Journal of

PERIODONTAL RESEARCH

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JOURNAL OF PERIODONTAL RESEARCH doi:10.1111/j.1600-0765.2012.01473.x

Evaluation of root surface microtopography following the use of four instrumentation systems by confocal microscopy and scanning electron microscopy: an *in vitro* study

Solis Moreno C, Santos A, Nart J, Levi P, Velásquez A, Sanz Moliner J. Evaluation of root surface microtopography following the use of four instrumentation systems by confocal microscopy and scanning electron microscopy: an in vitro study. J Periodont Res 2012; 47: 608–615. © 2012 John Wiley & Sons A/S

Background and Objective: The ideal instrument for initial periodontal therapy should enable the removal of all extraneous substances from the root surfaces without any iatrogenic effects. Because of that the objective of this study is to analyse and to compare the root surface roughness after using Gracey curettes, termination diamond burs (40 μ m), a piezo-ceramic ultrasonic scaler and a piezosurgery ultrasonic scaler using confocal microscopy and scanning electron microscopy.

Material and Methods: A 2 mm \times 2 mm interproximal root area of 20 teeth (n = 40 surfaces) was evaluated by confocal microscopy (\times 20 magnification) and scanning electron microscopy (\times 50 to \times 1000 magnification). Teeth were randomly assigned to the following four groups: Gracey curettes with 15 vertical strokes; termination diamond burs (40 µm) at 3000 r.p.m.; a piezo-ceramic ultrasonic scaler with a power of 11; and a piezosurgery ultrasonic scaler in mode ROOT with a power of two.

Results: Confocal microscopy revealed that curettes [mean changes in the value of surface roughness average reduced by 0.11 ± 0.3], piezo-ceramic ultrasonic scaler (roughness average reduced by 0.47 ± 0.93) and piezosurgery ultrasonic scaler (roughness average reduced by 0.62 ± 0.93) left a smoother surface than termination diamond burs (roughness average increased by 0.39 ± 0.18). Statistically significant differences were observed in roughness (p = 0.005) between piezosurgery and termination diamond burs (p = 0.005). No statistically significant differences were between piezosurgery and Gracey curettes (p = 0.140) and between piezosurgery and piezo-ceramic ultrasonic scalers (p = 0.745). Confocal microscopy and scanning electron microscopy showed that piezosurgery seems to leave the smoothest surface. Surfaces treated with termination burs appear to show more scratches and pits.

Conclusion: Three of the four instruments tested for root planing reduced surface roughness; however, the piezosurgery ultrasonic scaler produced the smoothest surface. The termination diamond burs (40 μ m) produced a rougher surface than the ultrasonic instruments and the hand curettes. Further clinical studies are needed.

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Key words: scaling and root planing; root roughness; curette; piezo-ultrasonic scaler; termination diamond bur

Accepted for publication February 4, 2012

The main goal of periodontal therapy is to eliminate infection and achieve health by the mechanical removal of bacterial deposits of plaque, calculus and their supragingival and subgingival endotoxins (1-3). It is important for the clinician to achieve an uncontaminated and smooth tooth surface to permit optimal oral hygiene control by patients. Instruction in oral hygiene technique, together with mechanical scaling and root planing, is the initial therapy carried out by the clinician to achieve this goal. Scaling and root planing can be performed with a variety of instruments.

Bacteria and endotoxins can penetrate both cementum and dentin, although there is no consensus regarding the depth of penetration. In vitro studies (4,5) have shown that gingival fibroblasts do not adhere to tooth surfaces contaminated by bacteria. Therefore, the objective of scaling and root planing is to provide a biologically acceptable surface for periodontal healing; however, it the amount of hard tissue that needs to be removed is contested (6.7). Nyman et al. (8) specified that their study did not answer the question of whether endotoxins are in fact present within or on the surface of the exposed cementum. The reason for this may be that endotoxins adhering to the surface are removed together with the bacteria by polishing or possibly that endotoxins within the cementum are neutralized by the inflammatory response of the host organism (8). Coldiron et al. (9) noted that the depth of root surface removal necessary to reach a healthy, disease-free area is unknown. The most recent recommendation is to remove as little tooth structure as possible in achieving a clean, smooth surface (10).

