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Analysis of the chemical interaction of polyhexanide with endodontic irrigants

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Abstract

INTRODUCTION. Chronic apical periodontitis (CAP) poses significant challenges in endodontics due to microbial resistance and inadequate disinfection protocols. Polyhexanide (PHMB) is a promising irrigant due to its antimicrobial properties. However, its interactions with other commonly used endodontic irrigants require further investigation to establish effective and safe clinical protocols.

AIM. To evaluate the chemical interactions of PHMB with sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), EDTA, and chlorhexidine and identify optimal irrigation protocols.

MATERIALS AND METHODS. The study utilized high-performance liquid chromatography (HPLC) to monitor reactions of PHMB with NaOCl, H_2O_2, EDTA, and chlorhexidine at intervals of 30 minutes, 1 hour, and 3 days. The analysis focused on detecting reaction products and assessing chemical stability.

RESULTS. PHMB formed a precipitate when combined with NaOCl, leading to its complete depletion in the solution. Reaction with H_2O_2 produced new chemical compounds, while EDTA demonstrated no significant negative reactions. Mixtures with chlorhexidine generated new products and precipitates. Sequential use of EDTA and PHMB showed compatibility and potential for effective irrigation.

CONCLUSIONS. Polyhexanide is a valuable irrigant for endodontics, particularly in combination with EDTA, as it provides smear layer removal and antimicrobial action without adverse interactions. Sodium hypochlorite and hydrogen peroxide require careful protocol adjustments to avoid chemical incompatibility. Further studies are necessary to confirm the clinical outcomes and refine protocols for safe and effective endodontic treatments.

Keywords: polyhexanide, irrigant, irrigation protocol, EDTA, NaOCl, interactions

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Анализ химического взаимодействия полигексанида с эндодонтическими ирригантами

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Резюме

ВВЕДЕНИЕ. Хронический апикальный периодонтит (ХАП) представляет сложность в эндодонтии из-за микробной резистентности и недостаточной эффективности протоколов дезинфекции. Полигексанид (PHMB) является перспективным ирригантом благодаря своим антимикробным свойствам. Однако взаимодействие PHMB с другими ирригантами требует дальнейшего изучения для разработки безопасных и эффективных протоколов.

ЦЕЛЬ. Оценить химическое взаимодействие PHMB с гипохлоритом натрия (NaOCl), перекисью водорода (H_2O_2), ЭДТА и хлоргексидином и выявить оптимальные протоколы ирригации.

МАТЕРИАЛЫ И МЕТОДЫ. Методом высокоэффективной жидкостной хроматографии (ВЭЖХ) изучались реакции PHMB с NaOCl, H_2O_2, ЭДТА и хлоргексидином через 30 минут, 1 час и 3 дня. Анализировались продукты реакции и химическая стабильность.

РЕЗУЛЬТАТЫ. PHMB образует осадок при смешивании с NaOCl, полностью исчезая из раствора. Реакция с H_2O_2 приводит к образованию новых соединений. ЭДТА не вызывает значительных негативных реакций. Смешивание с хлоргексидином сопровождается образованием новых продуктов и осадков. Последовательное применение ЭДТА и PHMB показало совместимость и эффективность.

ВЫВОДЫ. Полигексанид является перспективным ирригантом, особенно в сочетании с ЭДТА, обеспечивая удаление смазанного слоя и антисептическое действие без отрицательных взаимодействий. NaOCl и H_2O_2 требуют тщательной корректировки протоколов. Необходимы дополнительные исследования для подтверждения клинической эффективности и уточнения протоколов.

Ключевые слова: полигексанид, ирригант, протокол ирригации, ЭДТА, NaOCl, взаимодействие

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INTRODUCTION

Chronic apical periodontitis (CAP) is a common problem encountered by the general dental practitioner. The occurrence and progression of apical periodontitis are attributed to microbial factors. An intact root canal system is defined as sterile, meaning it is free of microorganisms and their by-products. However, certain factors, such as carious lesions (when the size of the dentinal bridge is less than 0.5–0.2 mm), inadequate direct or indirect restorations, dental treatment errors, or destroyed gingival attachment in the case of marginal periodontitis, may allow microorganisms and their toxic fractions to enter the pulp chamber. Prolonged irritation of the dental pulp by infectious agents can lead to its death [1–4]. Infectious apical periodontitis is primarily caused by the infection and necrotization of the neurovascular bundle of the tooth due to various bacterial factors [5].

As mentioned above, microbial infection of the root canal and the periapical tissues is considered to be one of the most important etiological factors in the development of apical periodontitis. Conservative treatment of pulpitis and periodontitis has a success rate ranging from 53% to 98%, with lower success rates reported in cases of retreatment. Endodontic treatment failure is commonly attributed to inadequate disinfection of the root canal space during treatment or re-infection caused by crown or apical microleakage [6–8].

The oral cavity harbours a greater variety of bacterial species compared to other parts of the gastrointestinal system. Two main groups of microorganisms can be distinguished in the oral cavity. The first group comprises transient flora that enter the mouth through air, liquids, and food. These microorganisms are not resistant to the protective factors present in the oral cavity and therefore cannot persist for long periods of time, eventually becoming extinct. The second group consists of resident (permanent) bacteria that form a stable ecosystem in the oral cavity. The microflora of the oral cavity is composed of various bacterial species that live in the oral cavity as an ecological niche. The oral cavity has different habitats that provide distinct ecological conditions for colonization and growth, including the mucosa of the lips, cheeks, palate, tongue, gums, and teeth [9–13].

However, due to the development of carious processes, which are caused by microorganisms (acid-producing cariogenic bacteria), an additional pathological niche can be formed, represented by the pulp chamber, into which bacteria and the products of their vital activity can penetrate. Endodontics distinguishes between primary and secondary infections. In cases of chronic apical periodontitis and after unsuccessful treatment, microorganisms may persist in the root canal, particularly in hard-to-reach areas such as isthmuses, fins, deltoid laterals, and deep within the dentinal tubules [14; 15]. The root canal system in cases of chronic apical periodontitis and unsuccessful endodontic treatment is often colonized by various genera of Gram-negative bacteria, such as Fusobacterium, Porphyromonas, Prevotella, Treponema, Tannerella, and Gram-positive bacteria, including Streptococcus, Enterococcus, Olsenella, Filifactor, Actinomyces, as well as fungi of the genus Candida [16–20].

The microflora present in primary and secondary endodontic infections differ, making it challenging to select a protocol for medicament treatment of root canals. It is important to note that bacteria within root canals exist not only as planktonic suspension filling the main lumen of the canal, but also as communities and consortia, known as biofilms. These biofilms can penetrate the thin lateral branches, isthmuses, and even the outer surface of the root, forming an extraradicular biofilm [21–23]. Bacterial communities are often more resistant to antiseptic agents due to various factors, such as quorum sensing, gene drift, antimicrobial drug efflux channels, and protective properties of the biofilm matrix [24; 25].

Mechanisms of bacterial resistance also include enzymatic modification or degradation of the drug, altering the antiseptic / antibiotic target in the bacterial cell, reducing membrane permeability to the drug or limiting drug accumulation by active transport of the drug out of the cell, creating metabolic bypass pathways [26–28]. It is important to distinguish between primary resistance of a bacterium and acquired resistance. The term “primary resistance” is defined as a natural property of a microorganism due to the absence of a target on which an antiseptic or antibiotic acts, whereas the term “acquired resistance” is the result of genetic changes and occurs either as a result of mutation or during the acquisition of new genetic material, for example through plasmids [29–31].

