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## **Diamond burs versus curettes in root planing: A randomized clinical trial**

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**Running title:** Burs versus curettes in root planing

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## **Diamond burs versus curettes in root planing: A randomized clinical trial**

**Running title:** Burs versus curettes in root planing

### **Abstract**

**Aim:** This study compares diamond burs and curettes by clinical, microbiological, biochemical, scanning electron microscopic parameters and treatment time data in the non-surgical periodontal treatment of patients with chronic periodontitis.

**Methods:** Two quadrants of each of the 12 patients received root planing with diamond burs whereas other 2 quadrants were treated with curettes. Clinical periodontal measurements were recorded at baseline and then 1, 3, 6 months after completion of non-surgical periodontal treatment. Subgingival plaque, gingival crevicular fluid samples were obtained at baseline and 1-month control. Twenty-one hopeless teeth received root planing with diamond burs or curettes or no treatment at all and then extracted for microscopic evaluations.

**Results:** Clinical periodontal parameters improved similarly with both treatment modalities. Microbiological analyses revealed similar findings for the bacterial load (16S gene copy numbers), ratio of each bacterium to the total bacterial count at baseline, 1-month control. Cytokine levels in the gingival crevicular fluid samples exhibited differences between the two treatments. Scanning electron microscopic analyses indicated that diamond burs were better in terms of calculus removal, loss of tooth substance indices, but roughness index values were better for curettes.

**Conclusion:** As a conclusion, diamond burs provide findings comparable with curettes in root planing.

**MeSH Keywords:** chronic periodontitis; dental plaque; non-surgical periodontal debridement; root planing/instrumentation

## INTRODUCTION

Periodontal treatment aims to eliminate infection and provide tissue healing by removing deposits from diseased root surfaces and improving patient's plaque control. Reducing bacteria and disturbing the biofilm with mechanical debridement result in reduction of inflammation.<sup>1</sup> Uncontaminated, smooth and biologically acceptable root surfaces are important to prevent progression and/or recurrence.<sup>2</sup>

Periodontal tissue healing and reattachment cannot be achieved unless necrotic cementum and endotoxin are eliminated by periodontal treatment.<sup>3,4</sup> However, there is no consensus on the required extent of root planing (RP). Endotoxin binds superficially to the cementum.<sup>5</sup> A conservative approach aims to remove only plaque and preserve as much of the root cementum as possible.<sup>5,6</sup> In vivo root surface roughness has been shown to have a minimal effect on healing of periodontal attachment apparatus, but a smooth root surface may be biologically more acceptable as it may reduce future bacterial accumulation.<sup>7</sup>

Curettes are the most widely used instruments for RP and regarded as the gold standard,<sup>8</sup> but their usage is technic sensitive and time consuming for the clinician and efficacy reduces in deep pockets with root irregularities and furcations.<sup>9</sup> Alternative sonic, ultrasonic and rotary instruments have been investigated for potential benefits in improving mechanical access for scaling.<sup>2</sup>

Rotating diamond bur systems have been developed for odontoplasty and scaling and root planing (SRP). There is no clinical study in humans evaluating microbiological, biochemical, and scanning electron microscopic (SEM) findings of non-surgical periodontal therapy with diamond burs. Different techniques and instrumentation used for SRP may lead to differences in surface properties that may affect treatment outcome by creating retention loci for bacteria. Tactile perception by the clinician may not be precise enough to determine the smoothness of a root surface. Therefore, *in vitro* evaluation techniques are frequently used in studies comparing root surface topography following SRP with various instruments. It is hypothesized that, RP with diamond burs provide comparable findings with curettes in pockets with probing depth (PD)  $\geq 5$  mm in a shorter time. Therefore, the present study was performed to compare diamond burs and curettes in non-surgical periodontal treatment of chronic periodontitis patients.

## MATERIALS and METHODS

### Study Population

Fifteen patients were recruited for this single-centred, randomized, prospective, split-mouth, controlled clinical trial between October 2013 and August 2014. The study was approved by the Ethics Committee of Ege University, İzmir, Turkey (Protocol number; 13-9/11) and registered to US National Institutes of Health Clinical Trials Registry site with the ID number of NCT02875470. Written informed consent was received from each patient. Inclusion criteria were: clinical diagnosis of generalized chronic periodontitis;<sup>10</sup> presence of at least 3 teeth with PD  $\geq 5$  mm and clinical attachment level (CAL)  $\geq 4$  mm in each quadrant. Exclusion criteria were: presence of any known systemic disease or using medications that affect periodontal tissues; antibiotic treatment

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and/or periodontal treatment within the past 6 months; tobacco usage during the last 12 months; being pregnant or in the lactation period. Third molars, maxillary first premolars (due to their frequently having 2 roots and root concavities), crown/bridge abutment teeth and teeth with enamel projections or pearls, excessive destruction of crown were excluded.

