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Scanning electron microscopy studies of biofilm in teeth with chronic apical periodontitis

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Abstract
INTRODUCTION. For a long time, the inflammatory process can be asymptomatic in the periapical space. Apical periodontitis is often associated with the presence of biofilm. The latter has a great influence on the prognosis of endodontic treatment.
AIM. Using scanning electron microscopy, to determine the presence and localization of biofilm and microflora in the roots of teeth with chronic apical periodontitis.
MATERIALS AND METHODS. Grins of 6 roots of teeth with chronic forms of apical periodontitis were studied for detection of microflora using the scanning electron microscopy method.
RESULTS. In 100% of cases, biofilm was found in the root canals of teeth with chronic forms of apical periodontitis. It was located focally, unevenly capturing different areas of both the root canal and the surrounding dentin of the tooth root.
DISCUSSION. Biofilm is observed in the necrotic dentin of the cervical third of the root canal, covers the pulp tissue of the tooth, penetrates into the thickness of the dentin. Microflora fills the lumen of the dentinal tubules, but does not penetrate through the intact cementum of the tooth root.
CONCLUSIONS. Microflora in the root canals of teeth with chronic forms of apical periodontitis is present not only in the form of individual cultures, but mainly in the form of biofilm, which covers not only the canal, but also penetrates to different depths into the thickness of the dentin of the tooth root. Biofilm covers dentin unevenly in different parts of the root.

Keywords: chronic periodontitis, microflora, biofilm, SEM

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Исследования при помощи сканирующей электронной микроскопии биопленки в зубах с хроническим апикальным периодонтитом

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Резюме
ВВЕДЕНИЕ. Длительное время воспалительный процесс может бессимптомно протекать в около верхушечном пространстве. Апикальный периодонтит чаще связывают с наличием биопленки. Последняя оказывает большое влияние на прогноз эндодонтического лечения.
ЦЕЛЬ. С помощью сканирующей электронной микроскопии определить наличие и локализацию биопленки и микрофлоры в корнях зубов с хроническим течением апикального периодонтита.
МАТЕРИАЛЫ И МЕТОДЫ. Изучены шлифы 6-ти корней зубов с хроническими формами апикального периодонтита на предмет обнаружения микрофлоры с использованием метода сканирующей электронной микроскопии.
РЕЗУЛЬТАТЫ. В 100% случаев в зубах с хроническим фирмами апикального периодонтита в корневых каналах была обнаружена биопленка. Она располагалась очагово, неравномерно захватывая разные площади как корневого канала, так и окружающего дентина корня зуба.

ОБСУЖДЕНИЕ. Биопленка наблюдается в некротизированном дентине пришеечной трети корневого канала, покрывает ткань пульпы зуба, проникает в толщу дентина. Микрофлора заполняет просвет дентинных канальцев, но не проникает через неповрежденный цемент корня зуба.
ВЫВОДЫ. Микрофлора в корневых каналах зубов при хронических формах апикального периодонтита присутствует не только в виде отдельных культур, но в основном в виде биопленки, которая покрывает не только канал, но и проникает на различную глубину в толщу дентина корня зуба. Биопленка покрывает дентин неравномерно в различных участках корня.

Ключевые слова: хронический периодонтит, микрофлора, биопленка, СЭМ

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INTRODUCTION

Apical periodontitis is one of the most common complications of dental caries and accounts for 50–80% of tooth extractions in clinical practice [1]. The complications associated with this condition can lead to the development of odontogenic inflammatory processes in the maxillofacial region [2] and may result in prolonged disability [3]. Even in the absence of clinical symptoms, chronic inflammation of necrotic pulp tissue and the periapical area may persist for years [4; 5]. The human body exists in constant interaction with a vast microbial environment, including the oral cavity, which may harbor up to 100 microbial species simultaneously, often organized into complex microbial communities [6]. Independent microbiological studies of the microbial flora (MF) isolated from root canals in various forms of apical periodontitis have not identified a single pathogenic species responsible for initiating periapical inflammation [7]. Instead, apical periodontitis is more commonly associated with the presence of a biofilm [8], which significantly affects the outcome of endodontic treatment. This is largely due to the biofilm's high resistance to adverse environmental conditions [9]. Numerous studies have demonstrated not only the clinical but also the social relevance of this condition [10].

