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The effect of the irrigation solutions on dentin organic components: Pilot study

Zurab S. Khabadze, Yulia A. Generalova ✉, Yulia A. Taptun, Liudmila A. Kozhevnikova, Fakhri Ya. Gadzhiev, Marina Yu. Dashtieva

Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Moscow, Russian Federation

✉ generalova_jua@rudn.university

Abstract

INTRODUCTION. Tooth decay and its complications are prevalent dental issues among adults. Endodontic treatment is the standard of care for complicated caries, specifically pulpitis and periodontitis. Endodontic irrigants, which are placed in the root canal lumen for a specific duration, can affect the intracanal dentin structure (organic and inorganic substances) in various ways. The use of polyhexanide-based antiseptic products in endodontics is a promising idea to overcome microbial resistance and minimize microbial-related endodontic treatment failures.

AIM. The aim of this study is to investigate the qualitative impact of standard irrigation solutions and a polyhexanide-based composition on the collagen matrix of dentin.

MATERIALS AND METHODS. The study investigated the effect of nine solutions on the dentin of the tooth root. Twelve intact maxillary third molars extracted for orthodontic reasons were taken for the study. The teeth were fixed in a 10% neutral formalin solution for at least one day and were decalcinated. 5–10 μm thick sample sections were made and stained with picrosirius (Picrosirius Red, Biovitrum, Russia) before being examined under polarized light. The histological slice was analyzed using a Carl Zeiss (Germany) software and hardware complex based on ZEN v3.0 and light microscope Axioimager M.1. All microphotos were loaded into the Fiji distribution program of ImageJ for microscopy pixel classification.

RESULTS. Upon analysis of the diagrams, it appears that there is a lower amount and density of stained dentin collagen array after exposure to sodium hypochlorite solutions of 3% and 1.5%, as well as polyhexanide.

CONCLUSIONS. The data obtained from the histogram analyzer of samples stained with picrosirius suggests that polyhexanide may have a qualitative effect on the organic structure of hard tissues, particularly collagen.

Keywords: polyhexanide, dentine, collagen fiber, picrosirius

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Влияние ирригационных растворов на органические компоненты дентина: экспериментальное исследование

З.С. Хабадзе, Ю.А. Генералова ✉, Ю.А. Таптун, Л.А. Кожевникова, Ф.Я. Гаджиев, М.Ю. Даштиева

Российский университет дружбы народов имени Патриса Лумумбы (РУДН), г. Москва, Российская Федерация

✉ generalova_jua@rudn.university

Резюме

ВВЕДЕНИЕ. Кариес и его осложнения являются распространенными стоматологическими проблемами среди взрослых. Эндодонтическое лечение является стандартом медицинской помощи при осложненном кариесе, в частности при пульпите и периодонтите. Эндодонтические ирриганты, которые помещаются в просвет корневого канала на определенное время, могут по-разному влиять на внутриканальную структуру дентина (органические и неорганические вещества). Использование антисептических средств на основе полигексанида в эндодонтии является перспективной идеей для преодоления устойчивости микроорганизмов и минимизации неудач эндодонтического лечения, связанных с микроорганизмами.

ЦЕЛЬ. Изучить качественное влияние стандартных ирригационных растворов и композиции на основе полигексанида на коллагеновый матрикс дентина.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании изучалось влияние девяти растворов на дентин корня зуба. Для исследования были взяты 12 интактных верхнечелюстных третьих моляров, удаленных по ортодонтическим показаниям. Зубы фиксировали в 10%-ном растворе нейтрального формалина не менее чем на сутки и подвергали декальцинации. Образцы толщиной 5–10 мкм окрашивали пикросириусом

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(Picrosirius Red, Biovitrum, Россия) и просматривали под поляризованным светом. Анализ гистологических срезов проводили с помощью программно-аппаратного комплекса Carl Zeiss (Германия) на базе ZEN v3.0 и светового микроскопа Axioimager M.1. Все микрофотографии загружались в дистрибутив Fiji программы ImageJ для классификации пикселей микроскопии.
РЕЗУЛЬТАТЫ. При анализе диаграмм видно, что после воздействия растворов гипохлорита натрия 3 % и 1,5 %, а также полигексанида наблюдается меньшее количество и плотность окрашенного коллагенового массива дентина.
ВЫВОДЫ. Данные, полученные с помощью анализатора гистограмм образцов, окрашенных пикросириусом, позволяют предположить, что полигексанид может оказывать качественное влияние на органическую структуру твердых тканей, в частности на коллаген.

