Microanalysis of Root Cementum in Patients with Rapidly Progressive Periodontitis

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Abstract

Objective: The objective of this study was to evaluate the microanalysis of various elements, and assess the surface characteristics of progressive periodontally diseased roots in comparison to sound root surface. Materials and Methods: 50 teeth were collected, 25 teeth from patients have progressive periodontitis, and 25 teeth from healthy patients. Measurements of probing depth and clinical attachment loss were taken prior to extractions. After the horizontal fracturing process of root specimens, healthy and diseased cementum layers of roots were evaluated by scanning electron microscopy (SEM) and energy dispersive X ray analysis (DXA). SEM and DXA. The collected data were statistically evaluated using t-test. The level of significance was set at $p<0.001$. Results: The results of this study showed a significant decrease in the calcium and phosphate contents along the entire cementum of root teeth of the progressive periodontitis and a significant increase in the magnesium and sulphur of the same root teeth in comparison to the control group. In addition, there were remarkable destructions of root cementum, crack lines and deep cavities reaching to the underlying dentin. Conclusion: In conclusion, the alteration in cementum structures and composition due to progressive periodontitis might have an important implication on periodontal therapy. The influence of alteration of cementum composition and structure on periodontal regeneration warrants further exploration.

Key Words: Root cementum, Periodontal disease, Periodontitis, Cavities, Microanalysis

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. Progressive periodontitis includes a group of rapidly progressive forms of periodontitis characterized by early onset of clinical manifestations at a young age and a distinctive tendency for cases to aggregate in families. Though once believed to be a rare condition, recent evidence suggests that aggressive periodontitis is more common than assumed [1]. Aggressive periodontitis is characterized by: (1) noncontributory medical history; (2) rapid attachment loss and bone destruction; (3) familial aggregation of cases; (4) lack of consistency between clinically visible bacterial deposits and severity of periodontal breakdown [2]. The treatment of such periodontally involved cementum by root planning has for long been considered an important part of periodontal therapy [3].

Root surface affected by periodontal disease may show various changes depending on the location of the root surface relative to the surroundings. When the exposed cementum comes into intimate contact with microbial dental plaque, changes occur in the diseased cementum including hypermineralization of the cement surface, degeneration of the collagen matrix and development of resorption lacunae due to penetration and / or absorption of bacterial endotoxins at the exposed cementum [4].

Chemical analysis of the exposed cementum has shown an increase in calcium, magnesium, and phosphorus with a depth of penetration 50 um or less into the cementum. The crystals of the hypermineralized surface zone were observed to be bigger than in the subjacent cementum [5].

Root surfaces have been assessed for clinical changes due to the influence of periodontal diseases. The reported results from such teeth indicated a higher Ca and P content than non-diseased root surfaces. Also, it has been notified that when root surfaces became bared to the oral cavity subsequently to periodontal disease, the swap of mineral at the cementum-saliva interface, reproduce a more highly mineralized surface region relatively 40 microns in depth [6]. In the contrary, to another study [7] it was noted that denuded root structures did not show Ca and P variance to a depth of 60 microns when evaluated by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis. They declared that earlier studies applied preparative processes such as precipitating fixatives, embedding medium or decalcifying solutions for withdrawal of organic matrix and dehydration, which modified the elemental content of the root surface.

The primary composition of root cementum is of a mineralized nature, but the basic elements present, besides calcium and phosphorus, have not been verified. Opinions differ concerning the changes in cementum associated with periodontal disease. In order to understand the nature of this calcified structure in health and disease, cognition of the elemental content of non-diseased as well as diseased root is required [8].

Aggressive periodontitis comprises of two phases, active and quiescent. During the active phase, the gingival tissues are intensely inflamed and there is hemorrhage, proliferation of the marginal gingiva, and exudation. Destruction is very rapid, with deprivation of much of the alveolar bone occurring within a few weeks. This phase perhaps associated with general malaise and weight loss, although these symptoms are not insured in all patients. The disease may advance, without remission, to tooth loss, or alternatively, it may subside and become quiescent with or without treatment. The quiescent phase is featured by the presence of clinically normal gingiva that may be firmly fitted to the roots of teeth with very progressive bone loss and deep periodontal pockets. The quiescent phase may be permanent, it may persist for an indefinite period, or the disease activity may return [9].

