Root cementum ultrastructure in healthy and periodontally diseased teeth

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Abstract
Relevance. Investigation of the root cementum ultrastructure in chronic generalized periodontitis is still relevant as changes in structure and composition of root cementum play a significant role in successful periodontal regeneration. Am is to study changes in the root cementum ultrastructure in patients with chronic generalized periodontitis.
Materials and methods. Scanning electron microscopy (SEM) was used to study the cementum surface of 9 teeth extracted due to severe chronic generalized periodontitis and 3 teeth with a clinically healthy periodontium extracted for orthodontic reasons. 3D visualization of the received SEM images was performed.
Results. The cementum of periodontally healthy teeth appeared homogeneous and regular,was covered in periodontal fibers and had a pebble-like or dome-shaped surface. In chronic periodontitis patients, the cementum surface was mostly irregular with multiple defects of various depth, areas of completely destroyed cementum, exposed dentinal tubules and a complete absence of periodontal fibers.
Conclusion. Loss of periodontal attachment and root cementum exposure to microbial biofilm may result in irreversible structural changes of the surface which may affect the regeneration of clinical attachment.
Key words: dental cementum, periodontitis, periodontium regeneration, scanning electron microscopy
For citation: Slazhneva, E.S., Tikhomirova, E.A., Elizova, L.A., Loboda, E.S., Orekhova, L.Yu., Atrushkevich, V.G. Root cementum ultrastructure in healthy and periodontally diseased teeth. Parodontologiya.2020;25(4):317-321. (in Russ.) https://doi.org/10.33925/1683-3759-2020-25-4-317-321.

INTRODUCTION

Chronic generalized periodontitis is an inflammatory disease resulting in periodontal tissue destruction and consequent tooth loss. Root cementum is located between root dentin and the periodontal ligament and while dentin is hard dental tissue, it functionally belongs to the periodontium. Periodontium includes the periodontal ligament, gingiva, alveolar bone and cementum. Cementum can be described as a mineralized tissue that covers tooth roots and provides attachment for the teeth to the alveolar bone by means of the collagen fibers of the periodontal ligament. Besides its essential role in tooth attachment to the surrounding bone, root cementum has important adaptive and reparative functions that play a major role in maintaining the occlusal relationship and root surface integrity, and in tooth support and anchorage [1]. Furthermore, cementum is a matrix rich in growth factors that may influence the activity of different periodontal cells and participate in periodontal regeneration [2, 3].

Chronic periodontitis progression causes loss of attachment of the periodontal ligament to the root surface, and exposure of the cementum to the periodontal pocket environment and oral cavity in general. All of the above can cause structural changes in cementum, as well as compositional changes in organic and inorganic components. Loss of collagen cross-linking and dissolution of mineral crystals can be observed in the cementum of patients with inflammatory periodontal diseases on SEM investigation. These changes may be irreversible and are of great significance for periodontal therapy and subsequent periodontal regeneration [4]. As the restoration of periodontal structure and functions along with the simultaneous formation of stable bone-PDL-cementum complex is one of the treatment objectives of chronic generalized periodontitis [5], it might be assumed that any damage to the cementum ultrastructure will complicate full regeneration or render it impossible.

There are morphological, histological and functional differences in cementum structure along the root length according to the presence or absence of cells and origin of matrix collagen fibers. They distinguish intermediate cementum (found in cementum-enamel junction), acellular cementum (found in coronal and middle thirds of a root) and cellular cementum (present only in the apical third and furcation area). Acellular extrinsic fiber cementum (primary or noncellular cementum) is found in the cervical area and in the upper two thirds of a root. It develops very slowly and is considered acellular as forming cells remain on its surface. A large number of the principal fibers of the periodontal ligament are incorporated into the acellular cementum. Sharpey's fibers as they are known are evidence of an important function of acellular cementum in the formation of periodontal attachment [2].

Cellular intrinsic fiber cementum (secondary cementum) is distributed along apical root and in furcation areas. Cellular intrinsic fiber cementum is characterized by the presence of cementoblasts, cementocytes and intrinsic collagen fibers produced by cementoblasts. Cellular intrinsic fiber cementum plays an important role as an adaptive tissue, it participates in cementum restoration and can be detected in root resorption defects and root fracture areas, although it does not provide tooth attachment directly [6, 7].

Formation of new cementum and repair of periodontal fiber attachment to the cementum are one of the objectives of regenerative periodontal treatment. This process requires cementoblasts though not enough is known yet of the origin of cementoblasts and of the molecular factors thatregulate their recruitment and differentiation [6].

