https://doi.org/10.36377/ET-0034

The investigation of endodontic irrigants and polyhexanide-based solution action on smear layer

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Abstract
AIM. The effect of irrigants on the smear layer on the intracanalicular dentin surface is one of the important issues in endodontics. The objective of this article is to determine the effect of different concentrations of polyhexanide-based solutions and standard irrigants on the smear layer using scanning electron microscopy (SEM).
MATERIALS AND METHODS. The present study was conducted on 42 extracted teeth (third molars, first and second premolars) with formed apices that had been extracted for orthodontic indications. The teeth were decoronated and samples were prepared. In order to create a smear layer, the lumen of the canals was instrumented with H-file #40 throughout. A simulation of root canal irrigation was conducted by immersing the specimens in a 5-ml container filled with the proper solution for 1 hour: group 1 – no treatment (*n* = 6); group 2 – saline (*n* = 6); group 3 – 2% chlorhexidine (*n* = 6); group 4 – 3% sodium hypochlorite (*n* = 6); group 5 – 17% EDTA; group 6 – polyhexanide 0.1% (*n* = 6); group 7 – polyhexanide 0.2% (*n* = 6). A ~30 nm thick platinum film was sputtered onto the surface of each sample using a magnetron deposition technique.
SEM microphotos were acquired on a Vega3 TESCAN scanning electron microscope (SEM) with an SE secondary electron detector, which enables imaging with high topographic contrast. The initial detector (in-camera) was employed for image acquisition, with an electron energy of 30 keV. Images were acquired at magnifications of x20 (overview image), x150, x250, x500, x1000, x2500, x7500 for each sample.
RESULTS. The samples from groups 1 and 5 demonstrated the absence of a smear layer on the dentin surface and the absence of smear plugs in the dentinal tubules. Groups 2 and 3 are distinguished by the deposition of a pronounced amorphous smear layer and the obturation of all dentinal tubules. Groups 4, 6, and 7 are distinguished by the deposition of a smaller volume of smear layer on the dentin surface, as well as the presence of semi-open or completely open entrances to the dentinal tubules.
CONCLUSION. The data obtained indicates that antiseptic compositions based on polyhexanide in concentrations of 0.1 and 0.2% do not have a pronounced effect on the smear layer. However, in visual evaluation of SEM micrographs, their effect is comparable to that of 3% sodium hypochlorite. In instances where enhanced dissolution of the smear layer and smear plugs from the tubules is necessary to facilitate decontamination of the wall dentin, the combination of antiseptics with chelate compounds, such as 17% EDTA, may be employed.

Keywords: polyhexanide, dentine, smear layer

Article info: received – 05.06.2024; revised – 01.08.2024; accepted – 18.08.2024

Conflict of interests: The authors declare no conflict of interests.

Acknowledgements: There are no financing and individual acknowledgements for declaration.

For citation: Khabadze Z.S., Generalova Yu.A., Kulikova A.A. The investigation of endodontic irrigants and polyhexanide-based solution action on smear layer. *Endodontics Today*. 2024;22(3):199–205. https://doi.org/10.36377/ET-0034

Исследование воздействия эндодонтических ирригантов и раствора на основе полигексанидов на смазанный слой

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Резюме
ЦЕЛЬ. Влияние ирригантов на смазанный слой, образующийся на внутриканальной поверхности дентина, является одним из важных вопросов в эндодонтии. Цель данной статьи – определить влияние различных концентраций антисептических композиций на основе полигексанида и стандартных ирригантов на смазанный слой с помощью сканирующей электронной микроскопии (СЭМ).
МАТЕРИАЛЫ И МЕТОДЫ. 42 удаленных по ортодонтическим показаниям зуба (третьи моляры, первые и вторые премоляры) со сформированными апексами были включены в исследование. Зубы были декоронованы и далее соответствующим образом были подготовлены образцы корневых каналов. Для создания смазанного слоя просвет каналов образцов был обработан Н-файлом № 40 на всем протяжении. Проводилась имитация ирригации корневого канала путем погружения образцов в 5-миллилитровый контейнер, заполненный соответствующим раствором на 1 час: группа 1 – без обработки (*n* = 6); группа 2 – физраствор (*n* = 6); группа 3 – 2% хлоргексидин (*n* = 6); группа 4 – 3% гипохлорит натрия (*n* = 6); группа 5 – 17% ЭДТА; группа 6 – полигексанид 0,1% (*n* = 6); группа 7 – полигексанид 0,2%