AIM

To determine the presence and localization of biofilm and microbial flora in the roots of teeth affected by chronic apical periodontitis using scanning electron microscopy.

MATERIALS AND METHODS

To investigate the presence of microbial flora, scanning electron microscopy (SEM) was employed to examine ground sections of six tooth roots affected by chronic apical periodontitis. The reliability and objectivity of the analysis were ensured through the use of a JSM-6490LV scanning electron microscope (JEOL, Japan) and an INCA Penta FETx3 energy-dispersive spectrometer (Oxford, UK), equipped with certified reference standards and appropriate analytical software.

At low magnification levels (×10 to ×50), the overall topography of the root surface was assessed. Higher magnifications (×100 to ×5000) were used to evaluate the presence and localization of biofilm and microbial structures within the root canal and radicular dentin, utilizing both secondary and backscattered electron imaging modes.

For the purposes of the study, each root section was divided into three regions: apical, middle, and cervical thirds. During the examination, each region was compared to the others to assess differences in microbial colonization across the root surface.

RESULTS

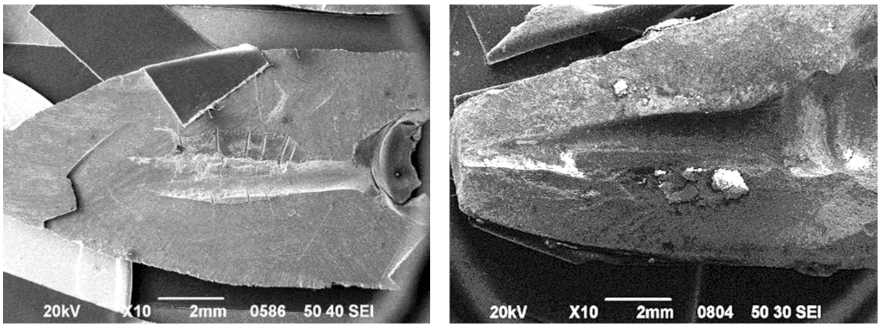
Upon examination of the ground sections of tooth roots affected by chronic apical periodontitis (Fig. 1), biofilm was identified in the root canals in 100% of the cases.

It should be noted that in all cases, the distribution of biofilm along the root surface was uneven. Certain areas of the root section were more extensively covered by biofilm than others. In two cases, a larger portion of the surface on one side of a single root section was covered by biofilm compared to corresponding areas on the opposite side of the same root. This heterogeneous distribution pattern was observed across all regions of the root, with no consistent correlation or identifiable pattern.

The analysis revealed that in the apical region, nearly the entire surface of the root canal was densely covered with biofilm (Fig. 2). The biofilm tightly – but unevenly – lined the lumens of the dentinal tubules. Although large areas of radicular dentin in the apical third were obscured by biofilm, some regions with exposed dentinal tubules remained. Microorganisms were frequently identified within the lumens of these tubules.

In the middle third of the root canal, areas free of biofilm predominated over those with biofilm coverage (Fig. 3). In regions where biofilm was present, it appeared less dense, with visible dentinal tubule openings not fully occluded. In biofilm-free zones, microbial structures were still observed inside the lumens of individual dentinal tubules (Fig. 4).

In the cervical third of the root canal, the biofilm also densely, yet unevenly, covers the dentin surface. In some areas, it exhibits a folded or wrinkled appearance (Fig. 5). The lumens of the dentinal tubules are more frequently filled with microbial flora; however, individual tubules free of microorganisms were also observed.



