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ЖУРНАЛ ВКЛЮЧЕН В РОССИЙСКИЙ ИНДЕКС НАУЧНОГО ЦИТИРОВАНИЯ

Эндодонтия Today – это научный рецензируемый журнал, включенный в Перечень ВАК рецензируемых научных изданий, в которых должны быть опубликованы основные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук, в соответствии с требованиями приказа Минобрнауки России. Журнал является информационным партнером Стоматологической Ассоциации России.

Журнал Эндодонтия Today является журналом с открытым доступом, что позволяет научному сообществу и широкой общественности получать неограниченный, свободный и немедленный доступ к статьям и свободно использовать контент. В журнале публикуются статьи практикующих врачей-стоматологов и научных сотрудников, подготовленные по материалам оригинальных научных исследований, обзоров научной литературы и клинических случаев в области терапевтической стоматологии и хирургической эндодонтической стоматологии, а также работы смежных стоматологических специальностей. Научная концепция журнала позволяет как врачам-стоматологам, так и врачам общих профилей узнавать о новых и передовых концепциях в лечении корневых каналов и последних достижениях в области эндодонтии.

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Identification of factors contributing to the appearance of inflammatory periodontal diseases preclinical symptoms in prison persons

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Abstract

AIM. The analysis of the main factors causing bleeding in the oral cavity in persons in places of deprivation of liberty, and the search for ways to eliminate this symptom, considering the limited capabilities of the respondents.

MATERIALS AND METHODS. The study involved 402 prisoners, 330 of whom were men and 72 women. The respondents were divided into age groups, the group of respondents under the age of 18 included 110 people, 18–25 years old – 7 people, 26–35 years old – 127 people, 36–45 years old – 94 people, 46–55 years old – 44 people and the group of 56 years and older consisted of 20 people. The respondents' responses were analyzed using the StatTech v 4.6.1 (developed by Stattech LLC, Russia).

RESULTS. 25 questions were analyzed, among which reliable correlations between bleeding and its development factors were established.

CONCLUSIONS. Based on the analyzed data, bleeding as an objective symptom was noticed by 157 respondents who noted brushing their teeth and eating hard food as factors provoking the development of this pathology. In this regard, it is necessary to develop a program aimed at improving the hygienic status and raising awareness of prisoners about proper oral care, considering their stay in places of deprivation of liberty. Such a program should primarily be based on the motivation of prisoners to adjust and improve their personal oral hygiene. In addition, in addition to preventive conversations, it is necessary to conduct master classes on the technique of proper dental cleaning and the use of additional personal oral hygiene products.

Keywords: bleeding, prisoners, oral hygiene, diabetes mellitus, periodontal diseases, penitentiary institutions.

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Выявление факторов, способствующих появлению доклинических симптомов развития воспалительных заболеваний пародонта у лиц, находящихся в местах лишения свободы

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Резюме

ЦЕЛЬ ИССЛЕДОВАНИЯ. Анализ основных факторов, вызывающих кровоточивость в полости рта у лиц, находящихся в местах лишения свободы, и поиск путей устранения этого симптома с учетом ограниченных возможностей анкетированных.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании принимали участие 402 заключенных, из которых 330 мужчин и 72 женщины. Респонденты были поделены на группы по возрасту, группа анкетированных

молоче 18 лет включала 110 человек, 18–25 лет – 7 человек, 26–35 лет – 127 человек, 36–45 лет – 94 человека, 46–55 лет – 44 человека и группа категории 56 лет и старше состояла из 20 человек. Ответы респондентов были проанализированы с использованием программы StatTech v. 4.6.1 (разработчик – ООО «Статтех», Россия).

РЕЗУЛЬТАТЫ. Проанализировано 25 вопросов, среди которых были установлены достоверные взаимосвязи между кровоточивостью и факторами ее развития.

ВЫВОДЫ. Исходя из проанализированных данных, кровоточивость как объективный симптом заметили 152 анкетированных, отметивших чистку зубов и прием жесткой пищи как факторы, провоцирующие развитие данной патологии. В связи с этим необходима разработка программы, направленной на улучшение гигиенического статуса и повышения информированности заключенных о правильном уходе за полостью рта с учетом нахождения их в местах лишения свободы. Такая программа в первую очередь должна основываться на мотивации заключенных корректировать и улучшать личную гигиену полости рта. Кроме того, помимо профилактических бесед необходимо провести мастер-классы по технике правильной чистки зубов и использования дополнительных средств индивидуальной гигиены полости рта.

Ключевые слова: кровоточивость, заключенные, гигиена полости рта, сахарный диабет, заболевания пародонта, пенитенциарные учреждения.

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RELEVANCE

In the Russian Federation, as of the beginning of 2024, about 430,000 people are in prison, which is about 300 people per 100 000 population. The penitentiary system in Russia consists of pre-trial detention centers, correctional colonies of various regimes, specialized open and closed type hospitals¹. Maintaining dental health in persons in places of detention is difficult due to living conditions and limited access to dental care, as well as low awareness of proper oral care. The condition of the oral cavity can also be a marker of general health [1]. Bleeding when brushing teeth is an objective symptom that a person can determine on their own.

It is not possible to achieve a good level of oral hygiene in the presence of several lifestyles features of prisoners but following simple and regular measures can significantly contribute to maintaining dental health.

Therefore, it is necessary to develop a set of measures for them, the implementation of which is possible in places of deprivation of liberty. It is also important to inform prisoners about the effectiveness of these manipulations, since it is known from the literature that awareness is an important link in forming a responsible attitude towards one's own health [2; 3].

INTRODUCTION

In this regard, the aim of the study is to analyze the main factors causing bleeding in the oral cavity in persons in places of detention and to find ways to eliminate this symptom, considering the limited capabilities of the respondents.

¹ Federal Penitentiary Service. Brief description of the penal enforcement system of the Russian Federation; 20.12.2024. Available at: <https://fsin.gov.ru/structure/inspector/iao/statistika/Kratkaya%20har-ka%20UIS/> (accessed: 02.03.2025).

To achieve the intended goal, the following tasks were set:

1. To identify the main factors causing bleeding in the oral cavity based on the data obtained from the statistical program.
2. To analyze reliable indicators.
3. Formulate conclusions based on the results of the analysis.

MATERIALS AND METHODS

A survey of 402 prisoners was conducted. The data on the respondents are shown in Table 1.

Table 1. Distribution of respondents by gender, length of stay in a penitentiary institution, age

Таблица 1. Распределение опрошенных по полу, длительности нахождения в пенитенциарном учреждении, возрасту

Parameter	Denotation	
Number of respondents	n = 402	
Gender, people (%)	Male	Female
	n = 330 (82.1%)	n = 72 (17.9%)
Duration of stay in a penitentiary institution, months (Me [Q ₁ –Q ₃] (min/max))	20 [9–39] (0/49)	
Age group, people, (%):		
Under 18 years of age	n = 110 (27.4%)	
18–25 years old	n = 7 (1.7%)	
26–35 years old	n = 127 (31.6%)	
36–45 years old	n = 94 (23.4%)	
46–55 years old	n = 44 (10.9%)	
56 years and older	n = 20 (5.0%)	

The questionnaire was provided in writing, which included 25 questions: 3 questions aimed at collecting demographic data, 2 questions aimed at assessing the general health of prisoners, 6 questions aimed at assessing dental status, 5 questions included collecting information about problems with the mucous membrane in the oral cavity, 5 questions about the presence of problems with periodontal tissues, including the presence of bleeding in the oral cavity under the influence of various factors, 4 issues, including the presence of bad habits and somatic pathology. The survey was conducted voluntarily, at the request of the prisoners. Each respondent was informed about the anonymity of his answers.