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Affected patients generally respond favourably to treatment by scaling and open or closed curettage, especially when accompanied by doses of antibiotics for regular periods. A small minority of patients does not react to any treatment, including antibiotics, and the disease progresses to tooth loss, even in the presence of aggressive periodontal therapy [10].

Energy dispersive X-ray spectroscopy (EDX) was run out in combination with SEM. The EDX-analysis separates the x-ray spectrum by energy with enough sensitivity to show x-ray spectral data at low beam currents. It is an analytical technique employed for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in great part to the underlying principle that each element possesses a unique atomic structure allowing a unique set of peaks in its X-ray emission spectrum. The EDX-analysis was applied to find out the chemical elemental content in the diseased cementum surface [11].

Objectives

Rapidly progressive periodontitis is one of the periodontal diseases that affect systemically healthy individuals. The disease is characterized by rapid bone destruction that is discrepant with the amount of bacterial plaque. The purpose of this study was to evaluate the microanalysis of various elements and assess the surface characteristics of the rapidly progressive periodontally diseased root surfaces in comparison to sound root surface by using scanning electron microscopy (SEM) and energy dispersive X ray analysis (DXA).

Materials and Methods

25 teeth affected by periodontitis and 25 healthy teeth extracted from patients attending King Abdulaziz University, Faculty of Dentistry were used. The selected patients were generally healthy, had no systemic diseases and did not receive any antibiotic nor periodontal therapy during the past 6 months. The teeth were divided into two groups according to the clinical and radiographic data:

- Group I (Control): 25 periodontally healthy sound teeth. These teeth required extraction for orthodontic reasons. There was neither loss of gingival attachment nor bone loss.
- Group II: 25 periodontally diseased teeth were collected from patients diagnosed with rapidly progressive periodontitis During extraction, care was taken to avoid instrumentation to the areas of the root to be studied. The teeth were collected in saline and store in the refrigerator.

Cross root sections were cut using diamond saw at more than 5 mm apical to the cementoenamel junction. The root surface opposite the surface to be evaluated was marked with shallow groove for proper identification of the examined surface. Areas for electron microscopic examination are selected to correspond to areas examined in the EDX-analysis. All tooth samples were mounted on specimen stubs and sputter with a 15 nm thick gold layer. The specimens are examined with a scanning electron microscope and analysed by using energy dispersive analyzer unit attached the scanning electron microscope.

Statistical Analysis

The collected data are statistically evaluated using t- test. The level of significance is set at $p < 0.001$.

Table 1. Descriptive statistics for the different parameters used in the current study.

Element	Groups	Site	Mean	SD
Na	Control	Apical	13.5	1.73
		Medium	15.5	5.45
		Cervical	18.63	3.86
		summation	47.63	8.06
	Periodontitis	Apical	16.97	5.69
		Medium	19.06	7.71
		Cervical	17.37	8.45
		Summation	52.58	19.97
Cl	Control	Apical	5	0
		Medium	7.5	0.71
		Cervical	3.5	0.71
		summation	16	0
	Periodontitis	Apical	21.5	6.75
		Medium	20.64	5.8
		Cervical	19.25	5.15
		summation	52.68	17.45
P	Control	Apical	25	2.16
		Medium	23.25	1.26
		Cervical	22	0.82
		summation	70.25	0.96
	Periodontitis	Apical	13.67	2.28
		Medium	14.89	3.29
		Cervical	14.36	2.96
		summation	42.92	6.63
Ca	Control	Apical	48.5	2.89
		Medium	46.25	4.35
		Cervical	44.75	3.4
		summation	139.5	5.26
	Periodontitis	Apical	26.33	4.55
		Medium	27.22	5.61
		Cervical	26.36	5.49
		summation	79.92	12.52
S	Control	Apical	6.88	2.95
		Medium	9.5	4.12

		Cervical	11	4.24
		summation	27.38	10.58
	Periodontitis	Apical	20.06	7.02
		Medium	20.11	9.59
		Cervical	23.69	12.84
		summation	63.86	25.32
Mg	Control	Apical	3.17	1.89
		Medium	1.75	0.5
		Cervical	1.88	0.85
		summation	6	3.19
	Periodontitis	Apical	10	2.86
		Medium	9.71	2.78
		Cervical	8.14	3.38
		summation	26.86	6.83

Table 2. t-test for the apical, medium, cervical and summation data in periodontitis cases versus control cases.