### **Clinical Measurements**

Plaque index (PI),<sup>11</sup> papilla bleeding index (PBI),<sup>12</sup> PD, and CAL were measured with UNC 15 periodontal probe (Hu-Friedy, Chicago, IL) at 6 sites/tooth, at baseline and 1, 3, 6 months after completion of SRP. All clinical periodontal measurements were performed by a single calibrated researcher (FT) and the intraexaminer kappa scores were 0.96 for PD and 0.85 for CAL.

### **Gingival Crevicular Fluid (GCF) and Subgingival Plaque Sampling**

Samples were obtained from the deepest site in each quadrant at baseline and then 1-month after completion of SRP. Sampling sites were air-dried gently, isolated with cotton rolls and supragingival plaque was gently removed with sterile curettes. GCF samples were collected by inserting filter paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA) 1 mm into the pocket and leaving for 30 sec. Care was taken to avoid mechanical injury and strips visually contaminated with blood were discarded. The absorbed GCF volume was estimated by a calibrated instrument (Periotron 8000, Oraflow Inc., Plainview, NY, USA). Subgingival plaque samples were obtained from the same sites by sterile paper points (Diadent Group International, Korea) from the base of the pockets. Samples were placed separately in polypropylene tubes, frozen immediately and kept at -40°C until the laboratory analyses.

### **Non-surgical Periodontal Therapy**

Patients were motivated and instructed to brush with modified Bass technique and use interdental toothbrushes, dental floss. A strict protocol was followed and the RP was completed during a single session. In brief, RP was performed under local anaesthesia (2% lidocaine, epinephrine 1:100.000). Diamond burs (Intensiv Perio Set, Swiss Dental Products, Switzerland) were used in the test quadrants and curettes (Hu-Friedy, Chicago, IL) were used in the control quadrants. Randomization was performed with a computer-generated list. In the test quadrants, 15 µm and 40 µm burs with long neck were used at 6000 rpm with low pressure. Tapered burs were used particularly at the anterior teeth to reach narrow subgingival sites, whereas, flame burs were used at premolars, molars to reach the furcation sites and root concavities. A new bur set and a standard curette set newly sharpened with *Arkansas* stone (SS4, *Hu-Friedy*, Chicago, IL, USA) was used for each patient. A single researcher (FT) performed SRP in all patients. The procedures were continued until a hard, smooth root surface was sensed with an explorer tip. The time spent for RP was recorded for both treatment types.

### **Microbiological Analyses**

To serve as polymerase chain reaction (PCR) assay standards and selected bacterial type strains were grown on agar plates prepared with the appropriate culture media and atmospheric conditions. Columbia agar with 5% horse blood (Oxoid UK) was used for *Streptococci* and these organisms were grown in a 5% CO<sub>2</sub>/air

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atmosphere. Fastidious anaerobic agar with 5% horse blood agar was used for anaerobes, e.g., *Fusobacterium nucleatum* and these were grown in an H<sub>2</sub>/N<sub>2</sub> atmosphere (10% H<sub>2</sub>, 80% N<sub>2</sub>, 10% CO<sub>2</sub>).

The Epicentre Masterpure Gram positive DNA isolation kit (Cambio, Cambridge, UK) was used to prepare genomic DNA. The amount of DNA and the purity was checked spectroscopically at 260 and 280 nm in a spectrophotometer (NANODROP 1000, Thermo Renfrew, UK) and the ratio of absorbance (260/280) determined (ratio=1.8-2.0 =good DNA purity). DNA amount in standard samples was measured by fluorometric analysis (CYquant, Invitrogen Paisley, UK) and the Copy number determined from the standard curves. A qRT-PCR assay using TaqMAN or SYBRgreen chemistry (ABI/Invitrogen Paisley, UK) was used for detection, quantification of bacterial gene copy numbers. The primers, probes selected for the following bacteria were as published in the sources: *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *P. micra*, *T. forsythensis*, *F. nucleatum*<sup>13-16</sup> *T. denticola*<sup>17</sup> *S. mutans*, *S. oralis*, *A. naeslundii*<sup>18</sup> and *S. sanguinis*,<sup>19</sup> (Invitrogen, Paisley, UK or Eurogentech, Liege, Belgium) Statagene MRX III thermal cycler, (Agilent Edinburgh, UK) the TaqMAN assay PCR cycling parameters used in the study were 10 min at 95°C, and 40 cycles of 30 s at 95°C and 1 min at 60°C. The cycles with SYBR green were as follows; 10 min at 95°C, and 40 cycles of 30 s at 95°C and 1 min at 60°C and 1 min at 72°C. All primer sets were validated by running four serial 1/10 dilutions of the standard DNA and calculating the efficiency of the reaction (E) where  $E=(10^{-1/\text{slope}})-1$ . All reaction efficiencies calculated were between 91-104% and deemed acceptable. All primer sets failed to produce amplicons from different DNA standards.