The roughness of the residual root surface, as the result of instrumentation, is another important consideration in periodontal therapy (4,11,12). Although root roughness *in vivo* has been shown to have a minimal effect on healing of the periodontal attachment apparatus, it may facilitate further bacterial accumulation and subsequent deposition of calculus (13); therefore, a smoothest root surface should be one goal of a successful scaling and root planing treatment. Root instrumentation with manual curettes is technically more difficult than other techniques; it is time consuming and causes fatigue to the clinician (14). In addition to curettes, however, there are other instruments for the mechanical preparation of the root surface, such as sonic, ultrasonic and rotary instruments (10).

Studies by Breininger *et al.* (15), Copulos *et al.* (16) and Drisko (17) have shown that ultrasonic instruments are superior to hand curettes. These studies concluded that ultrasonic instruments provided a surface biocompatibility, and they are more effective in removing endotoxin from periodontally affected root surfaces.

However, Santos et al. (18) investigated 35 single-rooted teeth that were assigned to four experimental groups: group 1, piezoelectric ultrasonic device; group 2, magnetostrictive ultrasonic device; group 3, hand instrumentation; and group 4, untreated teeth (control). After instrumentation, the teeth were extracted and the presence of residual deposits (roughness and characteristics of root surfaces) analysed. They concluded that curettes produced deep radicular sulci and removed more root surface material than the ultrasonic devices. The ultrasonic devices produced a smooth root surface.

A scanning electron microscopy study (19) showed that scaling and root planing with conventional hand curettes and termination diamond burs (Intensiv Perio Set[®], Grancia, Switzerland) resulted in a biologically acceptable root surface, which was free of bacterial contamination and endotoxin. This study acknowledged that Intensiv Perio Set[®] is an excellent supplement to curettes in root debridement. Other than this study, there are few published data concerning the use of termination diamond burs for scaling and root planing.

Regarding the use of piezoelectric ultrasonic scalers, there are only a few studies (20–24) that have analysed their use in nonsurgical periodontal therapy. The piezoelectric device appears to produce better results in terms of roughness and less damage to the root surface than the conventional magnetostrictive ultrasonic scaler.

Knowing that the ideal instrument should enable the removal of all extraneous substances from the root surfaces without any iatrogenic effects, the present study aimed to evaluate root surface topography after *in vitro* scaling and root planing with different instruments and to provide new and relevant data for its subsequent application at the clinical level.

Material and methods

A total of 20 extracted human teeth with 40 interproximal root surfaces, mesial and distal, were included in the study. Multiradicular teeth, teeth with root surface caries or external



Fig. 1. Surface roughness average value (A) Sample prepared in a block of silicone (A and B sides for each tooth) to be evaluated with confocal microscopy. (B) Sample prepared for observation with the Scanning Electron Microscope.



Fig. 2. Calculation of th surface roughness average value. For explanation, see main text.

resorption and teeth with restorations on the root surface were not included.

The study was conducted at the Research Laboratory of Universitat Internacional de Catalunya, Barcelona, Spain and at The Scientific-Technical Services UB (SCT-UB) of the Universitat de Barcelona, Barcelona, Spain.

Study design

This is a comparative, *in vitro*, blind study comparing Gracey curettes (Hu-Friedy[®], Chicago, IL, USA), termination diamond burs (40 µm; Intensiv Perio Set[®], Grancia, Switzerland), a piezo-ceramic ultrasonic scaler (Suprasson[®] P-5 Booster, Satelec, Barcelona, Spain) and a piezosurgery ultrasonic scaler (Mectron[®], Carasco, GE, Italy).

All teeth were free of calculus and were conserved in sodium chloride isotonic (0.9%) solutions (B. Braun Medical SA, Rubí, Barcelona, Spain). During the study, the solution was changed every 5 d. The teeth were numbered 1–20 for identification and were catalogued.

