

Microanalysis of Root Cementum in Patients with Aggressive Periodontitis

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Abstract

The purpose of this study was to evaluate the microanalysis of various elements, and assess the surface characteristics of aggressive periodontally diseased roots in comparison to sound root surface. 50 teeth were collected: 25 from patients with aggressive periodontitis, and 25 teeth from healthy patients. Measurements of probing depth and clinical attachment loss were taken prior to extractions. Healthy and diseased root cementum were evaluated by scanning electron microscopy and energy dispersive X-ray spectroscopy. The collected data were statistically evaluated using t-test. The level of significance was set at $p < 0.001$. The results of this study showed a significant decrease in the calcium and phosphate contents along the entire root cementum of the aggressive periodontitis and a significant increase in the magnesium and sulphur of the same root in comparison to the control group. In addition, there were remarkable destructions of cementum, crack lines and deep cavities reaching to the underlying dentin. In conclusion, the alteration in cementum structures and composition due to aggressive periodontitis might have an important implication on periodontal therapy. The influence of alteration of cementum .

Keywords

Aggressive periodontitis; Root cementum; Energy-dispersive X-ray spectroscopy; 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. Aggressive periodontitis is identified 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^[1].

Though once believed to be a rare condition, recent evidence suggests that aggressive periodontitis is more common than previously assumed^[2].

Several causes have been suggested for the aggressive periodontitis such as immunodeficiency of patients, bacterial invasion, genetic factors and defective cementogenesis of the involved teeth^[3-6].

Root surface influenced by periodontal disease that may display numerous changes, including hypermineralization of the cementum surface,

degeneration of the collagen matrix and development of resorption lacunae due to penetration of bacterial endotoxins into the exposed cementum^[7]. Abnormal cementum results in abnormal adhesion of teeth to the surrounding bone and provides an opportunity for invasion of pathogenic microorganisms. It may also be responsible for the localization of lesions^[8].

Chemical analysis of the exposed cementum presents an increase in calcium (Ca), magnesium (Mg), and phosphorus (P) with a penetration depth of about 50 microns or less into the cementum. The cementum crystals of the hypercalcified surface were detected to be bigger than in the underlying layer^[9].

The effect of periodontal disease on the root surface has been evaluated clinically. It has been observed that the contents of calcium and phosphorous were higher in diseased root surface than non-diseased. Exposure of the root surface to the oral environment as a result of periodontal disease leads to interchange of the minerals at the cementum–saliva interface resulting in a hypermineralized cementum surface with about 40 microns of depth^[10]. Another study, recognizing that previous studies had applied preparative methods which modified the chemical composition of the root surface, discovered that at the depth of 60 microns in the cementum of exposed root surface there were no differences in the calcium and phosphorous contents^[11].

The essential content of root cementum is formed of calcium and phosphorus, while the rest of the elements were not verified. There are different conceptions regarding the changes in cementum accompanied with periodontal disease. In order to clarify the feature of this mineralized cementum, knowledge of the elemental content of diseased and non-diseased root is required^[12].

Aggressive periodontitis comprises two stages, active and passive. During the active stage, the gingival tissues are severely inflamed and there is a profuse bleeding, overgrowth of the marginal gingiva, and exudation. There is a very rapid alveolar bone destruction within a few weeks or months. During this stage, most of the patients are suffering from general fatigue and weight loss. The progress of the disease is unpredictable. The passive stage is characterized by severe bone destruction and deep periodontal pockets, while the gingiva appears clinically normal and properly fitted to the root surface. This stage may remain for a long period of time, or the disease activity may return^[13]. Standard doses of antibiotics and

proper scaling with open or closed curettage improve the disease^[14].

Energy dispersive X-ray spectroscopy (EDS) was performed in combination with SEM. The EDS scatters the X-ray energy spectrum with enough sensitivity to show X-ray spectral data. Its effectiveness is derived from the fact that each element possesses a unique atomic structure allowing an exclusive set of peaks in its X-ray emission spectrum. It is an analytical technique used for chemical and elemental analysis and providing a full quantitative analysis of the sample composition^[15].