The statistical analysis was carried out using the Stat Tech v program. 4.6.1 (developed by Stattech LLC, Russia). The number of answers to each of the questions was calculated in absolute values and percentages. The statistical Pearson Chi-square method was used to determine the presence of correlations between the answers to the questionnaire questions.

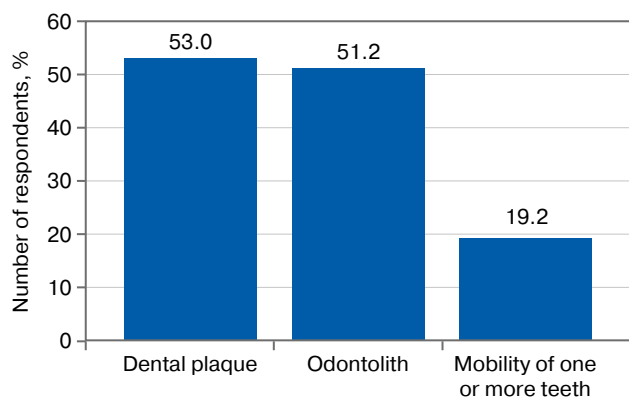


Fig. 1. The condition of the oral cavity according to the respondents

Рис. 1. Состояние ротовой полости по мнению респондентов

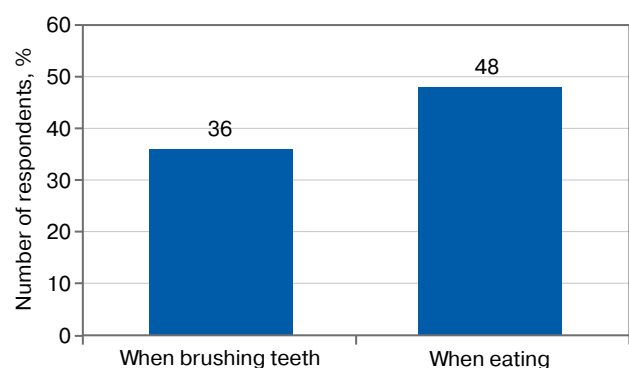


Fig. 2. Characteristics of bleeding when brushing teeth and eating when there is mobility of one or more teeth, according to respondents

Рис. 2. Характеристика кровоточивости при чистке зубов и приеме пищи при наличии подвижности одного или более зубов по мнению респондентов

RESULTS

Among the respondents to the questionnaire, 152 people (37.8%) reported bleeding in the oral cavity and, on the contrary, 250 people (62.2%) didn't experience bleeding.

Significant correlations between the presence of bleeding, as well as the degree of its severity, were identified for the following groups of issues:

- the condition of the oral cavity according to the respondents;
- hygiene habits;
- the presence of bad habits at the present time and in the anamnesis;
- several questions related to the overall health of the respondents.

Among the cohort who reported bleeding when brushing their teeth, 111 (27.6%) respondents had it, 25 (6.2%) had it when eating, and 37 (9.2%) respondents had spontaneous bleeding.

It was found that spontaneous bleeding, as well as bleeding when brushing teeth and eating, are significantly more common among the contingent who report poor oral health.

The characteristics of the oral cavity condition, according to the respondents, are shown in Fig. 1.

It was found that among the contingent who noted the presence of tartar, bleeding was significantly more common when brushing teeth and eating, compared with those who did not have bleeding. This may be due both to the presence of periodontal diseases in the respondents and to a disdainful attitude towards the condition of the oral cavity.

The characteristics of bleeding when brushing teeth and eating in the presence of mobility of one or more teeth, according to the respondents, are shown in Fig. 2.

Bleeding during meals is significantly more common among the population who report mobility of one or more teeth (12 (48.0%)), compared with those who experience bleeding while brushing their teeth (40 (36.0%)) ($p \leq 0.001$; $p \leq 0.001$)

The characteristics of hygiene habits according to the respondents are presented (Table 4, Fig. 3).

Table 4. Indicators of hygiene habits according to the respondents

Таблица 4. Показатели гигиенических привычек по мнению анкетированных

Parameter	Categories	People	%
Frequency of brushing teeth	1 time per day	113	28.1
	2 times a day	227	56.5
Frequency of toothbrush change	Once a month	18	4.5
	Regularly 1 time in 3 months	234	58.2
	Once every 6–12 months	150	37.3
Rinsing the mouth with water or saline solution	No	273	67.9
	Yes	129	32.1

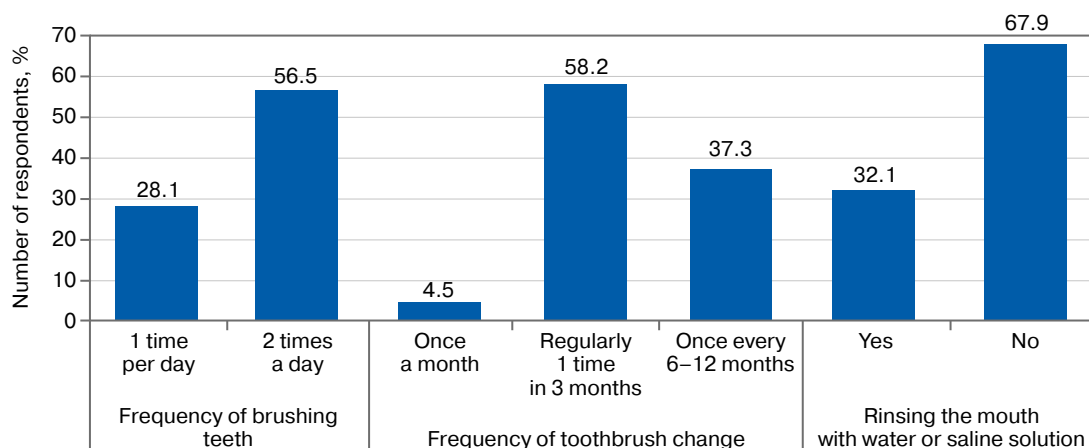


Fig. 3. Indicators of hygiene habits according to the respondents

Рис. 3. Показатели гигиенических привычек по мнению респондентов

There is a significant relationship between the habit of rinsing the mouth with water or saline solution and bleeding, respondents have a lower percentage of bleeding when brushing their teeth (26 (23.4%)) using this habit, compared with those who do not have the habit (103 (35.4%)) ($p = 0.022$).

It was also revealed that among those who brush their teeth once a day, bleeding occurs in more than half of the population, which is significant (43 (38.0%) versus 70 (24.1%)) ($p = 0.008$).

It was found that prisoners who could change their toothbrush regularly have a lower percentage of bleeding (9 (36.0%)), compared with those who change their toothbrush once every six months or less (16 (64.0%)) ($p = 0.014$), which may be due to the difficulty of regular change of toothbrush and lack of such.

The characteristics of the oral mucosa are presented in Table 5.

It was revealed that the studied contingent, who noted problems with the mucous membrane in the lip area, significantly more often noted bleeding when brushing their teeth (9 (8.1%)), compared with those who did not have bleeding (9 (3.1%)) ($p = 0.030$).

It was also revealed that bleeding during meals is significantly more common among prisoners who have problems with the mucous membrane in the lips, cheeks and tongue. (4 (16.0%)), (9 (36.0%)), (6 (24.0%)) accordingly, compared to those who have no bleeding (14 (3.7%), 10 (2.7%), 16 (4.2%) respectively) ($p = 0.004$; $p \leq 0.001$; $p \leq 0.001$, respectively). The same trend can be observed with spontaneous bleeding in the oral cavity. These indicators may be associated with chronic inflammation of the mucous membrane in the oral cavity, as a result, as well as due to malocclusion, poor-quality fillings with overhanging edges, together these causes may contribute to the development of chronic injury to the oral mucosa, and under the influence of external factors of its bleeding.