Microorganisms can develop resistance to antiseptics, especially cationic ones, through genetically determined mechanisms such as efflux pumps and changes in cell membrane hydrophobicity and permeability [32–34]. The emergence of chlorhexidine resistance is thought to be due to an increase in the expression of membrane proteins such as OprF, LptD, and Tol-Pal. Additionally, upregulation of PagL, flagellar proteins, chaperones, and proteins related to energy metabolism also contribute to bacterial resistance to cationic agents [25].

Irrigation is a crucial aspect of the endodontic CAP protocol. The conservative treatment of chronic apical periodontitis involves mechanical preparation of root canals with manual and mechanical steel and nickel-titanium instruments, pharmacological management, followed by three-dimensional obturation, according to the clinical guidelines for the treatment of periapical tissue diseases [35; 36].

The conditions for irrigation are created by adequate preparation of the root canal during mechanical treatment. In endodontics, even the most flexible instruments cannot fully contact all root canal walls, especially those with irregular and complex shapes, so medication is necessary. After preparation, pathogenic microorganisms remain on the canal walls, in dentin tubules, isthmuses, “fins” and other hard-to-reach places, and their further multiplication may lead to progression of the inflammatory process and a number of complications [37–41].

The issue of root canal irrigation within the field of endodontics has been widely discussed for a long time. The researchers aim to create a product with optimal antimicrobial activity and minimal cytotoxicity on periapical tissues and gingival fibroblasts. Practicing dentists and endodontists seek a pharmaceutical preparation with low reactivity to other irrigants, allowing for the use of different active substances in combinations without the risk of antagonistic interactions or toxic by-products.

Unfortunately, in endodontic practice there is no ideal irrigant that solves all the problems of medical treatment of endodontic infections. These tasks are – pronounced antibacterial action, including the effect on microbial biofilms, proteolytic action on necrotized tissues located in the root canal, complete elimination of the smear layer, no negative effect on the physical and mechanical properties of intra-root dentin and periradicular tissues [42–45]. Accordingly, it is important to combine products in order to achieve long-term positive results from conservative therapy.

Sodium hypochlorite (0.5–5.25%), ethylenediaminetetraacetic acid (17%) and chlorhexidine bigluconate aqueous solution (2%) are most commonly used in routine dental practice. The combination of NaOCl and EDTA is the “gold standard” for irrigation. However, sodium hypochlorite, EDTA and chlorhexidine have some negative and side effects, such as reduced physical and mechanical properties of dentin with NaOCl, dentin erosion with EDTA, acquired bacterial resistance and no effect on biofilms with chlorhexidine.

Each irrigant is a substance characterised by specific chemical reactions and interactions with other compounds [38; 46–48]. Spontaneous interaction of the above irrigants within the root canal (with sequential irrigation without separation of the solutions with distilled water or complete drying with paper pins) may result in chemical reactions with the formation of precipitates and toxic products.

The issue of root canal irrigation has been widely discussed in the field of endodontics for a long time. Researchers aim to develop a product with optimal antimicrobial activity and minimal cytotoxicity on periapical tissues and gingival fibroblasts [49–51]. Practicing dentists and endodontists are looking for a pharmaceutical preparation with low reactivity with other irrigants, allowing the use of different active ingredients in combinations without the risk of antagonistic interactions or toxic by-products.

Such a preparation as polyhexanide, a cationic antiseptic, is a promising irrigant in endodontic practice [52; 53]. Common bacterial resistance levels have not been documented.

It is important to detail the possible reactions between irrigants and new endodontic solutions, and their possible by-products in order to minimize complications during their use and to optimize treatment protocols for inflammatory pathologies of the pulpo-periodontal complex.

The aim of this study is to observe the reactions of polyhexanide compound with dental endodontic irrigants (sodium hypochlorite, chlorhexidine, EDTA, hydrogen peroxide) and to examine the course of possible reactions at room temperature for the presence and formation of new products.

MATERIALS AND METHODS

General design

1. The initial medications, including Lavasept (20% polyhexanide solution), 3% sodium hypochlorite (NaOCl), 3% hydrogen peroxide solution (H_2O_2), 17% ethylenediaminetetraacetic acid (EDTA), and 2% aqueous solution of chlorhexidine (CHX), were subjected to chromatographic control using high-performance liquid chromatography analysis (HPLC) to obtain qualitative and quantitative chemical composition and standard peaks of main ingredients of solutions.

2. The study of stability and reactivity of Lavasept (20% polyhexanide solution) to such reagents as:
- Sodium hypochlorite solution (3%);
- Hydrogen peroxide solution (3%);
- EDTA solution (ethylenediaminetetraacetic acid) (17%);
- Chlorhexidine solution (2% aqueous solution).

The progress of the reaction was investigated at 30 minutes, 1 hour, and 3 days. The reaction mixtures were analyzed in all 3 periods of time using HPLC to detect any new reaction products formed between the indicated components.

Chemicals and reagents

The following medicinal products were included in the present study:

1. Lavasept (Polyhexamethylene biguanide hydrochloride – 20% aqueous solution, B. Braun Melsungen AG, Germany). Lavasept contains polyhexamethylene

biguanide hydrochloride as active ingredient and auxiliary ingredients: macrogol-4000, water for injection (pH 5.0–7.0).

2. Hydrogen Peroxide (Hydrogen Peroxide – 3%, Ecotex, Russia). Components: medical hydrogen peroxide, stabilizer sodium benzoate, purified water

3. Belodez (Sodium hypochlorite – 3% solution, VladMiVa, Russia). Components: stabilized sodium hypochlorite solution.

4. MD Cleanser (EDTA – 17%, META, South Korea). Components: EDTA, water, ammonium water.

5. Liquid for antiseptic treatment of tooth root canals (Chlorhexidine bigluconate aqueous solution – 2%, Omega-dent, Russia). Components: chlorhexidine, water.

High-performance liquid chromatography (HPLC) analysis of initial reagents

HPLC is an effective method for separating complex mixtures of substances, commonly used in analytical chemistry and chemical technology. Chromatographic separation relies on the components of the mixture participating in a complex system of van der Waals interactions, primarily intermolecular, at the interface. This interaction allows for the separation of the components. It is important to note that chromatographic separation is based on objective scientific principles and not subjective evaluations [54–56].

The analysis was performed on an Agilent 1100 HPLC system with UV (VWD) detector (Agilent Technologies, Waldbronn, Germany) at 235 nm wavelength, Separon SGX CN column (Tessek, Czechoslovakia) on parameters (150×3.3mm, 5 μm). The mobile phase consisted of deionized water (solvent A) and acetonitrile (solvent B). The column temperature was maintained at 20°C using an oven. The analysis was conducted at a constant flow rate of 1.0 mL/min.

The samples were analyzed as standards:

- Lavasept solution (20% solution of Polyhexanide hydrochloride (Polyhexanide) (No. 1);
- Sodium hypochlorite solution (3%) (No. 2);
- Hydrogen peroxide solution (3%) (No. 3);
- Solution of EDTA (ethylenediaminetetraacetic acid) – 17% (No. 4);
- Chlorhexidine solution (2% aqueous solution) (No. 5).