Objectives

The aim of this study was to evaluate the microanalysis of various elements and assess the surface characteristics of the aggressive periodontally diseased root surfaces in comparison to sound root surface by using SEM and EDS.

Materials and Methods

This study included 50 teeth (25 teeth affected by aggressive periodontitis and 25 healthy teeth) extracted from patients attending King Abdulaziz University, Faculty of Dentistry. The patients' ages were between 17-25 years and they were generally healthy, with no systemic diseases and did not receive any antibiotic or periodontal therapy during the previous 6 months. Written informed consents were obtained from the participants. The diagnosis of the aggressive periodontitis was made according to the clinical and radiographic criteria described by the American Academy of Periodontology^[16]. A full-mouth series of periapical radiographs were obtained and in cases with more than 6 mm clinical attachment loss, the teeth were extracted due to their hopeless prognosis. Before tooth extraction, bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) were calculated. Each extracted tooth in patients with aggressive periodontitis had more than 8 mm in probing depth and more than 6 mm of clinical attachment level^[3].

Group I (Control)

25 periodontally healthy sound teeth. These teeth required extraction for orthodontic reasons. There was neither destruction of gingival attachment nor bone loss.

Group II:

25 periodontally diseased teeth were collected from patients diagnosed with aggressive periodontitis. A clinical distinction between aggressive periodontitis and chronic periodontitis as defined by the 1999 workshop on classification of periodontal diseases^[4] had been done, based on a number of significant clinical differences, including: (1) age of onset, (2) rates of progression, (3) patterns of destruction, (4) clinical signs of inflammation and (5) relative abundance of plaque and calculus^[10].

The teeth were collected and fixed in 2.5% buffered glutaraldehyde.

Cross root sections were cut at the cemento enamel junction. The selected root surface areas had been determined for examination by the scanning electron microscope and energy-dispersive X-ray analyzer unit.

Statistical Analysis

The collected data are statistically evaluated using "student's" *t*-test. The level of significance is set at $p < 0.001$.

Results

Energy-Dispersive X-ray Analysis

Statistical analysis for the energy dispersive analyzer showed that the control group differed from the periodontitis group regarding the concentrations of calcium, phosphorus, sulphur, and magnesium.

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 (Table 1).

The concentration of sodium showed no significant difference between control and periodontitis group.

Table 1. *t*-test for the apical, medium, cervical and summation data in periodontitis cases versus control cases.

Element	Groups	Control Mean	Periodontitis Mean	<i>t</i> ¹	Df ²	p-value
Sodium	Apical	13.50	16.97	-2.08	16.70	0.0529
	Medium	15.50	19.06	-1.07	6.43	0.3240
	Cervical	18.63	17.37	0.42	11.75	0.6806
	Summation	47.63	52.58	-0.76	13.26	0.4622
Phosphorus	Apical	25.00	13.67	9.40	4.61	0.0004
	Medium	23.25	14.89	8.38	13.52	<0.00000
	Cervical	22.00	14.36	9.45	18.40	<0.00000
	Summation	70.25	42.92	16.73	19.37	<0.00000
Calcium	Apical	48.50	26.33	12.33	6.86	<0.00000
	Medium	46.25	27.22	7.48	5.50	0.0004
	Cervical	44.75	26.36	8.60	7.05	0.0001
	Summation	139.50	79.92	15.07	11.97	<0.00000
Sulphur	Apical	6.88	20.06	-5.94	11.95	0.0001
	Medium	9.50	20.11	-3.47	11.59	0.0049
	Cervical	11.00	23.69	-3.43	15.97	0.0034
	Summation	27.38	63.86	-4.57	12.06	0.0006
Magnesium	Apical	3.17	10.00	-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	6.00	26.86	-9.20	10.34	<0.00000
Chloride	No valid data could be calculated as the N of control cases are less than 3					

Table 2. 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	Apical	–	<0.001	<0.001
R	Medium	0.85	–	0.83
P-value	Medium	<0.001	–	<0.001
R	Cervical	0.79	0.83	–
P-value	Cervical	<0.001	<0.001	–

The data collected for chlorides were insufficient to conclude a reliable statistical inference.

