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Dentinal Tubules of Attrited, Abraded and Eroded Teeth- A Comparative Evaluation of Scanning Electron and Light Microscope

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Authors' contributions

This work was carried out in collaboration between all authors. Author MC designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors BS and MK managed the literature searches and protocol of study and authors MC and SC managed the literature search and experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim was to study the ultrastructure of dentin exposed by attrition, abrasion and erosion separately and in combination and to visualize the lesions using scanning electron microscope and thereby establish a superior basis for evaluating dentinal changes at the ultra-structural level and to correlate the findings with changes seen at the light microscopic level.

Study Design: Tooth wear is often multifactorial. Loss and excessive wear of hard dental tissues is a permanent problem of the dentition, especially in present era, encompassing almost all age groups. In this study 40 extracted teeth were used.

Four groups were made and comparison was made between light microscope and scanning

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electron microscopic findings.
Place and Duration of Study: Department of oral and maxillofacial pathology, career post graduate institute of dental sciences, lucknow, (U.P.), India.
Methodology: Forty extracted permanent teeth (10 attrited, 10 abraded, 10 eroded and 10 normal premolars and molars) were sampled divided into four groups. After debridement and fixation in 10% formalin for 24-48 hours, the teeth were fractured along their longitudinal axes. Two halves of the teeth were studied under scanning electron and light microscope. The dentinal changes secondary to attrition, abrasion and erosion such as dead tracts, dentinal sclerosis and reparative dentin formation seen under light microscopy, were correlated with the ultra-structural findings. The data so obtained were tabulated and statistically analyzed.
Results: The light microscopic finding of 4 randomly selected teeth, showed frequency of tertiary dentin significantly more than dead tracts and dentinal sclerosis was absent, from each group. 10 samples scanned with electron microscope showed the tubular surface/margins smooth, tubular surface/margins rough and presence of crystals was highly significant ($p<0.001$) whereas peritubular dentin and intertubular dentin, was not significant ($p>0.05$) in each group. Similarly, mean density of dentinal tubules of normal group was the highest followed by abraded, attrited and eroded the least. The percentage of affected dentinal tubules was most in eroded & least in attrited teeth.
Conclusion: The scanning electron microscope is a powerful magnification tool which offers extremely high resolution. In the present study, scanning electron microscopic image complements the information available from the light microscope about the dentinal changes in abraded, attrited and eroded teeth.

Keywords: Attrition; abrasion; erosion; dead tracts; tertiary dentin; scanning electron microscope.

1. INTRODUCTION

Tooth wear is an inherent part of the aging process which occurs continuously but slowly throughout life. Loss and excessive wear of hard dental tissues is a permanent problem of the dentition, especially in modern man, encompassing almost all age groups. In some individuals tooth wear can be manifested to a greater extent thus leading to severe morphological, functional and vital damages to the dentition. Among individuals the regressive changes vary in etiology, extent and clinical presentation and are associated with physiologic or pathologic processes. Some of the regressive changes also known as the wasting diseases of teeth result from aging, and others occur due to chronic persistent tissue injury. Traditionally these entities have been classified as attrition, abrasion and erosion. These three are separate, distinct processes, each of which results in loss of tooth structure. Tooth wear is often multifactorial and the pattern and occurrence of tooth wear is related to dietary, occupational, cultural and geographic factors in a population. Other factors that can be considered include aging and occlusal relationship. There is general agreement in literature that changes occur in dentin exposed by attrition and abrasion [1-4]. The main cause of tooth wear in populations appears to have been due to some combination of friction of exogenous material forced over tooth surfaces and an increase in the number of power strokes during mastication when less refined, tougher foods are consumed.

The light microscopic changes secondary to attrition and abrasion (due to the exposure of dentinal tubules) include dead tracts, sclerosis and reparative dentin formation. A conspicuous finding under scanning electron microscope was irregularly angulated crystals. The crystals were identified as whitlockite and similar crystals have been observed by others as well. [1,5] however, there are only few ultra-structural studies on the changes of dentin secondary to erosion. Hence in spite of a number of reported studies, a clear consensus on the nature of these changes is not fully explained.

