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Prevention of the development of pulp pathology in teeth with endo-periodontal lesions: an in vitro study

Diana A. Ostanina ✉, **Sabina Sh. Alimukhamedova**, **Alexander V. Mitronin**
Russian University of Medicine, Moscow, Russia Federation
✉ dianaostanina@mail.ru

Abstract
AIM. To increase the effectiveness of prevention of development of pulp pathology in endo-periodontal lesions in patients with chronic periodontitis.
MATERIAL AND METHODS. In the course of the study, instrumental treatment of the outer surface of the extruded teeth was performed, which were divided into 3 groups: group 1 – ultrasonic skeleton "DTE D72" (n = 8); group 2 – Gracey curettes (n = 8); group 3 (control) – no treatment of the root surface (n = 8). Histological analysis of the extradited teeth, scanning electron microscopy of the teeth root surfaces treated, as well as energy dispersion X-ray spectroscopy of the examined nano-preparations were conducted.
RESULTS. In the coronal pulp, in 80% of cases, the presence of intrapulmonary calcificates was found, mainly in areas where cement integrity was impaired. Fractures and defects of the tooth root cement are 3 times more common in group 1 of the study than in groups 2 and 3, which correlates with the data of pigment penetration depth in the areas of cement destruction. The most effective obturation of the root outer surface was found in the first study group and amounted to 85%, which is 2 and 4 times more than the indicators of groups 2 and 3, respectively.
CONCLUSION. It has been shown that the best method of prevention of pulp pathology at endo-periodontal lesions is the use of Gracie curettes for carrying out hand scaling with subsequent impregnation of the treated tooth root surface with a "Dentin-sealing liquid".

Keywords: endo-periodontal lesions, pulp, periodontal, cement, dentin tubules, scaling, nano-preparations

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Профилактика развития патологии пульпы при эндо-пародонтальных поражениях: экспериментальное исследование in vitro

Д.А. Останина ✉, **С.Ш. Алимухамедова**, **А.В. Митронин**
Российский университет медицины, г. Москва, Российская Федерация
✉ dianaostanina@mail.ru

Резюме
ЦЕЛЬ. Повысить эффективность профилактики развития патологии пульпы при эндо-пародонтальных поражениях у пациентов с хроническим пародонтитом.
МАТЕРИАЛЫ И МЕТОДЫ. В ходе исследования была выполнена инструментальная обработка наружной поверхности корней экстрадированных зубов, которые были разделены на 3 группы: группа 1 – ультразвуковой скейлер «DTE D72» (n = 8); группа 2 – зоноспецифические кюреты Грейси (n = 8); группа 3 (контроль) – отсутствие обработки поверхности корня (n = 8). Был проведен гистологический анализ экстрадированных зубов, сканирующая электронная микроскопия обработанных поверхностей корней зубов, а также энергодисперсионная рентгеновская спектроскопия исследуемых нанопрепаратов.
РЕЗУЛЬТАТЫ. В коронковой пульпе в 80% случаев было выявлено наличие внутрипульпарных кальцификатов, преимущественно в областях, где целостность цемента была нарушена. В группе 1 исследования в 3 раза чаще наблюдалось повреждение наружной поверхности корня в сравнении с показателями групп 2 и 3, что коррелирует с данными глубины пенетрации пигмента в участки деструкции цемента. Наилучшая эффективность обтурации наружной поверхности корня была выявлена в группе 1 исследования и составила 85%, что в 2 и 4 раза больше показателей групп 2 и 3, соответственно.
ВЫВОДЫ. Выявлено, что наилучшим методом профилактики развития патологии пульпы при ЭПП является применение зоноспецифических кюрет Грейси для проведения ручного скейлинга с последующей импрегнацией обработанной поверхности корня зуба нанопрепаратом «Дентин-герметизирующий ликвид».

Ключевые слова: эндодонто-пародонтальные поражения, пульпа, пародонт, цемент, дентинные канальцы, скейлинг, нанопрепараты

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Конфликт интересов: А.В. Митронин является членом редакционной коллегии, однако, это было нивелировано в процессе двойного слепого рецензирования.

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INTRODUCTION

Despite recent advances in dentistry, the prevalence of inflammatory diseases of the pulp and periodontal tissues remains high [1]. This is due to the common embryonic, functional, and anatomical development of the periodont-endodont complex. Inflammation in the pulp tissues can contribute to the spread of infection both orthogradely from the tooth cavity in the direction of the apical periodontium and marginal periodontium, and in the opposite direction [2; 3]. The pathways of infection spread are usually divided into anatomical (apical foramen, lateral, accessory, lateral canals, which constitute the vascular pathway and the tubular pathway represented by dentinal tubules) and non-physiologic pathways associated with iatrogenic causes [4]. According to the studies conducted, endo-periodontal lesions (EPL) most often begin to develop with a primary periodontal lesion and subsequent, secondary lesions of the dental pulp [5].

