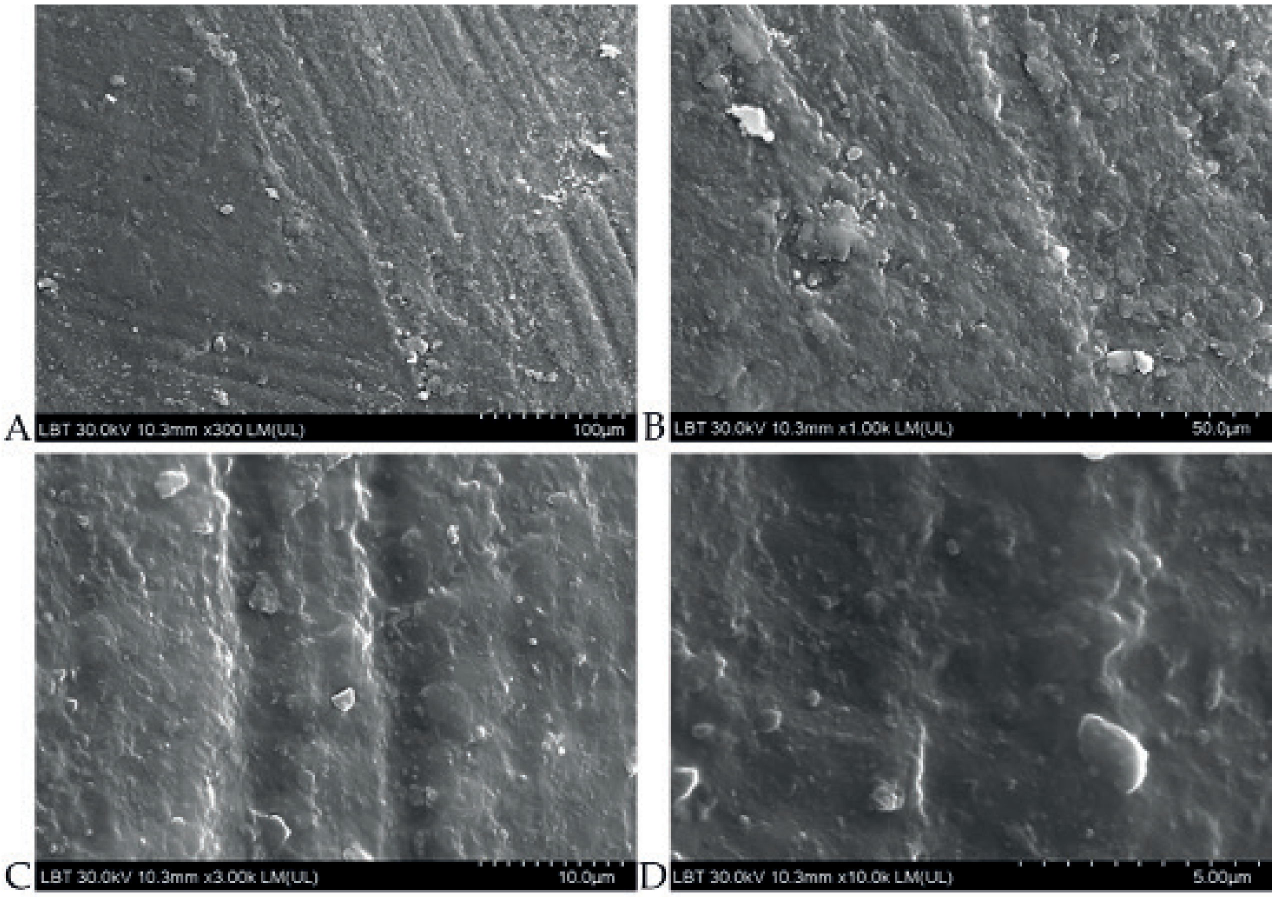

FIGURE 4. The microscopic aspect of the root surfaces after double ultrasonic mechanical instrumentation (1S tip and H4R/L tip) and *Perioflush®* applications, at different magnification: A. x300; B. x1000; C. x3000; D. x10000

application on the instrumented root surfaces with tip 1S plus tip H4R/L (Figures 4A, B). Applications of the antiseptic product did not reduce the smear layer deposits. The smear layer followed the striped pattern of the surface (Figure 4 C).