Regenerated cementum should ideally be very similar to acellular extrinsic fiber cementum as it has the greatest impact on attachment function. In the majority of studies devoted to periodontal regeneration however, the quality of attachment function was questionable as the newly-formed cementum was cellular intrinsic fiber cementum, but not the desired acellular extrinsic fiber cementum [5]. Thus, another strategy for the successful regeneration of periodontium is the delicate handling of cementum during subgingival treatment of the root by various instruments.

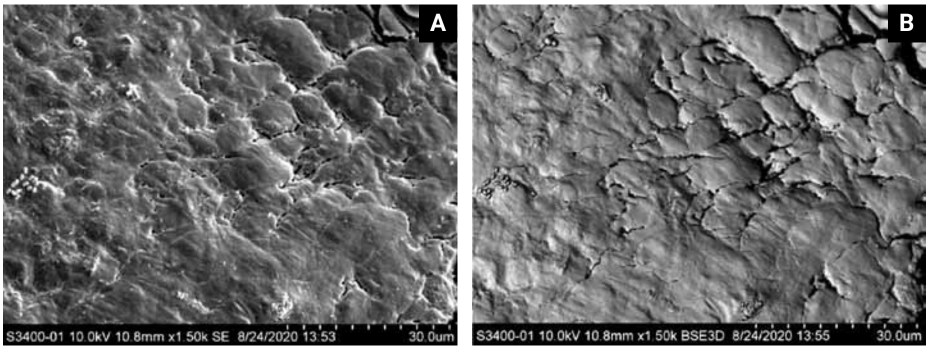

Fig. 1. **A – magnification x 1500. Surface of healthy root cementum of the upper and middle thirds. Cementum surface is pebble-like due to small tubercles. B – magnification x 1500. 3D visualization of the cementum surface**

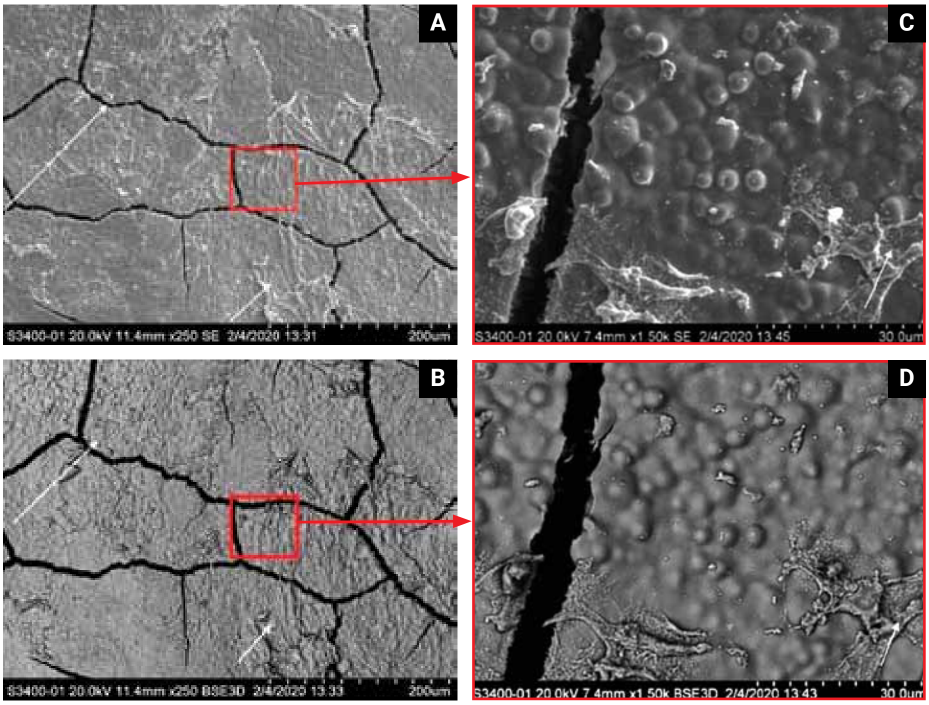

Fig. 2. **A – magnification x 250, C – magnification x 1500. Surface of healthy cementum of the apical third. Preserved fragments of periodontal fibers are indicated by white arrows. Cementum surface is dome-shaped and resembles the surface of aerated chocolate. B – magnification x 250, D – magnification x 1500. 3D visualization of healthy cementum surface**