The tooth root cementum is a barrier structure at the tooth-parodontium interface. The integrity of the cementum, on which the formation and maturation of the subgingival bacterial biofilm takes place, plays an important role in protecting the underlying dentin from bacterial contamination of the anatomical communication pathways of the endo-parodontal continuum [6; 7]. Excessive treatment of the root surface during professional hygiene, particularly scaling, can lead to cementum destruction and the formation of cracks and surface defects, thereby facilitating the penetration of pathogenic microflora through the numerous dentinal tubules to the dental pulp [8]. Based on the above, the urgent task is to increase the effectiveness of pulp disease prevention in EPL by obturation of dentinal tubules and surface defects of the root cement with nanopreparations.

AIM

Aim is improving the effectiveness of prevention of pulp pathology development in endo-parodontal lesions in patients with chronic periodontitis.

MATERIALS AND METHODS

In accordance with the objectives, a set of laboratory and experimental studies was carried out to evaluate the state of the pulpo-parodontal complex. The study included 30 patients with chronic periodontitis aged 45 to 59 years (according to WHO age group 2), whose teeth were extracted for medical reasons due to endo-parodontal lesions. The criteria for inclusion of patients in the study were: presence of supra- and sub-gingival dental deposits, presence of periodontal pockets up to 5 mm, bone resorption of ½ or more of the root length, presence of pathological mobility of 1–2 degree, absence of early endodontic treatment of the teeth under study and clinically expressed carious cavities.

Histologic study

Extracted teeth were fixed in 10% buffered formalin for 48 hours at room temperature. Then, the extracted teeth were washed under running water and placed in “Electrolyte decalcifying solution” (Biovitrum, Russia) for 2 weeks. Then the samples were subjected to standard histologic wiring: they were cast into paraffin blocks using the casting station “ESD-2800” (MedTekhnikaPoint, Russia) and histologic slices with a thickness of 4 μm, running along the axis of the tooth roots, were made using a semi-automatic rotary microtome “HM 340 E” (Thermo FS, UK). The obtained sagittal slices were stained with hematoxylin and eosin for further histologic analysis.

The study of the obtained histologic sections was performed using a microscope “Axio Scope. A1” (Zeiss, Germany) at low (×50) and high magnification (×100). Microphotographs were taken using a digital camera “Axiocam 105 color” (Zeiss, Germany), and computer analysis of the obtained images was performed in the “ZEN 3.0” software.

Methodology of instrumental treatment of tooth root surfaces

In the course of the study, we performed instrumental treatment of 24 roots of extracted teeth, which were divided into 3 groups according to the used instruments for performing scaling:

Group 1 – ultrasonic scaler “DTE D72” (Woodpecker, China), $n = 8$;

Group 2 – hand instruments – zone-specific Gracie curettes (Ekradent, Russia), $n = 8$;

Group 3 (control) – no root surface treatment, $n = 8$.

Scanning Electron Microscopy

To evaluate morphostructural changes in the external surface of the root after scaling, scanning electron microscopy (SEM) was performed using a Mira 3 FEF SEM microscope (Tescan, Czech Republic) at different magnifications: ×435, ×260, ×1.26. The obtained scanning electrographs were subjected to computerized assessment of the area of surface defects and cracks of the root cement using the software “ADID”.

Study of the depth of penetration of cracks and surface defects of root cementum

For experimental estimation of the depth of dye penetration into the formed cracks and surface defects of the root cement as a result of the instrumental treatment, an aqueous solution of methylene blue 1% was applied to the outer surface of the tooth root, then the samples were placed in an Eppendorf tube with physiological solution for 24 hours. Then, longitudinal sections of the stained teeth were made and analyzed using a Levenhuk digital microscope (DTX, USA).

Investigation of the effectiveness of methods for the prevention of surface defects of root cementum

To study the effectiveness of obturation of the formed cracks and surface defects of the root cementum the following preparations were used: "Dentin-sealing liquid" (HCH GmbH, Germany), suspension "Desensetin" (TechnoDent, Russia), preparation "Sensitab" (Omegadent, Russia). The preparations were applied to the treated tooth surface after application of ultrasonic scaler "DTE D72" (Woodpecker, China) according to the manufacturer's instructions.

The microstructure of the tooth root surface after application of preparations was studied using scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS) along the entire surface of the tested samples was performed to qualitatively and quantitatively determine the elemental composition of the studied preparations.