A “blank” test injection was made before the first sample analyzation to demonstrate the absence of impurity peaks from the column.

The method of polyhexanide hydrochloride study of was taken from the literature data published by the company – HPLC Method for Analysis of Polyhexanide (polyhexamethylene biguanide, PHMB) on BIST B+ by SIELC Technologies.

The HPLC chromatograms of analyzed initial samples No. 1, 4, and 5 were obtained using the same chromatography conditions as the polyhexanide hydrochloride standard sample (an Agilent 1100 liquid chromatograph with a diode array and an analytical column of Separon SGX CN (150×3.3 mm, 5 μm)). Initial samples No. 2 and 3 were not subjected to analyzation in first part of the experiment by injection because these drugs are not visualized on HPLC chromatograms.

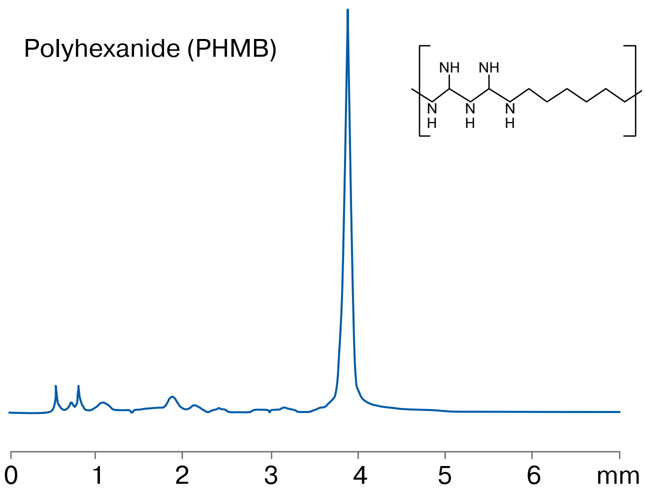
Analysis conditions for samples No. 1, 4, and 5:

- Liquid chromatograph – Agilent 1100 with diode array.
- Analytical column – Separon SGX CN (150×3.3 mm, 5 μm).
- Eluent: A – deionized water, B – acetonitrile (HPLC gradient grade).
- Gradient: 0–1 min 5% B, 1–7 min 50% B, 7–10 min 50% B.
- Flow rate – 0.72 ml/min, column thermostat temperature – 20°C, UV detection wavelength – 235 nm.
- Sample input No. 1 – 1 μl.
- Sample input No. 4 – 1 μl.
- Sample input No. 5 – 20 μl.

The HPLC analyzation of solutions interaction reactivity

The study of reactivity of Lavasept (20% polyhexanide hydrochloride solution) (No. 1) with solutions of 3% sodium hypochlorite (No. 2), 3% hydrogen peroxide (No. 3), 17% EDTA (No. 4), 2% chlorhexidine (No. 5).

The reactions of Lavasept (20% polyhexanide hydrochloride solution) with the reagents and compounds No. 2, 3, 4, 5 were monitored. Mixtures of Lavasept (20% polyhexanide hydrochloride solution) with sodium hypochlorite (3%), hydrogen peroxide (3%), EDTA (17%), and chlorhexidine (2%) were stirred on a magnetic stirrer for 30, 60 minutes and 3 days. For all the tests 100 μl of Lavasept (20% polyhexanide hydrochloride solution) was mixed with 2 ml of each solution mentioned above (No. 2, 3, 4, 5) and chromatographed.



Column:	BIST™ B+
Column size:	4.6 x 50 mm, 5 μm
Column part number:	TBP-46.50.0510
Mobile phase:	Solvent A: MeCN – 60%, H_2SO_4 – 0.2% for 2 min then Solvent B: MeCN – 0%, H_2SO_4 – 0.2%
Flow rate:	1.0 mL/min
Detection:	UV 200 nm
LOD:	0/05 ppm

Fig. 1. HPLC Method for Analysis of Polyhexanide (polyhexamethylene biguanide, PHMB) on BIST B+ by SIELC Technologies

Рис. 1. Метод ВЭЖХ для анализа полигексанида (полигексаметилен бигуанида, PHMB) на BIST B+ от SIELC Technologies

The investigation of possible reactions was conducted at room temperature to identify the presence and formation of new products. The reactions were monitored at 30 minutes, 1 hour, and 3 days after initiation. Chromatography was performed on the reaction mixtures to determine the presence of any new reaction products between the tested components.

The assay conditions were as follows:

- Liquid chromatograph – Agilent 1100 with diode array.
- Analytical column – Separon SGX CN (150×3.3 mm, 5 μm).
- Eluent: A – deionized water, B – acetonitrile (HPLC gradient grade).
- Gradient: 0–1 min 5% B, 1–7 min 50% B, 7–10 min 50% B.
- Flow rate – 0.72 ml/min, column thermostat temperature – 20°C, UV detection wavelength – 235 nm.

RESULTS

High-performance liquid chromatography (HPLC) analysis of initial reagents

Samples of solutions No. 1, 4, and 5 underwent high-performance liquid chromatography. The results are presented in Figures 2–4.

The retention time (chlorhexidine) – 9.547 min, peaks with retention times of 6.956 min and 7.475 min are impurities in the working standard. Based on the parameters and characteristics of the added peaks, they can be characterized as eugenol.

The HPLC analyzation of solutions interaction reactivity

Chromotograms of polyhexanide hydrochloride (Lavasept) (No. 1) reactions with the most widely used irrigants in endodontics, namely 3% sodium hypochlorite (No. 2), 3% hydrogen peroxide (No. 3), 17% EDTA (No. 4), 2% chlorhexidine (No. 5), at room temperature for the presence and formation of new products obtained.

Investigation of reactions progressing in time after 30 minutes, 1 hour and 3 days. Chromatograms of the obtained reaction mixtures for the presence of formation of new possible reaction products between the indicated components are presented in Figures 5–16.

Upon mixing, a yellowish precipitate formed and was subsequently centrifuged for 30 minutes at 12,500 rpm. Analysis of the data obtained reveals that the reaction mixture does not contain polyhexanide hydrochloride when two solutions of the initial sample were mixed. This is due to the hydrolysis of the hypochlorite ion, resulting in an alkaline reaction and the precipitation of polyhexanide in the form of a base. As a result, even residual amounts of polyhexanide are not observed in the reaction mixture.

In aqueous solutions, sodium hypochlorite undergoes hydrolysis and decomposition.

When dissolved in water, it dissociates into ions:

$NaOCl + H_2O \leftrightarrow Na+ + –OCl$

$–OCl + H_2O \leftrightarrow HOCl + –OH$.

Due to its weak nature (pKa = 7.5–7.6 [57]), hypochlorous acid (HOCl) undergoes hydrolysis in an aqueous medium (Fig. 6).