It was found that among the contingent noting the presence of an unpleasant taste in the mouth, bleeding is significantly more common when brushing

teeth (37 (33.3%)), compared with those who do not have bleeding (41 (14.1%)) ($p \leq 0.001$), this may be due to poor oral hygiene, mouth problems, as well as the presence of bad habits, as well as suppuration from periodontal pockets.

The characteristics of the general state of health and the presence of somatic pathology of the respondents are presented in Table 6.

Table 5. Indicators of the state of the oral mucosa

Таблица 5. Показатели состояния слизистой оболочки полости рта

Parameter	Categories	People	%
Problems with the oral mucosa	Lips	18	4.5
	Jowls	19	4.7
	Tongue	22	5.5
	The bottom of the oral cavity	8	2.0
	Unpleasant taste in the mouth (sour, bitter, metallic, sweet, etc.)	78	19.4

Table 6. Indicators of the general state of health and the presence of somatic pathology of the respondents

Таблица 6. Показатели общего состояния здоровья и наличие соматической патологии анкетированных

Parameter	Categories	People	%
Assessment of the general condition	Very bad	5	1.2
	The bad	32	8.0
	Satisfactory	148	36.8
	Good	156	38.8
	Very good	61	15.2
Symptoms	General weakness	296	73.6
	Malaise	36	9.0
	Reduced working capacity	47	11.7
Diabetes mellitus	Availability	21	5.2

It was also revealed that spontaneous bleeding and bleeding during meals are significantly more common among the contingent who report malaise. (9 (24.3%); (7 (28.0%) respectively), compared to those who do not have bleeding (27 (7.4%); (29 (7.7%) accordingly) ($p \leq 0.001$).

It has been established that spontaneous bleeding and bleeding during meals are significantly more common among the contingent noting a decrease in their working capacity. (8 (21.6%); 6 (24.0%) respectively), compared with those who have no bleeding (39 (10.7%); 41 (10.9%) accordingly) ($p = 0.049$); ($p = 0.048$), respectively).

It was also found that among prisoners who reported spontaneous bleeding, it was significantly more common to indicate the presence of general weakness (22 (59.5%)), compared with those with no general weakness (15 (40.5%)) ($p = 0.040$).

Analyzing the relationship between the presence of somatic pathology and bleeding, it was found that respondents with diabetes mellitus were significantly more likely to indicate the presence of bleeding during meals (4 (16.0%)), compared with those without bleeding (17 (4.5%)) ($p = 0.012$).

Analyzing the relationship between the presence of somatic pathology and bleeding, it was found that respondents with diabetes mellitus were significantly more likely to indicate the presence of bleeding during meals (4 (16.0%)), compared with those without bleeding (17 (4.5%)) ($p = 0.012$).

The characteristics of the presence of bad habits of the respondents are presented in Table 7.

Among the contingent who noted the presence of bad habits in the past, 273 (67.9%) of respondents were smokers, 21.1% of respondents had abused alcohol, and 89 (22.1%) of respondents had taken narcotic substances in the past.

It was revealed that among smokers, the vast majority noted bleeding in the oral cavity when brushing their teeth (89 (80.2%)).

It was also found that the studied population, who noted the use of alcoholic beverages and narcotic substances in the past, significantly more often indicated the presence of bleeding when brushing their teeth (35 (31.5%); 32 (28.8%) respectively), compared with those who have no bleeding (50 (17.2%); 57 (19.6%), respectively) ($p = 0.002$); ($p = 0.046$), respectively).

It was established that the studied contingent, who noted the use of alcoholic beverages and narcotic substances in the past, significantly more often noted bleeding during meals (12 (48.0%)) and (10 (40.0%)) accordingly, compared to those who have no bleeding (73 (19.4%); 79 (21.0%) accordingly) ($p \leq 0.001$ and $p = 0.026$, respectively).

It has been revealed that prisoners who have reported the use of alcoholic beverages and narcotic substances in the past are significantly more likely to report spontaneous bleeding. (16 (43.2%); (17 (45.9%) respectively), compared with those who have no bleeding (69 (18.9%); (72 (19.7%) accordingly) ($p \leq 0.001$).

Table 7. Indicators of the presence of harmful habits of the respondents

Таблица 7. Показатели наличия вредных привычек анкетирруемых

Parameter	Categories	People	%
Bad habits	Smoking	273	67.9
	Alcohol consumption	85	21.1
	Drug use	89	22.1

DISCUSSION

The problem of poor hygiene among persons in institutions of the federal Penitentiary Service can be traced not only in Russia, but also abroad, depending on the level of economic development of countries and the social status of prisoners.

In 2017, a study was conducted in Finland among prisoners of the Pelso correctional colony, during which a qualified dentist examined 100 prisoners: 89 men and 11 women, with an average age of 35 years. According to the survey results, it was found that half of the subjects had periodontal diseases, the maximum value of the general periodontal index or the periodontal disease needs index (CPI) among people under the age of 30 was 3 points (removal of dental deposits, curettage, hygiene, conservative therapy). in 33.3%, 2 points (removal of dental deposits, hygiene) in 67.7%, among people over 30 years of age, the maximum score corresponded to 4 points (removal of dental deposits, conservative therapy, flap surgery, orthopedic treatment, hygiene) in 9.1%, only 2.9% of subjects under the age of 30 had a healthy periodontal [4].

A 2014 study conducted in the Nigerian city of Enugu was also analyzed. This state is considered socially disadvantaged, which is attributed to the low level of medical care. To conduct the study, a questionnaire was compiled, and an examination of the oral cavity was performed by a specialist to assess the condition of the teeth and periodontal tissues. The study involved 230 prisoners, among them 5.2% of the surveyed had a CPI = 0 (12/230), 94.8% (218/230) had a CPI = 1/2/3/4. According to the results of the examination, more than half of the prisoners had caries with complications [5].

The data obtained by the authors during the survey of prisoners demonstrate a similar relationship, namely, based on the results of the analysis of respondents' responses, it can be concluded that in Russia the situation with the dental health of prisoners is comparable.

Patient awareness is achieved through preventive interviews, as well as the development of a program that includes sanitary and educational work with prisoners, training in dental cleaning techniques and quality control of oral hygiene, oral sanitation, and follow-up for the timely detection and treatment of dental diseases. An important aspect of the program should be the development of motivation among prisoners, since their interest in treatment contributes to the improvement of the oral cavity. There is a positive experience in the scientific literature with the development and implementation of a program based on the motivation of prisoners [4].

CONCLUSION

As a result of the analysis of the presence of bleeding in prisoners, which manifests itself under the influence of external stimuli or spontaneously without any prerequisites, reliable correlations between the presence of bleeding and the provoking factor of its development were revealed. This may be due to both the presence of periodontal diseases in the respondents and the neglect of the oral cavity. It may also be due to the inability

to regularly perform proper oral hygiene, as well as poor hygiene habits. Of course, the low awareness of prisoners about the importance of oral hygiene plays a key role here. Therefore, it is necessary to develop and implement a program that includes both preventive conversations with prisoners, their motivation, and master classes on brushing teeth, proper use of toothbrushes, mouthwashers, dental floss, and other additional personal oral hygiene products.

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A method for assessing changes in mandibular biomechanics using a software-hardware system

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Abstract

INTRODUCTION. The temporomandibular joint (TMJ) plays a crucial role in mastication, speech, and maintaining overall postural balance. Dysfunction in TMJ biomechanics can lead to local pain, functional limitations, and postural disturbances. Despite advances in digital technologies and the integration of osteopathy into dental practice, there remains a lack of accessible and validated tools for dynamic TMJ monitoring.

AIM. To develop and validate a method for quantitative assessment of mandibular biomechanics using craniometric measurements and digital technologies.