A B

Fig. 1. Root sections of teeth with destructive forms of apical periodontitis. SEM. Contrast in SEI. Magnification: 10x

Рис. 1. Шлифы корней зубов с деструктивными формами апикального периодонтита. СЭМ. Контраст в SEI. Увеличение: 10x

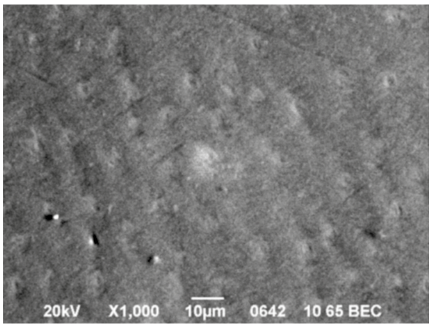


Fig. 2. Apical part of the tooth root canal. SEM. Contrast in BEC. Magnification: x1000

Рис. 2. Апикальная часть корневого канала зуба. СЭМ. Контраст в BEC. Увеличение: x1000

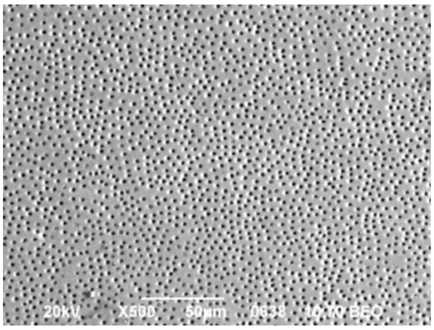


Fig. 3. Middle third of the root canal. SEM. Contrast in BEC. Magnification: x500

Рис. 3. Средняя треть корневого канала зуба. СЭМ. Контраст в BEC. Увеличение: x500

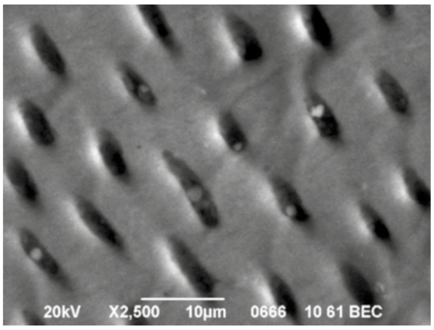


Fig. 4. The lumen of the dentinal tubules is partially filled with microflora in the middle third of the root canal. SEM. Contrast in BEC. Magnification: x2500

Рис. 4. Просвет дентинных канальцев частично заполнен микрофлорой в средней трети корневого канала зуба. СЭМ. Контраст в BEC. Увеличение: x2500

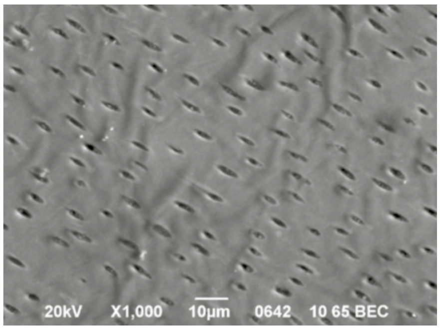


Fig. 5. Cervical part of the root canal. SEM. Contrast in BEC. Magnification: x1000

Рис. 5. Пришеечная часть корневого канала зуба. СЭМ. Контраст в BEC. Увеличение: x1000

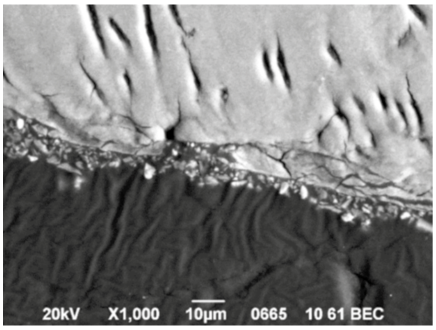


Fig. 6. Biofilm penetration from the root canal into necrotic dentin of the cervical area. SEM. Contrast in BEC. Magnification: x1000

