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,
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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 phosphorus 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[19].

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 was 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.

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

Cross root sections were cut at the cementoenamel 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 | t | Df | 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        | 52.38              | -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 | <0.0000 |
|         | Cervical | 22.00        | 14.36              | 9.45  | 18.40 | <0.0000 |
|         | Summation| 70.25        | 42.92              | 16.73 | 19.37 | <0.0000 |
| Calcium | Apical   | 48.50        | 26.33              | 12.33 | 6.86  | <0.0000 |
|         | 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 | <0.0000 |
| 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 | <0.0000 |
|         | Cervical | 1.88         | 8.14               | -6.78 | 18.57 | <0.0000 |
|         | Summation| 6.00         | 26.86              | -9.20 | 10.34 | <0.0000 |
| Chloride |          |              |                    |      |      |        |

No valid data could be calculated as the N of control cases are less than 3.
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...
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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).
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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 (1086x).

Figure 5. Apical third of the root of aggressive periodontitis showing multiple deep defect areas and complete absence of the periodontal fibers (1000x).
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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\(^5,17\). 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.

References
[1] Pradeep A, Patel SP. Multiple dental anomalies and aggressive periodontitis: a coincidence or an association? Indian J Dent Res. 2009; 20(3): 374-376.
[2] Levin L. Aggressive periodontitis: the silent tooth killer. Alpha Omegan. 2011; 104(3-4): 74-78.
[3] Ferraiolo DM. Predicting periodontitis progression. Evid Based Dent. 2016; 17(1): 19-20.
[4] Norderyd O, Hugoson A, Grusovin G. Risk of severe periodontal disease in a Swedish adult population. A longitudinal study. J Clin Periodontol. 1999; 26(9): 608-615.
[5] Alqutaibi AY, Algabri RS. Limited evidence suggests high risk of implant failure rates among people with generalized aggressive periodontitis. J Evid Based Dent Pract. 2015; 15(4): 187-189.
[6] Okte E, Unsal B, Bal B, Erdemli E, Akbay A. Histological assessment of root cementum at periodontally healthy and diseased human teeth. J Oral Sci. 1999; 41(4): 177-810.
[7] Nibali L, Carvalho Farias B, Vajgel A, Tu YK, Donos N. Tooth loss and disease progression in aggressive periodontitis: a systematic review. J Dent Res. 2013; 92(10): 866-875.
[8] Petruţiu S, Buiga P, Roman A, Danciu T, Mihu CM, Mihu D. Degenerative alterations of the cementum–periodontal ligament complex and early tooth loss in a young patient with periodontal disease. Rom J Morphol Embryol. 2012; 53(4): 1087-1091.
[9] Bimstein E, Wignall W, Cohen D, Katz J. Root surface characteristics of children teeth with periodontal diseases. J Clin Pediatr Dent. 2008; 32(2): 101-104.
[10] Armitage G, Cullinan M. Comparison of the clinical features of chronic and aggressive periodontitis. Periodontol. 2000. 2010; 53(1): 12-27.
[11] Ozden F, Ozgönencel O, Ozden B, Aydogdu A. Diagnosis of periodontal diseases using different classification algorithms: a preliminary study. Niger J Clin Pract. 2015; 18(3): 416-421.
[12] Onabolu O, Donos N, Tu Y, Darbar U, Nibali L. Periodontal progression based on radiographic records: An observational study in chronic and aggressive periodontitis. J Dent. 2015; 43(6): 673–682.
[13] Drisko CH. Nonsurgical periodontal therapy. Periodontal. 2000. 2001; 25: 77-88.
[14] Zhu XL, Meng HX. [Observation of the root surfaces and analysis of the mineral contents in cementum of patients with rapidly progressive periodontitis]. Zhonghua Kou Qiang Yi Xue Za Zhi. 2003; 38(2): 126-128.
[15] Kodaka T, Debari K. Scanning electron microscopy and energy-dispersive X-ray microanalysis studies of afibrillar cementum and cementicle-like structures in human teeth. J Electron Microsc. 2002; 51(5): 327-333.
[16] [No authors listed]. Parameter on aggressive periodontitis. American Academy of Periodontology. J Periodontol. 2000; 71(S Suppl): 867-869.
[17] Rex T, Kharbanda OP, Petocz P, Darendeliler MA. Physical properties of root cementum: Part 4. Quantitative analysis of the mineral composition of human premolar cementum. Am J Orthod Dentofacial Orthop. 2005; 127(2): 177-185.
[18] Shafik SS, Zaki AE, Ashrafi S. Comparative scanning electron microscopy of root surfaces in juvenile and adult periodontitis: a report of two cases. Saudi Dent J. 1992; 4(3): 115-119.
[19] Guzeldemir E, Toygar HU. From alveolar diffuse atrophy to aggressive periodontitis: a brief history. J Hist Dent. 2006; 54(3): 96-99.
[20] Silva N, Abuselme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernandez M, Gamonal J. Host response mechanisms in periodontal disease. J Appl Oral Sci. 2015; 23(3): 329-355.
[21] Yamamoto T, Kita M, Osekio F, Nakamura T, Imanishi J, Kanamura N. Cytokine production in human periodontal ligament cells stimulated with Porphyromonas gingivalis. J Periodontal Res. 2006; 41(6): 534-559.
[22] Watanabe H, Umeda M, Seki T, Ishikawa I. Clinical and laboratory studies of severe periodontal disease in an adolescent associated with hypophosphatasia. A case report. J Periodontol. 1993; 64(3): 174-180.
[23] Grzesik WJ, Narayanjan AS. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med. 2002; 13(6): 474-484.
[24] Paknejad M, Khorsand A, Yaghoobei S, Motahhari P, Etebarian A, Bayani M, Mehrfard A. Cementogenesis in patients with localized aggressive periodontitis. J Dent (Tehran). 2015; 12(5): 347-351.
التحليل المجهري لملاط الأسنان عند المرضى المصابين بالتهاب الغشاء السحاقى العدوى

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المستخلص: الغرض من هذا البحث هو تقييم التحليل المجهري للملاط المكونة لملاط جذور الأسنان عند المرضى المصابين بالتهاب الغشاء السحاقى العدوى، بالإضافة إلى مقارنة ملاط الجذور السليمة.

استخدم في هذه الدراسة خمسون سناً مقسمة كالتالي: خمسة عشرون سناً من مرضى مصابين بالتهاب الغشاء السحاقى العدوى، وعشرون سناً من أشخاص أصحاء. تم تجميع العينات وفحصها وذلك باستخدام المجهر الإلكتروني.

أسفرت النتائج عن إخفاق ملحوظ ذو دالة إحصائية في خصائص الكالسيوم والفوسيات وارتفاع ذو دالة إحصائية في عصري المغنيسيوم والكربون. وجدت أيضاً نتائج تشير إلى ملاط جذور الأسنان السليمة ملحوظاً أحياناً نشيج السحاقى. وأيضاً هناك تأثير عميق في بعض المناطق على سطح الجذور. وتنبئ من ذلك أن التغيرات في المكونات الأساسية في ملاط الأسنان السليمة وخصوصاً المعادن الأساسية والتحمير الشديد المصاحب لها لا بد من أن يوضع الاعتبار عند معالجة هذا المرض والذي يستدعي مزيداً من الاستكشاف في الطرق العلاجية.