Ключевые слова: полигексанид, дентин, коллагеновые волокна, пикрозириус

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INTRODUCTION

Tooth decay and its complications are prevalent dental issues among adults. Endodontic treatment is the standard of care for complicated caries, specifically pulpitis and periodontitis [1–3]. The interaction of mechanical root canal treatment, evacuation of necrotized pulp particles and infected dentin filings with various instruments, and antiseptic therapy with irrigation solutions impacts the microbial factor and inflammation [4; 5]. All of the above measures can affect intracanal dentin. Dentin is a complex, avascular structure composed of organic matter, mainly represented by a collagen fiber network, a mineral phase, and a small amount of water [6; 7].

The organic matrix of dentin consists primarily of type 1 collagen and proteoglycans. Its main function is to create an oriented fibrillar matrix for the deposition of hydroxyapatite and other inorganic components. Glycosaminoglycans regulate the permeability and water content of dentin. Collagen fibers in the dentin thickness are encapsulated by hydroxyapatite [8–10].

Endodontic irrigants can influence the organic and/or mineral phase of dentin to varying degrees. The treatment of inflammatory pathologies commonly involves the use of endodontic irrigants such as sodium hypochlorite in concentrations ranging from 1% to 5.25%, which has proteolytic and antimicrobial properties, chlorhexidine, which also possesses antimicrobial properties and the phenomenon of substantivity, and EDTA, which affects the smear layer [11–13].

Studies have shown changes in the chemical and structural composition of dentin when exposed to different regimens of NaOCl irrigation during root canal system therapy [14; 15]. Sodium hypochlorite, in any concentration, has a proteolytic effect on the organic matrix of dentin. This effect can lead to a decrease in the mechanical characteristics of dental tissues, such as elasticity and strength, which can result in excessive fragility of dentin and its fractures [15; 16]. Numerous research studies have determined the deleterious effect of different concentrations of NaOCl on collagen and other organic molecules in dentin. Factors such as reaction time, temperature, and NaOCl concentration can alter the proteolytic and dissolving power of this compound [17]. However, EDTA and chlorhexidine do not have pronounced proteolytic properties.

The use of polyhexanide-based antiseptic products in endodontics is a promising idea to overcome microbial resistance and minimize microbial-related endodontic treatment failures. According to authors [18], polyhexanide promotes wound healing significantly more than Ringer's solution and octenidine in combination with phenoxyethanol. However, its earlier effects on dentin collagen require further investigation.

AIM

The aim of this study is to investigate the qualitative impact of standard irrigation solutions and a polyhexanide-based composition on the collagen matrix of dentin.

MATERIALS AND METHODS

The study investigated the effect of nine solutions on the dentin of the tooth root. The study was conducted using a blind method, with each solution assigned a serial number in advance. Experimental work was conducted at the Department of Scientific and Laboratory Research of the Federal State Budgetary Institution "Central Research Institute of Dental and Maxillofacial Surgery" of the Russian Federation Healthcare Ministry.

I. Investigated antiseptics

The following antiseptic preparations used and intended to be used in the endodontic protocol for the treatment of inflammatory pulp and periodontal diseases were included in the present study:

1. Chlorhexidine bigluconate (2% aqueous solution – "Liquid for antiseptic treatment of tooth root canals", Omega-dent, Russia).