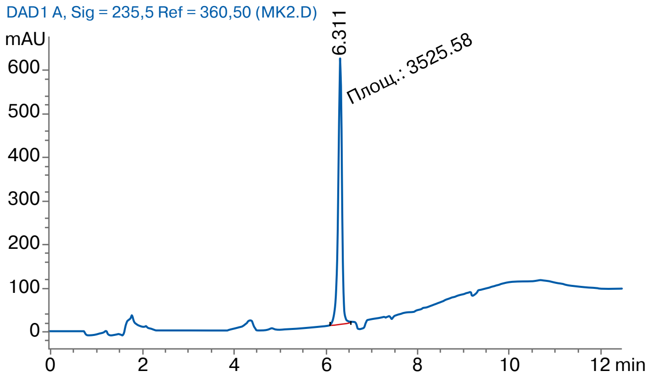


Fig. 2. Chromatogram of a sample of polyhexanide hydrochloride standard (No. 1), retention time (polyhexanide hydrochloride) – 6.311 min

Рис. 2. Хроматограмма образца стандарта полигексанида гидрохлорида (№ 1), время удерживания (полигексанида гидрохлорид) – 6,311 мин

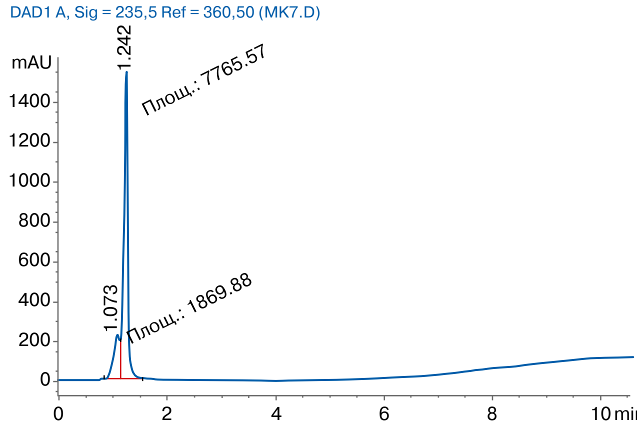


Fig. 3. Chromatogram of a sample of the standard EDTA solution (No. 4), retention time (EDTA, ethylenediaminetetraacetic acid) – 1.242 min

Рис. 3. Хроматограмма образца стандартного раствора ЭДТА (№ 4), время удерживания (ЭДТА, этилендиаминтетрауксусная кислота) – 1,242 мин

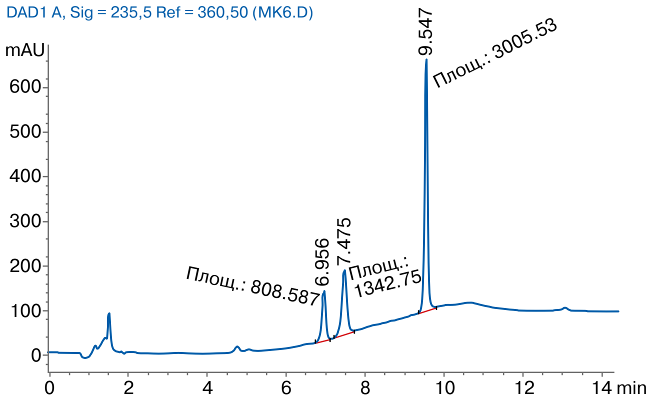


Fig. 4. Chromatogram of a sample of the chlorhexidine solution standard (No. 5)

Рис. 4. Хроматограмма образца стандартного раствора хлоргексидина (№ 5)

The chromatogram reflects all the same observations as at 30 min of reaction mixture (Fig. 7).

During the mixing of polyhexanide hydrochloride and hydrogen peroxide solution (3%) the following changes occur: after 30 min the reaction mixture still contains the original compound – polyhexanide hydrochloride (retention time slightly shifted to 5.816 min) and new compounds are formed with retention times of 2.639 min 3.121 min and 3.716 min, which indicates the reaction between the original sample polyhexanide and hydrogen peroxide (Fig. 7).

The chromatogram reflects all the same changes as at 30 min of reaction mixture (Fig. 8).

During the mixing of the solutions of polyhexanide hydrochloride solution with EDTA solution (17%) the following changes occur: after 30 min the reaction mixture still contains the original compound – polyhexanide hydrochloride (retention time has slightly shifted and is 5.819 min) and new conjugates are formed 5.819 min), and new conjugates are formed with retention times of 0.959 min and 1.082 min, and the presence of unreacted EDTA in the reaction mixture is also observed to be 1.253 min (the retention time of EDTA has a slight backlash) (unreacted EDTA) (Fig. 9).

The chromatogram reflects all the same changes as at 30 min of reaction mixture. After 60 min, the reaction mixture still contains the parent compound, polyhexanide hydrochloride (retention time has shifted slightly to 5.816 min), and new conjugates are formed with retention times of 0.951 min and 1.092 min, and the presence of unreacted EDTA in the reaction mixture is observed to be 1.272 min (retention time of EDTA has a slight backlash) (unreacted EDTA) (Fig. 10).

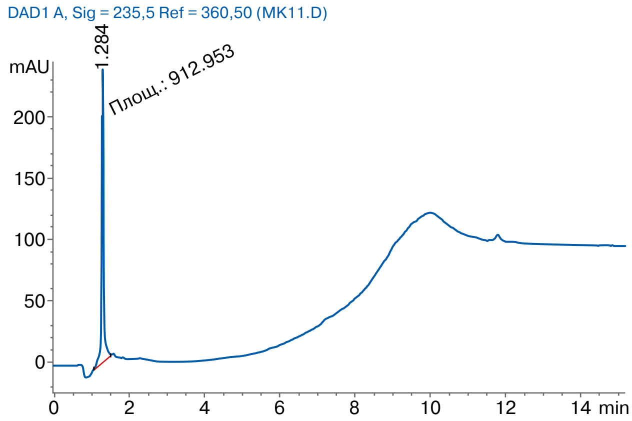


Fig. 5. Chromatogram of reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with 3% sodium hypochlorite (30 min)

Рис. 5. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с 3% гипохлоритом натрия (30 мин)

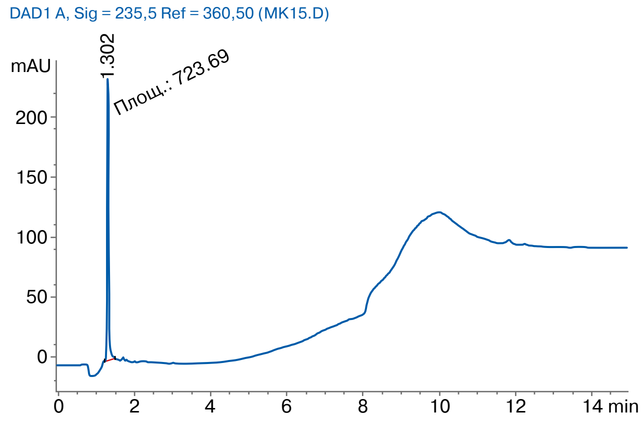


Fig. 6. Chromatogram of reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with 3% sodium hypochlorite (60 min)

Рис. 6. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с 3% гипохлоритом натрия (60 мин)

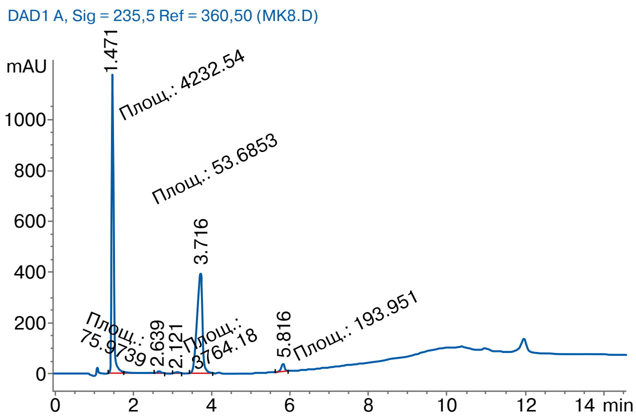


Fig. 7. Chromatogram of the reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with hydrogen peroxide solution (3%) (30 min)