(*n* = 6). На поверхность каждого образца методом магнетронного осаждения была напылена платиновая пленка толщиной ~30 нм. Микрофотографии получены на сканирующем электронном микроскопе (СЭМ) Vega3 TESCAN с детектором вторичных электронов SE, который позволяет получать изображения с высоким топографическим контрастом. Для получения изображений использовался первичный детектор (внутрикамерный) с энергией электронов 30 кэВ. Изображения были получены при увеличении x20 (обзорное изображение), x150, x250, x500, x1000, x2500, x7500 для каждого образца.
РЕЗУЛЬТАТЫ. Образцы групп 1 и 5 демонстрировали отсутствие смазанного слоя на поверхности дентина и отсутствие дентинных пробок в канальцах. Группы 2 и 3 отличаются наличием выраженного аморфного смазанного слоя и закупориванием дентинных канальцев. Для групп 4, 6 и 7 характерна визуализация меньшего объема смазанного слоя на поверхности дентина, а также наличие полуоткрытых или полностью открытых входов в дентинные канальцы.
ЗАКЛЮЧЕНИЕ. Полученные данные свидетельствуют о том, что антисептические композиции на основе полигексанида в концентрациях 0,1 и 0,2% не оказывают выраженного влияния на смазанный слой. Однако при визуальной оценке микрофотографий СЭМ их действие сопоставимо с действием 3% гипохлорита натрия. В случаях, когда для облегчения деконтаминации пристеночного дентина необходимо выраженной растворение смазанного слоя и раскрытие канальцев, рекомендуется использовать комбинацию антисептиков с хелатными соединениями, такими как 17% ЭДТА.

Ключевые слова: полигексанид, дентин, смазанный слой

Информация о статье: 05.06.2024; исправлена – 01.08.2024; принята – 18.08.2024

Конфликт интересов: Авторы сообщают об отсутствии конфликта интересов.

Благодарности: Финансирование и индивидуальные благодарности для декларирования отсутствуют

Для цитирования: Хабадзе З.С., Генералова Ю.А., Куликова А.А. Исследование воздействия эндодонтических ирригантов и раствора на основе полигексанидов на смазанный слой. *Эндодонтия Today*. 2024;22(3):199–205. (На англ. яз.) https://doi.org/10.36377/ET-0034

INTRODUCTION

In the context of endodontic therapy, the efficient removal of the smear layer is of critical importance for the successful outcome of root canal treatment. The smear layer (SL), which is composed of organic and inorganic debris, forms on the dentin surface during instrumentation and can obstruct the penetration of intracanal medicaments and sealers into dentinal tubules. Consequently, its elimination is essential to enhance disinfection and promote a fluid-tight seal within the root canal system [1–3].

The efficacy of different endodontic irrigants in affecting the smear layer varies considerably. Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX) are the most commonly used endodontic irrigants as part of medicational therapy. Polyhexanide is also a promising endodontic irrigant due to its broad-spectrum antibacterial activity and low cytotoxicity [3–6].

Sodium hypochlorite is a widely used irrigant due to its potent antimicrobial properties and ability to dissolve organic tissue. However, its role in smear layer removal is incomplete because it exhibits proteolytic activity only against the organic components of the smear layer, while the inorganic component is not affected [7–9].

EDTA, a chelating agent, is a standard for removing inorganic components of the smear layer, facilitating its detachment from the dentin surface. Various studies have demonstrated its effectiveness in cleaning and opening dentinal tubules from smear layer. However, for optimal results, it is recommended to use this irrigant in conjunction with sodium hypochlorite, which will be able to dissolve the organic constituents of the SL. Based on the aforementioned evidence, it can be concluded that EDTA enhances the penetration of intracanalicular drugs and sealers into the dentinal tubules [10; 11].

Chlorhexidine, which is known for its broad-spectrum antimicrobial activity, unfortunately lacks the ability to dissolve the smear layer. Chlorhexidine possesses the phenomenon of substantivity, and it is often used as a final irrigant rather than a primary agent, as its prolonged release helps to reduce the bacterial load in the root canal system [12; 13].