Statistical analysis

For each experimental group, the percentage of damaged area was calculated depending on the scaling tool used. In order to statistically evaluate the differences within each group, χ^2 Pearson's criterion (chi-square test) was used. The result was considered reliable at $p < 0.05$.

RESULTS

According to the results of the histologic study, 80% of cases showed the presence of free and attached intrapulpal calcifications in the crown pulp, mainly in the areas where the cement integrity was lost; there was also marked injection and full blood vessel hemorrhage (Fig. 1 A, B). Diffuse lymphoplasmacytic infiltration with abundance of segmented leukocytes and multiple acantholytic strands of multilayered squamous epithelium were detected in the periapical tissues (Fig. 1 C). Based on the above, pulp viability depends on the integrity of the cementum, which in turn is able to protect the underlying dentin from bacterial invasion of the anatomical communication pathways between the pulp and periodontium.

On computerized evaluation of the scan-electrograms, cracking and superficial defects of the root cementum were observed in 75% of the treated root surface in group 1 (Fig. 2 A). In group 2 of the study, the indices amounted to 25% (Fig. 2 B). The obtained results of root surface microstructure evaluation directly correlate with the data of pigment penetration depth into the formed areas of tooth root cement destruction, so in the 1st group of the study the pigment penetration depth was 3 and 1.5 times higher in comparison with the control and 2nd experimental group, respectively (Fig. 3). Thus, as a result of mechanical damage to the

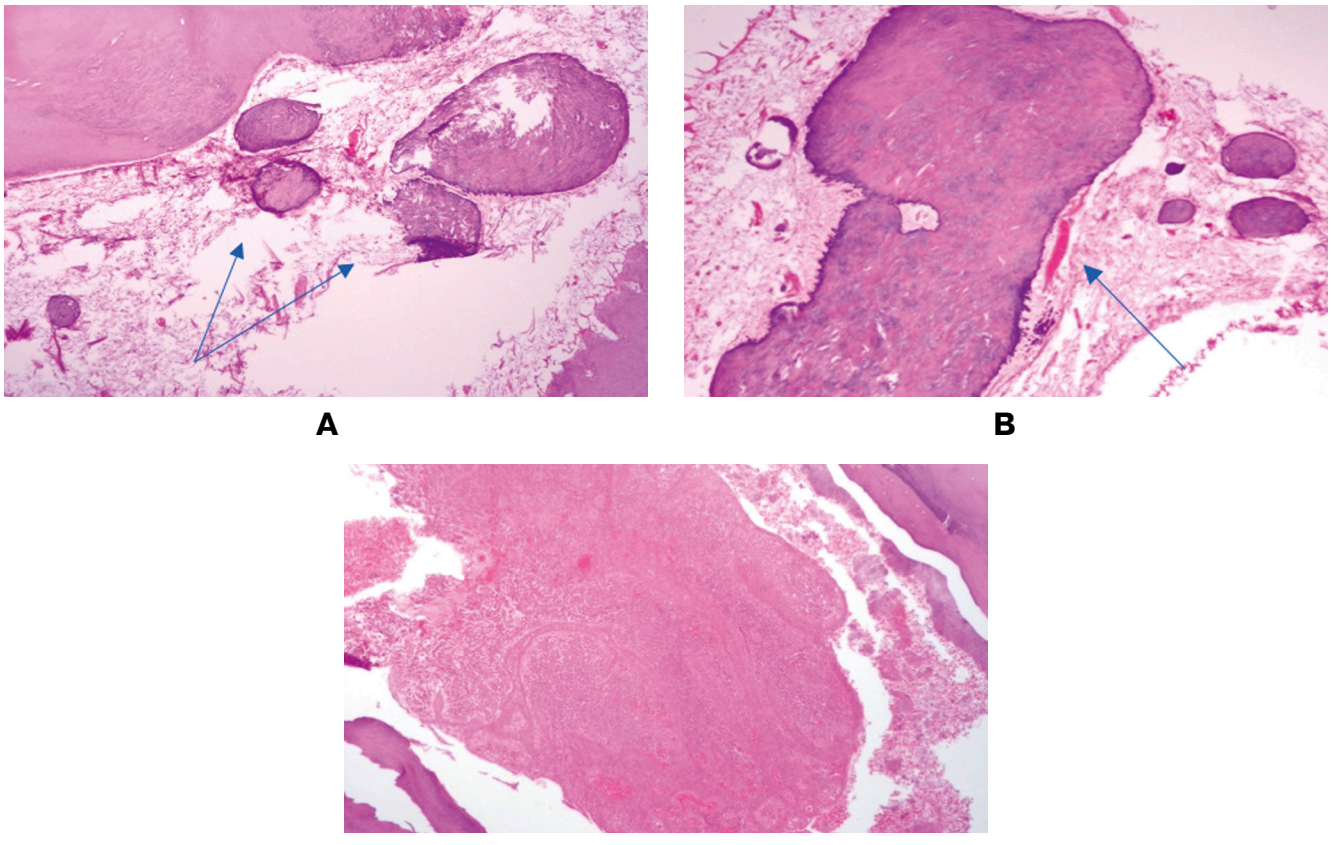
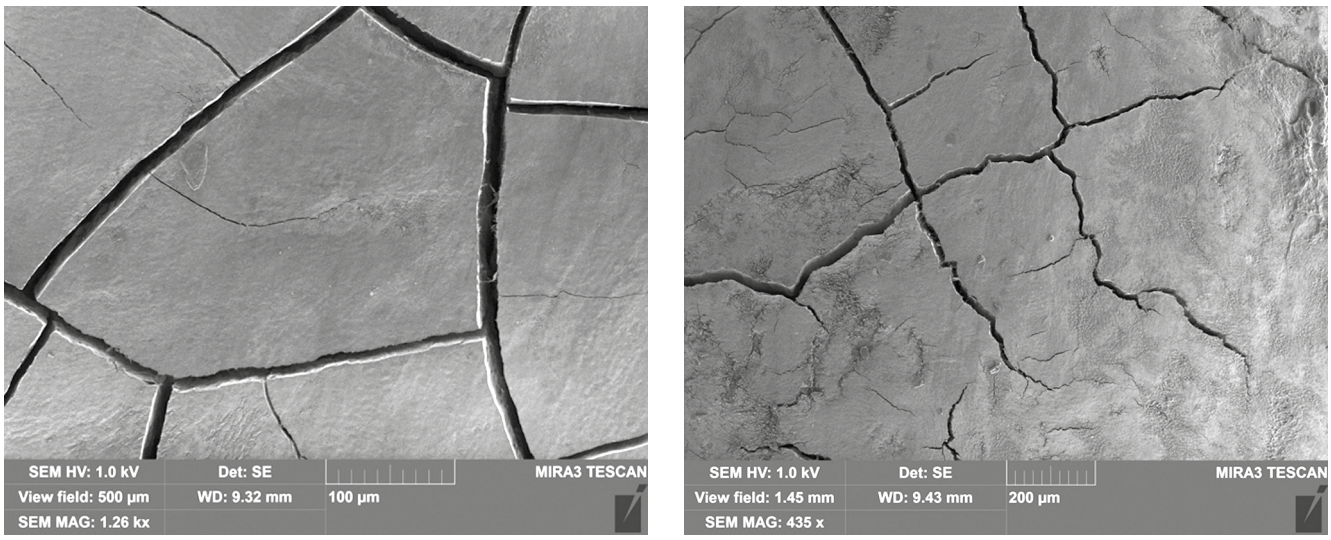