Рис. 6. Проникновение биопленки из корневого канала в некротизированный дентин пришеечной области. СЭМ. Контраст в BEC. Увеличение: x1000

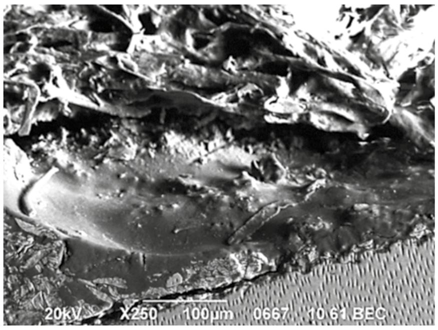


Fig. 7. Pulp chamber with necrotic pulp and biofilm in the root canal. SEM. Contrast in BEC. Magnification: x250

Рис. 7. Пульповая камера с некротизированной пульпой и биопленкой в корневом канале зуба. СЭМ. Контраст в BEC. Увеличение: x250

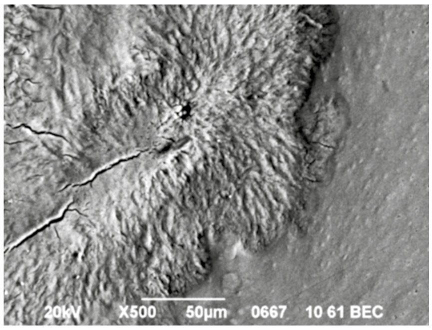


Fig. 8. Cementodentinal junction in the apical area. SEM. Contrast in BEC. Magnification: x500

Рис. 8. Цементно-дентинная граница в апикальной области. СЭМ. Контраст в BEC. Увеличение: x500

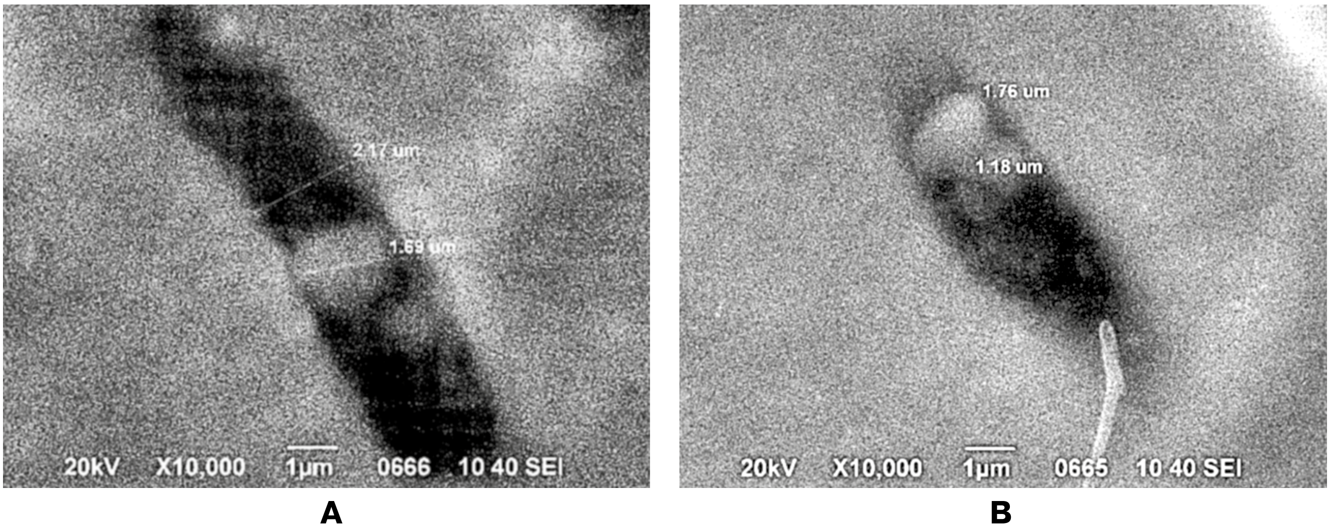


Fig. 9. Lumen of dentinal tubules with inclusions of microflora. SEM. Contrast in SEI. Magnification: x10,000

Рис. 9. Просвет дентинных канальцев с включениями микрофлоры. СЭМ. Контраст в SEI. Увеличение: x10 000

Both the biofilm and isolated microbial structures were found not only within the root canal but also extending into adjacent root areas, particularly in regions with necrotic dentin.

