Microscopic and Elemental Analysis of Periodontally Diseased Root Surfaces for Evaluation of Efficacy of Scaling and Root Planing Done Under Magnification

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Abstract

Background: A randomized controlled clinical study was undertaken to evaluate the effectiveness of scaling and root planing (SRP) by using Magnifying Loupes (ML) and Dental Operating Microscope (DOM) and compared it with electron probe analysis.

Materials and Methods: A total of 15 human teeth scheduled for extraction from 3 patients aged between 25 and 65 years suffering from generalized chronic severe periodontitis were randomly assigned to three treatment groups. Group 1 consisted SRP performed without using magnification (unaided), Group 2-SRP with ML and Group 3-SRP with DOM. Following extractions, samples were prepared for (i) presence of smear layer, debris by scanning electron microscopy (ii) elemental analysis by energy dispersive X-ray analysis. Data was subjected to statistical analysis using analysis of variance, post-hoc (Tukey-HSD) and Chi-square test.

Results: Statistically significant (P < 0.001) difference was found among the different treatment groups. Group 3 was the best while Group 1 was the least effective technique for SRP.

Conclusion: Magnification tools significantly enhance the efficacy of supragingival and subgingival SRP.

Keywords: Energy Dispersive X-Ray Spectroscopy; Magnification; Scaling and Root Planing; Scanning Electron Microscopy

Introduction

Progression of chronic inflammatory periodontal disease leads to loss of periodontal attachment from the root surface and exposure of cementum to the environment of the periodontal pocket. The treatment of such periodontally involved cementum by root planing has for long been considered an important part of periodontal therapy [1]. Over the years, various rationales have been proposed for root planing including the removal of "porous and infected" cementum, the removal of "foreign body" cementum, elimination of calculus, the removal of softened cementum, the preparation of smooth root surface to facilitate plaque control and inhibit further plaque accumulation [2] and the removal of hyper mineralized cementum [1]. However, many of this previous treatment based on clinical impression rather than scientific investigation so that there is DOMe confusion as to what is to be achieved clinically by root planing [3].

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Root surface affected by periodontal disease may show various changes depending on the location of the root surface relative to the environment. Chemical analysis of the exposed root surface has shown an increase in calcium, magnesium, and phosphorus with a depth of penetration 50 μm or less into the cementum. The crystals of the hypermineralized surface zone were observed to be larger than in the subjacent cementum. A limited number of studies have used an electron probe to analyze the distribution of various elements in cementum but no consensus could be reached regarding the occurrence or distribution of various elements and conflicting data were reported [4].

Materials and Methods

This clinical trial was conducted in post-graduate Department of Periodontics, Chandra Dental College and Hospital, Safedabad, U.P. The research protocol was reviewed and approved by the local Research Ethics Committee.

Study design

This is a prospective and randomized longitudinal study which evaluates efficacy of SRP under magnification. A sample size of 15 human teeth scheduled for extraction from 3 patients aged between 25 to 65 years suffering from generalized chronic periodontitis and one tooth which are indicated for orthodontic extraction was randomly assigned to following 3 treatment groups. In all the 3 groups, SRP was performed by Ultrasonic scaler in combination with hand instruments till the complete removal of calculus and diseased cementum was achieved.

Group 1: SRP performed by magnetostrictive ultrasonic scaler (Cavitron, Dentsply) followed by hand instruments with sharp Gracey curettes and Subgingival scalers (Hu-Friedy) without using any magnification device, i.e., unaided.

Group 2: SRP performed by magnetostrictive ultrasonic scaler (Cavitron, Dentsply) followed by hand instruments with sharp Gracey Curettes and Subgingival Scalers (Hu-Friedy) with the help of ML of magnification ×4.5 to ×5.5 (Microsurgery Instruments Co: Texas).

Group 3: SRP performed by magnetostrictive ultrasonic scaler (Cavitron, Dentsply) followed by hand instruments with sharp Gracey Curettes and Subgingival Scalers (Hu-Friedy) with DOM of magnification ×3.5, ×5.0, ×8.5, ×13.5, ×20.5 (3D Medical Systems, USA).

Inclusion criteria

- Patients with good general health, aged between 2 and 65 years suffering from generalized chronic sever periodontitis.

- Teeth scheduled for the extraction having 8 - 10m attachment loss.

- Caries free teeth.

- Teeth without wasting diseases or cervical restorations.

Exclusion criteria

- Patients allergic to local anesthesia.

- Endodontically involved teeth.

- Pregnant women.