### **Biochemical Analyses**

Enzyme-linked immunosorbent assay and specific kits were used for measurement of interleukin-1beta (IL-1β), tumour necrosis factor-alpha (TNF-α), osteoprotegerin (OPG), (R&D Systems, Abingdon UK) human soluble receptor-activator of kappa b ligand (sRANKL) (Peprotech, London UK) levels according to the manufacturers' recommendations in 96-well plates in duplicate (Nunc-ImmunoR MaxiSorpR, Nalge Nunc International, UK). Colour development was measured at 450 nm with wavelength correction set at 650 nm using a microplate reader (FLUOstar Omega; BMG Labtech, Germany). The minimum detection limits were as follows: TNF-α 1.6 pg/ml IL-1β 1.6pg/ml, OPG 8.4pg/ml; sRANKL 3.9pg/ml.

### **SEM Analyses**

Twenty-one hopeless teeth<sup>20</sup> (3 single-rooted, 18 multi-rooted) were randomly divided into three groups with seven teeth in each group: RP with diamond burs; RP with currettes; no treatment. A notch was made at the level of gingival margin before extraction for localization of the surface that will be examined. Extracted teeth were rinsed with saline for 2 min. The roots were separated from the crowns and rinsed with saline, stored in 2.5% glutaraldehyde (Aromas, İzmir) for 30-60 min, transferred to ethanol for dehydration in an ascending ethanol series (25%, 50%, 75%, 90%) and held in 100% ethanol over-night. Dehydrated samples were bonded to the

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bell metal, stored in a desiccator for 6 days, coated with 200 Å gold (Fisons Instruments, Polaron C502, Uckfield, UK). SEM analyses were performed with JSM-520 (JEOL, Tokyo, Japan) and microphotographs were taken at SEM Laboratory, School of Dentistry, Ege University. Ten photographs were taken from the approximal root surfaces and five from the furcations. The magnification of the photos was chosen from a pilot study of a series of photographs with magnification ranging between x50 and x2000. Then, the lowest magnification consistently showing dentin tubules, necrotic cementum, calculus, marks from instrumentation and smoothness of surface was chosen to analyse the widest possible area. In total 180 photos were taken at x350 magnification. A number was assigned to each photograph before blinding the treatment to the examiner. All photographs were divided into 9 equal regions by a standard grid and evaluated separately at full-screen resolution. Intraexaminer consistency was determined in 30 photographs for each index using the kappa statistic. The kappa values were as follows; “*Remaining Calculus Index*” (RCI)<sup>21</sup>=0.87±0.07, “*Loss of Tooth Substance Index*” (LTSI)<sup>21</sup>=0.88±0.06, “*SEM Roughness Index*” (SRI)<sup>22</sup>=0.94±0.03, “VAS evaluation”=0.90±0.05. The microphotographs were graded on a VAS scale ranging from 1 to 10 to harmonize measurement criteria. “0” was the surface without calculus, no loss of tooth substance and devoid of instrumental marks, while “10” denoted the maximum observed contrary score.

### **Statistical Analyses**

Microsoft Excel and commercially available statistical software (SPSS Inc. version 21 IBM, Chicago, USA and Graphpad Prism version 5, La Jolla, USA) were used to analyse the data. Statistical power calculation indicated that to exceed 80% statistical power where the effect size =1 a minimum of 23 individuals should be sampled for a non-parametric analysis using independent sample tests and for paired (dependent) sample tests the minimum number of participants N=12. With effect size =1.5, a statistical power of 80% was achievable in independent sample tests with N=12.

After determining the distribution, variance for each parameter on a Q-Q plot, non-parametric analyses were performed for skewed data sets, while parametric analyses were performed for normally distributed parameters. Clinical periodontal data were evaluated with ANOVA (repeated sample, for baseline and reassessment times and for treatment site comparisons) two-sided test and differences between treatments and control sessions were analysed. The non-parametric Wilcoxon test was used to investigate significant differences between the baseline and 1-month re-evaluations of the microbiological, biochemical analyses. Friedman and Kruskal-Wallis test was used for RCI and VAS analyses for matched and independent evaluations of the SEM data, respectively; the independent statistical test was required on a subset, where data were missing. Dunn’s test was used *post hoc*. All tests were performed at  $\alpha = 0.05$  significance level.