The SEM (scanning electron microscope) is a powerful magnification tool which offers extremely high resolution, three-dimensional images which provide intricate and minute detail of topographical, morphological and compositional information of the specimen making them invaluable in today's world of cutting edge research.

The present study was undertaken to study the ultrastructure of dentin exposed by attrition, abrasion and erosion separately and in combination and to visualize the lesions using scanning electron microscope and thereby establish a superior basis for evaluating dentinal changes at the ultra-structural level and to correlate the findings with changes seen at the light microscopic level.

2. MATERIALS AND METHODS

Forty extracted permanent premolars and molars teeth (Group-I-10 attrited, Group-II- 10 abraded, Group-III- 10 eroded and Group-IV-10 normal) constituted the study material. After debridement and fixation in 10% formalin for 24-48 hours, the teeth were fractured along their longitudinal axes. One half of each tooth was made into an approximately 100 μ thick ground section and the other half was utilized for scanning electron microscopy. Based on the microscopic findings observed in ground sections under transmitted light, corresponding areas were selected for study under the scanning electron microscope. The SEM half of each tooth was scanned from the lesional surface. The dentinal changes secondary to attrition, abrasion and erosion such as dead tracts, dentinal sclerosis and reparative dentin formation seen under light microscopy, were correlated with the ultra-structural findings. The points observed while scanning the lesional surfaces were:

1. Diameter and density of dentinal tubules in affected area,
2. Distinctness of peritubular and intertubular dentin,
3. Integrity of dentinal tubular surface and margins,
4. Presence of crystals within the tubules,
5. Occlusion of tubules.

The normal teeth were scanned first to form a base for the interpretation of samples with lesions. The nature of the normal dentin was observed in different areas and the findings noted down. To ensure uniformity, for each specimen in every group, the fields, closer to the lesional area were selected under x850, x2200 and/or x5000 magnification, among the photographs taken and the changes were interpreted. The findings were tabulated and statistically analyzed.

3. RESULTS

The frequency distribution of no of teeth/group is shown in Table 1.

3.1 Statistical Analysis

Continuous data were summarized as Mean ± SD while discrete (categorical) in %. Continuous groups were compared by one-way analysis of variance (ANOVA) after ascertaining the normality and homogeneity by Shapiro-Wilk and Levene's test respectively. Categorical data were analyzed with chi-square (χ^2) or Fisher's exact tests. A two-sided (α=2) $p<0.05$ was considered statistically significant.

3.1.1 Light microscopic findings

For each group, five random teeth were selected for light microscopic findings (dead tracts, Dentinal sclerosis and tertiary dentin). The light microscopic findings of four groups (Normal, Attrited, Abraded and Eroded) were summarized in Table 2.

3.1.2 Scanning electron microscopic findings

For each group, 10 teeth were scanned for peritubular and intertubular dentin, tubular surface/margins- smooth, tubular surface/margins- rough and presence or absence of crystals by electron microscope and the findings were summarized in Table 3.

3.1.3 Variations in diameter and density

The variation in diameter and density of dentinal tubules of four groups were summarized in Table 4. Table 4 showed that the mean diameter of dentinal tubules of normal group was the highest followed by eroded, abraded and attrited the least. Similarly, mean density of dentinal tubules of normal group was the highest followed by abraded, attrited and eroded the least.

Table 1. Frequency distribution of no of teeth of four groups

Study groups	Description	Sample size
Group I	Normal (premolars & molars)	10
Group II	Attrited (premolars & molars)	10
Group III	Abraded (premolars & molars)	10
Group IV	Eroded (premolars & molars)	10

3.1.4 Variations in affected tubules

The variation in density, number of affected tubules/field and % of affected tubules/field of three groups (attrited, abraded and eroded) were summarized in Table 7.