2. Chlorhexidine bigluconate (0.05% aqueous solution, Flora of Caucasus, Russia).

3. Sodium hypochlorite (3% solution – "Belodez", VladMiVa, Russia).

4. Sodium hypochlorite (1.5% solution – "Belodez", VladMiVa, Russia).

5. Octenidine dihydrochlorite (0.1% solution – "Octenisept", Schulke & Mayr, Germany).

6. Polyhexanide (0.1% solution – "Prontosan", B. Braun Melsungen AG, Switzerland).

II. Experimental groups

Experimental and control groups were formed accordingly.

K – Control group, physiologic solution (PanEco, Russia)

1 – Chlorhexidine 2%

2 – Chlorhexidine 0,05%

3 – Sodium hypochlorite 3%

4 – Sodium hypochlorite 1.5%

5 – Octenisept

6 – Octenisept

7 – Prontosan

8 – Prontosan

9 – Sodium hypochlorite 3%, heated to 55C.

Group 6 is identical to group 5 and group 8 is identical to group 7 in all respects except for the operator responsible for performing the experiment and the day of the experiment. This approach provides insight into the technical variability of the experimental results.

III. Histologic examination of dental hard tissues

Twelve intact maxillary third molars extracted for orthodontic reasons were taken for the study. The teeth were fixed in a 10% neutral formalin solution for at least one day. Then, decalcification was performed using a 4% solution of 1:1 hydrochloric and acetic acids, following the generally accepted method. After dehydration in a gradient of alcohols and xylene, the samples were encapsulated in paraffin. Finally, 5–10 μm thick sections were made and stained with picrosirius (Picrosirius Red, Biovitrum, Russia) before being examined under polarized light. Type I collagen and other tissues were labeled in red, while type III collagen or type I collagen with altered structure and packing were labeled in green. The histological slice was analyzed using a Carl Zeiss (Germany) software and hardware complex based on ZEN v3.0 and light microscope Axioimager M.1. The resulting panoramas provide a comprehensive view of the entire slice.

IV. Analysis

In order to determine the intensity of collagen staining zones by pricrosirius, all microphotos were loaded into the Fiji distribution program of ImageJ for microscopy pixel classification. 3D Surface Plot Diagrams of color distribution were constructed for each sample using the above software.

RESULTS

Upon microscopic examination in polarized light of the sections stained with picrosirius, it was observed that the dentin collagen did not undergo severe significant structural changes after treatment with the investigated substances (Fig. 1). Therefore, all dentin collagen appeared red in color. Green areas were only observed in the pulp and some areas of cementum. The lack of change in the permeability and bonding of type I collagen molecules with picrosirius can be attributed to the action of the investigated substances.

Solutions 3, 4, and 9 are expected to reduce collagen fiber network due to the organolytic (proteolytic) activity of sodium hypochlorite and its toxic effect, with heated solution having a greater effect. The following solutions were used in the experiment: This is illustrated in the graph for sample group No. 2, 3.

Figure 2 shows 3D surface plot diagrams of collagen staining color distribution. Upon analysis of the diagrams, it appears that there is a lower amount and density of stained dentin collagen array after exposure to sodium hypochlorite solutions of 3% and 1.5%, as well as polyhexanide.

DISCUSSION

Irrigation is a crucial aspect of endodontic treatment. Solutions present in the root canal lumen can affect not only the intended targets, such as microorganisms and components of the smear layer, but also the components of the dentin wall. Aggressive impact on dentin can compromise its physical and mechanical properties, leading to undesirable consequences such as root fracture [19–21]. Incorrect and excessive instrumentation of the root canal can increase the likelihood of this complication [22].

In endodontics, a combination of sodium hypochlorite and EDTA solutions is considered the standard for irrigation. However, high concentrations of NaOCl and long exposure time can negatively affect the collagen matrix of dentin, as noted by many authors [15–17; 23]. There are also data on the formation of dentin erosion in different irrigation protocols with EDTA, especially NaOCl-EDTA-NaOCl [24–26].