Рис. 1. Гистологическое исследование экстрадированного зуба вследствие эндо-пародонтального поражения. Окраска гематоксилином и эозином (×10; ×50): *A* – множественные кальцификаты в коронковой пульпе; *B* – выраженная инъекция и полнокровие кровеносных сосудов пульпы зуба; *C* – выраженная диффузная лимфоплазмоцитарная инфильтрация в периапикальных тканях

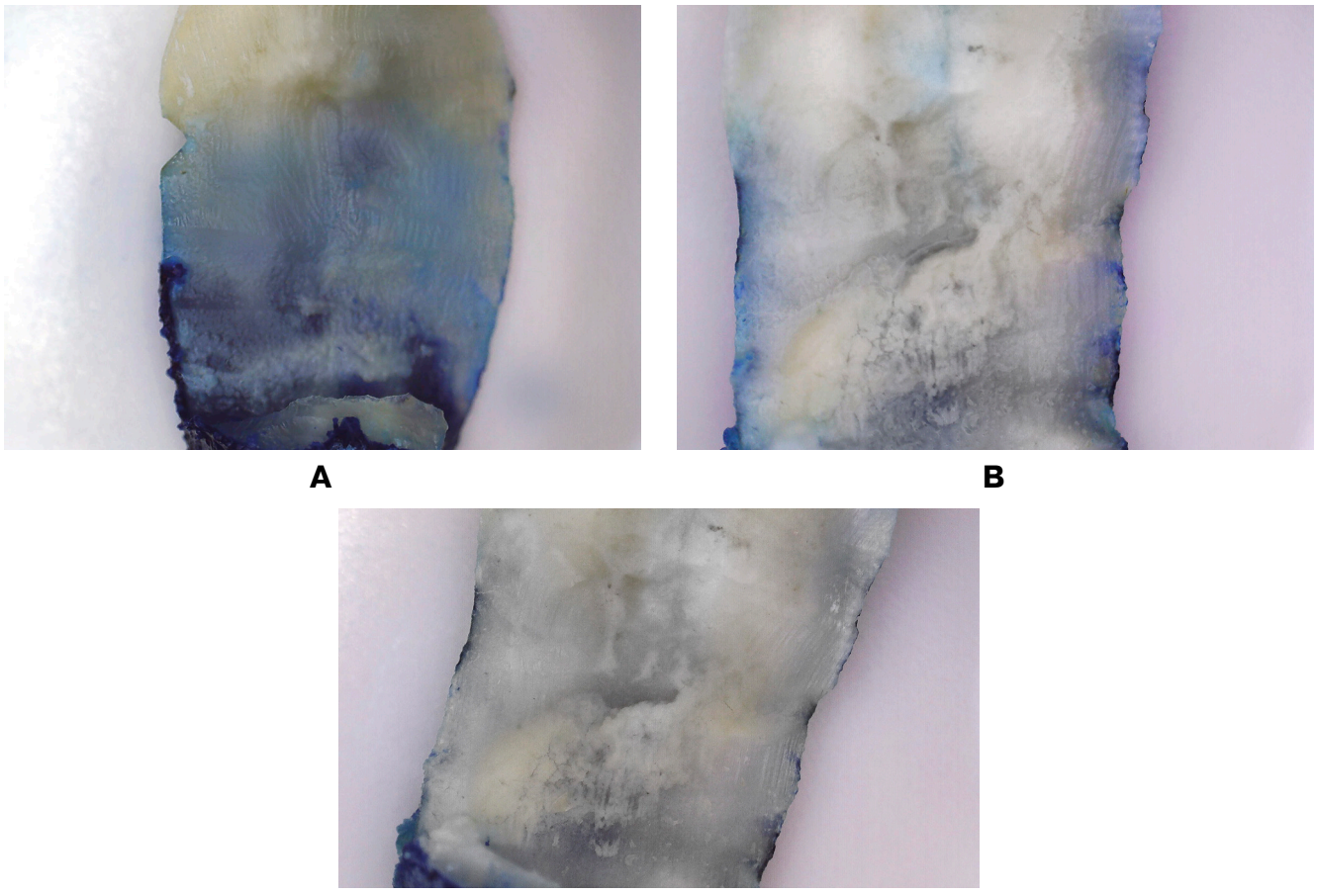
Fig. 1. Histological examination of an extracted tooth is the cause of endo-periodontal damage. Hematoxylin and eosin staining (×10; ×50): *A* – multiple calcifications in the coronal pulp; *B* – pronounced injection and congestion of blood vessels; *C* – pronounced diffuse lymphoplasmacytic infiltration in the periapical tissues



A **B**

Рис. 2. Сканирующая электронная микроскопия наружной поверхности корня зуба после инструментальной обработки (×1.26; ×435): *A* – ультразвуковой скейлер «DTE D72» (Woodpecker, Китай); *B* – ручные инструменты – зоноспецифические кюреты Грейси (Экрадент, Россия)

Fig. 2. Scanning electron microscopy of the external surface of tooth root cementum (×1.26; ×435): *A* – ultrasonic scaler «DTE D72» (Woodpecker, China); *B* – manual Gracey curettes (Ecradent, Russia)



A **B**

C

Рис. 3. Цифровая микроскопия продольного распила зуба после проникновения пигмента в образовавшиеся трещины и участки деструкции цемента вследствие механической обработки корня (×10): *A* – ультразвуковой скейлер «DTE D72» (Woodpecker, Китай); *B* – ручные инструменты – зоноспецифические кюреты Грейси (Экрадент, Россия); *C* – отсутствие обработки поверхности

Fig. 3. Digital microscopy of longitudinal tooth cutting after pigment penetration into formed cracks and cement destruction due to root machining (×10): *A* – ultrasonic scaler “DTE D72” (Woodpecker, China); *B* – manual Gracey curettes (Ecradent, Russia); *C* – no surface treatment