MATERIALS AND METHODS. Ninety patients aged 19 to 61 years with TMJ dysfunction and extra-occlusal disorders were enrolled. Participants were divided into a main group, which received both dental and osteopathic treatment, and a control group, which received dental treatment only. Standardized digital imaging was performed pre- and post-treatment. Mandibular deviation was assessed by measuring the angle and perpendicular distance from the sagittal plane using a mobile application. Statistical analysis was performed using IBM SPSS Statistics version 26.0, with significance set at $p < 0.05$.

RESULTS. A statistically significant reduction in mandibular deviation was observed in both groups ($p < 0.05$), with the main group showing greater improvements. The findings support the effectiveness of combining osteopathic correction with dental therapy in restoring mandibular biomechanics.

CONCLUSIONS. The proposed method provides an objective and efficient tool for assessing the outcomes of dental and osteopathic interventions in patients with TMJ dysfunction and offers potential for early detection of biomechanical impairments.

Keywords: osteopathy; dentistry; temporomandibular joint dysfunction; mandibular biomechanics

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Способ определения изменения биомеханики нижней челюсти с помощью программно-аппаратного комплекса

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Резюме

ВВЕДЕНИЕ. Височно-нижнечелюстной сустав играет ключевую роль в обеспечении функций жевания, речи и поддержания общего постурального баланса. Нарушения его биомеханики могут влиять не только на локальную боль и функциональные ограничения, но и на общее состояние осанки и подвижности всего опорно-двигательного аппарата. За последние годы в связи с активным развитием цифровых технологий и интеграции остеопатии в стоматологическую практику стали создаваться быстрые, малоинвазивные методы оценки выявления нарушений биомеханики височно-нижнечелюстного сустава. Тем не менее, пока нет доступных, общедоступных и валидизированных решений для динамического мониторинга.

ЦЕЛЬ ИССЛЕДОВАНИЯ. Разработать способ оценки биомеханики нижней челюсти на основе краниометрических измерений с использованием цифровых технологий.

МАТЕРИАЛЫ И МЕТОДЫ. Проведено исследование с участием 90 пациентов в возрасте от 19 до 61 года с дисфункцией височно-нижнечелюстного сустава и наличием экстраокклюзионных нарушений. Пациенты были разделены на основную и контрольную группы; основной группе помимо стоматологического лечения проводилась остеопатическая коррекция. Для сравнения результатов лечения делали фото пациента с открытым ртом, после чего проводится измерение отклонения нижней челюсти от сагиттальной линии по краниометрическим точкам. Измерялись угол отклонения и длина нормали до сагиттальной линии. Статистический анализ выполнялся с использованием IBM SPSS 26 при уровне значимости $p < 0,05$.

РЕЗУЛЬТАТЫ. У пациентов обеих групп отмечено статистически значимое уменьшение отклонения нижней челюсти после лечения ($p < 0,05$), при этом основная группа показала более выраженные улучшения. Данные подтвердили эффективность применения остеопатической коррекции в сочетании со стоматологическим лечением для восстановления биомеханики нижней челюсти.

ВЫВОДЫ. Предложенный способ позволяет объективно оценить эффективность стоматологического или остеопатического лечения пациентов с дисфункцией височно-нижнечелюстного сустава, а также предположить наличие дисфункции на ранних этапах.

Ключевые слова: остеопатия; стоматология; дисфункция височно-нижнечелюстного сустава

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INTRODUCTION

The temporomandibular joint (TMJ) plays a vital role in mastication, speech, and the maintenance of overall postural balance. Dysfunctions in TMJ biomechanics can result not only in localized pain and functional impairments but also in broader postural disturbances affecting the musculoskeletal system.

Currently, the primary diagnostic methods for assessing mandibular mobility and biomechanics include cone-beam computed tomography (CBCT), magnetic resonance imaging (MRI) with functional tests, and the fabrication of dental models on articulators. While these methods provide high diagnostic accuracy, they are time-consuming, require specialized expertise, and are not suitable for dynamic monitoring during the course of treatment [1]. Moreover, the use of ionizing imaging modalities necessitates justification due to associated risks.

In recent years, there has been a growing interest in integrating osteopathic approaches into dental practice. Interdisciplinary conferences, peer-reviewed publications, and doctoral research increasingly highlight the clinical synergy between these specialties [2]. Osteopathic practitioners play an active role in managing musculoskeletal dysfunctions of the TMJ, emphasizing the biomechanical interrelations between the joint and the broader postural system.

Additionally, emerging evidence points to the influence of extra-occlusal disturbances and overall postural balance on TMJ kinematics and mandibular biomechanics [3]. Nevertheless, there remains a lack of accessible, rapid, and validated methods for the objective dynamic assessment of these interactions in clinical practice.

In response to these challenges, we propose a novel method for the quantitative assessment of mandibular biomechanics using craniometric analysis supported by digital technologies.

AIM

This study focuses on the development and validation of a method for the dynamic evaluation of vertical mandibular movements based on digital craniometric measurements.

MATERIALS AND METHODS

According to the Helkimo Index, an acceptable mandibular deviation is defined as not exceeding 2 mm [4] (Fig. 1), measured at the gnathion – the point located at the lower border of the mandible where it intersects the midsagittal plane. The line connecting the craniometric landmarks nasion (N) and subnasale (Sn) is positioned along the sagittal plane. Since the angle formed between the N–Sn line and the N–Gn line (gnathion) is invariant to facial scaling in photographs, it is utilized by the “TMJ Master” mobile application to assess mandibular position (Fig. 2).

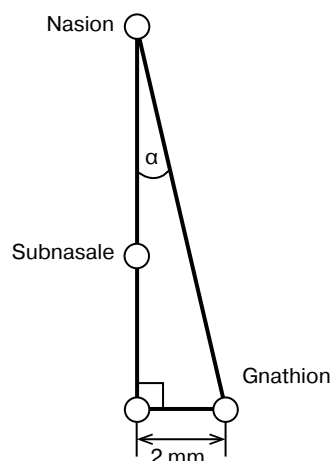


Fig. 1. Angle between the Sn–N and Gn–N lines

Рис. 1. Угол между линиями Sn–N и Gn–N

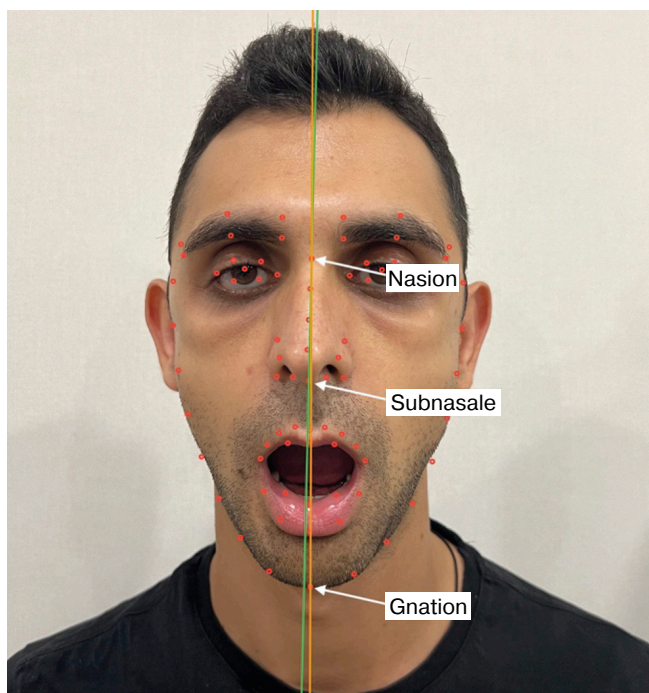


Fig. 2. Calculation of the angle between the Sn–N and Gn–N lines in the mobile application

Рис. 2. Расчет угла между линиями Sn–N и Gn–N в мобильном приложении

Table 1. Distribution of patients by sex and age

Таблица 1. Распределение пациентов по полу и возрасту

Parameter	Main group (n = 45)	Control group (n = 45)	p
Age			
M, SD	39.29 (11.35)	39.82 (12.43)	0.837
Me (Q1–Q3)	39 (29.5–48.0)	39 (29.5–51.0)	
Min, max	20; 60	19; 61	
Sex			
Male	17 (37.8%)	21 (46.7%)	0.393
Female	28 (62.2%)	24 (53.3%)	

Table 2. Mandibular deviation (mm)

Таблица 2. Отклонение нижней челюсти (мм)

Mandibular deviation	Main group (n = 45)		Control group (n = 45)	
	Before treatment	After treatment	Before treatment	After treatment
Me	3.01*	2.40* ^{**}	3.21*	2.92* ^{**}
Q1–Q3	2.86–3.18	2.27–2.56	2.83–3.67	2.53–3.37

Note. * Statistically significant difference ($p < 0.05$) before and after treatment within each group.

