Scanning electron microscopic evaluation of root surface morphology after root planing with curettes and ultrasonic perio mini-tip: An in vitro study

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Abstract
Mechanical removal of plaque and calculus along with bacterial toxins from the root surface by root planing is an effective means of altering the etiology of inflammatory periodontal disease which can be achieved by manual instruments (curettes) as well as ultrasonics. Curettes routinely leave a smear layer, remove more amount of root substance and take a longer time to achieve the desired root surface smoothness. On the other hand, ultrasonic instruments are easy to use, cause less operator fatigue and provide simultaneous flushing by the coolant spray. However, their only drawbacks include bulky working tips, risk of gouging the root surface if not used properly and poor tactile sensation. But the problem of bulky working tips have been overcome to a great extent by the introduction of micro-ultrasonic tips and slimline inserts, specially designed for root debridement purposes. Through this study, an attempt has been made to compare and evaluate the micro-topography of the root surface under scanning electron microscope, following instrumentation with curettes as well as ultrasonic perio mini-tip.

Keywords: Root planing, Curettes, Ultrasonic perio mini-tip, Scanning electron microscope.

Introduction
The role of microbial plaque in the aetiology of periodontal disease has been well established. Therefore, a major objective in the treatment of periodontal disease is to remove both supragingival as well as subgingival plaque and calculus and also to prevent recolonisation of periodontal pockets by pathogenic bacteria, thereby aiming at halting the disease progression as well as restoring the biological compatibility of the diseased cemental surface.[2,11]

Scaling and root planing procedures are concomitantly aimed at removal of bacterial toxins embedded within the cementum and to achieve a smooth, hard, clean and biologically acceptable root surface which is conducive to new cementum formation and biologically compatible for new collagen fibre insertion.[6,7,14] Mechanical therapy in routine clinical practice can be accomplished with both hand instruments and ultrasonics, used alone or in combination. Manual instruments like curettes routinely leave a smear layer, remove more amount of root substance and take a longer time to achieve the desired surface smoothness.[9] On the other hand, ultrasonic instruments are easy to use, cause less operator fatigue and provide simultaneous flushing by the coolant spray.[5] However, their only drawbacks include bulky working tips, risk of undue damage to the root surface if not used properly, poor tactile sensation and aerosol contamination.[12] But the problem of bulky working tips have been overcome to a great extent by the introduction of ultra-fine micro-ultrasonic tips and slimline inserts, specially designed for root debridement purposes.[16] With these tips, gaining access to the most difficult-to-reach areas of mouth like distal surfaces of second and third molars, furcation areas of multirooted teeth, pocket depths exceeding 5mm and areas having complex root anatomy has become much more easier.[15,16]

Aims and Objectives
1. To compare and evaluate the root surface morphology in-vitro, after instrumentation of cemental surface with hand instruments like Gracey curettes and with specialized ultrasonic piezoelectric root-planing tip, like the perio mini-tip.
2. To determine the optimum level of cemental surface instrumentation both with curettes and ultrasonic perio mini-tip towards obtaining a clean, hard, smooth and biologically acceptable root surface.
3. To determine the comparative efficacy of curettes and ultrasonic perio mini-tip towards obtaining a smooth, hard and clean cemental surface.

Materials and Methods
The present study was carried out on 120 selected numbers of periodontally involved, caries-free extracted human teeth. These teeth were extracted from 84 patients suffering from severely advanced periodontitis, attending the Out Patient Department (OPD). The study population consisted of both males and females in the age group of 28 to 70 years with a mean age of 45 years. All the teeth for experimental purpose were extracted randomly from the region of incisors, canines, premolars and molars the region of incisors, canines, premolars and molars of both the maxillary as well as mandibular arches. The cause behind the extraction for all the teeth was mobility (Miller's Grade III) with a clinical attachment loss of 6mm or more. Hence, these teeth were rendered hopeless for any type of conservative dental therapy. With the help of a William's periodontal probe, the amount of clinical attachment loss (CAL) was measured for all the teeth. Thereafter, each and every selected tooth was extracted with proper care so that the expected area of study which extends 5mm apically from the cemento-enamel

junction (CEJ) was not damaged or traumatized with surgical instruments (extraction forceps or elevators) under any circumstances. The ones that required such surgical instrumental intervention under any circumstances were discarded.