Рис. 7. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с раствором пероксида водорода (3%) (30 мин)

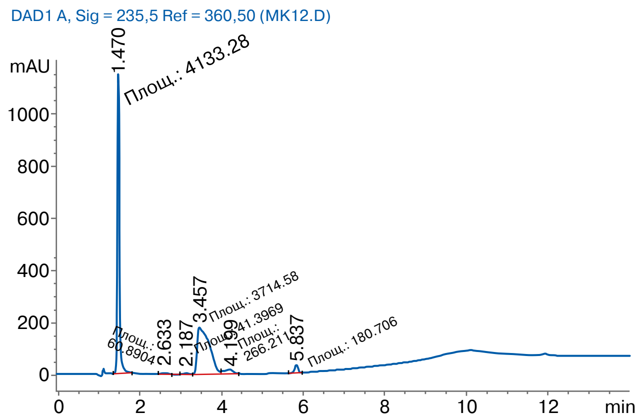


Fig. 8. Chromatogram of the reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with hydrogen peroxide solution (3 %) (60 min)

Рис. 8. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с раствором пероксида водорода (3%) (60 мин)

A white precipitate fell out on mixing, the precipitate was centrifuged for 30 min at 12500 rpm.

During the mixing of the solutions of polyhexanide hydrochloride and chlorhexidine (2%) the following changes occur: after 30 min, the reaction mixture still contains the starting compound – polyhexanide hydrochloride (retention time has slightly shifted and is 5.814 min), and the reaction mixture also forms the initial compound – polyhexanide hydrochloride 5.814 min), and new compounds with retention times of 3.955 min, 6.195 min, 9.035 min are formed, as well as the presence in the reaction mixture of an unreacted component of the reagent chlorhexidine – 6.975 min (Fig. 11).

The chromatogram reflects all the same changes as at 30 min of reaction mixture. After 60 min the reaction mixture still contains the initial compound – polyhexanide hydrochloride (retention time has slightly shifted and makes 5.805 min), and also new compounds with retention times of 4.063 min, 6.187 min, 9.026 min are formed, as well as the presence of unreacted component of the reagent chlorhexidine in the reaction mixture – 6.938 min (Fig. 12).

Upon examination of the reaction mixtures after three days, it is evident that there were no significant changes observed in the chromatographic patterns when compared to the reaction mixture at 30 minutes.

Comparative analysis of chromatograms during the 3 days experiment (Fig. 13).

A yellowish precipitate fell out when mixing (30 min reaction time). No significant changes were detected 60 min after mixing. However, a very weak polyhexanide signal appeared after 3 days of incubation of the solution mixture (Fig. 14).

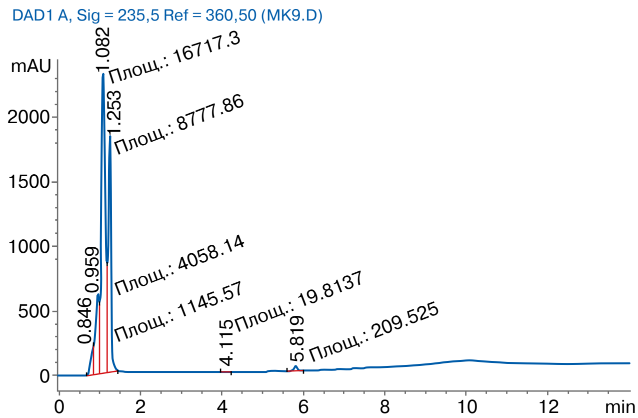


Fig. 9. Chromatogram of the reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with EDTA solution (17 %) (30 min)

Рис. 9. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с раствором ЭДТА (17%) (30 мин)

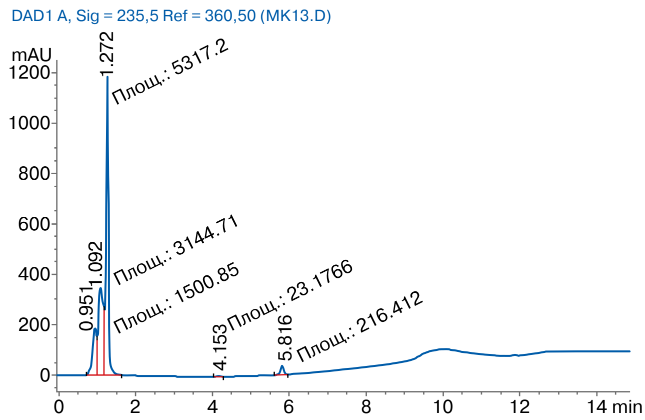


Fig. 10. Chromatogram of the reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with EDTA solution (17 %) (60 min)

Рис. 10. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с раствором ЭДТА (17%) (60 мин)

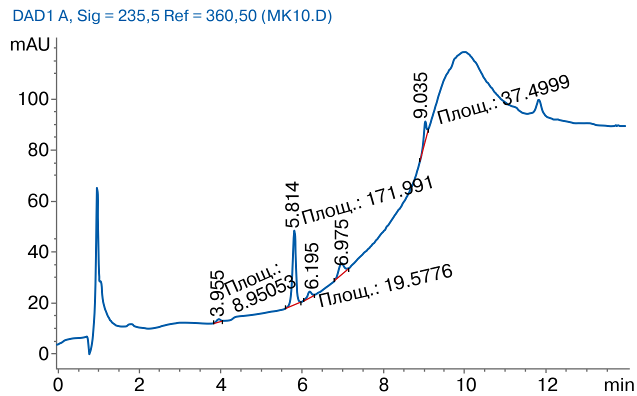


Fig. 11. Chromatogram of reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with chlorhexidine solution (2%) (30 min)

Рис. 11. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с раствором хлоргексидина (2%) (30 мин)

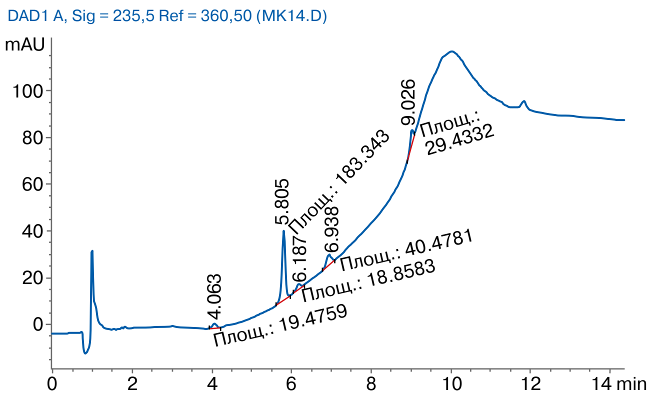


Fig. 12. Chromatogram of reaction mixture of polyhexanide hydrochloride (20% Lavasept solution) with chlorhexidine solution (2%) (60 min)

Рис. 12. Хроматограмма реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) с раствором хлоргексидина (2%) (60 мин)

Due to dilution, the polyhexanide hydrochloride signal shifted to 5.8 min. The area of the polyhexanide hydrochloride signal did not change at 3 days of incubation.