## RESULTS

### Clinical Measurements

This 6-month follow-up study was completed with 12 patients (7 males, 5 females, aged 37 to 60 years; mean age:  $46.41 \pm 6.22$  years) of the original 15 patients recruited, 3 patients had to be excluded due to non-compliance. The average duration of a RP session was 41.6 min for the burs and 36.5 min for the curettes ( $p > 0.05$ ). In total, 371 sites from single-rooted (Burs:185, Curettes:186) and 239 sites from multi-rooted (Burs:122, Curettes:117) teeth were evaluated. Distribution of various teeth was similar for the two treatment modalities ( $p > 0.05$ ). There was no significant difference in full-mouth clinical periodontal findings at any time point of the study between teeth treated with burs and those treated with curettes ( $p > 0.05$ ) (Table 1). The improvements in clinical periodontal findings were also similar with both treatment modalities. PD, CAL reductions from the baseline values were significant at single- or multi-rooted teeth with curettes and burs ( $p < 0.0001$ ). The reductions in PD were seen to be significantly greater at single-rooted teeth than at multi-rooted teeth at 1, 3 and 6 months when burs were used for RP ( $p < 0.05$ ) and at 3 and 6 months when the curettes were used for RP ( $p < 0.05$ ). Baseline and 1-month clinical periodontal parameters recorded at the sampling sites are presented in Table 2. Clinical improvements in PD, CAL, PI, and PBI were evident at 1 month at the selected GCF and microbial sample sites ( $p < 0.05$ ) and these improvements were similar for burs and curettes ( $p > 0.05$ ).

### Microbiological Parameters

Bacteria numbers -inferred from gene copy numbers- (Fig. 1) and percentage of each bacterium to the total bacteria present (Fig. 2) revealed similar findings at the two treatment sites at baseline and 1-month evaluation ( $p > 0.05$ ). The percentages of *F.nucleatum* and *T.denticola* were reduced at curette-treated sites, while percentages of *P.gingivalis* and *P.micros* were reduced at bur-treated sites of single-rooted teeth significantly at 1-month follow-up when compared with the baseline values ( $p < 0.05$ ).

### Biochemical Parameters

GCF sample volumes were similar in the two RP modalities both at baseline and 1-month after treatment (Table 2). Biochemical data are shown in Table 3. Total amount of IL-1 $\beta$  and TNF- $\alpha$  changed similarly following treatment. The cytokine levels were similar for the two RP modalities at baseline ( $p > 0.05$ ). At 1-month, significant differences were found in the total amounts of IL-1 $\beta$ , TNF- $\alpha$  between the GCF samples collected at diamond bur- and curette-treated single-rooted teeth ( $p < 0.05$ ).

### SEM Analyses on Extracted Teeth

Lower RCI values were found on all surfaces of RP treatment than the un-treated controls ( $p < 0.0001$ ). Bur-treated teeth exhibited less RCI values on all root surfaces except the furcation sites, but the difference between the two RP modalities was significant only on mandibular molar furcations ( $p < 0.0001$ ) (Fig. 3A). RP with burs revealed better results in the mandibular, maxillary furcations, whereas RP with curettes indicated better

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findings in the mandibular molar approximal root surfaces ( $p<0.0001$ ) (Fig. 3B). Curettes appeared to be more successful in SRI than the burs in single-rooted teeth and mandibular furcations without significant differences ( $p>0.05$ ). The burs were significantly better at maxillary furcational root sites ( $p<0.0001$ ) and curettes were better at molar approximal root surfaces ( $p<0.0001$ ) (Fig. 3C).

### VAS Evaluations

RCI, LTSI up to dentin and instrumental marks revealed no significant differences between the two treatment modalities ( $p>0.05$ ). Both treatment types showed improvements when compared with un-treated sites on all surfaces except for the furcations, but the differences were significant only for the furcational root surfaces of bur-treated mandibular molars ( $p<0.0001$ ) (Fig. 3D).