Element	Groups	Control Mean	Periodontitis Mean	t	df	p-value
Na	Apical	13.5	16.97	-2.08	16.7	0.0529
	Medium	15.5	19.06	-1.07	6.43	0.324
	Cervical	18.63	17.37	0.42	11.75	0.6806
	summation	47.63	52.58	-0.76	13.26	0.4622
P	Apical	25	13.67	9.4	4.61	0.0004
	Medium	23.25	14.89	8.38	13.52	<0.00000
	Cervical	22	14.36	9.45	18.4	<0.00000
	summation	70.25	42.92	16.73	19.37	<0.00000
Ca	Apical	48.5	26.33	12.33	6.86	<0.00000
	Medium	46.25	27.22	7.48	5.5	0.0004
	Cervical	44.75	26.36	8.6	7.05	0.0001
	summation	139.5	79.92	15.07	11.97	<0.00000
S	Apical	6.88	20.06	-5.94	11.95	0.0001
	Medium	9.5	20.11	-3.47	11.59	0.0049
	Cervical	11	23.69	-3.43	15.97	0.0034
	summation	27.38	63.86	-4.57	12.06	0.0006
Mg	Apical	3.17	10	-5.32	3.75	0.0072
	Medium	1.75	9.71	-11.07	18.81	<0.00000
	Cervical	1.88	8.14	-6.78	18.57	<0.00000
	summation	6	26.86	-9.2	10.34	<0.00000
Cl	No valid data could be calculated as the N of control cases are less than 3					

Statistical analysis for the energy dispersive analyzer *(Tables 1* and *2) (Figures 1* and *2)* showed that the control group was differed from the periodontitis group regarding the concentrations of calcium, phosphorus, sulphur, and magnesium.

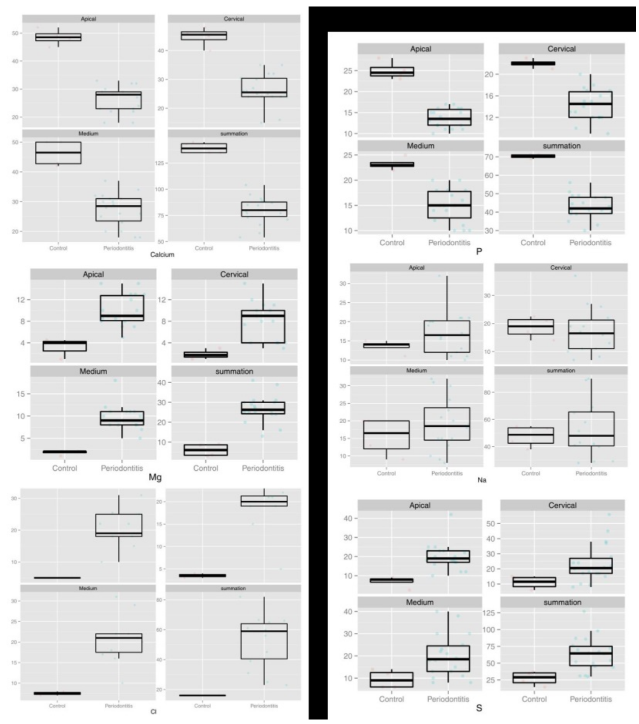

Figure 1. Box plots with whiskers showing the mean and the standard errors of different minerals at different localities.

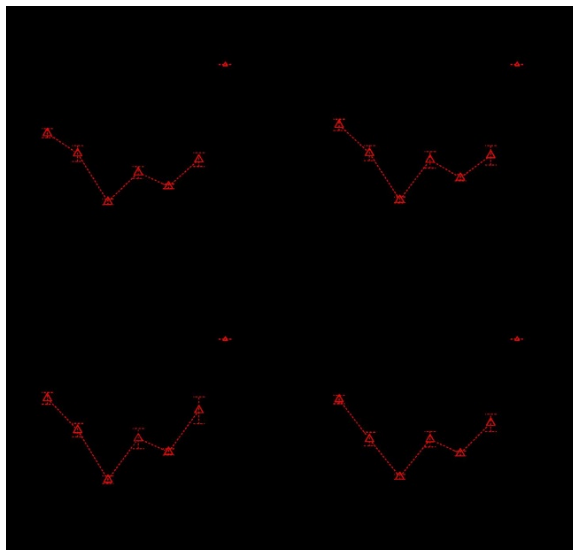

Figure 2. Plot of means for the different minerals at the cervical, medium, apical and summation sites.