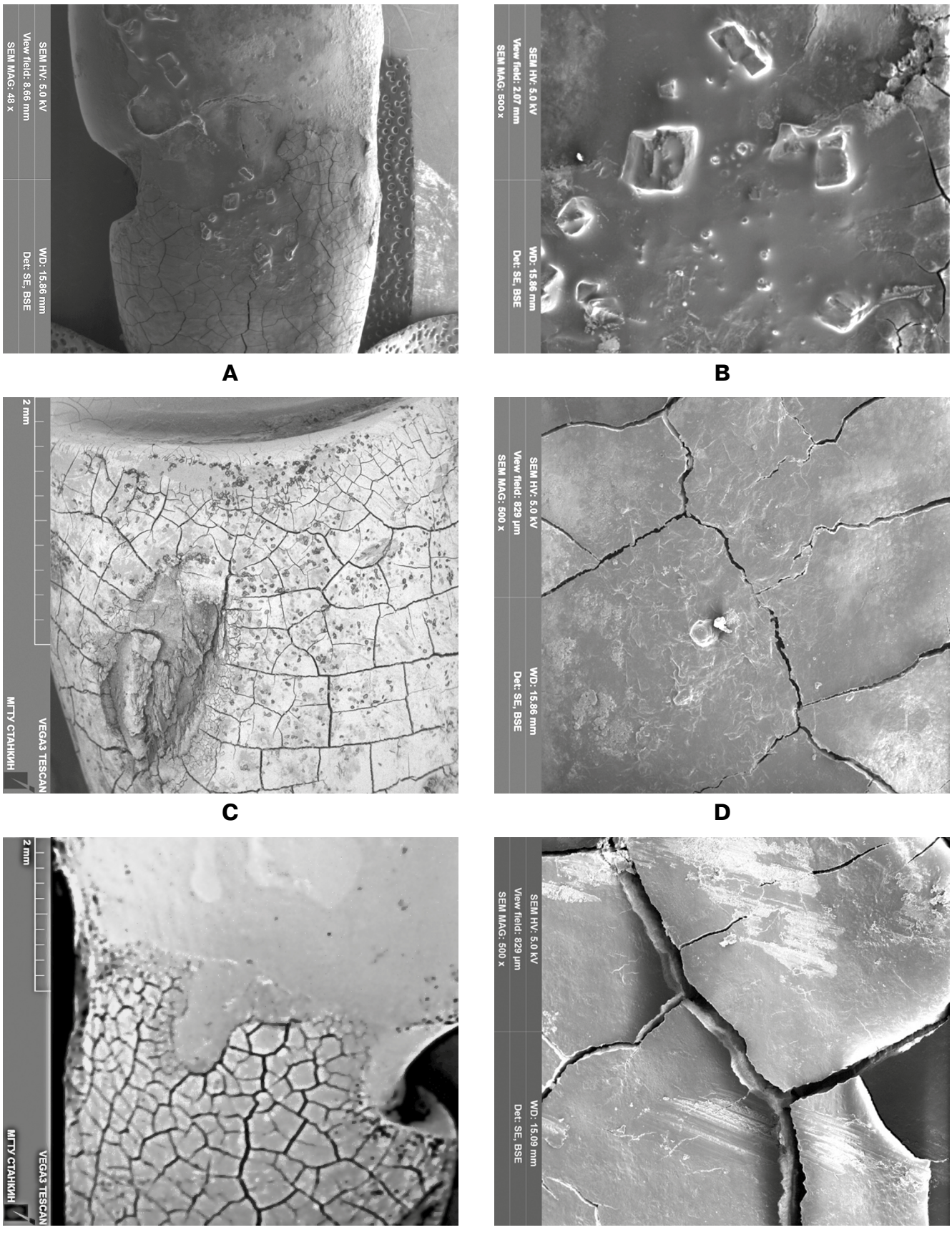


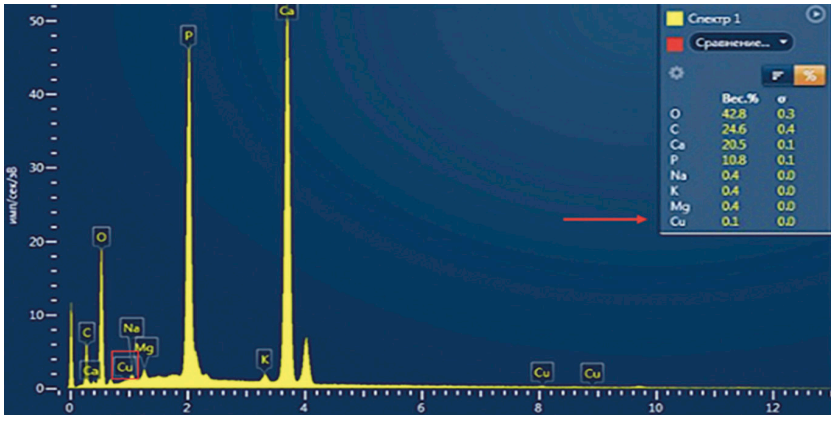
Рис. 4. Сканирующая электронная микроскопия обработанной поверхности корня зуба после нанесения нанопрепаратов (×48; ×500): *A–B* – «Дентин-герметизирующий ликвид» (HCH GmbH, Германия); *C–D* – препарат «Сенситаб» (Omegadent, Россия); *E–F* – суспензия «Десенсетин» (TechnoDent, Россия)