4. DISCUSSION

When the dentin of a tooth is exposed as a result of attrition, abrasion and erosion the chronic exogenous irritation that occurs causes various responses on and in the tissue. Hypermineralization of the intertubular dentin, deposits on inorganic substance in the dentinal tubules, Tomes fibers and development of peritubular dentin have been reported [6-10]. These responses have been studied histologically, historadiographically [11], and with the electron microscope. There are few studies in which the SEM was used to observe these responses of dentin [7,10]. Hence the present study was undertaken to observe the changes in and within dentin and dentinal tubules of attrited, abraded and eroded teeth.

Table 2. Frequency distribution of light microscopic findings of four groups

Variable	Characteristics	Normal (n=5)	Attrited (n=5)	Abraded (n=5)	Eroded (n=5)	χ^2 value	p value
Dead tracts	Absent	5 (100.0%)	0 (0.0%)	0 (0.0%)	3 (60.0%)	15.00	0.002
	Present	0 (0.0%)	5 (100.0%)	5 (100.0%)	2 (40.0%)		
	Distinct	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Dentinal sclerosis	Absent	5 (100.0%)	5 (100.0%)	5 (100.0%)	5 (100.0%)	NA	NA
	Present	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
	Distinct	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Tertiary dentin	Absent	5 (100.0%)	0 (0.0%)	0 (0.0%)	5 (100.0%)	20.00	p<0.001
	Present	0 (0.0%)	5 (100.0%)	5 (100.0%)	0 (0.0%)		
	Distinct	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		

Inference: *Frequency of tertiary dentin is significant more than dead tracts. Dentinal Sclerosis was absent*

Table 3. Frequency distribution of scanning electron microscopic findings

Variable	Characteristics	Normal (n=10)	Attrited (n=10)	Abraded (n=10)	Eroded (n=10)	χ^2 value	p value
Peritubular and Intertubular dentin	Absent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2.02	0.568
	Present	6 (60.0%)	3 (30.0%)	4 (40.0%)	5 (50.0%)		
	Distinct	4 (40.0%)	7 (70.0%)	6 (60.0%)	5 (50.0%)		
Tubular surface/margins- Smooth	Absent	0 (0.0%)	9 (90.0%)	7 (70.0%)	0 (0.0%)	30.50	p<0.001
	Present	7 (70.0%)	1 (10.0%)	3 (30.0%)	5 (50.0%)		
	Distinct	3 (30.0%)	0 (0.0%)	0 (0.0%)	5 (50.0%)		
Tubular surface/margins- Rough	Absent	10 (100.0%)	1 (10.0%)	3 (30.0%)	9 (90.0%)	24.87	p<0.001
	Present	0 (0.0%)	3 (30.0%)	3 (30.0%)	1 (10.0%)		
	Distinct	0 (0.0%)	6 (60.0%)	4 (40.0%)	0 (0.0%)		
Presence or absence of crystals	Absent	10 (100.0%)	1 (10.0%)	3 (30.0%)	8 (80.0%)	23.04	p<0.001
	Present	0 (0.0%)	4 (40.0%)	4 (40.0%)	2 (20.0%)		
	Distinct	0 (0.0%)	5 (50.0%)	3 (30.0%)	0 (0.0%)		

Inference: *The tubular surface /margins smooth & tubular surface /margins rough & presence of crystals was highly significant (p<0.001) & peritubular dentin & intertubular dentin, was not significant (p>0.05)*

Table 4. Variation (Mean ± SD) in diameter and density of dentinal tubules of four groups

Variable	Normal (n=10)	Attrited (n=10)	Abraded (n=10)	Eroded (n=10)
Diameter (µm)	2.28±0.36 (1.70-2.60)	1.36±0.34 (1.11-2.05)	1.37±0.34 (0.93-2.00)	2.08±0.34 (1.33-2.50)
Density (No. of tubules/field)	22.60±3.37 (15.00-27.00)	17.20±3.26 (13.00-23.00)	17.70±2.45 (14.00-21.00)	14.70±3.13 (10.00-20.00)