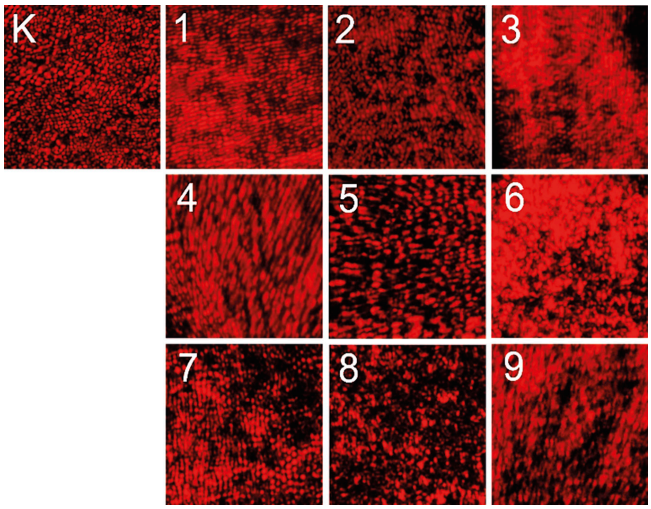

Fig.1. Enhanced collagen birefringence assessed by Picrosirius Red staining under polarized light shows dentin sections after treatment in tested solutions – K-Saline solution, Chlorhexidine 2% (*1*), Chlorhexidine 0.05% (*2*), Sodium hypochlorite 3% (*3*), Sodium hypochlorite 1.5% (*4*), Octenisept (*5*), Octenisept (*6*), Prontosan (*7*), Prontosan (*8*), and heated sodium hypochlorite 3% (*9*)

Рис. 1. Усиленное двулучепреломление коллагена, оцененное по окрашиванию Picrosirius Red в поляризованном свете, на срезах дентина после обработки растворами – К-Салин, Хлоргексидин 2% (*1*), Хлоргексидин 0,05% (*2*), Гипохлорит натрия 3% (*3*), Гипохлорит натрия 1,5 % (*4*), Октенисепт (*5*), Октенисепт (*6*), Пронтосан (*7*), Пронтосан (*8*) и нагретый Гипохлорит натрия 3% (*9*)