Fig. 4. Scanning electron microscopy of the processed tooth root surface after application of nanoprocessing (×48; ×500): *A–B* – “Dentin-sealing liqueur” (HCH GmbH, Germany); *C–D* – “Sensitab” (Omegadent,Russia); *E–F* – suspension “Desensitine” (TechnoDent, Russia)

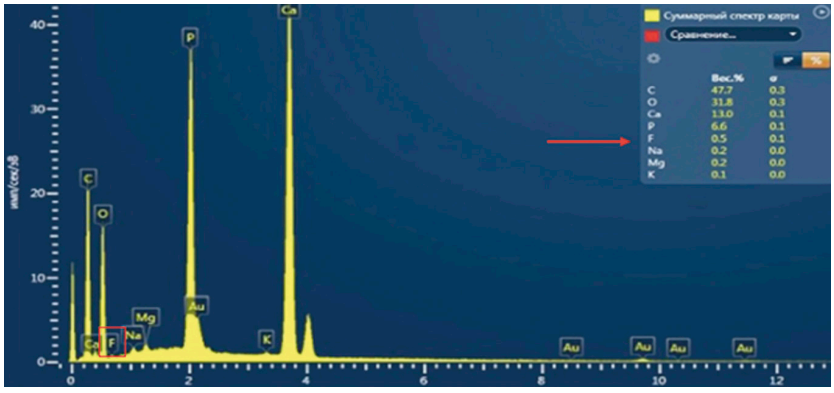
cement during professional oral hygiene in patients with chronic periodontitis, cracks and areas of destruction of the cement of the roots of the teeth were revealed, which are possible pathways of penetration of pathogenic microbiota from the periodontal pocket into the dentinal tubules, which significantly increases the risk of pulp pathology development in EPL.

To prevent the development of pulp pathology in EPL, it is necessary to reduce the permeability of dentin not only from the inside, but also from the outside; for this purpose, it is necessary to use preparations whose particles are able to penetrate into the lumen of dentinal tubules (diameter 50–1000 nm) and tightly obturate the surface defects of the root cement [9]. The SEM results demonstrated that the highest efficiency of obturation of the root surface was found when using the nanopreparation "Dentin-sealing liquid" and amounted to 85% of the entire root surface, which is 35% more in comparison with the use of the preparation "Sensitab". The sealing of the treated root surface with "Desensetin" suspension amounted to no more than 20% (Fig. 4).

Evaluation of the results of energy dispersive analysis revealed a similar distribution of elements for all materials with major peaks of calcium, phosphorus, oxygen, carbon, silicon, magnesium and sodium (Fig. 5).



A



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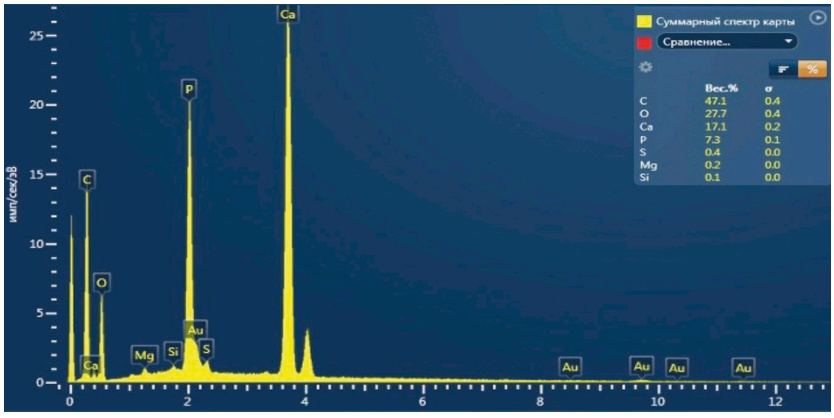

C

Рис. 5. Энергодисперсионная рентгеновская спектроскопия исследуемых нанопрепаратов:
A – «Дентин-герметизирующий ликвид» (HCH GmbH, Германия);
B – препарат «Сенситаб» (Omegadent, Россия); *C* – суспензия «Десенсетин» (TechnoDent, Россия)

Fig. 5. Energy-dispersive X-ray spectroscopy of tested nano-preparations: *A* – "Dentin-sealing liqueur" (HCH GmbH, Germany); *B* – "Sensitab" (Omegadent, Russia); *C* – suspension "Desensitine" (TechnoDent, Russia)

However, the presence of the element copper was detected only in the composition of the preparation "Dentin Sealing Liquid", which, according to the manufacturer, enhances its bactericidal effect. In the composition of the preparation "Sensitab" were detected peaks of the element fluorine, which tightly seals dentinal tubules with a mineral layer. In turn, the drug "Desensetin" has in its composition arginine, which only has a symptomatic effect, but does not solve the problem etiologically.