Inclusion Criteria

1. Patients with clinical attachment loss of 6mm or more.
2. Teeth having hopeless prognosis with grade III mobility.
3. Presence of subgingival calculus as detected by no.17 explorer (EXTU76—Hu Friedy).
4. Teeth exhibiting considerable radiographic bone loss.

Patients who had not received any periodontal treatment in past 6 months.

Exclusion Criteria

1. Teeth with subgingival restorations or root surface caries involving the cemento-enamel junction (CEJ).
2. Fractured teeth.
3. Root-canal treated teeth.

Grouping of Teeth

After extraction, the teeth were washed properly under running tap water with an ultra-soft bristled brush to remove visible debris, blood and tissue tags. Then they were treated with sodium hypochlorite and subsequently kept immersed in normal saline. Scaling was performed in vitro as the next step of experimental procedure to remove stains and calculus deposits in order to achieve a visibly clean tooth surface. Scaling was done by using an ultrasonic piezoelectric scaler (P5 Newtron, Suprasson Satelec). After scaling, all the teeth (total numbering to 120) were divided at random into two equal experimental groups—Group-A and Group-B, comprising of 60 teeth in each group. All the teeth in Group-A were instrumented with curettes and all the teeth in Group-B were instrumented with ultrasonic perio mini-tip.

Group-A (n=60) was further subdivided into 3 equal sub-groups: Ac_1, Ac_2, Ac_3, comprising of 20 teeth in each sub-group.

Sub-group 'Ac_1'—50 root planing strokes per surface per tooth.

Sub-group 'Ac_2'—100 root planing strokes per surface per tooth.

Sub-group 'Ac_3'—150 root planing strokes per surface per tooth.

Group-B (n=60) was subdivided into 3 equal sub-groups: Bu_1, Bu_2, Bu_3 comprising of 20 teeth in each subgroup.

Sub-group 'Bu_1'—15 seconds of ultrasonic root planing per surface per tooth.

Sub-group 'Bu_2'—30 seconds of ultrasonic root planing per surface per tooth.

Sub-group 'Bu_3'—45 seconds of ultrasonic root planing per surface per tooth.

Preparation of Experimental Site

Each caries-free surface of a tooth was examined visually and also with a magnifying glass to determine the anatomical location of cemento-enamel junction (CEJ) (Fig. 1). A tentative area on the root surface extending up to a length of 5mm apically from the CEJ was properly demarcated with a marker pen. All the proceedings of root planing were conducted within this demarcated area on the root surface.

Armamentarium

For Scaling

1. Ultrasonic piezoelectric scaler (P5 Newtron, Suprasson Satelec)
2. Universal scaler tip No. 1(Satelec Acteon)

For root Planning

1. Gracey's area specific curettes (SG 1/2 – SG 13/14, Hu-Friedy) (Fig. 2).
2. Ultrasonic piezoelectric perio mini-tip (H3, Satelec Acteon)(Fig. 3a & 3b).

Work Procedure

Root planing was done within the demarcated area on the root surface of each tooth by employing multidirectional strokes while using both hand instruments like Gracey's area specific curettes as well as ultrasonic perio mini-tip at a very low power setting of '4' as per manufacturer's recommendations. All the teeth after root planing were washed with water spray with the help of 3 way water syringe and were air dried with the same. During the course of root planing, the previously drawn demarcation line on the root surface got erased. So the planed root surface area which was to be viewed under microscope was demarcated again by a extra fine-tip marker pen (tip diameter 0.5mm). This time, the boundaries of the instrumented root surface area were delineated coronally by the CEJ, apically by a line which extends 5mm apical to CEJ and proximally by two lines corresponding to the proximal line angles of the root (Fig. 4). Each tooth was then preserved carefully in a plastic zip pouch and every sample was properly labelled with the respective sample group and serial number written on it.

Scanning Electron Microscopic (SEM) Study

Selected teeth from the experimental groups were fixed on the stub of the IB-2 Ion Coater gold-sputtering machine (Fig. 5) and the cover was put on to make the area air-tight. With the help of an air suction machine, air was taken out from the sputtering bottle to make the chamber vacuumised and sputtering was done with pure gold. The gold sputtered tooth surface (Fig. 6) was then transferred to the SEM machine (Hitachi S-530, Japan) (Fig. 7) and the area of interest on the root surface was examined at fixed magnifications of 50x and 100x. Suitable areas of observation were photographed and presented in Results and Observations.