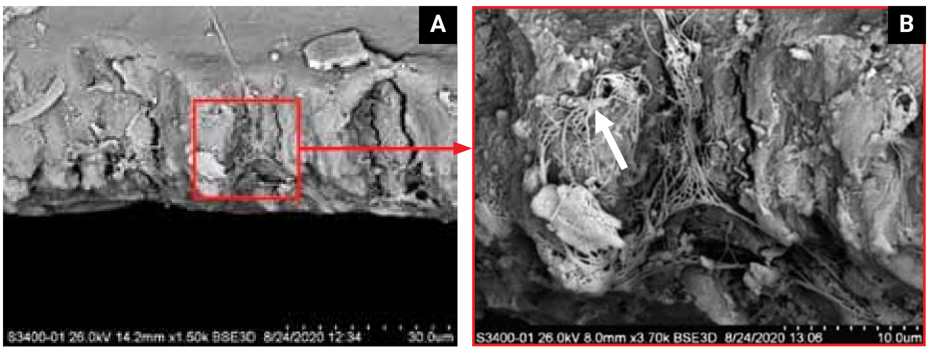

Fig. 3. **A – magnification x 1500. Cross-section of healthy root cementum. Closely-packed bundles of collagen fibers. B – magnification x 3700. Cementocyte (white arrow), some processes of which pass into the superficial cementum layer, and others incorporate with intrinsic fibers, forming irregular fibrous network. 3D visualization.**

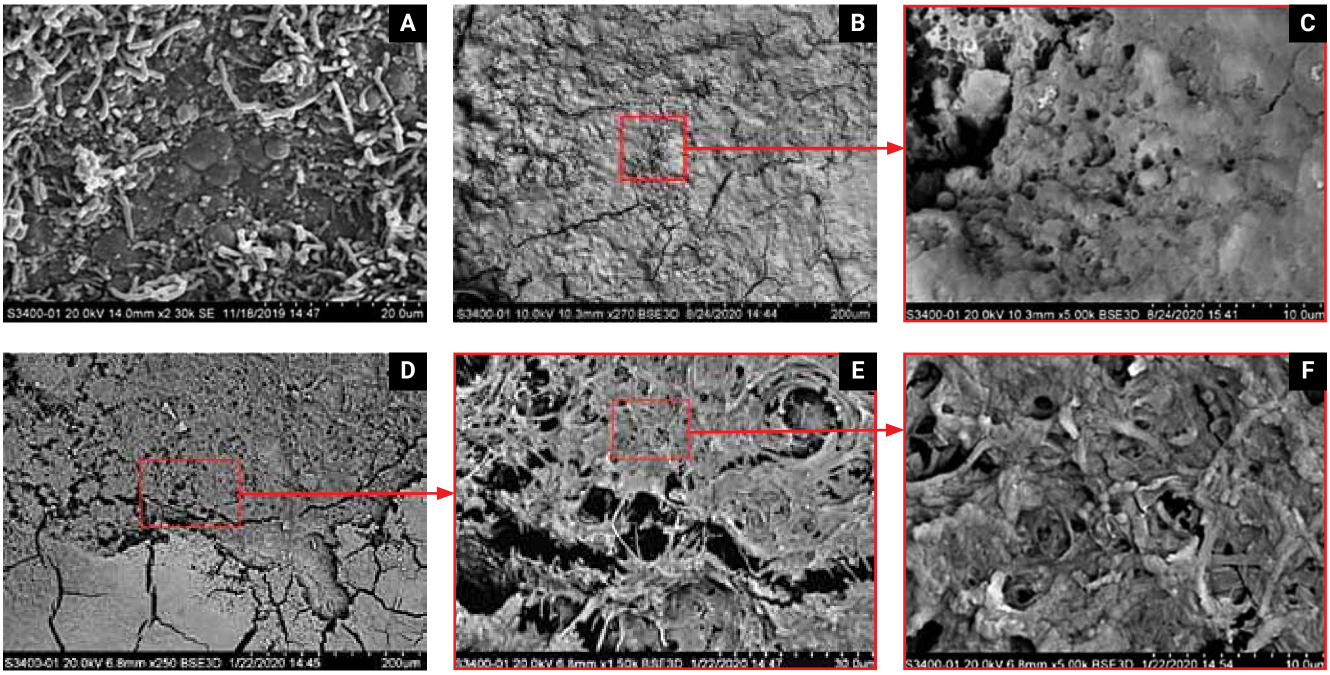

Fig. 4. **A – magnification x 2300. Root cementum surface of a tooth affected by chronic generalized periodontitis. Root surface covered by subgingival microbial biofilm.**
B – magnification x 270, C – magnification x 5000. Inhomogeneous cementum surface of a periodontally-diseased tooth with areas of possible cementum absence and exposed openings of dentinal tubules.
D – magnification x 250. Root surface with damaged cementum and exposed dentinal tubules. 3D visualization.
E – magnification x 1500, F – magnification x 5000. Demineralized inner layer of cementum, exposed intrinsic collagen fibers, cementum resorption lacunae extending to the underlying dentin. 3D visualization.