Inference: *The diameter of dentinal tubules was most in normal & least in abraded*
The density of dentinal tubules was most in normal & least in eroded
Numbers in parenthesis indicates the range (min-max)

Table 5. ANOVA summary

Variable	Source of variation (SV)	Sum of squares (SS)	Degrees of freedom (DF)	Mean square (MS)	F value	p value
Diameter	Groups	6.86	3	2.29	19.14	p<0.001
	Error	4.30	36	0.12		
	Total	11.16	39	2.41		
Density	Groups	327.70	3	109.23	11.56	p<0.001
	Error	340.20	36	9.45		
	Total	667.90	39	118.68		

Inference: *Diameter and density of dentinal tubules was highly significant*

Table 6. Comparison (p value) of mean difference between the groups by Tukey's test

Comparisons	Diameter	Density
Normal vs. Attrited	p<0.001	0.002
Normal vs. Adraded	p<0.001	0.006
Normal vs. Eroded	0.585	p<0.001
Attrited vs. Abraded	0.999	0.983
Attrited vs. Eroded	p<0.001	0.282
Abraded vs. Eroded	p<0.001	0.148

Inference: *Diameter of dentinal tubules was highly significant (normal vs attrited, normal vs abraded, attrited vs eroded & abraded vs eroded)*

Table 7. Variation (Mean ± SD) in density and density of affected dentinal tubules of three groups

Variable	Attrited (n=10)	Abraded (n=10)	Eroded (n=10)
Density (No. of tubules/field)	17.20±3.26 (13.00-23.00)	17.70±2.45 (14.00-21.00)	14.70±3.13 (10.00-20.00)
No. of affected tubules/field	7.60±1.43 (6.00-10.00)	9.00±1.49 (7.00-11.00)	7.60±1.96 (4.00-10.00)
% of affected tubules/field	44.94±8.55 (33.33-56.25)	51.15±7.46 (38.10-64.71)	52.04±11.36 (40.00-75.00)

Inference: *Percentage of affected dentinal tubules was most in eroded & least in attrited. Numbers in parenthesis indicates the range (min-max)*

Table 8. ANOVA summary

Variable	Source of variation (SV)	Sum of squares (SS)	Degrees of freedom (DF)	Mean square (MS)	F value	p value
Density	Groups	51.67	2	25.83	2.93	0.070
	Error	237.80	27	8.81		
	Total	289.47	29	34.64		
Density (No. of tubules/field)	Groups	13.07	2	6.53	2.42	0.108
	Error	72.80	27	2.70		
	Total	85.87	29	9.23		
% of affected tubules/field	Groups	299.04	2	149.52	1.74	0.195
	Error	2320.26	27	85.94		
	Total	2619.30	29	235.46		

Inference: *p value was not significant*

Table 9. Comparison (p value) of mean difference between the groups by Tukey's test

Comparisons	Density	Density (No. of Affected tubules/field)	% of affected tubules/field
Attrited vs. Abraded	0.925	0.156	0.308
Attrited vs. Eroded	0.163	1.000	0.219
Abraded vs. Eroded	0.079	0.156	0.975

The light microscopic findings revealed that all the attrited, abraded and eroded teeth showed the formation of dead tracts and variable amounts of reparative dentin on the pulpal side. This was consistent with findings of Richardson A (1966) who observed an association between exposure of dentin to the mouth by attrition and dead tract formation in the subjacent dentin [12]. Areas identified as dead tracts, dentinal sclerosis and reparative dentin were subjected to observation under the SEM, in addition to lesional surfaces. Normal teeth showed no evidence of dead tracts, dentinal sclerosis or reparative dentin formation. These three parameters were observed together exclusively in our study. Scanning electron microscopically, the mean tubular diameter, closer to lesional surface was most for normal, followed by attrited, then abraded and least for eroded teeth. The diameters of tubules were more in normal as compared to lesional surfaces of the other three groups. This was a unique finding in our study which compared the diameter of all the three groups (attrited, abraded and eroded) together against that of normal.