Qualitative assessment of root surface morphology was made by studying the SEM photomicrographs. The photomicrographs obtained were assessed individually under the following parameters—

Roughness and Loss of Tooth Substance assessed by taking help of the different criteria laid down by Roughness and Loss of Tooth Substance Index (RLTSI), proposed by Lie and Leknes, 1985.

Roughness and Loss of Tooth Substance Index

Grade 1→ Smooth and even root surface with no marks of instrumentation and no loss of tooth substance.

Grade 2→ Slightly roughened or corrugated local areas confined to the cementum.

Grade 3→ Definitely corrugated local areas where cementum may be completely removed although most of the cementum is still present.

Grade 4→ Considerable loss of tooth substance with instrumentation marks into the dentin; cementum is completely removed in large areas or it has considerable number of lesions from the instrumentation.

Estimation of remaining calculus assessed by taking help of the different criteria of Remaining Calculus Index (RCI), proposed by Lie and Meyer, 1977.

Remaining Calculus Index

Grade 1→ No calculus present on the root surface.

Grade 2→ Small patches of extraneous material, probably consisting of calculus.

Grade 3→ Definite patches of calculus confined to smaller areas.

Grade 4→ Considerable amounts of remaining calculus appearing as one or two voluminous patches or as several smaller patches scattered on the treated surface.

Degree of cleanliness of root surface assessed by taking help of Cleanliness Index, proposed by Dahiya and Pandit, 2011.

Cleanliness Index

Grade 1→Absence of visible debris and plaque with no evidence of remaining smear layer and good exposure of dentin.

Grade 2→ No visible debris, no exposure of dentin and presence of smear layer.

Grade 3→Presence of visible debris and plaque all over the scanned area, no visible dentin and smear layer present over the entire surface.

As because only two representative samples from each of the six experimental subgroups (each subgroup comprising of 20 teeth) were subjected to SEM analysis, therefore individual scoring of representative photomicrographs from each group according to the scoring criteria laid down by the above mentioned index systems, was not possible in this study. Instead, the above mentioned criteria for different index systems were taken help of, so as to get an overall impression of the quality of root surface instrumentation produced by both hand and ultrasonic instruments as manifested by the root surface topography under SEM.

Results and Observations

Scanning Electron Microscopic (SEM) Study

Results of the Scanning Electron Microscopic (SEM) evaluation of root surfaces after scaling and root planing, as done by visual qualitative analysis of individual SEM photomicrographs of selected samples from each of the six experimental subgroups (A_{C1}, A_{C2}, A_{C3}, B_{U1}, B_{U2}, B_{U3}), viewed under fixed magnifications of 50x and 100x, are hereby given with representative photomicrographs (Fig. 1 to Fig. 12).

Fig. 8 and Fig. 11 represent SEM photomicrographs of the root surface of a representative sample from subgroup-A_{C1} under experimental group-A (test side instrumented for 50 strokes with curette), viewed under 50x and 100x magnifications respectively. Under 50x magnification, the test side shows some rough and definitely corrugated patches where cementum is removed and instrumentation marks or scratches can be seen on dentin, although most of the cementum is still present on the root surface. Cemental cracks are evident on the residual cementum, which occurs as a result of dehydration process that the cementum undergoes due to alcohol treatment before it is gold-sputtered prior to viewing under SEM. In terms of cleanliness, plaque and smear layer are not evident under 50x magnification, except for a few isolated and scattered particles of debris. Residual embedded calculus particles in cementum cannot be appreciated under 50x magnification but under 100x magnification, some isolated dark particles can be seen scattered on the root surface along with one or two dark patches, which are suggestive of residual, calculus particles within the unremoved cementum. In addition, under 100x magnification, instrumentation scratches can be more clearly appreciated in the roughened or corrugated areas where cementum is completely removed.

Fig. 9 and Fig. 12 show SEM photomicrographs of root surface of a representative sample from subgroup-A_{C2} under experimental group-A (test side instrumented for 100 strokes with curette), viewed under 50x and 100x magnifications respectively. Under both 50x as well as 100x magnifications, the test side exhibits a relatively uniform smooth surface. There is no undue loss of tooth substance. Most of the root surface cementum has been conserved. Cementum exhibits cracks all over its surface. There is no evidence of any instrumentation marks or scratches even under higher magnification of 100x. There are no rough or corrugated patches where cementum has been aggressively removed. There is no evidence of any patches of remaining calculus. There is no evidence of plaque or smear layer except for a few scattered particles of debris. Overall, the root surface presents a relatively clean and smooth surface topography.