DISCUSSION

The provided study revealed that the treatment of the external root surface with ultrasonic scaler 3 times more often leads to the appearance of superficial defects and cracks of the tooth root cement, which is confirmed by the data of greater depth of pigment penetration into the formed cement defects during ultrasonic treatment of the tooth root. It was determined that in the pulp of the tooth in endo-parodontal lesions in 80% of cases there is diffuse lymphoplasmacytic infiltration and multiple calcifications in areas with exposed root cementum.

CONCLUSIONS

According to the data of laboratory and experimental study it was established that maintaining the integrity of the tooth root cement is necessary to preserve pulp viability in patients with EPL. It has been revealed that the best complex of measures aimed at preventing the development of pulp pathology in EPL is: 1 – professional oral hygiene in patients with CGP using hand instruments – Gracie's curettes, due to their less mechanical impact on the cement surface and 2 – mandatory subsequent impregnation of the treated tooth root surface with the nanopreparation "Dentin-sealing liquid" for obturation of the cracks and surface defects of the tooth root cement.

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INFORMATION ABOUT THE AUTHORS

Diana A. Ostanina – Cand. Sci. (Med.), Associate Professor of the Department of Therapeutic Dentistry and Endodontics, Russian University of Medicine; 4, Dolgorukovskaya St., Moscow 127006, Russian Federation; https://orcid.org/0000-0002-5035-5235

Sabina Sh. Alimukhamedova – Student of the Department of the Faculty of Dentistry, Russian University of Medicine; 4, Dolgorukovskaya St., Moscow 127006, Russian Federation; https://orcid.org/0009-0005-0906-5665

Alexander V. Mitronin – Dr. Sci. (Med.), Professor, Dean of the Faculty of Dentistry, Head of the Department of Therapeutic Dentistry and Endodontics, Honored Doctor of Russian Federation, Russian University of Medicine; 4, Dolgorukovskaya St., Moscow 127006, Russian Federation; https://orcid.org/0000-0002-3561-6222

ИНФОРМАЦИЯ ОБ АВТОРАХ

Останина Диана Альбертовна – к.м.н., доцент кафедры терапевтической стоматологии и эндодонтии, ФГБОУ ВО «Российский университет медицины» Министерства здравоохранения Российской Федерации; 127006, Российская Федерация, г. Москва, ул. Долгоруковская, д. 4; https://orcid.org/0000-0002-5035-5235

Алимухамедова Сабина Шухратбековна – студент стоматологического факультета, ФГБОУ ВО «Российский университет медицины» Министерства здравоохранения Российской Федерации; 127006, Российская Федерация, г. Москва, ул. Долгоруковская, д. 4; https://orcid.org/0009-0005-0906-5665

Митронин Александр Валентинович – д.м.н., профессор, декан стоматологического факультета, заведующий кафедрой терапевтической стоматологии и эндодонтии, Заслуженный врач РФ, ФГБОУ ВО «Российский университет медицины» Министерства здравоохранения Российской Федерации; 127006, Российская Федерация, г. Москва, ул. Долгоруковская, д. 4; https://orcid.org/0000-0002-3561-6222

AUTHOR'S CONTRIBUTION

Diana A. Ostanina – has made a substantial contribution to the concept or design of the article; the acquisition, analysis, or interpretation of data for the article; drafted the article; revised the article critically for important intellectual content.

Sabina Sh. Alimukhamedova – the acquisition, analysis, or interpretation of data for the article; drafted the article.

Alexander V. Mitronin – has made a substantial contribution to the concept or design of the article; revised the article critically for important intellectual content; approved the version to be published.

ВКЛАД АВТОРОВ

Д.А. Останина – существенный вклад в замысел и дизайн исследования, сбор данных, анализ и интерпретация данных, подготовка статьи, критический пересмотр статьи в части значимого интеллектуального содержания.

С.Ш. Алимухамедова – сбор данных, анализ и интерпретация данных, подготовка статьи.

А.В. Митронин – существенный вклад в замысел и дизайн исследования, критический пересмотр статьи в части значимого интеллектуального содержания; окончательное одобрение варианта статьи для опубликования.