In normal teeth, many of the dentinal tubules showed distinct peritubular and intertubular dentin. The margins/walls of the tubules were smooth and there was no evidence of any crystals within the tubules. The peritubular dentin was distinctly denser and finely crystalline, in comparison to coarse crystalline nature of intertubular dentin (Fig. 1). This was in accordance with the study done by Tronstad L (1972) on human coronal dentin [1].

In attrited and abraded teeth, distinct peritubular and intertubular dentin was observed in many tubules in the dead tract area. The peritubular dentin was denser in the area closer to the lesional surface. Many of the tubules showed roughened margins/surface and irregular tubular wall due to small globular calcifications. Crystals of a variety of shapes (including rhomboid and cuboidal) and sizes, were seen in many of the dentinal tubules (Figs. 2,3) [13-15].

It was obvious that these have originated from within the tubules and not been introduced from outside. The two possibilities could be 1) the mineralization induced by the damaged odontoblastic process, 2) The acidic media penetrating the tubules might have caused demineralization of the tubular wall and when neutralized, the supersaturated solution might have induced mineralization on the walls of the tubules and crystal formation around central nidi. Interspersed between the affected were unaffected dentinal tubules with smooth margins and tubular walls. This was in agreement with SEM investigation by Yagi T, (1990) on human sclerosed dentinal tubules secondary to attrition and abrasion lesions and study by Isokawa S, et al. (1973) on dentin exposed by contact facets and cervical abrasion [7,16].

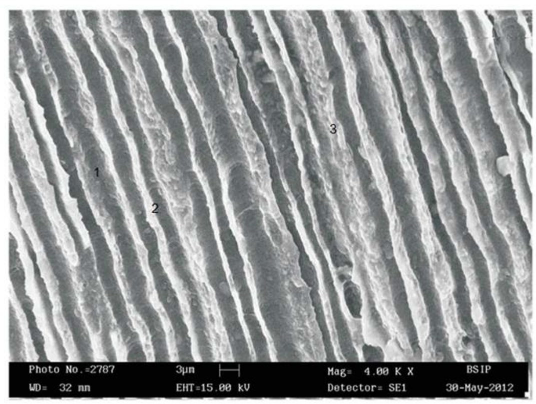

Fig. 1. Scanning electron micrograph of a normal tooth showing (1) dentinal tubules (2) peritubular dentin and (3) intertubular dentin & smooth margins of the tubules (mag: 4.00 kx)

In eroded teeth, distinct peritubular and intertubular dentin was seen in all of the tubules and majority of tubules showed smooth surface margins/walls. The dentinal tubules were patent with intermediate plugging found between their lengths which probably explain the frequent symptom of erosion, the painful sensitivity of the affected teeth, first reported by Miller in 1907 (Fig. 4).

This was consistent with more recent studies which have shown that acid erosion is associated with dentin hypersensitivity and acids readily remove the dentin smear layer to expose the tubules [17-19].

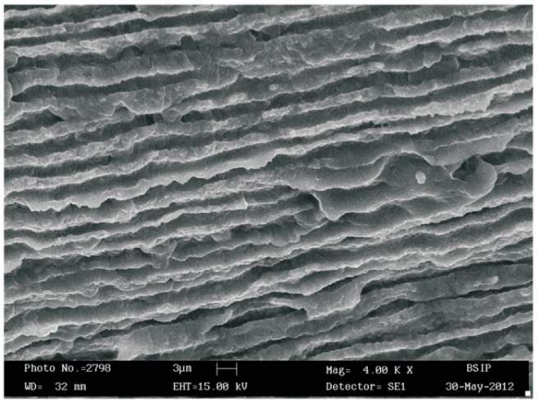

Fig. 2. Scanning electron micrograph of an attrited tooth showing, roughening of the tubular walls margins with some irregularly shaped crystals within the tubules & variation in the diameter of the tubules can be noted (mag: 4.00kx)