Microanalysis of root planed surface was done with SEM along with Elemental Analysis with Energy Dispersive Spectroscopy to evaluate the surface topography and/or presence/absence of debris, smear layer, calculus, scratches and/or the opening of dentinal tubules respectively.

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Clinical procedure

Scaling and Root planing was performed on the teeth to be extracted, on all the four surfaces magnetostrective ultrasonic scaler, followed by hand instruments with sharp subgingival Gracey curettes, hoes and periodontal files according to Group I, II and III.

Before extraction of the teeth, area was marked from cementoenamel junction to gingival margin with the help of bur, making a groove at the location of gingival margin.

Teeth were then extracted after suitable anesthesia with the beak of extraction forceps, placed above the cementoenamel junction, avoiding any trauma to the root surface. Teeth were rinsed thoroughly under running tap water and brushed lightly with an ultra-soft bristle brush for removal of soft tissue tags. Teeth in three different groups were then placed in normal saline solution and transported SEM along with EDAX analysis.

Samples for SEM analysis and EDAX evaluation

Section cutting of all the samples of selected teeth were stored in normal saline to avoid dehydration and taken to Birbal Sahini Institute of Palaeobotany, Lucknow for SEM along with EDAX evaluations. Then sections were mounted on Aluminium Stub with the help of double slide tape, electro conductive material Dotite (called as Silver Paint) applied on ends of the samples to make contact with the stub. The mounted stub were place in Polaron Sputter Coater in which sample were coated with gold pallidium for 160 seconds at 18 mA current. Coater stubs were examined under SEM and exposure were taken on desired location and magnification. Photographs of the central portion of each specimen were taken at of 3200X magnification by Scanning Electron Microscope (Company LEO 430 USA).

SEM examination was performed by a single blinded examiner. The following parameters were evaluated: surface morphology (regular, irregular or flaky surface), presence or absence of smear layer, debris, calculus, scratches and the opening of dentinal tubules.

Grading: The grades are given as follows [5]:

- Grade I: Absence of visible debris and plaque, with good exposure of dentinal tubules and no evidence of remaining smear layer.

- Grade II: No visible debris, no exposure of dentinal tubules, and presence of smear layer.

- Grade III: Presence of visible debris and plaque all over the scanned area, no visible tubuli and smear layer present on the entire surface.
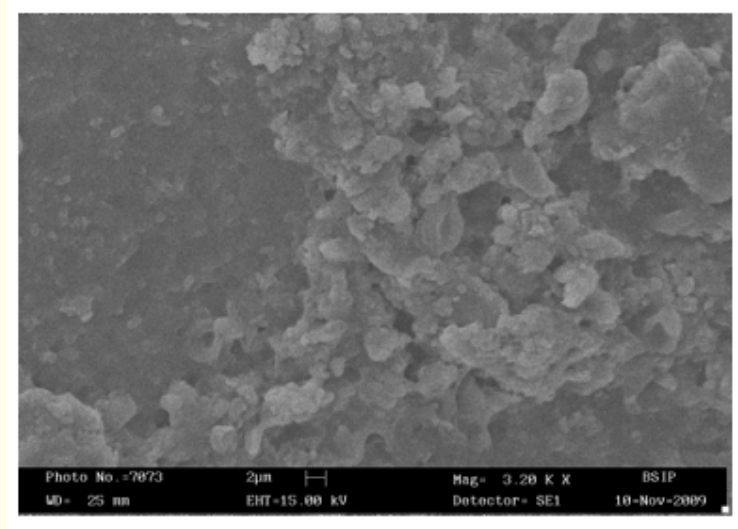
The elements analyzed and compared in electron probe study were those with an atomic number of 11 or higher, having enough intensity that the EDAX could discern them from background or scatter radiation. Minerals most often detected in the specimens were phosphorus (P), calcium (Ca), copper (Cu), zinc (Zn), magnesium (Mg) and occasionally sodium (Na).

All the observation were tabulated by one observer only and they were subjected to statistical analysis. The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean ± SD.

Results

Group 1 sample

SEM analysis of this group showed the presence of visible debris all over the scanned area, smear layer present on the entire surface and no visible dentinal tubules at magnification ×3200 (Figure 1) and EDAX analysis revealed maximum levels of mineral contents for magnesium (Mg), potassium (K) and minimum P on the surface in weight %.

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Figure 1: Presence of visible debris all over the scanned area, smear layer present on the entire surface, and no visible dentinal tubules at magnification 3200X.