Fig. 10 and Fig. 13 show SEM photomicrographs of root surface of a representative tooth sample from subgroup-A_{C3} under experimental group-A (test side instrumented for 150 strokes with curette), viewed under 50x and 100x magnifications respectively. Here, the root surface appears extremely smooth and very well polished. There are no visible cemental cracks. There are no rough patches or corrugated areas over the root surface, suggestive of the fact that all the necrotic cementum has been uniformly removed and root surface exhibits a very clean, smooth and uniform surface topography, probably with good exposure of dentin. There is no evidence of any patch or flecks of remaining calculus, no evidence of plaque, smear layer or debris.

Fig. 14 and Fig. 17 show SEM photomicrographs of the root surface of a representative tooth sample from subgroup-

$B_{U1,}$ under the experimental group-B (test side instrumented for 15 seconds with ultrasonic perio mini-tip), viewed under 50x and 100x magnifications respectively. Here, the root surface presents definitely corrugated areas or rough patches confined to the cementum. This suggests that all the necrotic cementum is not uniformly removed, resulting in certain degree of root surface roughness. Cemental cracks are also evident, although lesser in number. Root surface also shows isolated particles scattered all over the cementum as slight projections, probably suggestive of debris or particles of smear layer. Minute particles of embedded residual calculus are also evident, scattered as isolated dark particles over the root surface cementum. However, scratches due to instrumentation are not seen.

Fig. 15 and Fig. 18 show SEM photomicrographs of the root surface of a representative tooth sample from experimental subgroup-$B_{U2,}$ under Group-B (test side instrumented for 30 seconds with ultrasonic perio mini-tip), viewed under 50x and 100x magnifications respectively. Here, the root surface exhibits slightly corrugated local areas confined to the cementum. Cemental cracks are evident in abundance. Cemental surface does not appear to be uniformly smooth. Localised areas of undulations are evident within the cementum just below the CEJ, suggestive of the fact that probably all the affected cementum has not been removed. Therefore, it indicates insufficient removal of affected cementum. However, scratches due to ultrasonic instrumentation are not seen. Cemental surface is devoid of any debris, smear layer or isolated flecks or patches of remaining calculus.

Fig. 16 and Fig. 19 show SEM photomicrographs of root surface of a representative sample tooth from experimental subgroup-$B_{U3,}$ under Group-B (test side instrumented for 45 seconds with ultrasonic perio mini-tip), viewed under 50x and 100x magnifications respectively. Here, the cemental surface shows relatively smooth and uniform surface topography. Cemental cracks are evident in abundance. There is no substantial loss of tooth structure. Root surface is devoid of calculus, plaque or smear layer, except for a few isolated particles of debris.