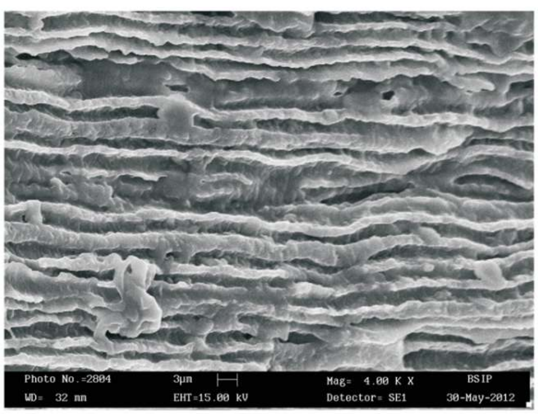

Fig. 3. Scanning electron micrograph of an abraded tooth showing, roughening of the tubular walls margins with some irregularly shaped crystals within the tubules & variation in the diameter of the tubules can be noted (mag: 4.00kx)

Thus, in our study correlating the findings of light microscopy with that of SEM, areas of dead tracts which were observed under light microscopy, revealed the presence of diseased tubules showing varying degree of changes under SEM. These areas of dead tract formation in relation to attrition, abrasion and erosion appear, dark under transmitted light and bright under reflected light. These diseased or damaged tubules predominated within the dead tract area. However completely unaffected tubules, without any degenerative changes as in the case of normal dentin were found occasionally adjacent to the affected tubules. In the dead tract areas of attrited, abraded and eroded teeth, the percentage of affected tubules was more in the area closer to the lesion surface as compared to the area closer to the pulp [20]. The cause of the appearance of the dead tracts in the exposed dentinal tubules has been controversial. The most common explanation has been that the sealed off dentinal tubules contain air and this results in the appearance of dead tracts [20,21]. The other explanation is the accumulation of crystals within the dead tracts (Toda et al.) [4]. The changes in diseased tubules appeared to affect the entire length of the tubules, even though individual tubules could not be traced to its full length. The affected tubules showed irregularities in the walls of the tubules which obviously had resulted in rough irregular margins of the tubules and same can be seen in topography of these lesions as discussed by B. Hur et al. in 2011[22].

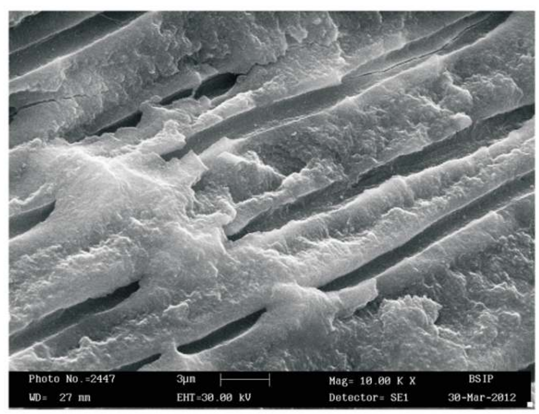

Fig. 4. Scanning electron micrograph of an eroded tooth showing smooth surface margins with plugging in between and absence of any crystals (mag: 10.00 kx)

Variable amounts of reparative dentin formation under light microscopy was also found on the pulpal aspect of all attrited teeth but only in few of abraded and eroded teeth. This was in accordance with the features of reparative dentin which is deposited at sites of the pulpal aspects of primary or secondary dentin, corresponding to areas of external irritations and due to the absence of tubules, and appears bright in transmitted light and dark in reflected light [4].

5. CONCLUSION

To conclude, the present study revealed that all cases of attrition, abrasion and erosion with dentin exposure showed the formation of dead tracts and variable amounts of reparative dentin, under light microscopy. Under SEM, the predominant finding in the areas of dead tracts of attrited, abraded and eroded teeth was the presence of diseased or affected dentinal tubules. The diseased tubules were identifiable because of the presence of crystals and globular calcifications on the walls of the tubules.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical committee of the institute had approved the study.

Career post graduate institute of dental sciences, Lucknow, Uttar Pradesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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