Polyhexanide, a polymeric biguanide compound, may represent a promising alternative to traditional endodontic irrigants. It has been observed to exhibit antimicrobial activity against a wide range of microorganisms, including endodontic pathogens [14]. However, there has been no previous study into the effect of this agent on the smear layer components.

AIM

The objective of this article is to determine the effect of different concentrations of polyhexanide-based solutions and standard irrigants on the smear layer using scanning electron microscopy (SEM).

MATERIALS AND METHODS

1. Sample Preparation

The present study was conducted on 42 extracted teeth (third molars, first and second premolars) with formed apices that had been extracted for orthodontic indications. To ensure the teeth were free from any confounding factors, a preliminary screen was conducted to exclude those with root fractures, carious lesions, or previously done endodontic treatment. Subsequently, all teeth were stored in an isotonic solution containing gentamicin sulfate at a temperature of +4°C prior to being utilized.

The teeth included in the study were decoronated using a diamond spade-shaped bur (FO-11 ISO 299/013, Mani, Japan). Subsequently, 1-mm deep slackening cuts were made in the bi- or trifurcation zone with a diamond spade-shaped bur. The specimens were then split longitudinally in vestibulo-oral plane into two halves with a chisel, taking sufficient care not to damage the dentin. Every fragment was marked half of the root canal lumen.

In order to create a smear layer, the lumen of the canals was instrumented with H-file #40 throughout.

A simulation of root canal irrigation was conducted by immersing the specimen in a 5-ml container filled with the proper preparation.

Initial solutions:

1. Sodium chloride (BIEFFE MEDITAL, S.A., Spain).
2. Liquid for antiseptic treatment of root canals of teeth (chlorhexidine bigluconate – 2% aqueous solution, Omega-Dent, Russia).
3. Belodez (sodium hypochlorite – 3% solution, VladMiVa, Russia).
4. MD Cleanser (EDTA – 17%, META, South Korea).
5. Lavasept (polyhexanide hydrochloride – 20% aqueous solution, B. Braun Melsungen AG, Germany).

A 0.2% polyhexanide solution was prepared by diluting the original 20% polyhexanide solution. One milliliter of the polyhexanide 20% solution was placed in a 100-milliliter measuring flask, and the volume was brought to the mark with distilled water. A 0.1% polyhexanide solution was prepared by diluting the original 0.2% polyhexanide solution. Twenty-five milliliters of the 0.2% polyhexanide solution was placed in a 50-milliliter measuring flask, and the volume was brought to the mark with distilled water.

After exposure of the specimens in the solutions, they were washed with distilled water to remove the residues of irrigants and placed in a 5 ml container with physiological solution in order to prevent drying of dentin.

2. Testing groups

The 42 samples were divided into seven groups of six samples each, according to the treatment done:

– group 1 – specimens without instrumental and medical treatment (no smear layer);

– group 2 – instrumental treatment to create a smear layer, exposure in physiological solution for 1 hour;

– group 3 – instrumental treatment to create a smear layer, exposure in 2% aqueous chlorhexidine solution for 1 hour;

– group 4 – instrumental treatment to create a smear layer, exposure in 3% sodium hypochlorite solution for 1 hour;

– group 5 – instrumental treatment to create a smear layer, exposure in 17% EDTA solution for 1 hour;

– group 6 – instrumental treatment to create a smear layer, exposure in 0.1% polyhexanide solution 1 hour;

– group 7 – instrumental treatment to create a smear layer, exposure in 0.2% polyhexanide solution for 1 hour.

3. Preparation of Samples for SEM

A ~30 nm thick platinum film was sputtered onto the surface of each sample using a magnetron deposition technique. This method allowed for the avoidance of the accumulation of electric charge in the near-surface layer of the sample and the associated distortions of the image.

4. Scanning electron microscopy

Images were acquired on a Vega3 TESCAN scanning electron microscope (SEM) with an SE secondary electron detector, which enables imaging with high topographic contrast. The initial detector (in-camera) was employed for image acquisition, with an electron energy of 30 keV.