### DISCUSSION

Sonic, ultrasonic and rotary systems have some disadvantages like decreased tactile sensitivity, uncontrolled damage and inadequate adaptation to the root surface.<sup>23</sup> Mengel et al.<sup>24</sup> compared scalers, curettes, rotating systems with 40  $\mu\text{m}$  and 15  $\mu\text{m}$  diamond burs and found better results with the rotating system and 15  $\mu\text{m}$  diamond burs in root areas that were difficult to access mechanically. It was reported that rotating instruments form a thicker smear layer and remove greater amounts of tooth substance and diamond instruments produce typical traces. Moreno et al.<sup>25</sup> compared burs, curettes and different ultrasonic tips on extracted teeth and found more pronounced irregularities following the use of 40  $\mu\text{m}$  diamond burs. This might be explained if the practitioner ignored the manufacturer's instructions, such as using 15  $\mu\text{m}$  burs prior to 40  $\mu\text{m}$  ones. Schlageter et al.<sup>26</sup> achieved smoother surfaces with 15  $\mu\text{m}$  diamond burs in comparison to curettes, piezoultrasonic scalers, periplaner curettes, sonic scalers and 75  $\mu\text{m}$  diamond burs. Smoother root surfaces have been reported with 15  $\mu\text{m}$  burs when used after curettes than with curettes alone.<sup>27</sup> In the present study, SEM comparisons of diamond burs and curettes in terms of surface roughness revealed comparable results which were partly in agreement with previous studies. Unfortunately the, different methodologies used and different tooth types investigated in the different studies tend to prevent comparison of the findings as a whole.

To the best of our knowledge, this is the first study reporting clinical human data with burs used for RP. Efficacy of diamond burs on RP has been investigated on either extracted teeth<sup>25,28,29</sup> or after reflection of full-thickness flaps in an open debridement manner.<sup>26,27,30</sup> Lie & Meyer<sup>21</sup> performed RP in an experimental model by mounting extracted teeth and partly covering the roots with rubber to simulate gingiva. There is no published study evaluating clinically difficult-to-reach areas like narrow and deep pockets and anatomical factors like tongue, cheek have not been considered in previous studies. We performed RP before extraction and without flap reflection in order to compare the efficacy of these instruments within the real framework of non-surgical periodontal therapy.

Studies on RP have primarily used visual inspection and secondarily contour changes by light microscopy. We preferred to use SEM, as it can differentiate irregularities caused by instrumental marks and calculus remnants.<sup>22</sup> Lie & Leknes<sup>31</sup> used roughness and loss of tooth substance indices and formulated a modification and combination of SRI and LTSI.<sup>21</sup> Meyer & Lie<sup>22</sup> compared the scanning roughness index with prophylometer and

found analogous results suggesting the index as an objective method. In the present study, we divided the SEM photos into 9 equal areas to standardize the method and increase the reliability of visual microscopic inspection. We used the RCI for all surfaces; however, SRI and LTSI could not be applied to the surfaces that have roughness due to presence of cementum, since SRI is valid for reporting the roughness on dentin surface only. Therefore, we used SRI and LTSI separately and complemented these indices with VAS evaluation on all surfaces. SRI and LTSI were not implemented to the sections, which contain calculus and necrotic cementum, as dentin tubules and instrumental marks on dentin would not be seen without appropriate RP.

The greater reduction of PD obtained at single-rooted than multi-rooted teeth can be explained by anatomical factors of multi-rooted teeth making it more difficult to clean for the patient and the clinician. Thinner biotype of anterior teeth leading to a greater contraction after treatment may be another factor explaining this difference as suggested previously.<sup>32</sup> The present PD, CAL changes obtained by SRP are in agreement with previous clinical studies<sup>4,33</sup> and the ranges reported in a review.<sup>34</sup>

Haffajee et al.<sup>35</sup> found significant decreases only in red complex bacteria and significant increase in *A. viscosus* 3 months after non-surgical periodontal therapy. The rest of the investigated bacteria revealed similar levels before and after periodontal treatment and the authors concluded that periodontal therapy may decrease the total number of bacteria only transiently for 1-2 weeks, after which recolonisation allows the numbers of bacteria to approach the baseline values. Periodontal therapy has been suggested not to affect the majority of the subgingival microbiota but rather alter the equilibrium between the host and microbiota.<sup>35</sup> Moreover, superinfections by an unwanted microbial complex might occur if periodontal therapy could alter the equilibrium to a large extent. Eick et al.<sup>36</sup> and Iannou et al.<sup>4</sup> also found complex effects of non-surgical periodontal treatment on the mean levels of microorganisms at 3- and 6-month follow-ups. In some respects our present findings are similar to the previous studies. PD and presence of supragingival plaque are the major determinants and the interactions of different bacteria within the biofilm may be more important than their sole presence or absence. It is neither necessary nor possible to eradicate all bacteria from the oral cavity. The realistic goal should be to disturb the complex interactions and alter the balance towards an oral health compatible microbial community.