After incubating polyhexanide solution with hydrogen peroxide for three days, the reaction product that formed during the 30 and 60-minute experiments with a retention time of 3.716 minutes disappeared. A new peak with a retention time of 2.486 minutes appeared on the chromatogram, indicating a transformation of one compound into another (Fig. 15).

No significant changes occur after 60 minutes and 3 days after mixture of mentioned solutions (Fig. 16).

After three days of incubation, small changes in the formation of new peaks corresponding to new products are still observed.

DISCUSSION

Endodontics is a rapidly improving branch of dentistry. The significant progress in this field is attributed to the creation of numerous materials and the introduction and application of new technologies by scientists and researchers. Today, an apex locator, operating microscope, biodentine and bioceramics, and irrigants are essential tools for practical therapeutic purposes (and not only) [42–44; 58–62].

The success of endodontic treatment largely depends on the medicament treatment of root canals. Irrigants play a crucial role in this process [44; 45; 59; 60; 63; 64]. It is important to note that irrigating solutions should have antimicrobial properties, dissolve organic elements, and provide mechanical washing without causing damage to

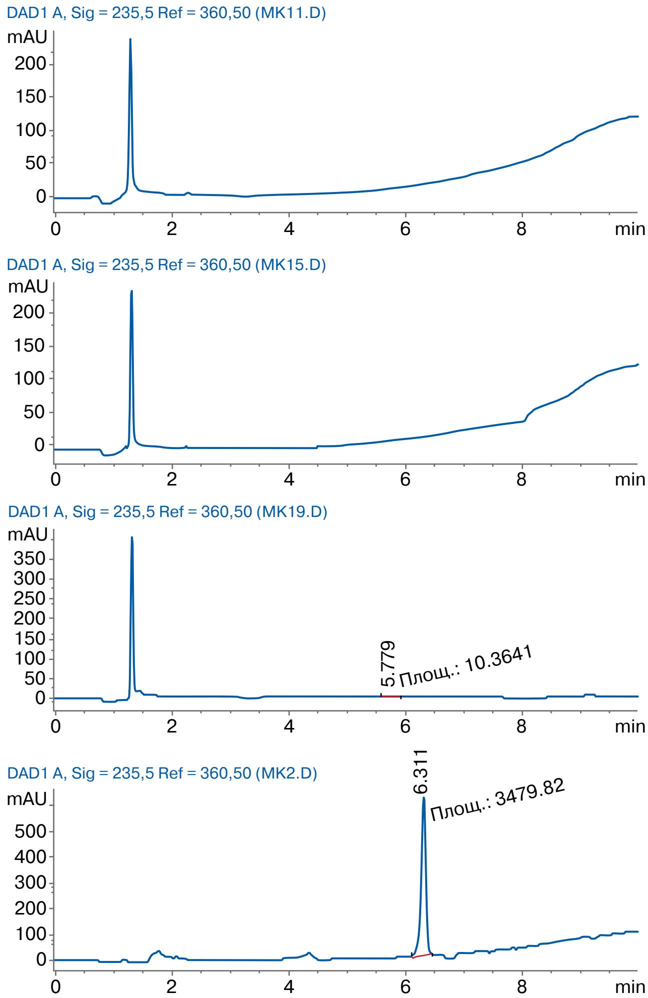


Fig. 13. Chromatograms of reaction mixture polyhexanide hydrochloride (20% Lavasept solution) and sodium hypochlorite (3%) (from top to bottom: 30 min, 1 hour, 3 days and polyhexanide hydrochloride standard, sample volume injected everywhere – 1 μl)

Рис. 13. Хроматограммы реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) и гипохлорита натрия (3%) (сверху вниз: 30 мин, 1 час, 3 дня и стандарт полигексанида гидрохлорида, объем вводимого образца везде – 1 мкл)

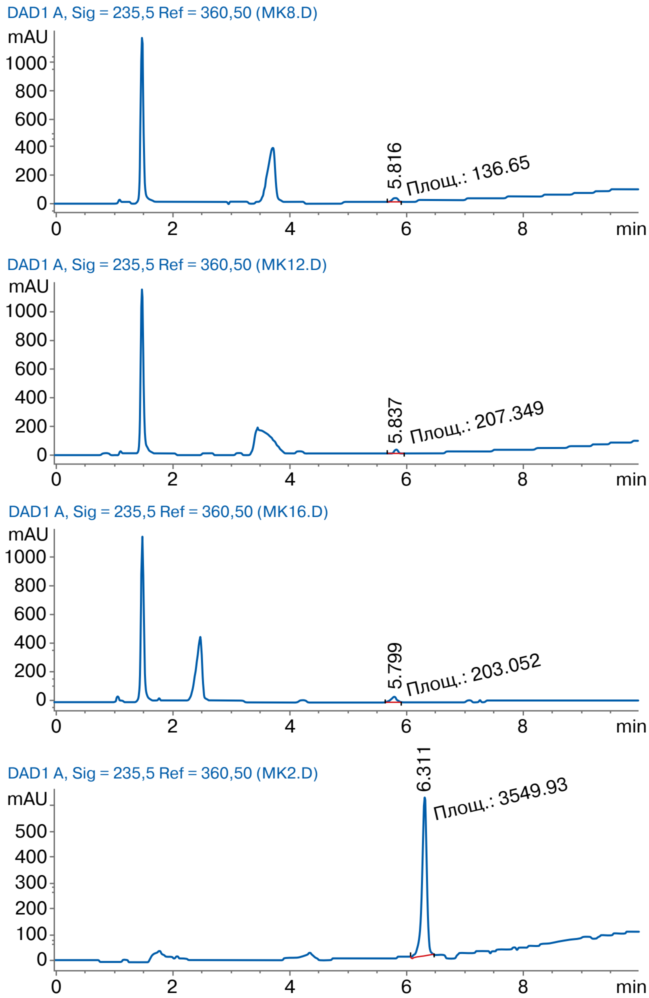


Fig. 14. Chromatograms of reaction mixture polyhexanide hydrochloride (20% Lavasept solution) and hydrogen peroxide solution (3%) (from top to bottom: 30 min, 1 hour, 3 days and polyhexanide standard, sample volume injected everywhere is 1 μl)

Рис. 14. Хроматограммы реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) и раствора пероксида водорода (3%) (сверху вниз: 30 мин, 1 час, 3 дня и стандарт полигексанида, объем вводимого образца везде 1 мкл) periradicular tissues in case of extrusion into periodontal tissues. However, not all irrigants are capable of fulfilling these functions, which often results in the development of secondary endodontic infections [49–51; 65–68].

Regarding persistent infection, it is important to note that microorganisms that contaminate root canals enter them directly, either during or after treatment [69–71]. Inadequately treated instruments are a common source of contamination. Microbial penetration into the root canal system of root canals can also occur due to saliva entering the tooth cavity as a result of improper placement of the cofferdam. It is important to ensure proper placement of the cofferdam and use of stable temporary fillings to prevent these issues. Microbial penetration into the root canal system of root canals can also occur due to saliva entering the tooth cavity as a result of improper placement of the cofferdam [29–34]. Additionally, microleakage through an unstable temporary filling can also be a significant cause.

As previously mentioned, microorganisms within root canals are primarily located as biofilms, which are more resistant to medication. Due to the development of acquired drug resistance of bacteria and the frequent complications of endodontic treatment, the search for a new irrigant is promising. Polyhexanide has the potential to address the challenges of endodontic treatment.