DISCUSSION

Contemporary nonsurgical periodontal therapy is centered on the control of calculus and bacterial biofilm to achieve a favorable tissue response. However, in daily clinical practice it is a challenge to separate mechanical instrumentation in three distinct stages: debridement, scaling and root planning. Current protocols recommend tools and techniques that correspond to scaling as well as to surface planning. A smooth root surface provides the premise for a clean surface, associated with a reduced bacterial load, resolution of local inflammation and the restauration of the periodontal health [5]. Furthermore, a smooth root surface delays bacterial recolonization and biofilm formation, thus preventing the recurrence of inflammation and reactivation of local destructive processes [5,10]. Hence, it is essential to select the instruments and technique to minimize the morphological alterations of the root surface and to prevent the excessive removal of the cementum [6].

In the present study, SEM examinations of the treated root surfaces revealed no notable differences between the study groups. The analyses of groups 2, 3 and 4 of root surface samples displayed traced surfaces with the disposition of the grooves parallel to the direction of action of the ultrasound tips mostly observable at higher magnifications (x1000), with terminal areas with a more irregular appearance. Samples from group 1 presented far fewer distinct traces left by Gracey curettes, with most samples revealing only the presence of an abundant smear layer. These findings align with the results of other studies, which concluded that instrumentation with Gracey curettes leaves behind the smoothest surface, unlike the roughness created by the passage of ultrasonic instruments [5,6,10,11].

The amount of smear layer is also important in the periodontal healing process: the smear layer prevents the adhesion of the fibroblasts to the cementum or dentin surface, thus having a negative impact on local healing [10]. In the samples examined by the present study, at the lowest magnification (x20), no notable differences between instrumented root surfaces were observed. As the magnification increased (x500-1000), the smear layer became more evident. Other studies also highlighted through SEM the abundance of the smear layer resulting from manual or ultrasonic instrumentation of the root surfaces, without statistically significant differences between the treatments [12,13].

The indication of adjuvant agents in the non-surgical treatment of periodontitis is especially valuable in hard-to-reach areas. The additional smear layer-removing effect of these products would be beneficial. According to the observations of the present study, the adjunctive application of *Perioflush*® solution did not affect the morphology of the root surfaces or the amount of smear layer, the samples treated with the antiseptic preparation being similar to the root surfaces resulting from simple ultrasound instrumentation. Other in vitro studies investigated the effect of various solutions on the smear layer. A single application of a chemical agent (*Carisolv*®) on root surfaces after mechanical instrumentation, did not result in root surface changes, and the smear layer was not removed [14]. Multiple applications of the product, however, induced a significant reduction of the thickness of the smear layer [14]. By comparing the effectiveness of three chemical decalcifying agents (*SofScale*®, *Carisolv gel*®, *QMix*®) on the removal of the smear layer resulted after mechanical instrumentation, statistically significant differences were obtained in favor of *QMix*® [15]. To our knowledge, the present in vitro study is the first to investigate the effect of *Perioflush*® on the mechanically instrumented root surfaces by ultrasonic scaling and planning action. Other authors reported the presence of significant roughness and an abundant smear layer after manual scaling and Perioflush® applications [16].

Conflict of interest: none declared
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The present study is based only on SEM observation of root surfaces treated by different instrumentation methods, with or without the application of Perioflush®, an analysis that is difficult to quantify. Additional studies are planned to quantify the effect of different regimens of Perioflush® applications on the root morphology and the smear layer.

CONCLUSIONS

According to our observations, manual instrumentation with Gracey curette created a smoother radicular surface compared to the ultrasonic instrumentation. At higher magnifications, SEM analysis highlighted the presence of linear superficial grooves covered in smear layer on the surface of the root samples treated with ultrasonic instruments. Root planning with H4R/L ultrasonic tip did not improve the surface morphology. No matter the treatment approach, the smear layer was evident on the surface of all examined samples. The additional application of *Perioflush*® did not influence the surface morphology or the quantity of smear layer.

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