Group 2 sample

SEM analysis of the same group showed no visible debris, with DOM opening of dentinal tubules and presence of smear layer on the surface at magnification ×3200 (Figure 2) and EDAX analysis revealed mineral contents on the surface of ML sample in weight%.
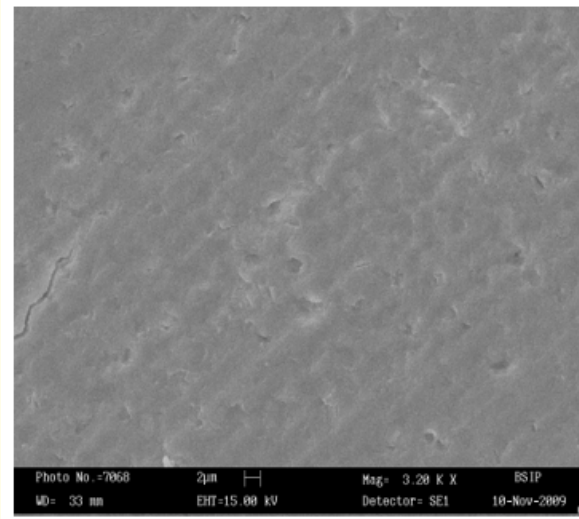
Figure 2: No visible debris, with some opening of dentinal tubules, and presence of smear layer on the surface at magnification 3200 X.

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Group 3 sample

SEM analysis of the same group showed absence of visible debris with good exposure of dentinal tubules and no evidence of remaining smear layer at magnification ×3200 (Figure 3) and EDAX analysis revealed complete elimination of diseased cementum.
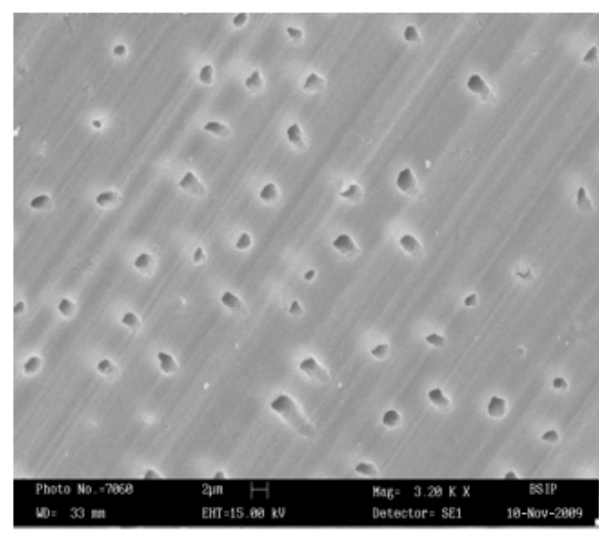
Figure 3: Absence of visible debris with good exposure of dentinal tubules and no evidence of remaining smear layer at magnification 3200 X.

EDAX results using ANOVA for mineral levels in different groups is shown in table 1, revealing statistically significant intergroup differences ($P < 0.05$) for magnesium-potassium (Mg-K) and calcium/magnesium (Ca/Mg). Multiple comparisons using Tukey HSD test in table 2, for phosphorus-potassium (PK), calcium-potassium (CaK) and calcium/phosphorus (Ca/P), didn't reveal a statistically significant difference ($P > 0.05$).

		Sum of Squares	df	Mean Square	F	Sig.
MgK	Between Groups	4.506	2	2.253	19.067	< 0.001
	Within Groups	1.418	12	.118		
	Total	5.924	14			
PK	Between Groups	6.615	2	3.307	1.840	0.201
	Within Groups	21.570	12	1.798		
	Total	28.185	14			
CaK	Between Groups	2.611	2	1.306	2.081	0.168
	Within Groups	7.528	12	.627		
	Total	10.140	14			
Ca/P	Between Groups	.065	2	.032	2.554	0.119
	Within Groups	.152	12	.013		
	Total	.217	14			
Ca/Mg	Between Groups	885.754	2	442.877	11.779	0.001
	Within Groups	451.171	12	37.598		
	Total	1336.925	14			

Table 1: Analysis of variance revealed statistically significant intergroup differences (p < 0.05) for MgK and Ca/Mg only.