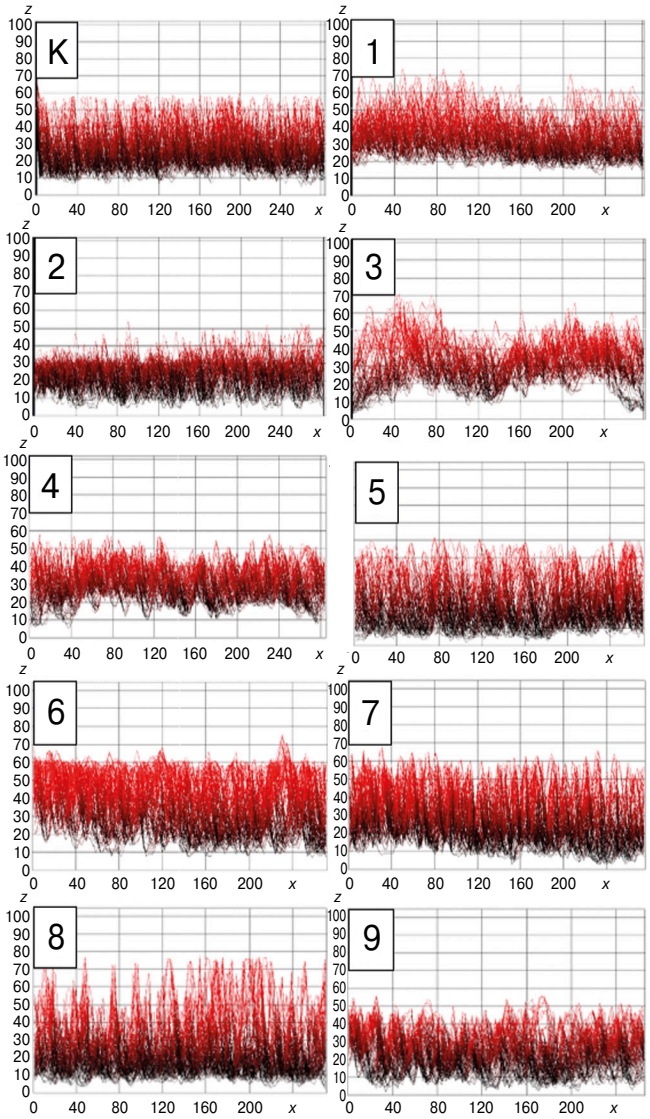

Fig. 2. 3D surface plot diagrams of collagen staining color distribution. Red zones – stained collagen I type, black zones – absence of collagen fibers. K-Saline solution, Chlorhexidine 2% (*1*), Chlorhexidine 0.05% (*2*), Sodium hypochlorite 3% (*3*), Sodium hypochlorite 1.5% (*4*), Octenisept (*5*), Octenisept (*6*), Prontosan (*7*), Prontosan (*8*), and heated sodium hypochlorite 3% (*9*)

Рис. 2. Поверхностные 3D диаграммы распределения цвета окрашивания коллагена. Красные зоны – окрашенный коллаген I типа, черные зоны – отсутствие коллагеновых волокон. Раствор K-Saline, Хлоргексидин 2% (*1*), Хлоргексидин 0,05 % (*2*), Гипохлорит натрия 3 % (*3*), Гипохлорит натрия 1,5 % (*4*), Октенисепт (*5*), Октенисепт (*6*), Пронтосан (*7*), Пронтосан (*8*), подогретый Гипохлорит натрия 3 % (*9*)

New endodontic preparations and protocols are being sought to achieve more predictable results in the conservative treatment of inflammatory pulp and periodontal diseases. The use of polyhexanide, a polymeric biguanide-based irrigant, shows promise in this regard. However, there is limited experimental data on its effectiveness in endodontics, but it has demonstrated efficacy against endodontic pathogens [27; 28].

The aim of this study was to demonstrate the presence or absence of an effect of a 0.1% polyhexanide-based preparation on dentin collagen by staining extracted tooth specimens with picrosirius after exposure to the solutions. Picrosirius red staining enables qualitative and quantitative assessment of collagen distribution, maturity, and fibrillar packing through collagen birefringence. Polarizing microscopy facilitates the visualization of anisotropic structures that appear bright or glossy on a dark background [29].

The data obtained suggests that different concentrations of sodium hypochlorite, particularly when heated to 55 degrees Celsius, exhibit the most qualitatively pronounced proteolytic effect. However, the data obtained from the histogram analyzer of samples stained with picrosirius indicates a potential effect of polyhexanide on the organic structure of hard tissues, specifically collagen. This finding expands the potential indications for the use of this antiseptic solution in irrigation protocols.

Studies on the effect of PHMB on dentin collagen structures are a pressing issue in modern endodontics. Further studies are necessary to determine the qualitative and quantitative effects of polyhexanide on the structural organic components of dentin. To even it out, it is necessary to examine a set of teeth from the same group of patients of the same age. It is also important to standardize the procedure and region of dentin sampling for histologic examination in further scientific researches.

CONCLUSIONS

The data obtained from the histogram analyzer of samples stained with picrosirius suggests that polyhexanide may have a qualitative effect on the organic structure of hard tissues, particularly collagen. This finding broadens the potential indications for the use of this antiseptic solution in irrigation protocols and highlights an important issue that will be addressed in future scientific studies. To conduct a detailed quantitative and qualitative assessment of the effect of polyhexanide on dentin collagen, further studies with a larger number of tooth samples and taking samples from similar locations of dentin are necessary.