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 $P < 0.001$) and apical concentrations ($R = 0.79$ and $P < 0.001$). Similarly, medium concentrations correlated positively and significantly in the apical concentrations ($R=0.85$ and $P < 0.001$) (Table 2).

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 (Fig. 1), while the cementum of aggressive periodontitis teeth (Group

II) showed an irregular, uneven surface with multiple defect areas of varying sizes and depths at cervical and middle thirds of the roots (Figs. 2, 3, and 4). In addition to the presence of deep crack lines widely distributed over the entire cementum surface was a complete absence of periodontal fibers and numerous resorption areas extending deep into the underlying dentin at the apical third of the root (Fig. 5).

Discussion

Elemental analysis revealed a significant difference between the two groups in terms of the amount of mineral content along the three root thirds. These findings confirmed previous studies that identified modifications in the mineral content of roots affected by periodontitis^[16,17]. The variation in mineral content of periodontally involved roots could be clarified

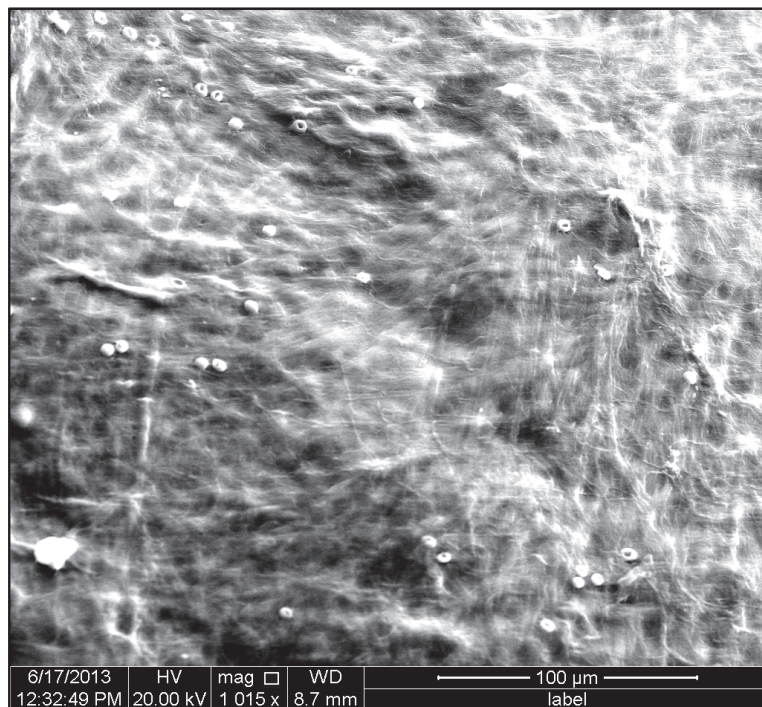


Figure 1. Scanning electron micrograph of group I (Control) at the cervical third of the root showing a smooth homogenous cementum surface covered by periodontal fibers. (1015 x).