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Multiple comparisons (Tukey HSD)							
Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Upper Bound	Lower Bound
MgK	NE	Loupe	0.428	0.217	0.162	-0.152	1.008
		SOM	1.316	0.217	< 0.001	0.736	1.896
	Loupe	NE	-0.428	0.217	0.162	-1.008	0.152
		SOM	0.888	0.217	0.004	0.308	1.468
	SOM	NE	-1.316	0.217	< 0.001	-1.896	-0.736
		Loupe	-0.888	0.217	0.004	-1.468	-0.308
PK	NE	Loupe	-0.402	0.848	0.885	-2.664	1.860
		SOM	-1.566	0.848	0.197	-3.828	0.696
	Loupe	NE	0.402	0.848	0.885	-1.860	2.664
		SOM	-1.164	0.848	0.385	-3.426	1.098
	SOM	NE	1.566	0.848	0.197	-0.696	3.828
		Loupe	1.164	0.848	0.385	-1.098	3.426
CaK	NE	Loupe	0.892	0.501	0.217	-0.444	2.228
		SOM	0.878	0.501	0.227	-0.458	2.214
	Loupe	NE	-0.892	0.501	0.217	-2.228	0.444
		SOM	-0.014	0.501	1.000	-1.350	1.322
	SOM	NE	-0.878	0.501	0.227	-2.214	0.458
		Loupe	0.014	0.501	1.000	-1.322	1.350
Ca/P	NE	Loupe	0.052	0.071	0.751	-0.138	0.242
		SOM	0.158	0.071	0.108	-0.032	0.348
	Loupe	NE	-0.052	0.071	0.751	-0.242	0.138
		SOM	0.106	0.071	0.331	-0.084	0.296
	SOM	NE	-0.158	0.071	0.108	-0.348	0.032
		Loupe	-0.106	0.071	0.331	-0.296	0.084
Ca/Mg	NE	Loupe	-5.002	3.878	0.427	-15.348	5.344
		SOM	-18.216	3.878	0.001	-28.562	-7.870
	Loupe	NE	5.002	3.878	0.427	-5.344	15.348
		SOM	-13.214	3.878	0.013	-23.560	-2.868
	SOM	NE	18.216	3.878	0.001	7.870	28.562
		Loupe	13.214	3.878	0.013	2.868	23.560

***Table 2:** For PK, CaK and Ca/P, multiple comparisons using Tukey HSD test did not reveal a statistically significant difference (p > 0.05). For MgK, the mean value obtained for NE was significantly higher as compared to both Loupe and SOM; no statistically significant difference was seen between NE and Loupe groups. For Ca/Mg too, mean value of SOM was significantly higher as compared to NE and Loupe while there was no significant difference between NE and Loupe groups.*

For Mg-K, the mean value obtained for unaided was significantly higher as compared to both Loupe and DOM; no statistically significant difference was seen between unaided and Loupe groups. For Ca/Mg too, mean value of DOM group was significantly higher as com-

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pared with unaided and Loupe groups while there was no significant difference between unaided and ML groups. For Mg-K, all the three groups had significantly higher mean value as compared with negative control ($P < 0.05$), the significance of difference was very highly significant for unaided and ML groups ($P < 0.001$) while for DOM group it was just significant ($P = 0.020$).

As compared with positive control, all three groups had significantly lower mean values. The AQ1 difference from positive control was highly significant for unaided group (P = 0.003) while for ML and DOM groups it was very highly significant (P < 0.001). For PK, all three groups had no statistically significant difference from negative control (P > 0.05). However, as compared with positive control, DOM group had significantly higher mean value (P = 0.006) while the other two groups did not show a statistically significant difference. Table 2 reveals for PK, CaK and Ca/P, multiple comparisons using Tukey HSD test did not reveal a statistically significant difference (p>0.05). For MgK, the mean value obtained for NE was significantly higher as compared to both Loupe and DOM; no statistically significant difference was seen between NE and Loupe groups. For Ca/Mg too, mean value of DOM was significantly higher as compared to NE and Loupe while there was no significant difference between NE and Loupe groups.

For Ca/K, all the three groups had significantly lower mean value as compared with negative control ($P < 0.05$), however for positive control, unaided group did not show a significant difference ($P = 0.775$) while the mean value of ML and DOM groups were significantly lower ($P < 0.05$).

For Ca/P, the mean value of unaided group and ML group was not significantly different from either positive control or negative control. However, for DOM group the mean value showed no significant difference from negative control, but the difference from positive control was significant statistically ($P = 0.022$).

For Ca/Mg, all the three groups had statistically significant differences from both positive and negative controls ($P < 0.05$) as seen in table 3.