** Statistically significant difference ($p < 0.05$) between the main and control groups after treatment.

Примечания. * Статистически значимая разница ($p < 0,05$) до и после лечения внутри каждой группы.

** Статистически значимая разница ($p < 0,05$) между основной и контрольной группами после лечения.

The α angle was automatically calculated by the application for each uploaded image during the treatment course. A reduction in this angle was anticipated following effective therapy.

For the final evaluation, the real-world length of the perpendicular line from the gnathion to the N–Sn line was measured. A scaling factor was applied, determined by the ratio between the physical distance of standard anthropometric reference points on the patient and the corresponding distance on the photograph.

Standardized digital imaging was performed under consistent conditions: patients stood on a level surface with their mouths open, ensuring that only the head was within the frame.

To validate the hypothesis, a cohort of 90 patients aged 19 to 61 years with temporomandibular joint dysfunction (TMJD) and extra-occlusal disorders was enrolled. Participants were randomly assigned to either a main group or a control group, with no significant differences in sex or age between groups (Table 1). Both groups received dental treatment, while only the main group underwent additional osteopathic intervention. Dental therapy consisted of splint therapy, while osteopathic treatment targeted the correction of extra-occlusal imbalances. Standardized digital imaging was conducted before and after the treatment period to assess changes in mandibular deviation from the sagittal line. Statistical analysis was performed using IBM SPSS Statistics version 26.0, with a p -value < 0.05 considered statistically significant [5; 6].

RESULTS

Following the treatment, a statistically significant reduction in mandibular deviation ($p < 0.05$) was observed in both groups (see Table 2). Greater improvements were noted in the main group, which received additional osteopathic intervention.

Analysis of variance (ANOVA) revealed a significant interaction between group and treatment ($p < 0.05$), confirming that the addition of osteopathic correction had a more pronounced effect on the restoration of mandibular biomechanics.

Additionally, a tendency toward improved symmetry in mouth opening was observed, as evidenced by a reduction in patient-reported joint clicking and subjective improvement in symptoms recorded through patient questionnaires.

DISCUSSION

Analysis of the obtained data revealed that in 84% of cases, the direction of mandibular deviation corresponded to the side of the local somatic dysfunction of the TMJ identified during osteopathic examination. These findings were also consistent with electromyographic results, which demonstrated hyperactivity of the masticatory and temporalis muscles on the same side as the osteopathically diagnosed dysfunction.

This observation supports the existing osteopathic literature, which suggests that somatic dysfunction tends to localize where mobility is restricted – specifically, in the hypomobile temporomandibular joint. Pre-

viously, objective instrumental data confirming this hypothesis had not been presented [7].

Hypomobility in the TMJ region can manifest at multiple levels, including the ligamentous structures, muscular system, intra-articular components, fascial tissues, or even within the osseous structures.

Typically, in such cases, the contralateral TMJ adopts a compensatory function, becoming hypermobile, “overstretching”, and progressively losing stability. As a result, asymmetry develops, characterized by one joint remaining hypomobile while the other becomes hypermobile. The range of motion of the hypomobile joint is reduced compared to the compensatorily overloaded joint, leading to mandibular deviation during mouth opening relative to the midline. This biomechanical disturbance is often clinically manifested as an asymmetric smile or deviation during active mandibular movements, which is considered an unfavorable clinical finding.

CLINICAL CASES

Case 1

Patient A., a 25-year-old male, presented to the Osteopolyclinic with complaints of asymmetric mouth opening, clicking in the right temporomandibular joint (TMJ), and thoracic spine pain.

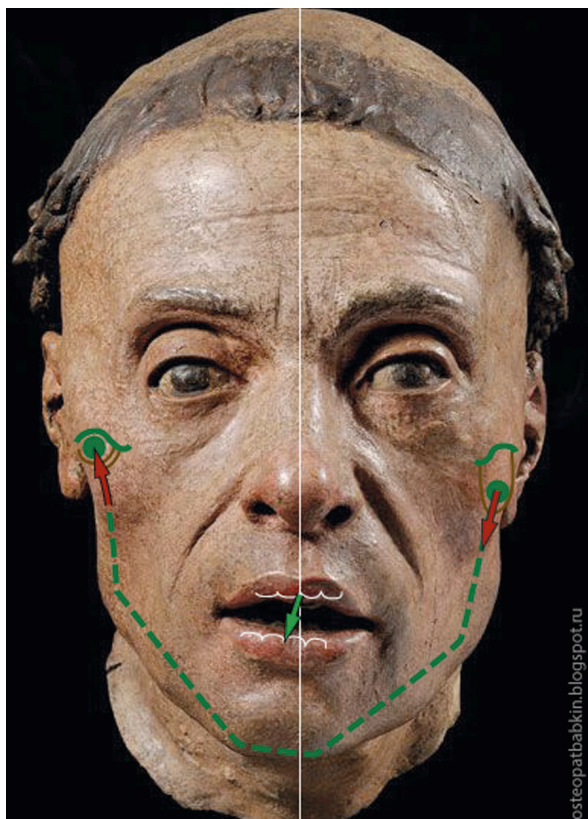


Fig. 3. The right TMJ is hypomobile, and the left TMJ is hypermobile

Source: [7]

Рис. 3. Правая ВНЧС – гипомобильна, левая ВНЧС – гипермобильна

Источник: [7]

A previous dental examination had diagnosed him with a dentoalveolar anomaly and right-sided TMJ dysfunction. The diagnoses according to ICD-10 were K07.20 (Distal occlusion) and K07.6 (TMJ disorders).

Osteopathic examination revealed a dominant somatic dysfunction of the thoracic region, a local somatic dysfunction at the TMJ level, and extra-occlusal influences of the primary somatic dysfunction on the chronic local TMJ dysfunction. The ICD-10 code was M99.0 (Segmental or somatic dysfunction).

To objectively assess mandibular biomechanics before and after osteopathic correction, the “TMJ Master” mobile application was used. Craniometric points were identified via photodocumentation. Analysis results demonstrated a statistically significant improvement: after osteopathic correction, the angle between the gnathion and the sagittal line decreased.

Case 2

Patient S., a 27-year-old female, presented to the “Dilos” dental clinic with complaints of asymmetric mouth opening, clicking in the left TMJ, and thoracic spine pain.

A dental examination diagnosed her with a dentoalveolar anomaly and left-sided TMJ dysfunction. The ICD-10 codes were K07.20 (Distal occlusion) and K07.6 (TMJ disorders).

Orthodontic and prosthetic treatment was recommended. During the preparation phase, cone-beam computed tomography (CBCT) was performed for TMJ visualization, dental impressions were taken for model fabrication on an articulator, and an initial biomechanical assessment was conducted using the “TMJ Master” application.