Many studies reported increases in GCF levels of IL-1 $\beta$ , TNF- $\alpha$ , sRANKL and decrease in OPG in relation with periodontal disease,<sup>37-39</sup> whereas others found contradicting findings such as decreased IL-1 $\beta$ , unchanged TNF- $\alpha$  levels.<sup>40</sup> GCF total amount of IL-1 $\beta$  remained unchanged in a study of Gamonal et al.<sup>41</sup> A slight but not significant increase in GCF total amount of IL-1 $\beta$  has also been reported following non-surgical periodontal therapy in chronic periodontitis patients.<sup>32</sup> Buduneli et al.<sup>42</sup> reported a significant decrease in GCF total amount and concentration of OPG whereas sRANKL values were similar to those of baseline 4 weeks after non-surgical periodontal therapy in chronic periodontitis patients. Lu et al.<sup>43</sup> compared OPG and sRANKL levels at healthy and diseased sites and failed to find any relationship between OPG, sRANKL levels and degree of periodontal disease. The discrepancy between the findings of different studies including the present one may be explained by the complex and episodic character of periodontal disease, individual variability in the response to therapy, as well as by the methodological differences.

SEM investigations revealed less residual calculus with burs than curettes on all surfaces and might suggest that diamond burs can access the frequent root concavities on mandibular molars better than curettes. This was true for all sites except for the furcation sites of the mandibular molar teeth. The better results obtained with curettes at furcation areas may be explained by the difficulties of correct positioning of diamond burs to avoid trauma to soft tissues where the entrance of a furcation area is closed by gingiva.

Burs provided better results in LTSI, whereas, curettes gave better results in SRI. It may be suggested that 40 µm burs did not cause tissue loss because of their non-aggressive conformation, but 15 µm burs were inferior to curettes in terms of providing smooth surfaces. SEM data of the present study were similar to those of a previous study.<sup>30</sup> The burs resulted in cleaner surfaces with less calculus and smear layer than did the curettes, and tissue loss was less, but there were more instrumental marks and roughness was greater.<sup>30</sup> Accordingly, in a recent in vitro study, a specific set of curettes, a piezo-ceramic ultrasonic scaler, and a sonic scaler were compared and the smoothest root surfaces were obtained with the ultrasonic device.<sup>44</sup>

The shorter -although not significantly different- time required for RP with curettes might be explained by the fact that the whole mass of calculus can be removed by placing curette to the bottom of the calculus mass, whereas, diamond burs can only abrade the calculus starting from the outer surface. The impact this may have on the results is difficult to state, but the increased time required for efficient RP might deter some clinicians from the use of burs.

The present study has some limitations; a greater number of patients, including the RP of more teeth with deep pockets, and analysing more GCF, plaque samples would increase the statistical power of the study. Moreover, the gold coating utilized for the SEM investigation did not allow the comparison of the root surfaces before and after SRP.

## **Conclusion**

Within the limits of the present study, it can be concluded that RP with diamond burs provided no better but did result in similar clinical, microbiological, and biochemical data when compared with curettes. Larger scale studies with inclusion of more patients as well as more periodontists may be required to support these findings.

**Conflict of Interest:** All authors explicitly declare that they have no conflict of interest.

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- Accepted Article
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- Accepted Article
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## Figure Legends

**Figure 1:** Copy numbers of subgingival plaque bacteria before and after RP-treatment with burs and curettes. The 16S rRNA gene copy numbers of *A. actinomycetemcomitans*, *Fusobacterium spp.*, *P.gingivalis*, *P. intermedia*, *P. micros*, *S. mitis*, *S. mutans*, *S. sanguinus*, *T. forsythensis*, and *T. denticola*: (A) at single rooted teeth and (B) at multi-rooted teeth. Data are shown as means  $\pm$  SD.

**Figure 2:** Proportions of *A. actinomycetemcomitans*, *Fusobacterium spp.*, *P. gingivalis*, *P. intermedia*, *P. micros*, *S. mitis*, *S. mutans*, *S. sanguinus*, *T. forsythensis*, and *T. denticola* of the sum of the copy numbers of these bacteria in subgingival plaque before and after RP-treatment with burs and curettes: (A) at single rooted teeth and (B) at multi-rooted teeth. Data are shown as means  $\pm$  SD.

\* Significant reduction compared to baseline following RP ( $p < 0.05$ ).

**Figure 3.** Evaluation of tooth surfaces following RP: (A) Calculus index of un-treated teeth and of teeth following RP. (B) Loss of tooth substance index of teeth following RP. (C) Roughness index of teeth following RP (D) VAS evaluation of un-treated teeth and teeth following RP. Data are shown as means  $\pm$  SD.