S. No.	Mineral	-ve Control	+ve Control	Significance of Difference from Control ("p" value)					
				Naked Eye (n = 5)		Loupe (n = 5)		SOM (n = 5)	
				-ve Control	+ve Control	-ve Control	+ve Control	-ve Control	+ve Control
1.	MgK	1.01	4.08	< 0.001	0.003	< 0.001	< 0.001	0.020	< 0.001
2.	PK	30.17	29.4	0.172	0.783	0.271	0.801	0.289	0.006
3.	CaK	63.9	61.33	0.036	0.775	< 0.001	0.005	0.001	0.048
4.	Ca/P	2.04	2.09	0.217	0.531	0.449	0.881	0.111	0.022
5.	Ca/Mg	63.2	15.32	< 0.001	0.002	< 0.001	0.001	0.006	0.005

Table 3: Comparison of mean values of minerals in different groups as compared to normal root (without calculus - negative control) and subgingival calculus (positive control) values. One sample "t" test was used to calculate significance of difference from control.

Grade distribution in three groups is depicted in table 4. The entire specimen in DOM group were graded as I, all the Loupe group were graded as II and all the specimen in Naked eye group were graded as III. There was a statistically significant difference among the groups (p < 0.001).

SN	Grade	Naked Eye (n = 5)		Loupe (n = 5)		SOM (n = 5)	
		No.	%	No.	%	No.	%
1.	I	0	0	0	0	5	100
2.	II	0	0	5	100	0	0
3.	III	5	100	0	0	0	0

Table 4: Analysis for the grades comparison in three groups.
$\chi^2 = 1440; p < 0.001.$

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Discussion

The present study evaluates the effectiveness of supragingival and subgingival scaling and root planing (SRP) under different magnifications using ML and DOM. Microanalysis of root surface was done by using SEM, EDAX to study the root surface characteristics, elements following SRP under magnification.

The results of the SEM of the diseased cementum surface revealed the presence of rough irregular surface with multiple resorption lacunae of variable depths. This is in agreement with Adriaens., *et al*. [6] and they explained that these lacunae may display a route of entry for bacteria into root cementum and radicular dentin. Daly., *et al*. [7] also showed cracks within cementum from periodontally- involved root surfaces. These surfaces topographical features may be of importance in therapeutic interventions aimed at rendering the root surface biologically acceptable. Eide., *et al*. [8] have observed a mineralized surface coating on dental cementum incident to periodontal disease and they stated that this coating is derived from components of inflammatory exudates within periodontal pockets and that this might be a reservoir of cementum-associated LPS. In addition, results obtained by other investigators strongly suggested that improved cellular attachment can be promoted on areas of root surface previously damaged by periodontitis if superficial cementum is first removed by mechanical curettage.

Results of this study revealed that DOM group was the best while Naked eye group was the least effective as far the cleaning efficacy was concerned, Mandibular teeth were more efficaciously managed as compared to Maxillary teeth, Anterior teeth were more efficaciously managed as compared to Posterior teeth, Buccal aspects were most efficaciously cleaned then Mesial, Distal, Lingual and least effective was Palatal. No significant difference in efficacy was seen for subgingival/supragingival areas in higher magnifications in DOM. The findings depict the order of efficacy of the three techniques for all the parameters to be in the order: DOM > Loupe > Naked Eye.

This study was also concerned with mineral or element changes on and within the root structure attributable to chronic, destructive periodontal disease. The electron microprobe analysis appears to be the most suitable technique for examining elemental composition within localized small areas of mineralized tissue. Its main advantage is its capability to analyze in situ volumes on the order of a few cubic microns.

The findings of Ca, P, and Mg in cementum agree with the studies of Neiders [9], who found them in healthy young permanent teeth. The significantly higher values for Mg in the diseased teeth were agreed from the report by Selvig., *et al* [10]. They showed that Ca^+ and Mg content in supragingival cervical cementum of periodontally diseased teeth were DOM what higher than in healthy teeth. In an electron probe study, Selvig and Hals [11] found Mg content to be the same for diseased and non-diseased teeth. Rita M Khounganian., *et al.* [12] also found Ca and P and Mg contents of the diseased cementum surface were higher in comparison to non-diseased cementum.

In EDAX results for MgK, Group NE had maximum levels followed by Group Loupe and then Group DOM. However, for PK, the order was just reverse with Group NE showing minimum and Group DOM showing maximum values. For CaK, there was not much difference yet Group NE had maximum value and Group Loupe had minimum value. For Ca/P, Groups NE and Loupe had very close values while Group DOM had minimum value. For Ca/Mg the differences were most evident, showing minimum value in Group NE and maximum value for Group DOM.

Conclusion

1. It was proved from the analysis of results that magnification tools significantly enhance the efficacy of supra gingival and sub gingival scaling and root planing.

2. Microanalysis by SEM revealed that the DOM proved to be the best aid in producing smooth root surface free of debris, calculus, scratches etc.

3. In elemental analysis EDAX, revealed that DOM eliminated diseased cementum.

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