Table 1. Mean scores of smear layer on microphotographs with x7500 magnification

Таблица 1. Средние оценки смазанного слоя на микрофотографиях с увеличением x7500

Groups ($n = 6$ each)	Mean ± SD
1	1 ± 0
2	3 ± 0
3	2.833 ± 0.372
4	2.5 ± 0.500
5	1.333 ± 0.471
6	2.833 ± 0.372
7	2.667 ± 0.471

Images were acquired at magnifications of x20 (overview image), x150, x250, x500, x1000, x2500, x7500 for each sample.

The microphotographs were analyzed by two independent investigators in a blinded manner to assess the effect of the irrigant on the dentin surface. The observations were recorded in points, according to M. Torabinejad et al. [15], which were given to every image:

– 1 point – no smear layer. No smear layer on the surface of the root canals and no smear plugs inside tubules;

– 2 point – moderate smear layer. No smear layer on the surface of root canal, but tubules contained smear plugs inside;

– 3 point – heavy smear layer. Smear layer covered the root canal surface and the tubules.

5. Statistical analysis

The one-way ANOVA test was provided with StatPlus 6 (AnalystSoft, CA, USA) for the average data obtained in each testing group, followed by a post hoc Tukey's test. The correlation test was applied to assess the correlation of smear layer presence. The significance level was set at $p < 0.05$.

RESULTS

By analyzing the SEM micrographs, certain patterns and characteristics describing the intracanal dentin surface of each group were obtained. Mean scores of smear layer on microphotographs are shown in Table 1.

In Group 1, where the specimens were neither medicated nor instrumented, the micrographs at all magnifications demonstrated the absence of a smear layer and open dentinal tubules. The standard histological picture is depicted in great detail, showing oval-shaped dentinal tubes surrounded by an array of intertubular dentin (Fig. 1).

All specimens of the second group exhibited the presence of a pronounced smear layer on the dentin, as well as the obturation of dentinal tubules with smear plugs. In this instance, the smear layer array was not affected, as the saline solution lacks both organolytic and chelate properties. Consequently, the SL was not dissolved, resulting in the formation of a single amorphous mass that covers the surface of intracanal dentin and occludes the entrance to the majority of dentinal tubules (Fig. 2).

In the third group, the microphotographs revealed the presence of smear layer formation, with the majority of dentinal tubes exhibiting plugging. Nevertheless, some tubules remain unobstructed. It is evident that chlorhexidine is unable to affect the organic components of the smear layer. With regard to the interaction of chlorhexidine with inorganic SL part, it should be noted that the antiseptic does not eliminate mineral conglomerates; rather, it binds to them in the form of crystals. Consequently, the dentin surface is coated with a substantial volume of the smear layer. The minimum number of dentinal tubule entrances was observed in all specimens, indicating that they are filled with smear plugs. Mentioned above information precludes the use of this antiseptic as a standalone agent in the protocol for medicament treatment of root canals (Fig. 3).

The samples of the fourth group exhibited a smaller amount of smear layer on the surface of dentine compared to the two previous groups. A greater number of dentinal tubule openings were observed. It can be posited that the smaller volume of the smear layer and the greater number of visible dentinal tubule entrances visible on the intercanal dentin surface are a consequence of the dissolution of the organic portion of the SL and the loss of a small amount of associated mineral particles (Fig. 4).

In the majority of cases, the absence of a smear layer and smear plugs in the dentinal tubules was observed on the dentin surface of root canal samples from group 5. EDTA forms a stable complex with calcium and exerts a pronounced effect on the inorganic components of the smear layer. The elimination of inorganics by the chelating agent results in the opening of dentinal tubules from smear plugs, a process that is well documented in microphotographs (Fig. 5).

The formation of a smear layer and dentin plugs was also observed in the dentin of samples from groups 6 and 7. However, in group 7, the smear layer on the surface of intracanal dentin was less pronounced. Nevertheless, no statistically significant differences were observed between the 6th and 7th groups. The microphotographs of dentin following exposure to polyhexanide solutions demonstrate the opening of some dentinal tubule entrances, with some exhibiting an oval morphology, comparable to that observed in the native control photo (prior to exposure to solutions) (Fig. 6).