\* Significant difference between RP with burs or curettes on the same surface types ( $p < 0.0001$ )

† Significant difference between RP with burs or curettes on the same surface types ( $p < 0.0001$ ).

**Table 1.** Clinical parameters at baseline and at 1, 3 and 6 months following RP (all sites).

<b>Tooth type</b>	<b>Clinical periodontal parameter</b>	<b>Time point</b>	<b>Diamond burs</b>	<b>Curettes</b>
<b>Single-rooted teeth</b> n=371	<b>PD (mm)</b>	Baseline	5.8 ± 0.3	5.5 ± 0.3
		1. month	3.3 ± 0.3*†	3.4 ± 0.4*
		3. month	3.0 ± 0.5*†	3.0 ± 0.4*†
		6. month	3.1 ± 0.6*†	2.9 ± 0.5*†
	<b>CAL (mm)</b>	Baseline	6.5 ± 0.5	6.1 ± 0.8
		1. month	5.0 ± 0.8*	4.78 ± 1.0*
		3. month	4.6 ± 1.0*	4.2 ± 1.0*
		6. month	4.8 ± 0.8*	4.0 ± 0.9*
	<b>PI (0-5)</b>	Baseline	3.8 ± 0.6	3.6 ± 0.7
		1. month	1.6 ± 0.8*	1.2 ± 1.0*
		3. month	1.1 ± 0.8*	0.8 ± 0.5*
		6. month	1.1 ± 0.8*	0.9 ± 0.8*
	<b>PBI (0-4)</b>	Baseline	2.5 ± 0.5	2.2 ± 0.5
		1. month	0.3 ± 0.3*	0.4 ± 0.4*
		3. month	0.3 ± 0.3*	0.3 ± 0.3*
		6. month	0.3 ± 0.3*	0.4 ± 0.5*
<b>Multi-rooted teeth</b> n=239	<b>PD (mm)</b>	Baseline	5.9 ± 0.5	5.7 ± 0.3
		1. month	4.1 ± 0.7*	4.0 ± 0.4*
		3. month	3.7 ± 0.6*	3.9 ± 0.5*
		6. month	3.6 ± 0.6*	4.0 ± 0.5*
	<b>CAL (mm)</b>	Baseline	6.2 ± 0.83	6.5 ± 0.7
		1. month	5.0 ± 1.2*	5.0 ± 0.6*
		3. month	4.8 ± 1.2*	5.0 ± 1.00*
		6. month	4.6 ± 0.9*	5.0 ± 0.7*
	<b>PI (0-5)</b>	Baseline	3.8 ± 0.5	4.1 ± 0.6
		1. month	1.4 ± 1.0*	2.0 ± 1.2*
		3. month	0.9 ± 0.9*	1.1 ± 0.6*
		6. month	1.3 ± 0.9*	1.2 ± 0.7*
	<b>PBI (0-4)</b>	Baseline	2.55 ± 0.37	2.8 ± 0.7
		1. month	0.7 ± 0.4*	1.0 ± 0.6*
		3. month	0.6 ± 0.4*	0.7 ± 0.4*
		6. month	0.5 ± 0.3*	1.0 ± 0.4*

Data are shown as means ± SD.

There was no significant difference at any time point between teeth treated with burs and those treated with curettes.

\* Significant reduction compared to baseline following RP ( $p < 0.0001$ ).

† Significantly greater reduction at single rooted teeth at the same assessment session when compared with multi-rooted teeth ( $p < 0.05$ ).

**Table 2.** Clinical parameters at baseline and 1 month following RP with burs or cures (sampled sites).

Tooth type	Clinical periodontal parameter	Time point	Diamond burs	Curettes
Single-rooted teeth n=24	PD (mm)	Baseline	5.9 ± 0.7	5.9 ± 0.9
		1. month	3.6 ± 0.7*	3.3 ± 0.6*
	CAL (mm)	Baseline	6.6 ± 1.4	6.7 ± 1.5
		1. month	5.1 ± 1.9*	5.1 ± 1.4*
	PI (0-5)	Baseline	3.7 ± 0.6	3.7 ± 1.1
		1. month	1.6 ± 1.2*	1.9 ± 1.4
	PBI (0-4)	Baseline	2.3 ± 1.0	2.1 ± 0.7
		1. month	0.2 ± 0.4*	0.2 ± 0.3*
	GCF volumes (µl)	Baseline	0.6 ± 0.2	0.6 ± 0.2
		1. month	0.4 ± 0.2*	0.5 ± 0.2
Multi-rooted teeth n=24	PD (mm)	Baseline	6.1 ± 0.9	5.2 ± 0.3
		1. month	4.1 ± 0.9*	3.5 ± 0.5*
	CAL (mm)	Baseline	6.8 ± 1.6	5.8 ± 0.9
		1. month	5.2 ± 1.5*	4.5 ± 0.9
	PI (0-5)	Baseline	3.8 ± 0.8	3.6 ± 0.8
		1. month	1.0 ± 1.4*	1.1 ± 1.3*
	PBI (0-4)	Baseline	2.5 ± 0.7	2.3 ± 0.9
		1. month	0.8 ± 1.0*	0.1 ± 0.5*
	GCF volumes (µl)	Baseline	0.6 ± 0.2	0.7 ± 0.2
		1. month	0.5 ± 0.2	0.5 ± 0.1*