An individual occlusal splint was fabricated and prescribed. After one month of regular use, the patient returned for a follow-up assessment.

Biomechanical evaluation of the mandible before and after osteopathic correction of extra-occlusal disturbances was again performed using the “TMJ Master” application. Craniometric measurements based on photodocumentation revealed a statistically significant improvement: a reduction in the angle between the gnathion and the sagittal line was observed post-correction.

CONCLUSION

The application of the described method based on a software-hardware system and a mobile application enabled a quantitative assessment of changes in mandibular biomechanics in patients with TMJ dysfunction. The data obtained confirm the clinical relevance of deviation measurements based on craniometric landmarks as an objective criterion for evaluating the effectiveness of dental and osteopathic treatments. The methodology demonstrated high sensitivity and may be used not only for monitoring treatment dynamics but also as a tool for the early detection of dysfunctions. However, further research with larger sample sizes and control of additional variables is required to enhance the validity of the results.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

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The possibility of full-arch rehabilitation for edentulous patients supported by 4 dental implants

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Abstract

AIM. The objective of this study is to review possible methods of fixed prosthesis for patients with complete edentulousness using 4 dental implants.

RESULTS. Patients with complete edentulousness and significant alveolar bone atrophy often complain about unsatisfactory fixation of complete removable dentures. Dental implantation is used to make prostheses with stable fixation. Full fixed prostheses supported by 6–8 dental implants have the greatest stability and strength indicators, however, in some cases, with significant bone atrophy, the placement of a large number of implants is impossible. The “All-on-4” technique was proposed to accelerate the rehabilitation of elderly edentulous patients, which allows making fixed prosthesis supported by 4 dental implants without bone grafting procedures. This treatment method involves the installation of distal implants at an angle to the frontal plane in order to position the prosthetic platforms in the premolar area, which reduces the length of the distal console elements and distributes the occlusal load more evenly.

CONCLUSIONS. The “All-on-4” technique has become widespread among dentists due to its relative ease of performance, low cost and quick rehabilitation of edentulous patients. This treatment method shows high survival and success rates, and thus can be an effective alternative to placing more implants. However, this method of treatment requires further study from the point of view of functional changes of the dento-alveolar system in patients with complete absence of teeth and justification using the methods of electromyography of masticatory muscles and digital analysis of occlusion, as well as determination of critical values of angles of inclination of distal implants using the method of mathematical modeling.

Keywords: implant, edentulousness, atrophy, prosthesis, All-on-4

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Возможности несъемного протезирования пациентов с полным отсутствием зубов с опорой на 4 дентальных импланта

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Резюме

ЦЕЛЬ. Обзор метода несъемного протезирования пациентов с полным отсутствием зубов с использованием 4 дентальных имплантатов.

РЕЗУЛЬТАТЫ. Пациенты с полным отсутствием зубов и выраженной атрофией альвеолярной кости при использовании полных съемных протезов часто предъявляют жалобы на их неудовлетворительную фиксацию. С целью улучшения фиксации протезов применяется метод дентальной имплантации. Наибольшей устойчивостью и прочностными свойствами обладают полные несъемные протезы с опорой на 6–8 дентальных имплантатов, однако в ряде случаев при выраженной атрофии костной ткани установка большого количества имплантатов невозможна. С целью более быстрой реабилитации беззубых пациентов с выраженной атрофией альвеолярной кости, а также пациентов с соматической патологией, была предложена методика «All-on-4», позволяющая изготовить несъемный протез с опорой на 4 дентальных имплантата, не прибегая к обширным костно-пластическим операциям. Данный метод лечения предполагает установку дистальных имплантатов под углом к фронтальной

плоскости с целью расположения ортопедических платформ в области премоляров, что позволяет уменьшить длину дистальных консольных элементов и более равномерно распределить окклюзионную нагрузку.

ВЫВОДЫ. Методика «All-on-4», получила широкое распространение среди стоматологов вследствие относительной простоты выполнения, дешевизны и быстроты реабилитации беззубых пациентов. Этот метод лечения демонстрирует высокие показатели выживаемости и успешности, а значит, может быть эффективной альтернативой установке большего количества имплантатов. Однако, данный метод лечения требует дальнейшего изучения с точки функциональных изменений зубочелюстной системы у пациентов с полным отсутствием зубов и обоснования с использованием методов электромиографии жевательных мышц и цифрового анализа окклюзии, а также определения критических значений углов наклона дистальных имплантатов с помощью метода математического моделирования.

Ключевые слова: имплантат, адентия, атрофия, протезирование, All-on-4

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INTRODUCTION

According to the World Health Organization, 29% of individuals aged 65–74 worldwide suffer from complete edentulism. This condition can be addressed through two principal prosthetic options: the fabrication of complete removable mucosa-supported dentures or the placement of prosthetic constructions supported by dental implants. The absence of teeth may lead to difficulties or inability to chew food, impaired social integration, and psycho-emotional discomfort due to compromised speech and esthetic deficiencies. Furthermore, long-term edentulism results in functional disturbances of the stomatognathic system, including temporomandibular joint dysfunction and altered masticatory muscle activity. The most significant disadvantage of removable mucosa-supported dentures is their inadequate retention, particularly in cases of pronounced alveolar ridge atrophy [1]. This can lead not only to mechanical damage of the prosthesis—such as fracture or increased wear of artificial teeth—but also to exacerbation of functional impairments. Nevertheless, the relatively low cost of such prostheses and the absence of additional surgical interventions make them an attractive solution, although every prosthetic method presents both advantages and significant limitations.

In contemporary dental practice, dental implants are widely used for the replacement of missing teeth to provide support for both removable and fixed prosthetic constructions. This has greatly expanded treatment possibilities for patients with complete edentulism [2]. Numerous studies have been published to determine the optimal number of dental implants required for full-arch rehabilitation. In a 2010 study, V.V. Korkin conducted a comparative analysis of the survival of prosthetic constructions with different numbers of supporting implants using the Schleicher–Nadan criterion, which evaluates the combined effects of tensile, compressive, and shear stresses. The highest biomechanical strength in the “fixed prosthesis–endosseous implants–alveolar bone” system was observed when six to eight vertically placed implants were used [3].

However, several anatomical characteristics of the maxilla, such as extensive pneumatization of the maxillary sinus and bone atrophy due to periodontitis and long-term tooth loss, often result in insufficient bone volume for the placement of six to eight dental implants, necessitating sinus augmentation procedures. On the mandible, vertical alveolar bone resorption progresses at an average rate of 0.2 mm per year. When combined with periodontal bone defects, this often requires bone grafting before implant placement. Performing extensive bone augmentation procedures in elderly patients with comorbidities carries an increased risk of complications. Moreover, in many cases, the actual bone volume gain after augmentation is minimal, which may necessitate repeated surgical intervention. For example, I. Urban, the author of the guided bone regeneration technique known as the “Sausage technique”, reports that additional augmentation is often required at the stage of implant placement within the previously regenerated area to ensure long-term volume stability [4].

Given these considerations, alternative treatment options to the placement of six to eight dental implants should be explored in specific clinical scenarios – one such alternative being the use of four implants in regions with favorable anatomic conditions. The fabrication of a fixed prosthesis supported by dental implants in cases of severe alveolar ridge atrophy requires careful planning of implant positioning and prosthetic design. One of the complications associated with fixed prostheses supported by four implants in fully edentulous patients is bone resorption around the implants caused by excessive loading resulting from altered stomatognathic function. According to D. Wismeijer, P. Casentini, G. Gallucci, and M. Chiapasco, the majority of cases of bone resorption and implant failure occurred in the distal segments, underscoring concerns regarding the long-term survival of fixed full-arch prostheses supported by only four dental implants [5].