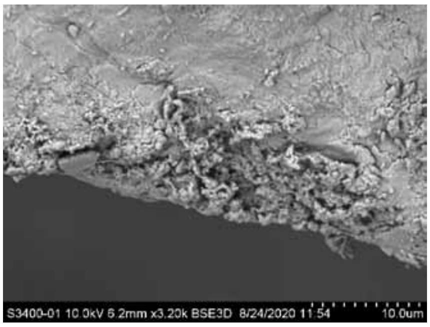

Fig. 5. **Magnification x 3200. Root cementum cross-section in a tooth of a patient with chronic generalized periodontitis. Almost completely lost root cementum, irregular fibers and demineralization of the residual cementum. 3D visualization.**

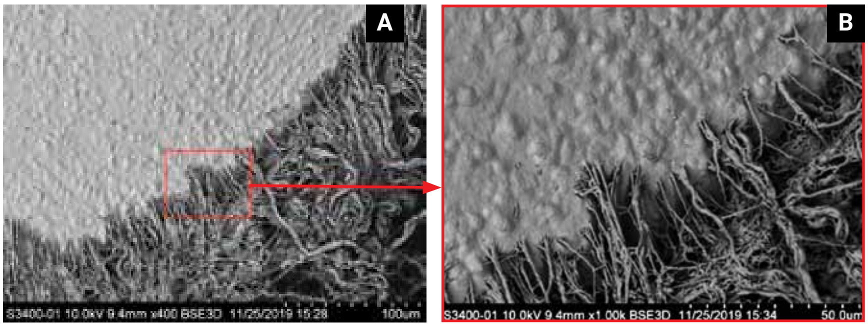

Fig. 6. **A – magnification x 400. Preservation of normal dome-shaped structure of cementum at the bottom of a periodontal pocket.**
B – magnification x 2500. Periodontal fibers incorporate with tops of tubercles. 3D visualization.

Our study aimed to investigate changes in the ultrastructure of root cementum in patients with chronic generalized periodontitis.

MATERIALS AND METHODS

Sample collection and preparation for SEM

For the study, 9 teeth with grade 3 mobility and poor prognosis were extracted in patients with severe chronic generalized periodontitis without previous scaling and root planning. A further 3 teeth with healthy periodontium were extracted for orthodontic reasons in patients treated in the Periodontology Department of MSUMD University clinic. Informed consent was received. Tooth surfaces were delicately cleansed of blood and other debris with saline. Extracted teeth were fixed in 2.5% glutaraldehyde in Hank's Balanced Salt Solution (pH = 7.2) at 4˚C. Samples were placed in 5.25% Sodium hypochlorite for 48 hours to remove organic deposits from the root surface.

Standard sample preparation for SEM investigation was then performed. Teeth were dehydrated in a series of alcohol solutions of increasing concentration (30-100% ethanol, 5-10 minutes in each solution) and processed in a critical point dryer. Prepared samples were mounted on the aluminum stub with conductive carbon glue and coated with a thin layer of gold by evaporation.

SEM imaging and 3D visualization

The scanning electron microscope S 3400N Hitachi (Japan) was used to examine the prepared samples at accelerating voltage in high vacuum. Root surface was studied longitudinally and cross-sectionally from the cemento-enamel junction to the apex. Real-time 3D micrographs were produced by simultaneous capture of images by built-in 4-quadrant secondary electron detectors and Hitachi PC-SEM software.

RESULTS

SEM images of the cementum surface of the healthy teeth and teeth extracted due to chronic generalized periodontitis were received at different magnifications.

Healthy cementum

SEM images of healthy teeth demonstrate homogeneous regular cementum surface covered by fragments of periodontal fibers indicated (white arrows) (Fig. 1, 2). Interestingly, the root cementum surface of the upper and middle thirds was pebble-like due to multiple small tubercles (Fig. 1A), and dome-shaped in the apical root and furcation area, resembling aerated chocolate (Fig. 2A, C). Conventional SEM images and 3D visualization images are shown for comparison (Fig. 1B, 2B, D). Microcracks on the surface of the studied cementum were caused by dehydration of the samples according to the protocol of SEM.