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INFORMATION ABOUT THE AUTHORS

Zurab S. Khabadze – Cand. Sci. (Med.), Associate Professor, Department of Therapeutic Dentistry, Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Medical Institute, 6 Miklukho-Maklaya Str., Moscow 117198, Russian Federation; https://orcid.org/0000-0002-7257-5503

Yulia A. Generalova – Assistant, Department of Therapeutic Dentistry, Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Medical Institute, 6 Miklukho-Maklaya Str., Moscow 117198, Russian Federation; https://orcid.org/0000-0003-1926-7162

Yulia A. Taptun – Assistant, Department of Therapeutic Dentistry, Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Medical Institute, 6 Miklukho-Maklaya Str., Moscow 117198, Russian Federation; https://orcid.org/0000-0002-0733-2687

Liudmila A. Kozhevnikova – Cand. Sci. (Med.), Associate Professor, Department of Therapeutic Dentistry, Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Medical Institute, 6 Miklukho-Maklaya Str., Moscow 117198, Russian Federation; https://orcid.org/0000-0002-3298-2104

Fakhri Ya. Gadzhiev – Postgraduate Student, Department of Therapeutic Dentistry, Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Medical Institute, 6 Miklukho-Maklaya Str., Moscow 117198, Russian Federation; https://orcid.org/0000-0003-1796-3594

Marina Yu. Dashtieva – Department of Therapeutic Dentistry, Peoples' Friendship University of Russia named after Patrice Lumumba (RUDN University), Medical Institute, 6 Miklukho-Maklaya Str., Moscow 117198, Russian Federation; https://orcid.org/0000-0001-8903-2487

ИНФОРМАЦИЯ ОБ АВТОРАХ

Хабадзе Зураб Суликоевич – к.м.н., доцент, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (РУДН); 117198, Российская Федерация, г. Москва, ул. Миклухо-Маклая, 6; https://orcid.org/0000-0002-7257-5503

Генералова Юлия Алексеевна – ассистент, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (РУДН); 117198, Российская Федерация, г. Москва, ул. Миклухо-Маклая, 6; https://orcid.org/0000-0003-1926-7162

Таптун Юлия Александровна – ассистент, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (РУДН); 117198, Российская Федерация, г. Москва, ул. Миклухо-Маклая, 6; https://orcid.org/0000-0002-0733-2687

Кожевникова Людмила Алексеевна – к.м.н., доцент, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (РУДН); 117198, Российская Федерация, г. Москва, ул. Миклухо-Маклая, 6; https://orcid.org/0000-0002-3298-2104

Гаджиев Фахри Яшар оглы – аспирант, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (РУДН); 117198, Российская Федерация, г. Москва, ул. Миклухо-Маклая, 6; https://orcid.org/0000-0003-1796-3594

Даштиева Марина Юзбеговна – ассистент, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (РУДН); 117198, Российская Федерация, г. Москва, ул. Миклухо-Маклая, 6; https://orcid.org/000-0001-8903-2487

AUTHOR'S CONTRIBUTION

Zurab S. Khabadze – 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.

Yulia A. Generalova – the acquisition, analysis, or interpretation of data for the article; drafted the article.

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Liudmila A. Kozhevnikova – the acquisition, analysis, or interpretation of data for the article; drafted the article.

Fakhri Ya. Gadzhiev – the acquisition, analysis, or interpretation of data for the article; drafted the article.

Marina Yu. Dashtieva – the acquisition, analysis, or interpretation of data for the article; drafted the article.

ВКЛАД АВТОРОВ

З.С. Хабадзе – существенный вклад в замысел и дизайн исследования, критический пересмотр статьи в части значимого интеллектуального содержания; окончательное одобрение варианта статьи для опубликования.

Ю.А. Генералова – сбор данных, анализ и интерпретация данных, подготовка статьи.

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

Л.А. Кожевникова – сбор данных, анализ и интерпретация данных, подготовка статьи

Ф.Я. Гаджиев – сбор данных, анализ и интерпретация данных, подготовка статьи

М.Ю. Даштиева – сбор данных, анализ и интерпретация данных, подготовка статьи.