For calcium and phosphorus, the concentrations of the two minerals were significantly lower in the periodontitis group compared to the control group. This was apparent in the cervical, medium and apical regions as well as in the summation of these areas. The reverse was observed for the magnesium and sulphur, where their concentrations in the periodontitis groups were statistically higher than that of the control group. Standardized to the calcium and phosphorus trend the concentrations of magnesium and sulphur were higher in the cervical, medium and apical regions. Of course, the summation of these regions was also higher in the periodontitis group compared to that of the control group.

The concentration of sodium showed no significant difference between control and periodontitis groups. The data collected for chlorides were insufficient to conclude a reliable statistical inference.

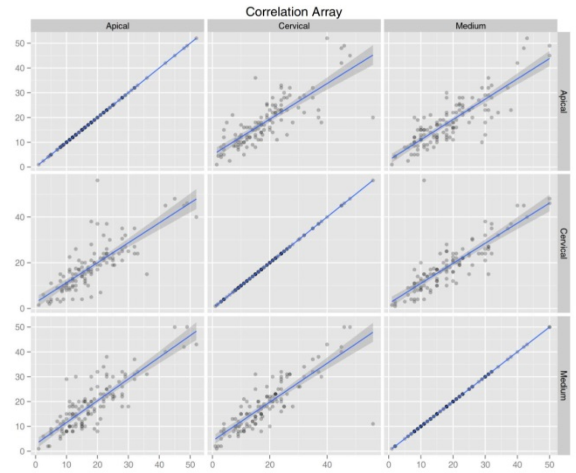

Figure 3. Correlation array for the concentration of different minerals in different study regions.

Correlation analysis revealed that for all elements studied and in all groups, the cervical concentrations of elements correlated positively and significantly in the medium (R=0. 83 and P-value <0.001) and apical concentrations (R=0. 79 and P-value <0.001). Similarly, medium concentrations correlated positively and significantly with the apical concentrations (R=0. 85 and P-value <0.001). These findings are presented in *Table 3* and illustrated in *Figure 3*.

Table 3. Correlation array of the mineral composition in the apical, medium and cervical examination sites.

		Apical	Medium	Cervical
R	Apical	_	0.85	0.79
P-value			<0.001	<0.001
R	Medium	0.85	_	0.83
P-value		<0.001		<0.001
R	Cervical	0.79	0.83	_
P-value		<0.001	<0.001	

Scanning Electron Microscope Examination: The cement surface of the sound teeth (Group I) had a homogenous regular smooth appearance and was embraced by the periodontal fibers *(Figure 4)*, while the cementum of progressive periodontitis teeth (Group II) showed an irregular, uneven surface with multiple defects areas of varying sizes and depths at cervical and middle thirds of the base *(Figures 5-7)*.

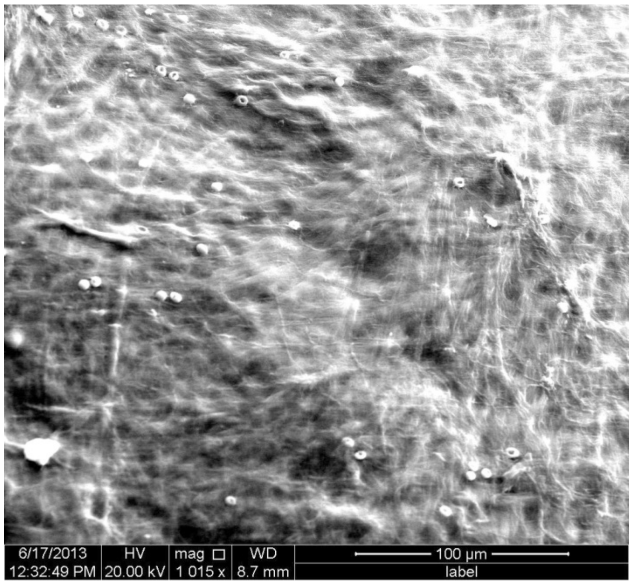

Figure 4. Scanning electron micrograph of group I (Control) at the cervical third of the root showing a smooth homogenous cementum surface covered by periodontal fibers (1015 x).