Fig. 1: Viewing the CEJ under magnifying glass

Fig. 2: A set of Gracey curettes

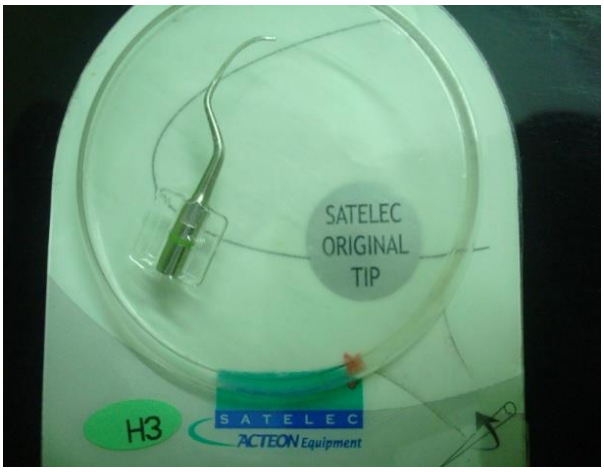

Fig. 3a: Ultrasonic perio mini-tip in sealed pack

Fig. 3b: Enlarged view of the perio mini-tip

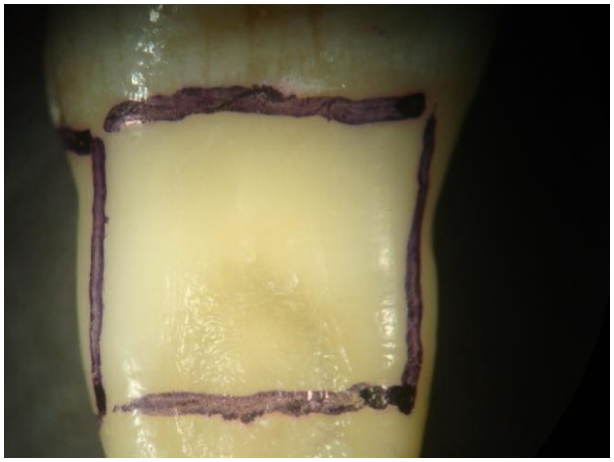

Fig. 4: Demarcated area of instrumentation

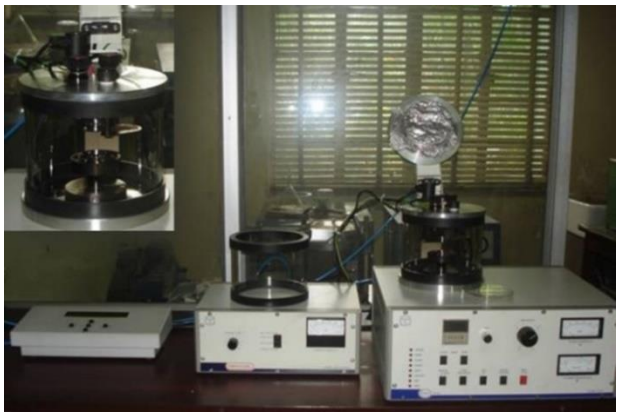

Fig. 5: Gold-sputtering machine (IB-2 ION COATER). Inset picture shows enlarged view of the sputtering chamber

Fig. 6: Tooth samples after gold coating

Fig. 7: Scanning electron microscope (HITACHI S-530)