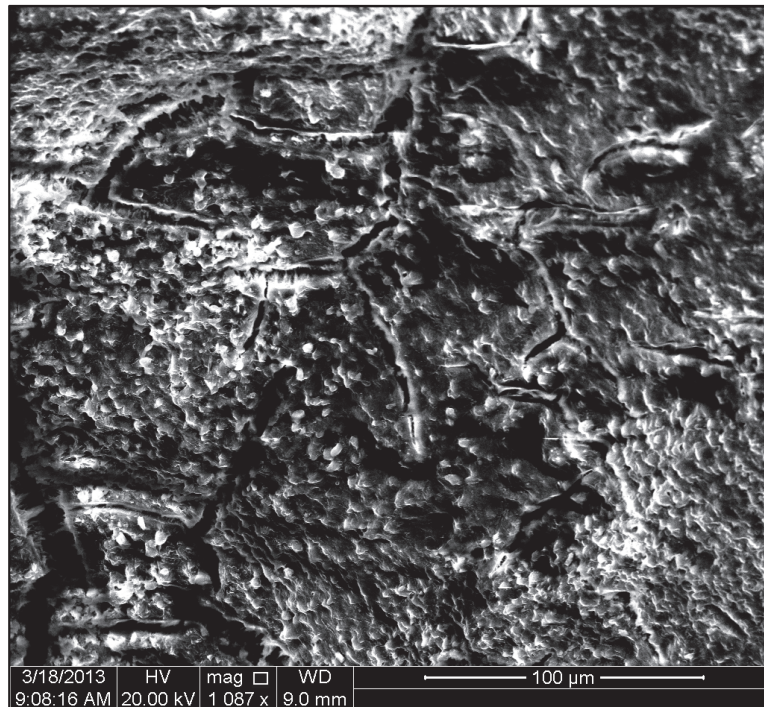


Figure 2. Aggressive periodontitis at the cervical third of the root showing irregular, rough surface with the presence of multiple deep crack lines (1078 x).

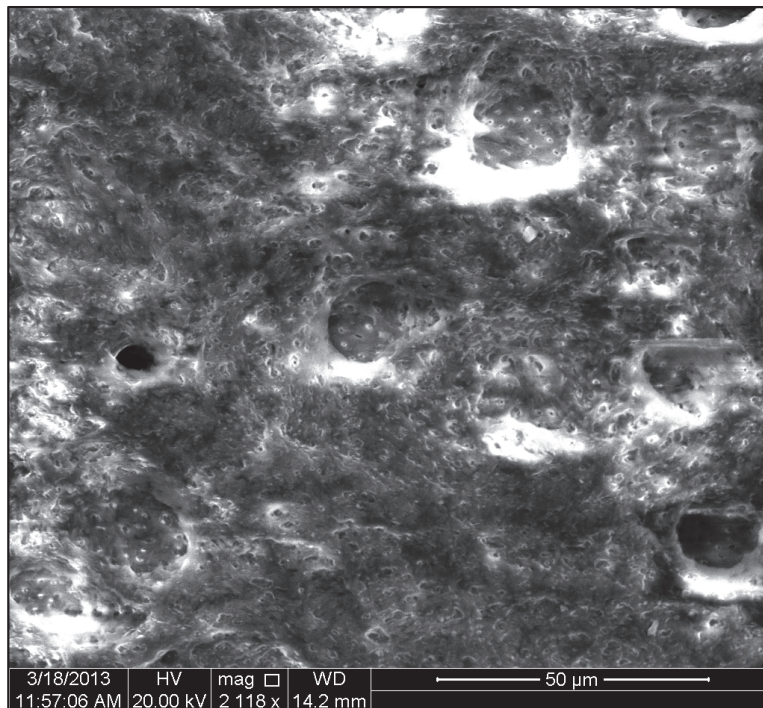


Figure 3. Middle third of the root of aggressive periodontitis showing severe destruction of cementum surface with the exposure of the underlying dentin with the presence of multiple craters and deep multiple resorption area (2118x).