The 40 interproximal root surfaces were randomly assigned and divided into four groups of 10 surfaces each. In order to be consistent and precise, an area of 2 mm \times 2 mm was drawn on the coronal third of each root surface, and a mark was made in the upper right corner of the box (area 2 mm \times 2 mm) with a thin cylindrical bur (Komet[®], Lemgo, Germany) to produce a defined reference point when using the light microscope for analysis. This mark defined the control and test areas.

- In group 1, Gracey curettes were used to make 15 vertical strokes with movements from the most apical point to the most coronal root surface point (25).
- In group 2, termination diamonds burs (40 μm) were used with irrigation for 15 s at 3000 r.p.m. Movements were made parallel to the axis of the tooth.
- In group 3, the piezo-ceramic ultrasonic scaler, with a universal insert, was applied at a medium power of 11 and with irrigation for 15 s (15). Movements were parallel to the tooth axis and the working strokes were perpendicular to the tooth axis.

In group 4, the piezosurgery ultrasonic scaler set on function On/Mode Periodontics (ROOT), with the insert PS1, was applied at a medium power of two for 15 s using the same movements as with the piezo-ceramic ultrasonic scaler.

One operator (C.S.M.) performed all scaling and root planing procedures. A second blinded operator (A.V.) evaluated the samples with confocal microscopy (Leica Microsystems, Barcelona, Spain) and also scanning electron microscopy (SEM Stereoscan S-360, Leica Microsystems) at the Scientific-Technical Services UB (SCT-UB) of Universitat de Barcelona.

Confocal microscopy

This technique was used because it is a nondestructive method that is highly accurate at an extremely high speed of acquisition. Vertical resolution of the lens used is 15 nm and lateral resolution is 280 nm. Heights in these samples were at least 10 times higher, making this method suitable for the study. The equipment was calibrated in accordance with the manufacturer's specifications (Leica Microsystems).

A control measurement was made using confocal microscopy before scaling and root planing each root surface. After the treatment, a test measurement was made at the same point as the control measurement on the coronal third of each root.

For evaluation with confocal microscopy, the samples were dried and placed horizontally in a block of silicone. The silicone molds were originally designed for all interproximal root surfaces (A and B proximal sides, previously assigned to each group) of each tooth selected in the study groups (modification of the protocol used by Busslinger et al.; 23; Fig. 1). Then each surface was observed at a magnification of ×20. In order to distinguish surface A from surface B, a mark was made with a round bur (Komet[®], Lemgo, Germany) on the crown of all B surfaces.

Confocal microscopy enables the reconstruction of three-dimensional structures from the images obtained and offers quantitative roughness values. Five profiles for each sample were randomly assigned in order to obtain a mean roughness average.

The roughness average value was selected from these quantitative roughness values because it is the specific arithmetical mean roughness (26). Roughness average (shown as Ra in Fig. 2) is obtained from an arithmetical formula (in micrometers), when the roughness curve is expressed by y = f(x), taking the *x*-axis as the mean line direction and the *y*-axis as the vertical magnification of the roughness curve in the range of sampled reference lengths 'l' (Fig. 2).

Scanning electron microscopy

As the scaling and root planing were performed only on the coronal third of each interproximal root surface, to evaluate the sample for the scanning electron microscope, a mark with a cutting dental disc (Komet[®]) was made on the middle of the root to delineate two areas; the inferior part corresponded to the control (untreated tooth surface) and the superior part to the test (treated tooth surface with scaling and root planing).

The control and test measurements for the scanning electron microscopy were made after completing all the examinations with confocal microscopy, because different sample preparations are needed for each of the microscopes (Fig. 1).

The specimens were first dried completely and gold sputtered. After that, the surfaces were examined at magnifications ranging from $\times 50$ to $\times 1000$.

Statistical analysis

The means and standard deviations were calculated, and statistical analysis between means was performed with factorial analysis of variance. The statistical analysis was done with an available statistics computer program (SOFA statistics software version 1.0.2, Paton-Simpson & Associates Ltd, Auckland, New Zealand) on a Macintosh computer. The level of significance was determined at 1%.