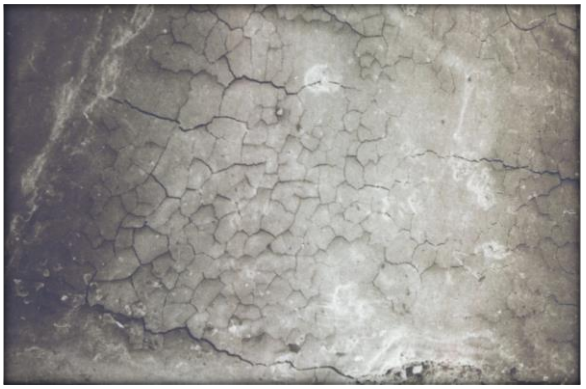

Fig. 8: SEM view of test side instrumented for 50 strokes with curette. 50x

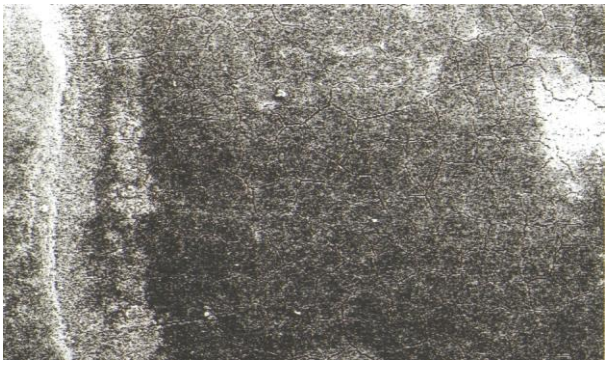

Fig. 9: SEM view of test side instrumented for 100 strokes with curette. 50x

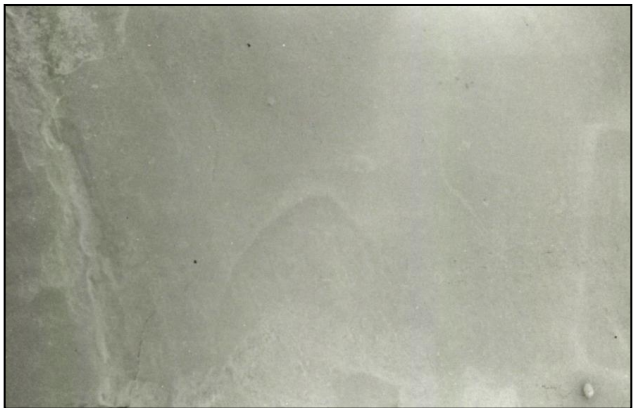

Fig. 10: SEM view of test side instrumented for 150 strokes with curette. 50x

Fig. 11: SEM view of test side instrumented for 50 strokes with curette. 100x

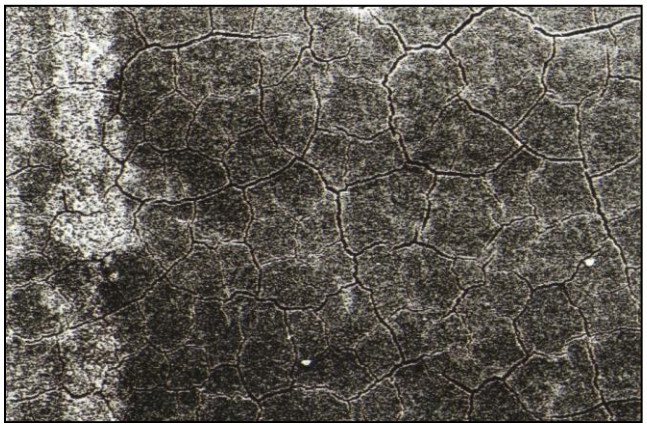

Fig. 12: SEM view of test side instrumented for 100 strokes with curette. 100x

Fig. 13: SEM view of test side instrumented for 150 strokes with curette. 100x

Fig. 14: SEM view of test side instrumented for **15 seconds** with ultrasonic perio mini-tip. **50x** magnification.

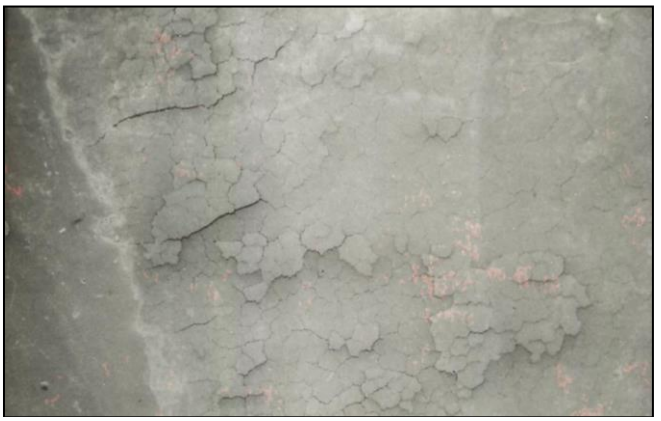

Fig. 15: SEM view of test side instrumented for **30 seconds** with ultrasonic perio mini-tip. **50x** magnification.

Fig. 16: SEM view of test side instrumented for 45 seconds with ultrasonic perio mini-tip. 50x magnification.

Fig. 17: SEM view of test side instrumented for 15 seconds with ultrasonic perio mini-tip. 100x magnification.

Fig. 18: SEM view of test side instrumented for 30 seconds with ultrasonic perio mini-tip. 100x magnification.

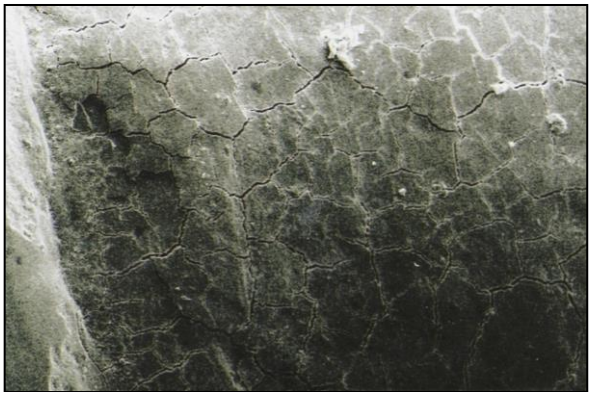

Fig. 19: SEM view of test side instrumented for 45 seconds with ultrasonic perio mini-tip.100x magnification

Discussion

The degree of root surface smoothness achieved by root planing with hand instrument like curette, is influenced by several determining factors like number of root planing strokes used, degree of sharpness of curette blade, tilting angle of the instrument, pressure or force exerted during root planing by individual operators, manual dexterity and expertise of the clinician while instrumenting hard to reach areas of the dentition like distal surfaces of last molars, deep tortuous pockets, furcation areas of molars.[15] While using ultrasonic instruments, the determining factors are the time devoted for root planing, pressure applied during instrumentation, the displacement amplitude of the tip of the ultrasonic device and the instrument design used.[10] The design of the present study is such that the root surface characteristics of a group of teeth (n=20) instrumented for a fixed number of strokes (e.g., 50/100/150 strokes) per surface per tooth with curette is compared to that of a group of teeth (n=20) instrumented for a fixed number of seconds (e.g., 15/30/45 seconds) per surface per tooth by ultrasonic perio mini-tip.