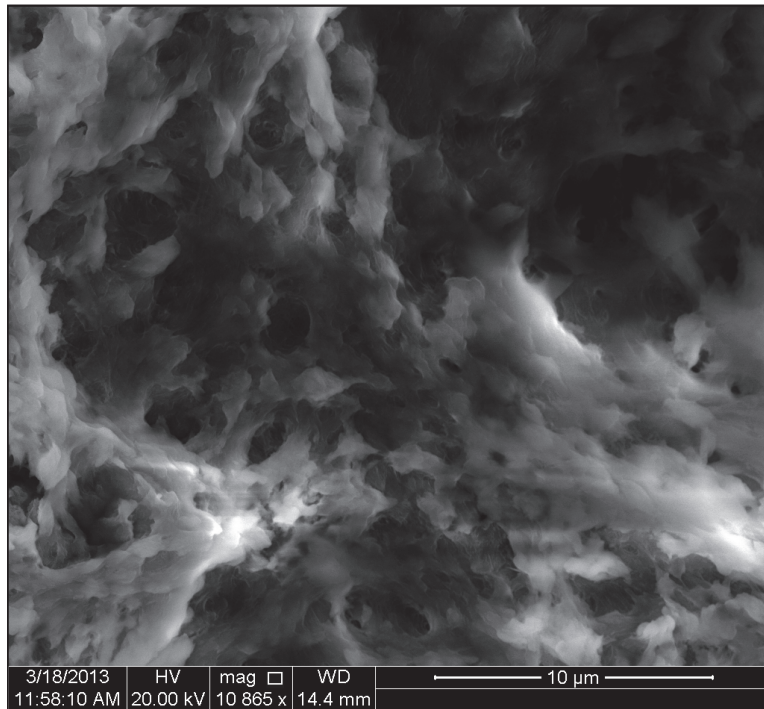


Figure 4. Middle third of the root showing severe destruction of cementum surface with the exposure of the underlying dentin with the presence of multiple craters (10865x).

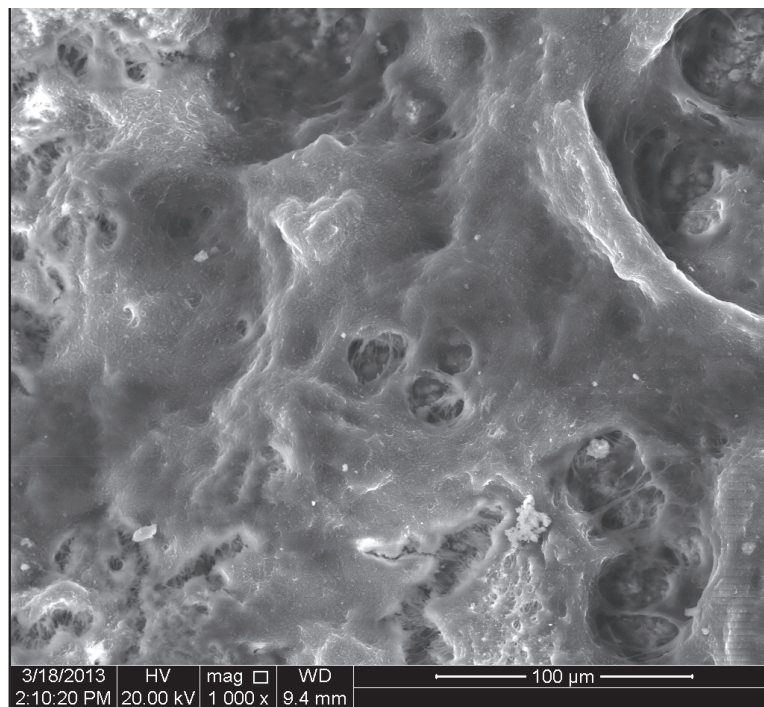


Figure 5. Apical third of the root of aggressive periodontitis showing multiple deep defect areas and complete absence of the periodontal fibers (1000 x).

based on the exposure of the root to saliva and the surrounding infected environment through gingival recession, bone loss and pocket formation in the aggressive periodontitis group.