The primary outcome variable was surface roughness average. This vari-



Fig. 3. Mean surface roughness average. Mean surface roughness average (in micrometers) immediately before and after scaling and root planing using Gracey curettes (group 1), termination diamond burs (40 μ m; group 2), a piezo-ceramic ultrasonic scaler (group 3) and a piezosurgery ultrasonic scaler (group 4).

able was tested between the four instruments for scaling and root planing. The change in surface roughness for each instrument after treatment was also considered.

Note that the statistical analysis was performed with the surface roughness value obtained through the confocal microscopy. The reductions of roughness average values amongst the groups and within the groups were tested before and after instrumentation.

Results

There were 20 teeth included for evaluation, providing 40 root surfaces for the analysis.

Confocal microscopy

The initial mean \pm SD roughness average values for groups 1, 2, 3 and 4 were 0.47 \pm 0.31µm, 0.41 \pm 0.16µm, 1.12 \pm 0.93µm and 1.11 \pm 1 µm, respectively. After scaling and root planing with the different test instruments, the mean \pm SD roughness average values were reduced only by three of the four instruments, as follows: to 0.35 \pm 0.12 in group 1; to 0.65 \pm 0.34 in group 3; and to 0.49 \pm 0.15 in group 4 (Fig. 3).

The results revealed that group 2, termination diamond burs (40 μ m), with a mean \pm SD roughness value of

 0.8 ± 0.17 , was the only instrument that created a rougher surface after scaling and root planing than before (Fig. 3).

The mean changes in the value of surface roughness average were as follows: Gracey curettes reduced by 0.11; termination diamond burs ($40 \mu m$) increased by 0.39; piezo-ceramic ultrasonic scaler reduced by 0.47;and piezosurgery ultrasonic scaler reduced by 0.62 (Table 1). The piezosurgery ultrasonic scaler created the smoothest surface.

There was no statistically significant reduction in roughness after treatment in group 1 (p = 0.019; Fig. 4). For groups 3 and 4, there was a statistically significant reduction in roughness. The reduction of roughness in group 4 was slightly more significant (p < 0.001) than in group 3 (p = 0.001; Fig. 4). For group 2, in contrast, there was no reduction of roughness after instrumentation, and in fact there was an increased of roughness average, with the results being highly statistically significant (p < 0.001; Fig. 4).

Regarding the mean final changes in the value of surface roughness average amongst the four groups, there were nonstatistically significant differences between Gracey curettes, the piezoceramic ultrasonic scaler and the piezosurgery ultrasonic scaler (Gracey curettes vs. piezo-ceramic ultrasonic scaler, p = 0.287; Gracev curettes vs. piezosurgery ultrasonic scaler, p =0.140; and piezo-ceramic ultrasonic scaler vs. piezosurgery ultrasonic scaler, p = 0.745). Use of the two different ultrasonic scalers produced similar degrees of roughness after treatment. In addition, there were nonstatistically significant differences for termination diamond burs (40 µm) compared with the piezo-ceramic ultrasonic scaler (p = 0.014) and with the piezosurgery ultrasonic scaler (p = 0.005). Termination diamond burs (40 µm) compared with Gracey curettes produced significantly different results (p < 0.001; Fig. 5).

Scanning electron microscopy

After instrumentation of all specimens, we observed that the surfaces of groups 1, 3 and 4 had smooth areas (Fig. 6), although all surfaces contained gouges. These results appear to correspond with the roughness average values obtained with confocal microscopy. There were, however, fewer gouges in the surfaces that showed a smaller roughness average value.

In group 2, we observed parallel grooves running in the direction of the instrumentation in all surfaces (Fig. 6).

In group 3, after instrumentation with the piezo-ceramic ultrasonic scaler, smooth root surfaces with small pits were obtained in the most of specimens (Fig. 6).

Scanning electron microscopy revealed that the piezosurgery ultrasonic scaler seems to leave a smoother surface compared with the other methods. Surfaces treated with termination diamond burs (40 µm) appeared to result in more scratches and pits than the other methods of instrumentation.