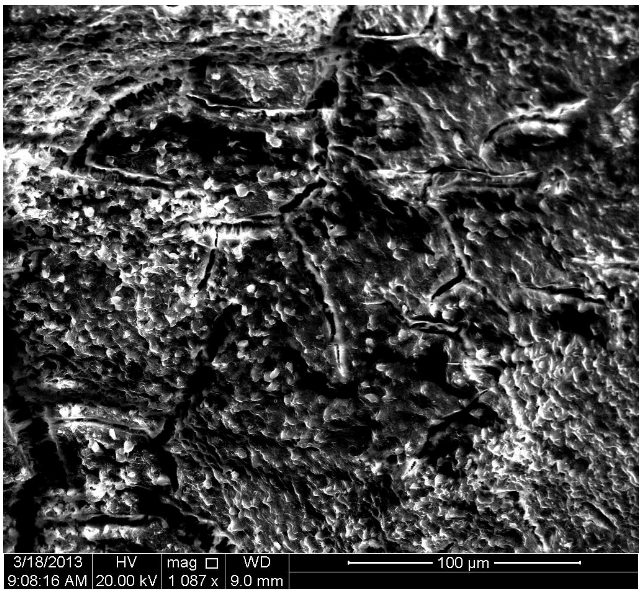

Figure 5. Progressive periodontitis at the cervical third of the root showing irregular rough surface with the presence of multiple deep crack lines (1078 x).

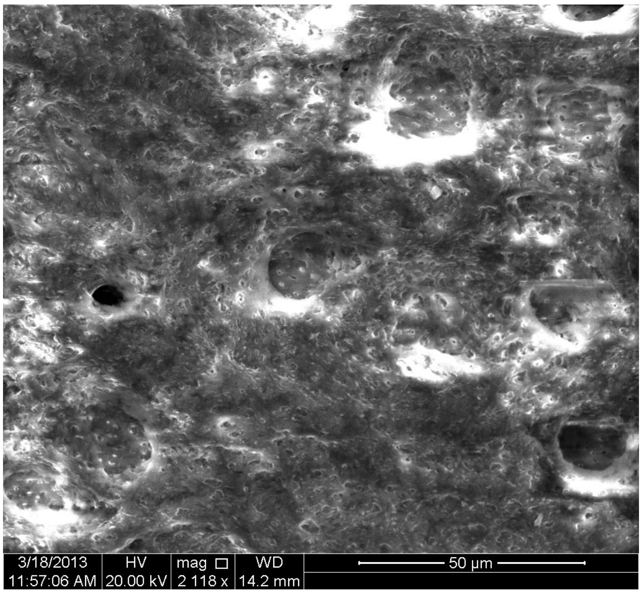

Figure 6. Middle third of the root of progressive periodontitis showing severe destruction of cementum surface with the exposure of the underlying dentin with the presence of multiple craters and deep multiple resorption area (2118x).

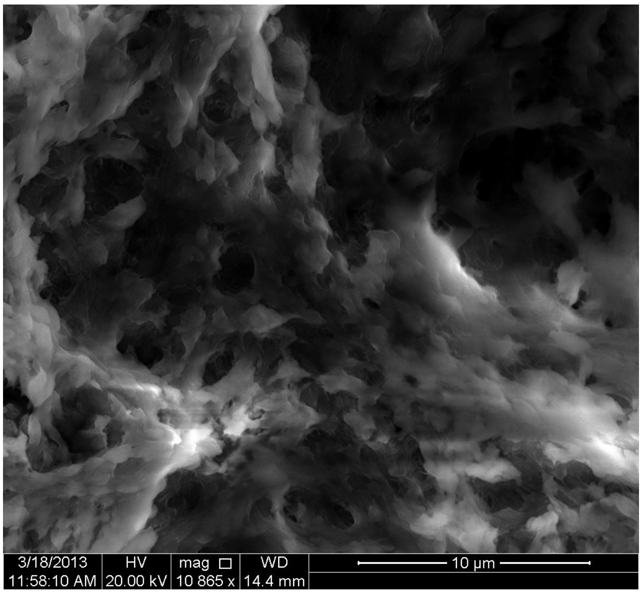

Figure 7. Middle third of the root showing severe destruction of cementum surface with the exposure of the underlying dentin with the presence of multiple craters (10865x).