In the current study, the cementum surface in affected teeth with aggressive periodontitis showed multiple areas of hypoplasia. These hypoplastic areas were seen on all the examined teeth and all over the root surfaces. These alterations are likely to be due to the vulnerability of the cementum to the oral environment by periodontal disease. Our results were in agreement with the observations of previous studies regarding cementum hypoplasia in teeth affected with aggressive periodontitis^[1,17]. These findings further confirm the hypothesis that abnormal cementum and its defective formation can enhance the invasion of pathogens and exacerbate extensive bone loss^[3,18]. It has been demonstrated that cementum matrix structural integrity and biochemical composition are severely compromised in periodontal disease and the provisional matrix produced in the process of periodontal regeneration is different from that in normal cementum^[14]. It bears to be mentioned, nevertheless, that there are some reports that implicated defective cementum as a predisposing factor in the loss of periodontal attachment and development of aggressive periodontal destruction by rendering the periodontium more susceptible to bacterial infection^[14,15]. Studies have demonstrated that aggressive periodontitis patients have a defect in their immune system^[19] where the cytokines and the inflammatory mediators are able to stimulate periodontal breakdown and collagen destruction via tissue-derived matrix metalloproteinases, a characterization of the progression of periodontitis as a stage that presents a significantly host immune and inflammatory response to the microbial challenge under the influence of multiple environmental and genetic factors^[20,21]. In agreement with the aforementioned study, it is not reliable to evaluate the reason for symmetric dissemination pattern of aggressive periodontitis just from the bacterial origin aspect without considering immunological conditions^[22]. Thus both the local microbiota and immune response play an essential role in the pathogenesis of aggressive periodontitis.

The alteration in cementum structures and composition due to periodontal disease might have

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 initiates and encourages 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. Nevertheless, these methods have major limitations. For example, root conditioning exposes molecules, such as type-I collagen, that have poor cell specificity and, more importantly, it does not reestablish the unique composition of the cementum local environment^[23]. Utilization of the barrier membranes is also not a likely method to re-establish the unique composition of the cementum local environment that assists in cellular differentiation, although it might facilitate population of the treated site by desired cells^[24]. 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 assist their differentiation^[24].

Conclusion

In 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 initiates and encourages new cementum formation.

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Conflict of Interest

The authors have no conflict of interest.

Disclosure

None of the authors received any type of commercial support either in forms of compensation or financial for this study. They have no financial interest in any of the products or devices, or drugs mentioned in this article.

Ethical Approval

Obtained.

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التحليل المجهرى لملاط الأسنان عند المرضى المصابين بالتهاب الغشاء السمحاقى العدواني

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المستخلص. الغرض من هذا البحث هو تقييم microanalysis of various elements, and asses the surfaces التحليل المجهرى للعناصر المختلفة المكونة لملاط جذور الأسنان عند المرضى المصابين بالتهاب الغشاء السحقاقى العدواني بالمقارنة مع ملاط الجذور السليمة. استخدم في هذه الدراسة خمسون سينة مقسمة كالاتي: خمسة وعشرون سينة من مرضى مصابين بالتهاب الغشاء السحقاقى العدواني وخمسة وعشرون سينة من أشخاص أصحاء. تم تحضير العينات وفحصها وذلك باستخدام المجهر الإلكتروني المسح وجهاز تحليل الأشعة السينية باستخدام الطاقة المتفرقة وأيضاً تم تقييمها إحصائياً. أسفرت النتائج عن إنخفاض ملحوظ ذو دلالة إحصائية في عنصرى الكالسيوم والفوسفات وارتفاع ذو دلالة إحصائية في عنصرى المغنسيوم والكبريت في ملاط جذور الأسنان المصابة بالتهاب الغشاء السحقاقى السريع المدمر. أيضاً لوحظ وجود تصدعات وتدمير شديد في سطح ملاط جذورا لأسنان مع اختفاء كامل لألياف النسيج السحقاقى وأيضاً تآكل عميق في بعض المناطق على سطح الجذور. ويستنبط من هذى النتائج أن التغيرات في المكونات الأساسية في ملاط الأسنان المصابة وخصوصاً المعادن الأساسية والتدمير الشديد المصاحب لها لابد من أن يوضع الاعتبار عند معالجة هذا المرض والذى يستدعى مزيداً من الاستكشاف في الطرق العلاجية.