Discussion

This study was designed to compare the surface roughness before and after scaling and root planing with Gracey curettes, termination diamond burs ($40 \mu m$), a piezo-ceramic ultrasonic scaler and a piezosurgery ultrasonic scaler.

To evaluate the surface roughness we used scanning electron microscopy

Table 1. Mean changes in the value of surface roughness average.

Mean change in roughness average (µm)				
	Group 1	Group 2	Group 3	Group 4
Mean	-0.11	0.39	-0.47	-0.62
SD	0.3	0.18	0.93	0.93
Minimum	-0.9518	-0.0062	-2.6298	-3.3242
Maximum	0.1966	0.7312	0.4182	-0.0042
Range	1.1484	0.7374	3.048	3.32

Group 1, Gracey curettes; group 2, termination diamond burs ($40 \mu m$); group 3, piezoceramic ultrasonic scaler; and group 4, piezosurgery ultrasonic scaler. Note that a minus sign indicates that the roughness reduced in the group.

and confocal microscopy. With confocal microscopy we obtained a threedimensional view of the surface and a quantitative value of roughness that complemented the results obtained with scanning electron microscopy. In order to achieve the quantitative value of roughness, we applied the roughness average surface variable. There is no consensus about the optimal surface roughness or the importance of surface variables other than roughness average. Edblad *et al.* (27) found large variations in surface topographical factors. They evaluated the topographical characteristics in dental enamel and root cementum in the cervical region of healthy teeth and concluded that the natural variations in surface topography, within and between teeth, are considerable for root cementum and enamel, although the roughness (roughness average) of root cementum shows smaller variations than those of enamel.

Periodontal root planing procedures aimed at removing dental plaque and calculus from the root surface also, by design, leave a root surface with a degree of roughness, which is hopefully less than what it was prior to therapy (28). Some studies (13,29,30) concluded that a smooth root surface is not a critical factor for a successful



Fig. 4. Confocal microscopy results (orignal magnification \times 20). Three-dimensional reconstruction from confocal miscrocopy data, of a 2 mm \times 2 mm area of root surface treated with Gracey curettes (group 1), termination diamond burs (40 µm; group 2), a piezo-ceramic ultrasonic scaler (group 3) and a piezosurgery ultrasonic scaler (group 4) before (B) and after instrumentation (A). According to the color scale, note that the closer cool and warm colors turn green and shadows tend to disappear as the surface becomes smoother. Also appreciate the relief in all specimens, which decreased after treatment. In panel 2A, (termination diamond burs after treatment), note the persistent instrument marks.



Fig. 5. Mean values \pm SD of change of surface roughness average (in micrometers). Group 1, Gracey curettes; group 2, termination diamond burs (40 µm); group 3, piezo-ceramic ultrasonic scaler; and group 4, piezosurgery ultrasonic scaler.

treatment result. Interestingly, during conventional periodontal flap surgery, Oberholzer and Rateischack (30) root planed the teeth and then used coarse diamond stones to roughen the root surfaces, and they compared the healing with teeth where Gracey curettes were used to achieve a root surface as smooth as possible. Clinical healing was the same for both groups.

A smooth root surface may, however, be advantageous near the gingival margin, because a smooth surface is less likely to accumulate plaque than a rough surface, and plaque removal will be more efficient with a smooth rather than a rough surface. In animal studies by Leknes *et al.* (31,32) and also in the study of Quirynen *et al.* (33), the authors concluded that roughness resulting from subgingival instrumentation significantly influenced the subgingival microbial colonization.

In our study, we did not standardize the pressure on flattened root surfaces, because we were evaluating the effects of instrumentation on the layered structure of cementum and dentin root surface to simulate the situation *in vivo* when the human hand applies the instruments. This experimental protocol allows for comparisons between the instruments, because it is necessary to determine optimal working parameters (standardized speed and strokes, scaling and root planing by one operator) before clinical use.