Since the 1960s, standard Toronto-type implant-supported prostheses have been widely used in the treatment of edentulous patients, particularly in cases

of severe jaw atrophy. When four dental implants are used to support a fixed prosthesis, they are typically placed vertically in the anterior region of the mandible between the mental foramina. To avoid implant placement in the molar area, the distal cantilever extensions of such prostheses often reach lengths of up to 20 mm. However, cantilever lengths exceeding 15 mm are associated with a higher risk of complications, such as screw loosening, chipping of the veneering ceramic, framework fracture, significant bone resorption around the implants, and loss of osseointegration.

In his monograph, E. Agliardi cites Tulasne et al., who in 1989 proposed a protocol for placing 20–22 mm long implants into the pterygoid process of the sphenoid bone at an angle of 35–55 degrees. This approach was later modified: implants were instead placed in the pterygoid-maxillary region, parallel to the distal wall of the maxillary sinus, often without engaging the pterygoid process itself.

In 2000, Krekmanov et al. analyzed the effect of extending the prosthetic span of full-arch fixed restorations on both jaws by tilting the distal implants. This angulated implant placement allowed for better distribution of occlusal forces, reduction in the length of distal cantilevers, and improved implant survival rates within fixed prosthetic constructions. The survival rate of tilted implants reached 95.7%, compared to 90.2% for vertically placed implants, with no significant differences found in force and bending moments at the level of each implant [6]. As a result, tilted implant placement has become a well-established clinical practice and a viable alternative to bone augmentation procedures.

AIM

To review current approaches to fixed prosthetic rehabilitation in completely edentulous patients using four dental implants, including protocols involving the placement of tilted implants.

RESULTS

Between 2003 and 2005, P. Maló introduced the “All-on-4” protocol, which allows for the fabrication of a fixed full-arch prosthesis supported by four dental implants. In this technique, the two anterior implants are placed vertically in the region of the central or lateral incisors, while the two posterior implants are tilted at an angle of 30–45 degrees to the frontal plane in order to position the implant platforms in the region of the first or second premolars. This configuration avoids the need for bone grafting procedures and minimizes the risk of injury to critical anatomical structures. Tilting the posterior implants significantly reduces biomechanical forces and distributes occlusal load more efficiently than the use of five vertically placed implants. The prosthetic structure typically consists of a 12-unit acrylic prosthesis reinforced with a titanium framework, screw-retained onto multi-unit abutments.

In 2019, P. Maló published a retrospective long-term follow-up study on the “All-on-4” concept with an observation period ranging from 10 to 18 years. The study included 471 patients who received 1884 implants and

471 fixed prostheses. The primary outcome measures were prosthesis and implant survival rates, while secondary outcomes included marginal bone loss at 10 and 15 years, as well as biological and mechanical complications. A total of 176 patients (37%) were lost to follow-up. The cumulative prosthesis survival rate was 98.8%, and implant survival and success rates were 93% and 91.7%, respectively, over the 18-year observation period [7].

The relative simplicity of the procedure, reduced cost due to the absence of bone grafting materials, and growing global adoption of the “All-on-4” technique have inspired continued research. In a 2014 study by M. Taruna, B. Chittaranjan, and colleagues, which focused on the prosthetic success of the “All-on-4” method, particular attention was given to the angulation limits of distal implants, the length of cantilever extensions, and the importance of prosthetic framework reinforcement. When an implant is part of a splinted multi-implant framework, the rigidity of the prosthesis helps reduce implant bending. A more distal position of the posterior implant and a shorter cantilever can reduce stress on the implant. Theoretical models suggest that angulated implants allow for a longer prosthetic framework, which in turn reduces the forces acting on implants. From a biological perspective, the position of the prosthetic platform may be more critical than the actual angle of implant insertion.

Cantilever loading can create a hinge effect, generating high stress levels on the implants closest to the load. Excessive cantilever length may lead to deformation of the prosthetic framework and subsequent complications such as screw loosening, fracture of acrylic teeth, or even framework fracture. Splinted tilted implants demonstrate lower stress levels compared to vertically placed implants supporting a cantilever. Therefore, reducing prosthesis-induced stress may improve the longevity of full-arch fixed prostheses relative to traditional implant positioning. When vertical force is applied to the first premolar area of a tilted implant, adjacent implants share the load. Since the prosthesis is loaded between the anterior and posterior implants, the stress is distributed across both, without overloading the tilted implant.

Long-term studies have shown no significant differences in implant survival between maxillary fixed prostheses supported by four versus six implants. Stress distribution and loading patterns were comparable in four- and six-implant models. Cantilever length should be minimized, as long extensions significantly increase stress on distal implants regardless of the number of supporting implants. Finite element models examined deformation around distal implants angled at 0°, 15°, 30°, and 45°. No substantial differences were observed between the 0°, 15°, and 30° groups, although deformation increased at 45°. The recommended maximum cantilever length is 10–12 mm in the mandible and no more than 6–8 mm in the maxilla due to lower bone density [8].

Modern implant systems offer various implant–abutment connection designs, with the most common

being conical, internal flat-to-flat, and external hexagonal connections. In a 2023 study, Pei-Shuang Wang, Ming-Hsu Tsai, and colleagues performed a biomechanical evaluation of full-arch prostheses supported by four implants with different connection types. The implant–abutment connection type was identified as a factor influencing occlusal load distribution. Two groups were compared: one using implants with an external hex connection (4.0 mm diameter) and the other using conical connections (4.3 mm diameter). All components of the “All-on-4” protocol was included in the analysis—custom titanium frameworks, multi-unit abutments, fixation screws, and implants.

Precise measurements were taken using calipers and a digital microscope, and the models were additionally scanned with a 3D optical system (Aicon SmartScan-HE). CAD software and finite element analysis tools were used to create 3D models, which were embedded into a bone block model (50 mm × 30 mm × 40 mm) designed to mimic human bone, with a 3 mm dense outer cortical layer and an inner trabecular core. A vertical force of 190 N was applied at the distal cantilever of the framework.

Both groups showed similar stress levels and distribution on distal implants under identical load conditions. The highest von Mises stresses were recorded in the fixation screws, followed by the multi-unit abutments, indicating that these components are the most vulnerable in the distal implant zone. The lowest stress values were consistently found in the bone, with slightly higher bone stress in the external hex group compared to the conical group. Both groups showed a gradual decrease in von Mises stress from the multi-unit abutment to the implant collar. In the external hex group, stress was mainly concentrated on the screws and abutments, whereas in the conical group, the stress was more evenly distributed and slightly lower across the framework, screws, abutments, and bone tissue. Marginal bone resorption was higher in the external hex group, though the difference was not statistically significant. Therefore, both connection types are considered clinically suitable for the “All-on-4” protocol [9].

Despite the numerous studies devoted to the “All-on-4” technique—many of which emphasize its versatility and high clinical success—not all meet the criteria for methodological rigor and objectivity. This concern was highlighted by David Soto-Peñaloza, Regino Zaragoza-Alonso, María Peñarrocha-Diago, and colleagues in their 2017 systematic review of the “All-on-4” concept. An initial screening yielded 728 articles, of which only 24 met the inclusion criteria. Methodological quality assessment revealed that sample size calculation was performed in only one study, and follow-up periods generally included small sample sizes – a limitation that may introduce bias and lead to misleading interpretations. The reported implant survival rate beyond 24 months was 99.8%. However, current evidence remains limited due to insufficient methodological detail, lack of long-term follow-up, and small participant cohorts. Biological complications, particularly peri-implantitis, were reported in a minority of patients after an average follow-up period of two years. Therefore, the authors emphasized the need for clearer success and survival criteria, given the high prevalence of peri-implant diseases [10].