As shown in Fig. 6, the biofilm forms a dense layer along the root canal wall and penetrates into the necrotic, structurally compromised dentin of the cervical third, especially near the base of the carious cavity. It is important to emphasize that microbial infiltration into the dentinal tissue occurs from the root canal side outward, rather than in the reverse direction. The biofilm also covers the necrotic pulp tissue (Fig. 7). At the same time, along the entire root surface, the biofilm does not cross the cementodentinal junction in areas where the cementum remains intact (Fig. 8).

To confirm that the structures observed within the dentinal tubules were indeed of microbial origin, morphometric analysis was performed. As shown in Fig. 9, *A*, the diameter of the dentinal tubule in its widest portion measured 2.17 µm, while the lumen was occupied by a rounded structure with a diameter of 1.69 µm. Given that cocci typically range in size from 0.5 to 2 µm, it may be assumed that the observed structure is of coccal origin.

In the lumens of other dentinal tubules, we identified oval-shaped bodies whose morphology and dimensions were consistent with diplococci. Additionally, filamentous fragments were detected, morphologically resembling fungal hyphae or mycelial threads (Fig. 9, *B*).

DISCUSSION

Considering the uneven, focal distribution of biofilm within the dentin layers of the tooth root, an important question arises regarding the underlying mechanisms of this phenomenon. The observation that biofilm not only covers the root canal surface but also infiltrates necrotic dentin in the cervical third of the canal supports the earlier statement by J.F. Siqueira and I.N. Rôças, who concluded that microbial flora colonizes the necrotic root canal system [11].

Microorganisms penetrate through perforations at the base of carious lesions in the cervical third of the root canal and enter the pulp chamber. Even when the microbial flora infiltrates the pulp chamber via necrotic dentin, it does not immediately spread into adjacent healthy dentin. Instead, the microorganisms initially localize within the pulp tissue. From there, they gradually disseminate through the root canal and infiltrate the dentin matrix, colonizing increasingly larger areas over time.

The intact cementum acts as a natural barrier preventing microbial penetration into the periapical tissues. Upon reaching the cementodentinal junction, microbial organisms do not cross beyond the boundary of undamaged cementum, thereby failing to infect the external root surface and the surrounding periodontal tissues. This raises a critical question: why is microbial colonization limited to dentin and pulp tissues, but not extended to intact cementum?

Given that dentin can be extensively and deeply colonized during the chronic course of the inflammatory process, these findings call into question the effectiveness of current root canal disinfection protocols and highlight potential causes of endodontic treatment failures.

CONCLUSIONS

In teeth with chronic forms of apical periodontitis, microbial flora is present not only as isolated cultures but predominantly in the form of biofilms. These biofilms cover the root canal walls and penetrate to varying depths into the radicular dentin. The distribution of biofilm is uneven across different regions of the root, with the highest accumulation observed in the apical and cervical thirds. In the cervical third, biofilm is most commonly found in areas of necrotic dentin.

Using scanning electron microscopy (SEM), we confirmed not only the presence of microbial flora in roots affected by destructive apical periodontitis, but also identified its morphological characteristics suggestive of specific microbial taxa. In chronic inflammatory conditions, biofilm can extensively colonize large areas of the root dentin, posing significant challenges to endodontic disinfection and treatment efficacy.

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AUTHOR'S CONTRIBUTION

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