DISCUSSION

The data obtained from the results of scanning electron microscopy permit the conclusion that the efficacy of medicament treatment of root canals should be verified and standardized in the form of certain protocols.

The micrographs obtained clearly demonstrate that the greatest quantitative reduction in the smear layer from the dentin surface and from the dentin tubules was achieved through the use of a chelate compound, 17% EDTA. The mechanism of action is the binding of the inorganic component of SL. These data are corroborated by other authors [16–18]. However, it should be noted that EDTA does not possess pronounced antibacterial properties. Consequently, it should be used in combination with antiseptic agents.

Of the antiseptic substances tested, it was observed that certain effects were produced on the smear layer when samples were exposed to 3% sodium hypochlorite and polyhexanide solutions. Upon examination of microphotographs of specimens treated with the aforementioned solutions, the presence of semi-open or open entrances to dentinal tubules was observed. Furthermore, a greater number of rounded tubules is observed in groups 6 and 7.

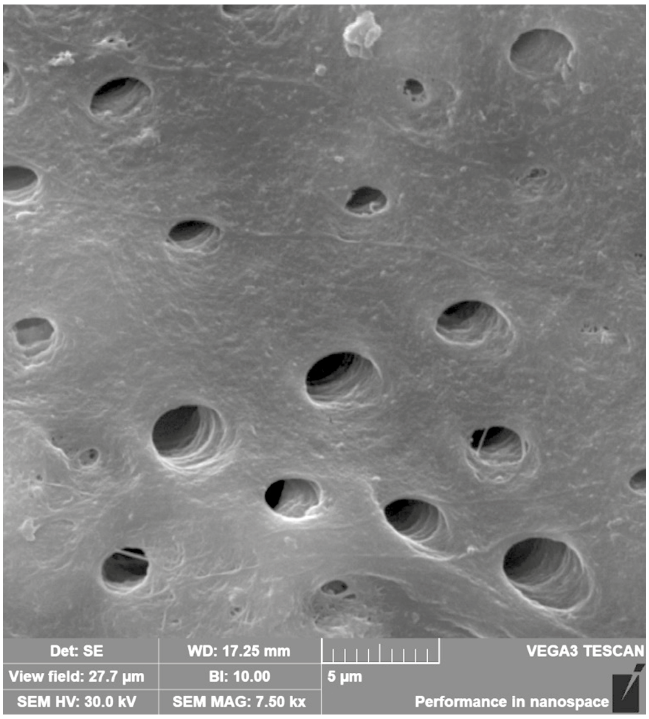


Fig. 1. Microphotograph of Group 1 – no treatment. x7500 magnification

Рис. 1. Микрофотография группы 1 – без обработки. Увеличение x7500

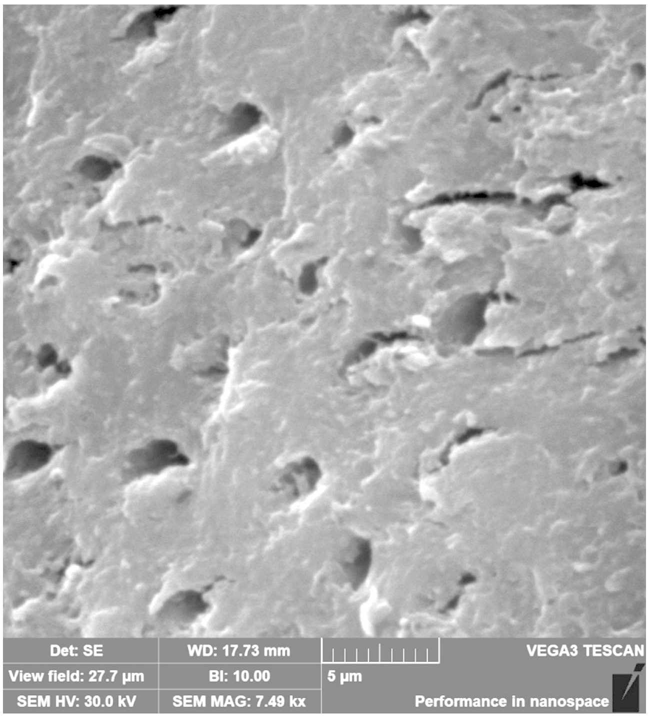
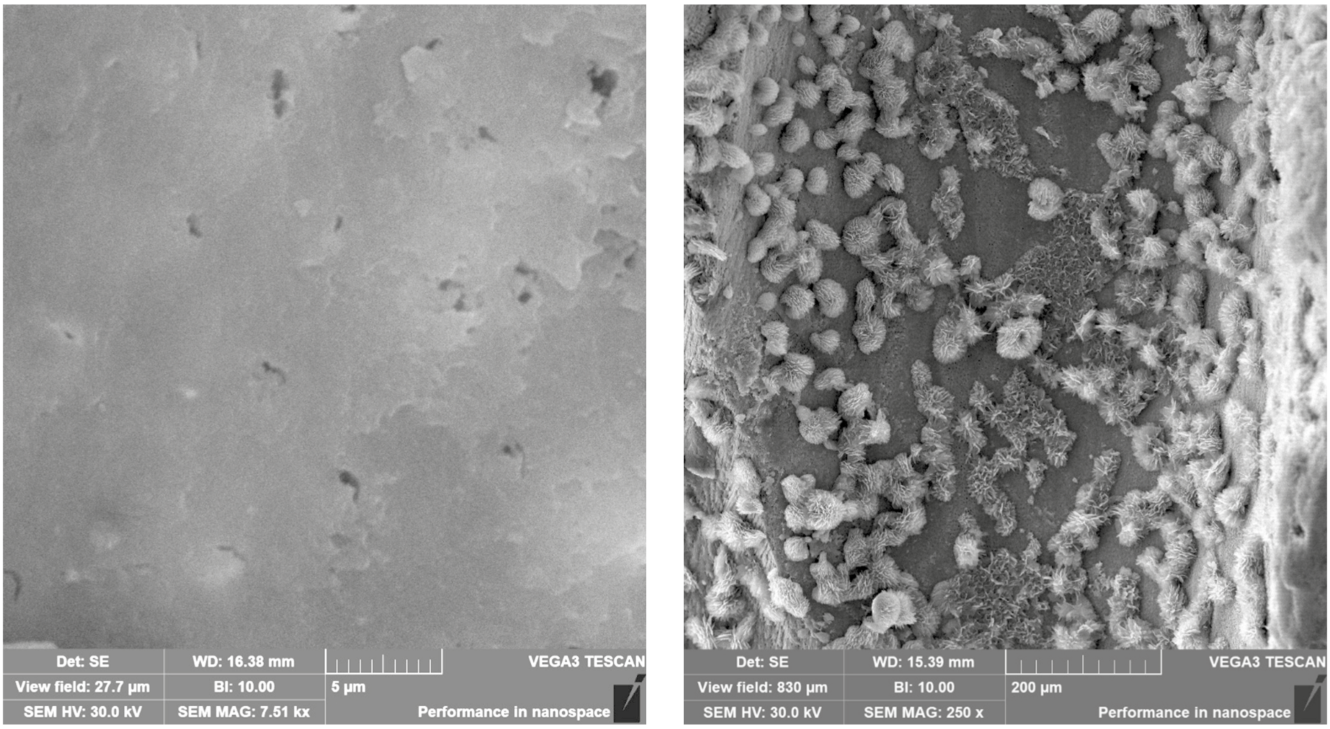


Fig. 2. Microphotograph of Group 2 – instrumentation + exposition in saline. x7500 magnification

Рис. 2. Микрофотография группы 2 – обработка инструментом + воздействие физиологическим раствором. Увеличение x7500



A **B**

Fig. 3. Microphotograph of Group 3 – instrumentation + exposition in 2% chlorhexidine. x7500 magnification (*A*). Crystals on intracanal dentine surface after exposure with 2% chlorhexidine. x250 magnification (*B*)

Рис. 3. Микрофотография группы 3 – обработка инструментом + воздействие 2% хлоргексидином. Увеличение x7500 (*A*). Кристаллы на поверхности внутриканального дентина после воздействия 2% хлоргексидином. Увеличение x250 (*B*)