Data are shown as means ± SD.

There was no significant difference at any time point between teeth treated with burs and those treated with cures.

\* Significant reduction compared to baseline following RP ( $p < 0.0001$ ).

**Table 3.** Total amount (pg/30 sec) and concentration (pg/ $\mu$ l) of the cytokines.

C y t o k i n e	Tooth type	Time point	Diamond burs		Curettes	
			Total amount	Concentration	Total amount	Concentration
IL-1 $\beta$	Single rooted teeth	Baseline	11.35 $\pm$ 2.36	20.35 $\pm$ 8.29	14.31 $\pm$ 3.36	25.21 $\pm$ 11.29
		1. month	13.05 $\pm$ 9.28	30.20 $\pm$ 19.60	4.20 $\pm$ 1.97* $\dagger$	11.28 $\pm$ 9.70*
	Multi rooted teeth	Baseline	8.59 $\pm$ 2.08	14.47 $\pm$ 6.45	10.50 $\pm$ 3.01	16.71 $\pm$ 6.43
		1. month	14.94 $\pm$ 3.60*	32.42 $\pm$ 14.97*	14.80 $\pm$ 1.80	35.32 $\pm$ 8.14*
TNF- $\alpha$	Single rooted teeth	Baseline	10.52 $\pm$ 1.96	18.81 $\pm$ 7.31	12.78 $\pm$ 3.19	22.39 $\pm$ 10.04
		1. month	11.80 $\pm$ 8.51	27.4 $\pm$ 18.80	3.79 $\pm$ 2.12* $\dagger$	10.32 $\pm$ 9.85* $\dagger$
	Multi rooted teeth	Baseline	8.16 $\pm$ 1.95	13.6 $\pm$ 5.70	10.10 $\pm$ 2.81	15.86 $\pm$ 5.78
		1. month	14.68 $\pm$ 4.12*	35.38 $\pm$ 31.30*	13.75 $\pm$ 2.07	32.75 $\pm$ 7.76
sRANKL	Single rooted teeth	Baseline	140.92 $\pm$ 10.24	248.20 $\pm$ 73.50	159.26 $\pm$ 15.12	282.55 $\pm$ 130.23
		1. month	175.50 $\pm$ 23.97*	473.21 $\pm$ 223.11*	171.63 $\pm$ 6.29	456.78 $\pm$ 294.63*
	Multi rooted teeth	Baseline	240.40 $\pm$ 16.70	402.20 $\pm$ 130.10	269.25 $\pm$ 21.91	424.80 $\pm$ 105.00
		1. month	246.39 $\pm$ 86.95	554.01 $\pm$ 396.03	165.14 $\pm$ 9.05*	403.18 $\pm$ 128.72
OPG	Single rooted teeth	Baseline	82.19 $\pm$ 10.77	145.40 $\pm$ 47.00	72.73 $\pm$ 10.51	123.17 $\pm$ 36.26
		1. month	124.84 $\pm$ 46.65	352.96 $\pm$ 232.00 $\ddagger$	163.19 $\pm$ 9.99*	437.42 $\pm$ 295.05 $\ddagger$
	Multi rooted teeth	Baseline	96.90 $\pm$ 15.90	160.96 $\pm$ 51.75	109.91 $\pm$ 20.43	172.98 $\pm$ 49.59
		1. month	155.11 $\pm$ 12.10*	350.53 $\pm$ 209.39 $\ddagger$	142.88 $\pm$ 14.93	350.64 $\pm$ 125.00 $\ddagger$

Data are shown as means  $\pm$  SD.

\* Significant difference compared to baseline ( $p < 0.0001$ ).

$\dagger$  Significantly lower compared to RP with curettes at single-rooted teeth ( $p < 0.05$ ).

$\ddagger$  Significant increase compared to baseline ( $p < 0.05$ ).