In the present study, it seems that the piezoelectric instrument reduced the surface roughness more than the piezo-ceramic ultrasonic scaler, curettes and termination diamond burs (40 µm). Surprisingly, the termination diamond burs (40 µm) increased the roughness after treatment. To our knowledge, there is no previously published study comparing these four instruments by confocal microscopy and scanning electron microscopy, and no study that compares the termination diamond burs (40 µm) with other instruments for the scaling and root planing procedure.

It is noteworthy that, according to the manufacturer's specifications, the Perio Set contains 12 instruments with conical spear-shaped working parts, ISO sizes 012, 014 and 016, grain 75, 40 and 15 µm, with a short and a long neck. The coarse-grain instruments (75 µm) are used only for odontoplasty, to access or open bifurcations and root constraints; the average-grain instruments (40 µm) for scaling and root planing; and the fine-grain instruments (15 µm) for the final polishing of root surfaces. For these reasons, the termination diamond burs (40 µm) were selected for our study, because they are indicated for scaling and root planing.

There are few studies (20,21,23,24) that have analysed the use of piezoelectric devices in nonsurgical periodontal therapy. The piezoelectric device appears to produce better results in terms of minimal roughness and less damage to the root surface than the conventional ultrasonic scalers as showed by Cross-Poline *et al.* (20) and Flemmig *et al.* (21,22).

On the contrary, Busslinger et al. (23) concluded that the piezoelectric ultrasonic scaler was more efficient than the magnetostrictive ultrasonic device in removing calculus, but left the instrumented tooth surface rougher than with the magnetostrictive scaler and curettes. As with the study by Busslinger et al. (23), we applied a medium power, and only one operator performed all scaling and root planing. Our results were different from theirs. We found with examination by scanning electron microscopy and confocal microscopy that the piezosurgery ultrasonic scaler leaves the surface smoother than the Gracey curettes, piezo-ceramic ultrasonic device and the termination diamond burs.

Busslinger *et al* (23) noted that although a medium power setting was selected, there was no way of knowing whether the two electric devices delivered similar power at the same settings. Thus, the power of the piezoelectric device could have been higher than that of the magnetostrictive device, causing more root damage, which was interpreted in the higher roughness average value. It is noteworthy that



Fig. 6. Photomicrographs presenting scanning electron microscopy results (original magnification \times 500) of roots treated with Gracey curettes (group 1), termination diamond burs (40 µm; group 2), a piezo-ceramic ultrasonic scaler (group 3) and a piezosurgery ultrasonic scaler (group 4) before (B) and after instrumentation (A). Note that for termination diamond burs (2A) there are accentuated instrument marks with peaks and valleys. In panels 1A, 3A and 4A, note a few instrument marks.

Flemmig *et al.* (21,22) established a relationship between the power setting, the tip angulation and lateral force on defect volume.

Comparisons with other studies should be made with caution. Different methodologies could lead to divergent conclusions, because the type of evaluation (profilometer, laser Doppler or scanning electron microscopy evaluation) and the determination of the analysis area have been shown to affect the results directly (34,35).

Conclusions

Within the limits of this *in vitro* study, we can conclude that Gracey curettes,

the piezo-ceramic ultrasonic scaler and the piezosurgery ultrasonic scaler leave a smoother root surface after the scaling and root planing procedure than before therapy. The piezosurgery ultrasonic scaler created the smoothest surface, with a statistically significant difference in roughness after a nonsurgical periodontal treatment. The root surface roughness with termination diamond burs ($40 \ \mu m$) was higher than before treatment, which suggests that they should be used with caution for scaling and root planing. Further clinical studies are needed.

Acknowledgements

The authors are grateful to Curaden Swiss for providing the materials used in the study and for their attention and availability provided throughout the investigation. The authors are grateful to Intensiv SA, Grancia, Switzerland for their support of the study by providing their termination diamond burs; and to Incotrading SA, Madrid, Spain for providing the piezosurgery ultrasonic scaler (Mectron[®]) and the inserts for the study. Their attention and availability provided throughout the research were invaluable.

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