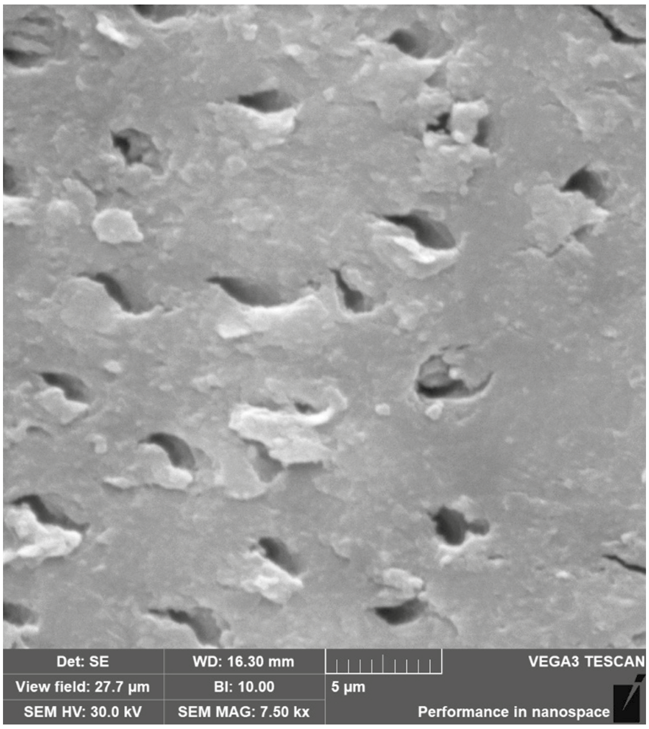


Fig. 4. Microphotograph of Group 4 – instrumentation + exposition in 3% sodium hypochlorite. x7500 magnification

Рис. 4. Микрофотография группы 4 – обработка инструментом + воздействие 3% гипохлоритом натрия. Увеличение x7500

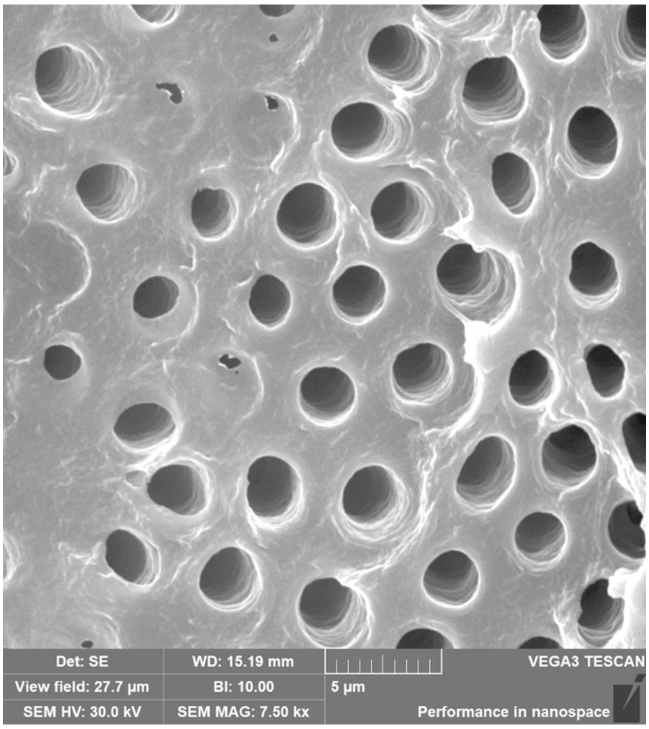
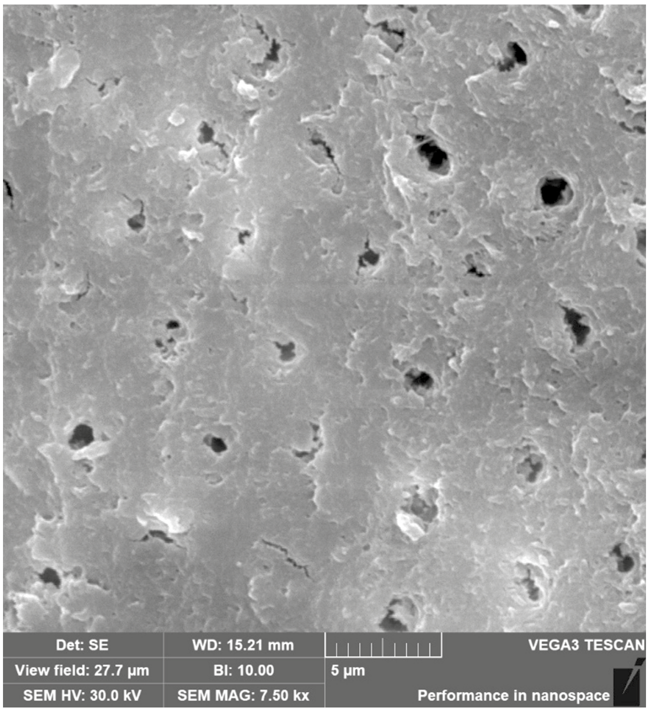
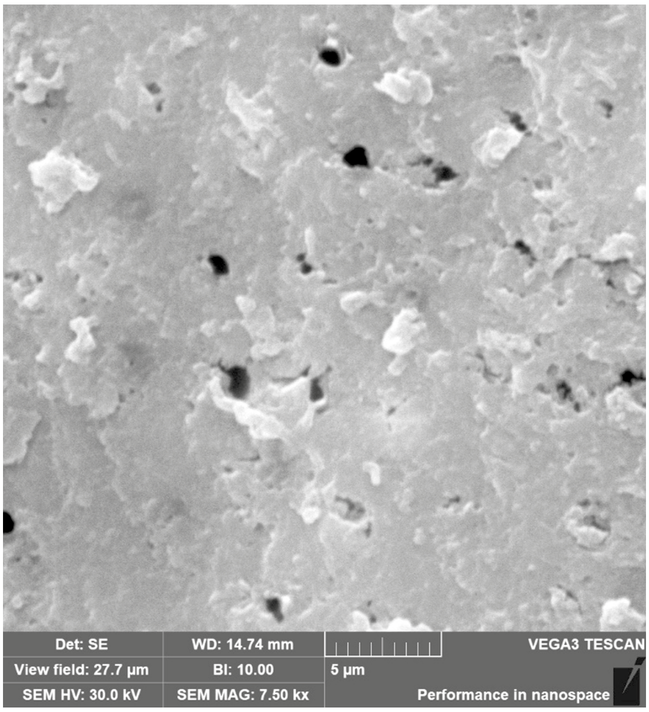