In addition to the presence of deep crack lines were widely distributed on the entire cementum surface with complete absence of periodontal fibers and numerous resorption areas extended deep into the underlying dentin at the apical third of the root *(Figure 8)*.

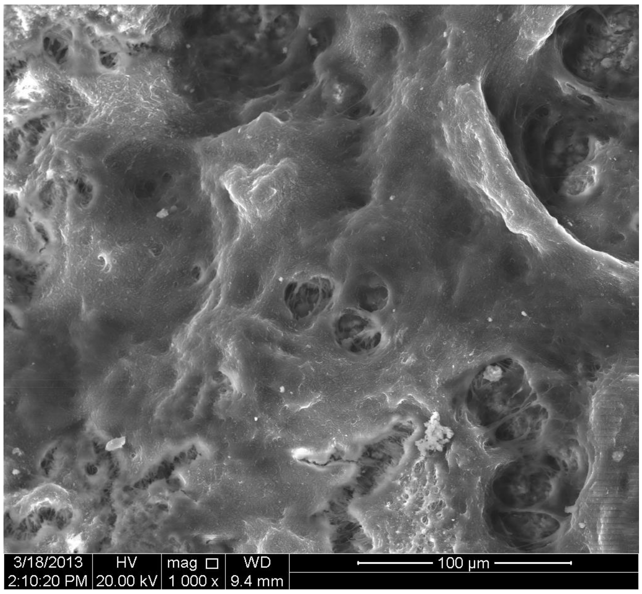

Figure 8. Apical third of the root of progressive periodontitis showing multiple deep defect areas and complete absence of the periodontal fibers (1000x).

Discussion

The outcomes of this study demonstrated variations in the mineral contents of periodontally involved roots and sound controls. The concentrations of calcium and phosphorus were lower in the periodontitis group compared to the control group, whereas the concentration of magnesium and sulphur were higher in the periodontitis than the control group. This change in the mineral content was noticed in all three sections of the roots, namely, apical, middle and cervical. These findings confirmed earlier studies that identified a modification in the mineral content of roots affected by periodontitis [12,13]. The variation in mineral content of periodontally involved roots and healthy controls could be ascribed to exposure of the root to saliva and to an infected environment through the recession and pocket formation in the periodontitis group.

The results of the electron microscope assessment revealed that the cementum surface of periodontally affected teeth had an irregular, uneven surface with multiple defects, areas of variable sizes and depths, whereas the cementum of the sound teeth showed a homogenous regular smooth appearance and was covered by the periodontal fibres. These alterations in the periodontally affected teeth are likely due to the vulnerability of the cementum to the oral environment by periodontal disease. It bears to be mentioned, nevertheless, that there are some reports that implicated defective cementum as a predisposing factor in loss of periodontal attachment and development of aggressive periodontal destruction by rendering the periodontium more susceptible to bacterial infection [14,15].

The alteration in cementum structures and composition due to periodontal disease might cause an important implication on periodontal therapy. An essential objective of periodontal regeneration is the establishment of new cementum and restoration of connective tissues and epithelial adhesion to the cementum. The integrity of cementum is altered by periodontal disease, as demonstrated in this work. The influence of alteration of cementum composition and structure on periodontal regeneration warrants further exploration. Furthermore, future research should concentrate on establishing a cementum microenvironment that initiate and encourage new cementum formation. Current methods to assist in this aspect include: root conditioning, application of some growth factors and enamel proteins and utilization of barrier membranes. These methods, nevertheless, have major limitations. For example, root conditioning expose molecules, such as type-I collagen, that has poor cell specificity and more importantly it does not re-establish the unique composition of cementum local environment [16]. Utilization of the barrier membranes is also not a likely method to re-establish the unique composition of cementum local environment that assist in cellular differentiation although it might facilitate population of the treated site by desired cells [17]. Enamel matrix protein on the other hand might have the ability to assist in early cementogenesis but it lacks the ability to recruit cementoblasts progenitors in adults and for their differentiation [18].

Conclusion

The outcomes of this study showed alteration in the cementum composition and structure of teeth that were involved with aggressive periodontitis compared to healthy teeth. Specifically the affected teeth showed a lower concentration of calcium and phosphorus and a higher concentration of magnesium and sulphur. Future research should focus on establishing a cementum microenvironment that initiate and encourage new cementum formation.

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