Polyhexamethylene biguanide (PHMB) is a bisbiguanide with broad spectrum antimicrobial activity [57; 71; 73]. Antiseptic compositions containing only the active ingredient PHMB are not available on the pharmaceutical market. In this study, a 20% PHMB concentrate named Lavasept solution (B. Braun Melsungen AG, Germany) was used as a polyhexanide standard.

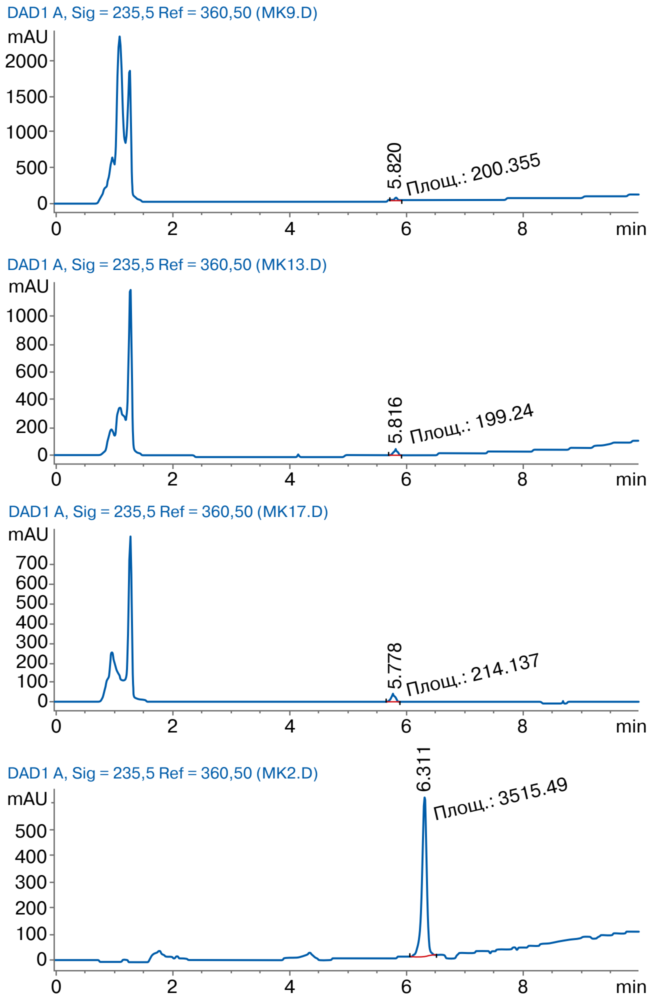


Fig. 15. Chromatograms of reaction mixture polyhexanide hydrochloride (20% Lavasept solution) and EDTA (17%) (from top to bottom: 30 min, 1 hour, 3 days and polyhexanide hydrochloride standard, sample volume injected everywhere – 1 µl)

Рис. 15. Хроматограммы реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) и ЭДТА (17%) (сверху вниз: 30 мин, 1 час, 3 дня и стандарт полигексанида гидрохлорида, объем вводимого образца везде – 1 мкл)

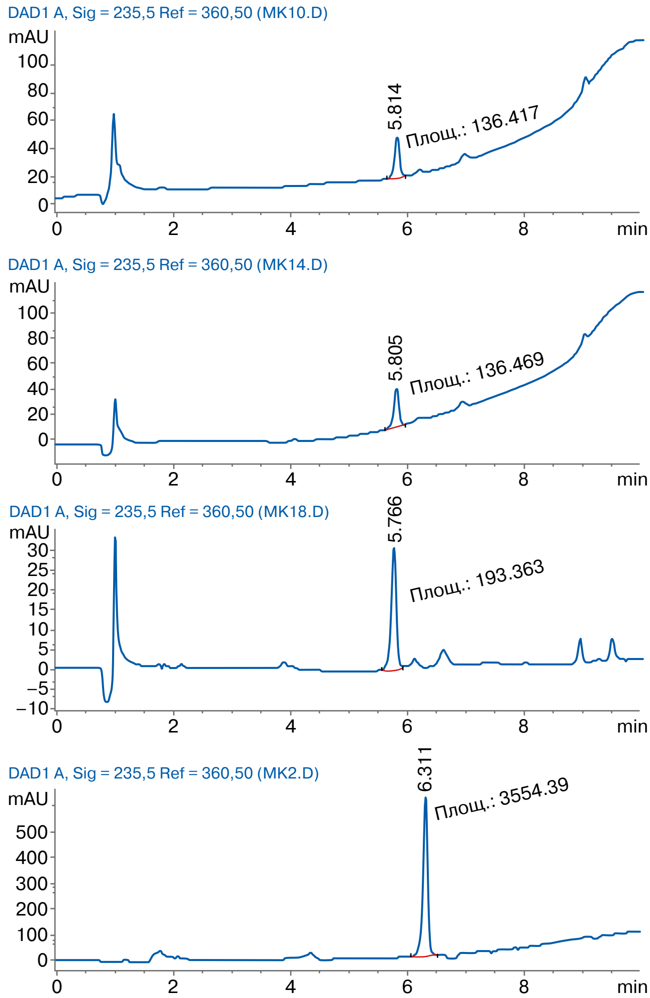


Fig. 16. Chromatograms of reaction mixture polyhexanide hydrochloride (20% Lavasept solution) and chlorhexidine (2%) (from top to bottom: 30 min, 1 hour, 3 days and polyhexanide hydrochloride standard, sample volume injected everywhere – 1 µl)

Рис. 16. Хроматограммы реакционной смеси полигексанида гидрохлорида (20% раствор Лавасепта) и хлоргексидина (2%) (сверху вниз: 30 мин, 1 час, 3 дня и стандарт полигексанида гидрохлорида, объем вводимого образца везде – 1 мкл)

Macrogol 4000 was added to the antiseptic composition to influence the surface tension of the solution and reduce cytotoxicity, thereby improving the characteristics of the compound [54–56].

Polyhexanide has been extensively studied for its antimicrobial properties in various medical fields, including traumatology, ophthalmology, urology, treatment of burns, long non-healing wounds, and ulcers [57; 74–78]. However, its use in dentistry is not yet widespread. This substance is commonly applied as a component of solutions for mouthwashes for the prevention of caries and diseases of the mucous membranes [79–82]. A few studies on the effectiveness of this substance against microorganisms-colonizers of the root canal system are presented [83–84]. However, further clinical and laboratory studies are needed to determine its antimicrobial activity against endodontic pathogens that cause inflammatory diseases of the pulpo-periodontal complex.

Clinicians often use combinations of irrigants in the endodontic treatment protocol because there is currently no single solution that can solve all the problems associated with irrigation. The gold standard in dentistry is the use of sodium hypochlorite, which has antibacterial and proteolytic activity, and EDTA, which can affect the components of the smear layer. Additionally, final irrigation of the root canal system with an aqueous chlorhexidine solution is widespread due to its prolonged antimicrobial action. However, as chemical substances, all of the aforementioned agents can interact with each other if used in succession without separation by water or complete drying in the root canal. Chemical interaction can drastically reduce the effectiveness of the pharmaceutical therapy. Since polyhexanide is a cationic antibacterial agent, similar to chlorohexidine, it is practical to use it in combination with other irrigants that enhance its antimicrobial and chelating properties. This assumption is based on the idea that polyhexanide does not have a pronounced effect on the smear layer.