Fig. 5. Microphotograph of Group 5 – instrumentation + exposition in 17% EDTA. x7500 magnification

Рис. 5. Микрофотография группы 5 – обработка инструментом + воздействие 17% ЭДТА. Увеличение x7500



A



B

Fig. 6. Microphotograph of Group 6 (*A*), group 7 (*B*) – instrumentation + exposition in polyhexanide 0.1% and 0.2%. x7500 magnification

Рис. 6. Микрофотография группы 6 (*А*) и группы 7 (*В*) – обработка инструментом + воздействие полигексанидом 0,1% и 0,2%. Увеличение x7500

With regard to sodium hypochlorite, an organolytic effect on organic components of SL was determined [10; 11; 19]. However, further studies are necessary to ascertain the proteolytic activity of polyhexanide, as it is impossible to obtain exact values according to SEM data.

A polyhexanide-based antiseptic composition may be employed as an irrigation solution in a root canal medication protocol. In the case of pathologies in which infection of the root canal walls has not occurred, such as acute focal pulpitis, or in pre-prosthetic endodontic root canal preparation, the polyhexanide solution can be used alone, as the presence of a residual smear layer will not adversely affect the prognosis of treatment. If additional decontamination of the dentin wall is necessary, it can be combined with chelate compounds to enhance the efficacy of the process, particularly in the case of chronic apical periodontitis.

CONCLUSION

The data obtained indicates that antiseptic compositions based on polyhexanide in concentrations of 0.1 and 0.2% do not have a pronounced effect on the smear layer. However, in visual evaluation of SEM micrographs, their effect is comparable to that of 3% sodium hypochlorite. In instances where enhanced dissolution of the smear layer and smear plugs from the tubules is necessary to facilitate decontamination of the wall dentin, the combination of antiseptics with chelate compounds, such as 17% EDTA, may be employed.

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Alena A. Kulikova – 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.

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