Scanning electron microscope represents a qualitative method of assessing the root surface characteristics.[1,4,8] Evaluation of the degree of cleanliness, amounts of remaining calculus and roughness and loss of tooth substance was based on the visual inspection of standardized photomicrographs of the representative samples from all their respective experimental subgroups. SEM assessment of the standardized photomicrographs of representative samples within and across all experimental groups within the limits of this study showed that hand instrumentation with curette employing 150 strokes per surface produced the smoothest, cleanest, calculus free and most even root surface among all experimental groups as per visual perception.

A number of studies have evaluated the influence of presence or absence of smear layer on treated root surfaces. This amorphous irregular surface layer is composed of tooth substance debris, dentinal fluid, grinding dust, water. It has a negative effect on soft tissue attachment and impedes binding of fibroblasts to cementum and dentin.[7,14] The presence of smear layer was more in surfaces treated with

ultrasonic tip than in root surfaces instrumented with curette.

Influence of root surface roughness after instrumentation on postoperative healing has been extensively studied. Ruben et al. (1994) stated that a roughened yet debrided root surface is needed for new attachment. Stahl et al. has highlighted the need for the presence of a mineralized microroughness cementum layer for initiating cementogenesis in the healing process but a significantly rough residual root surface post instrumentation could obviously constitute a potential danger of colonization by periodontal pathogens.[11,13]

Damage to root surface is a major concern to the dental clinician. If the operator gouges the root surface, a new environment may be created which facilitates the retention of subgingival microbial plaque along with micro-niches for colonization by periodontopathic microorganisms.[11] Instruments used mechanically to prepare root surfaces, therefore, should not excessively damage, gouge and trough the root surface or remove injudicious amounts of tooth structure.[9,12] In this regard, it was seen in this study that ultrasonic instrumentation with perio mini-tip at a low power setting of '4', causes much less damage to the root surface when compared to hand instrumentation using curettes.

After comparing all SEM photomicrographs of representative sample teeth from the respective experimental subgroups, it can be concluded that within the limits of this study, hand instrumentation with curettes consistently produces the most smooth and even root surfaces, albeit at the cost of loss of excess tooth substance, when compared to ultrasonic instrumentation. Hand instrumentation can produce large number of scratches on root surface whereas ultrasonic root planing with perio mini-tip produce fewer scratches, and at the same time it does not cause undue loss of excess tooth substance and preserves cemental structure while producing a relatively smooth root surface. As because, in this study, ultrasonic instrumentation of root surface was time bound in terms of fixed number of seconds as against a fixed number of strokes with curette, it seems apparent from all the SEM photomicrographs that the result produced by 100 strokes of curette may be equivalent to the result produced by 45 seconds of ultrasonic instrumentation. However, since the sample tooth is different in different experimental criteria, the topography of the root surface mineral content as produced by the periodontal disease process might have influenced the topographical view of the surface under SEM. This, in turn, yielded the difference in surface smoothness after instrumentation.

Conclusion

1. It seems apparent after comparing all SEM photomicrographs of representative samples from all experimental groups that within the limits of this study, hand instrumentation with curette employing 150 strokes per surface produced the smoothest, cleanest, calculus free and the most even root surface topography.
2. The result produced by 100 strokes of curette seems to be equivalent to 45 seconds of ultrasonic instrumentation using microultrasonic perio mini-tip, as per visual inspection and comparison of SEM photomicrographs of the representative samples.
3. Amount of remaining calculus flecks and smear layer embedded in cementum even after scaling and root planing seems to be more in surfaces treated with ultrasonic perio mini-tip when compared to root surfaces treated with curettes.
4. Instrumentation marks or scratches are more in root surfaces treated with curettes when compared to surfaces instrumented with ultrasonic perio mini-tip.
5. Manual instrumentation with curettes consistently produces the smoothest and cleanest root surfaces albeit at the cost of loss of extra tooth substance, which may sometimes become a matter of concern for the clinicians because of the damage produced on the root surface due to injudicious removal of tooth structure. In contrast, ultrasonic instrumentation with the modern-day microultrasonic tips like the perio mini-tip, does not cause injudicious removal of tooth substance, preserves cemental structure and at the same time produces a relatively smooth root surface.

Conflict of Interest: None.

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