Root cementum surface affected by periodontitis

Micrographs of periodontally involved cementum demonstrated a wide variety of surface characteristics (Fig. 4). Firstly, subgingival accumulation of bacterial biofilm was frequently revealed on the root surface; a cementum surface with characteristic dome-shaped or pebble-like patterns was found in some areas under the biofilm (Fig. 4A).

Secondly, the cementum surface frequently lost its rolling structure and appeared inhomogeneous and rough, scaly subgingival plaque was recorded. There were areas with cementum disturbance and exposed openings of dentinal tubules. Periodontal fibers were completely absent (Fig. 4B, C).

Areas with significant surface destruction were also detected, evidenced by demineralization, multiple craters and deep resorption, exposure of intrinsic fiber layers or underlying dentin and openings of dentinal tubules (Fig. 4D, E, F).

At the same time, areas of cementum with preserved normal structure could be seen at the bottom of a periodontal pocket (Fig. 5).

In cross-section periodontally healthy teeth had a thicker layer of root cementum in comparison with extracted periodontally diseased teeth (Fig. 3, 5), while teeth with a clinically healthy periodontium demonstrated cementum with closely-packed fibers and cementocytes with processes extending to the outer and inner layers of cementum (Fig. 3A, B). Cross-sections of teeth with chronic periodontitis frequently showed an absence of cementum or its demineralization and softening.

DISCUSSION

The results show that SEM is a valuable tool for a detailed study of the cementum surface. Current SEM visualization technology, when real-time imaging is simultaneously received from 4 secondary electron detectors and 3D visualization is produced, provides a better understanding of the surface of a sample. 3D micrographs give a better impression of the spatial relationships between the outer and inner layers of cementum.

The cementum surface of periodontally-healthy teeth samples was homogeneous, regular and covered by fragments of periodontal fibers after extraction. At high magnification it was evident that the surface of the intact cementum was not absolutely smooth, but was pebble-like or dome-shaped (or resembled aerated chocolate) depending on the studied root region. These data were confirmed by other studies describing similar cementum surface structure [8-11]. It appears that differences in cementum surface structure was based on fiber attachment of the periodontal ligament, for example, Sharpey's fibers.

As for the cementum surface of periodontally-diseased teeth, other data concerning surface characteristics of cementum were received. It should be underlined that modification of conventional SEM images into 3D gave a better understanding of the pattern of the damaged cementum.

Remnants of microbial biofilm represented by a combination of various morphological bacterial types were found on the cementum surface of teeth extracted due to periodontitis. In addition, foliated or scaly subgingival dental deposits were registered on the cementum surface of periodontally-diseased teeth. Bacterial enzymes and acid metabolites can be present in sufficiently high concentrations to induce demineralization and localized damage to the cementum. This may result in bacterial invasion of the deeper layers of cementum. Lacunar defects were previously described as a general characteristic of root cementum surface in patients with chronic periodontitis; periodontal pathogens may colonize there, and treatment by different instruments may be difficult, which can decrease the regenerative ability of the cementum. Bacteria that remain in these lacunae can represent a reservoir for recolonization by microbial biofilm, and can lead to a relapse of periodontal disease. Areas of damaged cementum with exposed openings of dentinal tubules are an entry point for periodontal pathogens that may infect the dental pulp and cause retrograde pulpitis in patients with severe chronic generalized periodontitis [12].

Thus, the SEM results demonstrate that the cementum surface of periodontally-diseased teeth is usually irregular with multiple defects of various depth, areas of complete destruction of cementum and dentin exposure (exposure of openings of dentinal tubules), and a complete absence of periodontal fibers on the damaged surface. At the same time, areas of preserved cementum surface were revealed, e.g. on the apical root where preserved periodontium was detected radiographically [13].

Data suggests that damaged cementum can be a predisposing factor responsible for clinical attachment loss and the development of aggressive periodontal destruction which in turn increases the susceptibility of the periodontium to bacterial infection [2]. Changes in cementum structure and composition due to periodontal diseases may be of primary importance for the effectiveness of periodontal therapy. Minor damage to the root cementum is usually reversible and heals by reparative cementum formation, which appears to repeat cementogenesis. Irreversible damage to the root cementum may occur when its surface is subject to an invasion of periodontal pathogens which form microbial biofilm on the cementum surface. This may influence the regeneration capacity of tooth-supporting structures [1, 10]. Further investigations are needed to confirm the present conclusion.

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Conflict of interests:
The authors declare no conflict of interests
Article received 19.04.2020
Revised 28.05.2020
Accepted 10.09.2020

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