High-performance liquid chromatography was used to determine the optimal clinical protocol and potential interactions between polyhexanide and commonly used irrigants. The initial standards of endodontic medications, including 20% polyhexanide, 3% sodium hypochlorite, 3% hydrogen peroxide, 17% EDTA, and 2% chlorhexidine, were analyzed, as well as mixtures of polyhexanide with each of these solutions at 30, 60 minutes, and 3 days after initial contact.

High-performance liquid chromatography (HPLC) is an effective method for separating complex mixtures of substances. It is widely used in both analytical chemistry and chemical technology. Chromatographic separation is based on the participation of mixture components in a complex system of van der Waals interactions, mainly intermolecular, at the interface. HPLC is a method of analysis that involves separating complex mixtures into simpler ones before analyzing them using physicochemical or chromatography-specific methods.

The HPLC method has a wide range of applications in fields such as chemistry, petrochemistry, biology, biotechnology, medicine, food industry, environmental protection, and drug production. High-performance liquid chromatography with mass spectrometric detection is a promising method for identifying and quantifying drug substances in various biological objects. The method is highly specific and accurate, capable of detecting substances in minimal concentrations. This makes it suitable for quantitatively determining drugs in pharmacokinetic studies and drug monitoring, which is significant for clinical laboratory diagnostics [65–67].

Based on the results of our study, it is not recommended to mix sodium hypochlorite and polyhexanide solutions during sequential irrigation in the root canal. This is due to the formation of a yellowish flake-like suspension that precipitates and causes the formation of sediment. Sedimentation when mixing irrigants in root canals is a serious complication of pharmaceutical therapy, resulting in the depressurization of the root canal filling – intracanal dentin system. Insufficient penetration of sealer into the dentinal tubules, due to flakes of sediment blocking the canal lumen obturation hermeticism, can result in persistent infection and endodontic treatment failure.

When sodium hypochlorite and polyhexanide are mixed, the latter precipitates as a base, leaving no residual amount of PHMB in the chromatogram. It is assumed that PHMB, like chlorhexidine, is a cationic antimicrobial agent with a similar structure (except for polymeric structure and absence of chlorine-substituted groups) and interacts generally similar with sodium hypochlorite. When NaOCl and CHX are mixed, a brick-red precipitate is formed, which many authors refer to as parachloraniline. This substance is formed as a result of chlorination of chlorhexidine guanidinoazotes under the action of a strong oxidizing agent. The quantity of precipitate produced is directly proportional to the concentration of sodium hypochlorite. As the concentration of NaOCl increases, more parachloraniline is formed when in contact with chlorhexidine [37; 45]. However, some authors have suggested that the mixture of NaOCl and CHX does not contain free parachloraniline. The difference in opinions may be due to misinterpretation of the para-chloramide moiety of CHX or any CHX derivative in the precipitate [85; 86]. Due to oxidation in a sodium hypochlorite medium, PHMB may exhibit similar activity to chlorhexidine.

Therefore, it is not advisable to use sodium hypochlorite and polyhexanide sequentially in an endodontic medication protocol without strictly separating the irrigants. This can be achieved by rinsing the root canal with distilled water, inactivating NaOCl with sodium thiosulfate, or completely drying sodium hypochlorite with paper absorber pins while aspirating any residual solution [87; 88]. However, it is necessary to conduct further investigation of the centrifuged precipitate, forming while PHMB and NaOCl contact, to determine its chemical composition.

Hydrogen peroxide was previously used in endodontics, but its use is now limited due to its lack of antimicrobial efficacy against periopathogens and insufficient action on the smear layer and intracanalicular dentin [89; 90]. The chromatogram indicates the formation of new reaction products with retention times of 2.639 min, 3.121 min, and 3.716 min, suggesting a reaction between the original polyhexanide sample and hydrogen peroxide. Therefore, it is not recommended to sequentially apply these solutions into the root canal.

EDTA is a molecule with a claw-like structure that captures divalent and trivalent metal ions, such as calcium and aluminum, forming a stable ring structure. It is commonly used in endodontics due to its ability to affect the mineral components of dentin and remove the smear layer formed after root canal preparation, facilitating the sliding of instruments in the lumen of the root canal [91–93].

The chromatogram did not show any significant negative interactions between polyhexanide and EDTA. After 30 minutes from the initial contact, the reaction mixture still contains the original compound, polyhexanide hydrochloride. The retention time has shifted slightly to 5.819 minutes due to dilution. Additionally, new conjugates have been formed with retention times of 0.959 and 1.082 minutes. Unreacted EDTA is also present in the reaction mixture with a retention time of 1.253 minutes. It is our opinion, that in this case PHMB is forming salts with EDTA rather than undergoing a chemical reaction, and that these salts are able to dissolve upon pH shift. Additional experiments are required to confirm this. According to the information provided, the combination of EDTA and PHMB is reasonable method of root canal irrigation, due to the fact that there are no harmful interactions when mixing these solutions in the canal. It is important to note that EDTA may not be completely removed from the canal through reintroduction of polyhexanide. However, it is sufficient to eliminate excess solution using the aspiration system.

Chlorhexidine is a drug that exhibits a broad spectrum of antimicrobial activity. It is commonly available in the form of salts, including diacetate, digluconate, and dihydrochloride. Aqueous solutions are most stable within a pH range of 5–8 [94]. In modern medicine, it is frequently used as a final irrigant due to its substantivity, which allows it to be gradually released over an extended period when in contact with the substrate, such as enamel, dentin, or glycoprotein groups. After 30 minutes a white precipitate was dropped on mixing, and the chromatogram shows that the reaction mixture of CHX and PHMB still contains the initial compound, polyhexanide hydrochloride. The retention time has slightly shifted to 5.814 minutes. Additionally, new compounds with retention times of 3.955, 6.195, and 9.035 minutes have formed. There is also unreacted chlorhexidine present in the reaction mixture, with a retention time of 6.975 minutes. Considering the formation of new products during a chemical reaction, it is not rational to sequentially introduce polyhexanide after chlorhexidine without evacuating the latter. Therefore, it is necessary to conduct further investigation of the precipitate to determine its chemical composition.

CONCLUSION

Root canal irrigation with polyhexanide is a promising approach in endodontics. The protocol for using this substance should be combined with other irrigants, that have an ability to affect the smear layer. PHMB can act as a main antibacterial irrigation solution in combination with a popular EDTA-based chelating agent. Clinical protocol of polyhexanide and EDTA usage had shown positive results. To eliminate the smeared layer, EDTA solution should be used sequentially, followed by polyhexanide as an antiseptic agent. It is reasonable to use a dental suction system to partially remove EDTA from the root canal prior to PHMB, as there are no significant chemical interactions between these solutions.

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А.Ю. Умаров – анализ и интерпретация данных, подготовка статьи, критический пересмотр статьи в части значимого интеллектуального содержания.

Ф.В. Бадалов – подготовка статьи, критический пересмотр статьи в части значимого интеллектуального содержания, финальная коррекция.

А. Вехби – подготовка статьи, критический пересмотр статьи в части значимого интеллектуального содержания, финальная коррекция.

Э.М. Какабадзе – существенный вклад в замысел и дизайн исследования, сбор данных.