CONCLUSION

The “All-on-4” technique – which involves the placement of four dental implants (including tilted posterior implants) and the fabrication of a screw-retained, reinforced fixed prosthesis – represents an effective alternative to the placement of a greater number of implants with bone grafting procedures in cases of significant alveolar bone loss. This protocol substantially reduces treatment time, facilitates postoperative recovery, and lowers patient costs by eliminating the need for grafting materials. However, in our view, this method requires further investigation concerning the functional changes of the stomatognathic system in fully edentulous patients. Specifically, it warrants evaluation using electromyographic analysis of masticatory muscles, digital occlusion analysis, and mathematical modeling to define the critical angulation thresholds for distal implant placement.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

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Morphological evaluation of the fibrin framework in the treatment of dental pulp hyperemia: experimental study

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Abstract

INTRODUCTION. The complexity of the prognosis in the early and late stages of the conservative method of pulpitis treatment has limited its wide application. The article is aimed at studying and analyzing the potential of regenerative dentistry, which consists in restoring the function and structure of the dental pulp using innovative technologies. It is considered how biomaterials, fibrin framework (PRF) can be used to achieve this goal based on an experimental study and morphological assessment of the effect of these materials on the dental pulp. Platelet-rich fibrin (PRF) is a concentrate of platelets, which in recent years has become increasingly popular for regenerative procedures. An analysis of the materials used with appropriate conclusions is carried out.

AIM. To conduct a comparative morphological assessment of the use of modern bioactive materials and fibrin (PRF) as a scaffold in the treatment of pulp hyperemia.

MATERIALS AND METHODS. The experimental part of the study was performed on 8 white laboratory rats of 64 molars of teeth, Wistar lineage, of both sexes, with a body weight of 350–600 g, quarantined for at least 10–14 days, and kept in standard vivarium conditions of the Federal State Budgetary Educational Institution of Higher Medical Education of the Ministry of Health of the Russian Federation. The animals were divided into 4 groups – 2 individuals and 16 teeth in each group. Group 1 animals used fibrin (PRF) + “Trioxidant” “Vladmiva”, Group 2 collagen membrane Geistlich Bio-Gide® + “Biodentine”, Group 3 fibrin (PRF) + “Biodentine” “Septodont”, Group 4 “Dycal” “Dentsply”. The experiment was conducted under anesthesia (protocol of the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education of the Ministry of Health of the Russian Federation No. 125 dated 09/12/2023). On the chewing surfaces of the 1st and 2nd molars, diamond carbide spherical borons were used to open the tooth cavity with partial pulp exposure. The formed cavity was treated with 0.05% chlorhexidine solution and dried. The test materials were then applied to the autopsy area. Experimental animals were removed from the experiment on days 3, 14, and 30. The resulting biological material was fixed in a 10% neutral formalin solution, decalcified, then poured into a histological medium and stained with hematoxylin and eosin according to Van Gieson. The sections were obtained on an Accu-Cut SRM 200 rotary microtome.

RESULTS. In the course of an experimental study in group 1 using fibrin (PRF) + Trioxidant in the area of contact with the therapeutic material, activation of reactive and compensatory processes in the tooth pulp tissue was detected while maintaining its viability for 30 days, which indicated the most pronounced regenerative potential among all the studied groups. As a result of a morphological assessment of micro-preparations of pulp with direct coating with “Dycal” material, group 4 showed that on the 30th day the pathological process developed in the pulp is irreversible, because morphological changes were most pronounced: inflammatory infiltration with the presence of lymphocytes and neutrophils in the infiltrate, sclerotic changes, as well as granulation tissue and necrosis focus in the area of contact with the therapeutic material were noted.

CONCLUSIONS. The results of an experimental study conducted to compare histological changes in the pulp state with the use of various groups of materials in the near and long-term follow-up periods showed that the combined use of bioactive materials and fibrin (PRF) for direct coating of pulp increases the effectiveness of dental treatment with diagnoses of pulp hyperemia, and is also a promising direction in regenerative dentistry, due to the high the potential to influence the stimulation of reparative processes in the tooth pulp.

Keywords: dental pulp, fibrin skeleton, pulp hyperemia, regenerative dentistry

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Морфологическая оценка фибринового каркаса при лечении гиперемии пульпы зуба: экспериментальное исследование

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Резюме

ВВЕДЕНИЕ. Сложность прогноза в ранние и отдаленные сроки консервативного метода лечения пульпита ограничил его широкое применение. Статья направлена на изучение и анализ потенциала регенеративной стоматологии, который заключается в восстановлении функции и структуры пульпы зуба с помощью инновационных технологий. Рассмотрено, как биоматериалы, фибриновый каркас (PRF) могут быть использованы для достижения этой цели на основании экспериментального исследования и морфологической оценки влияния данных материалов на пульпу зуба. Богатый тромбоцитами фибрин (PRF) представляет собой концентрат тромбоцитов, который в последние годы становится все более популярным для регенеративных процедур. Проведен анализ используемых материалов с соответствующими выводами.

ЦЕЛЬ. Провести сравнительную морфологическую оценку применения современных биоактивных материалов и фибрина (PRF) в качестве каркаса при лечении гиперемии пульпы.

МАТЕРИАЛЫ И МЕТОДЫ. Экспериментальная часть исследования выполнена на 8 белых лабораторных крысах 64 моляров зубов, линии Вистар, обоего пола, с массой тела от 350–600 г., прошедших карантин не менее 10–14 дней, находящихся в стандартных условиях вивария ФГБОУ ВО КубГМУ Минздрава России. Животных разделили на четыре группы – по 2 особи и 16 зубов в каждой группе. У животных 1-й группы применялись – фибрин (PRF) + «Триоксидент» «Владмива», 2-й группы – коллагеновая мембрана Geistlich Bio-Gide® + «Biodentine», 3-й группы – фибрин (PRF) + «Biodentine» «Septodont», 4-й группы – «Dycal» «Dentsply». Эксперимент проводился под наркозом (протокол этического комитета ФГБОУ ВО КубГМУ Минздрава России №125 от 12.09.2023 г.). На жевательных поверхностях 1 и 2 моляров алмазным твердосплавными шаровидным борами производили вскрытие полости зуба с частичным обнажением пульпы. Сформированную полость обрабатывали 0,05% раствором хлоргексидина, высушивали. Затем на область вскрытия наносили исследуемые материалы. Экспериментальных животных выводили из эксперимента на 3, 14 и 30-е сутки. Полученный биологический материал фиксировали в 10% нейтральном растворе формалина, подвергали декальцинированию, затем заливали в гистологическую среду и окрашивали гематоксилином и эозином по Ван-Гизону. Срезы получали на ротационном микротоме Accu-Cut SRM 200.

РЕЗУЛЬТАТЫ. В ходе экспериментального исследования в 1-й группе при использовании фибрин (PRF) + «Триоксидент» в зоне контакта с лечебным материалом была обнаружена активизация реактивных и компенсаторных процессов в ткани пульпы зуба с сохранением ее жизнеспособности на 30 сутки, что свидетельствовало о наиболее выраженном регенеративном потенциале среди всех исследуемых групп. В результате морфологической оценки микропрепаратов пульпы при прямом покрытии материалом «Dycal» 4-й группе, показал, что на 30 сутки развившийся в пульпе патологический процесс носит необратимый характер, так как морфологические изменения были наиболее выражены: отмечалась воспалительная инфильтрация с наличием в инфильтрате лимфоцитов и нейтрофилов, склеротические изменения, а так же определялась грануляционная ткань и очаг некроза, в зоне контакта с лечебным материалом.

ВЫВОДЫ. Результаты проведенного экспериментального исследования с целью сравнительного анализа гистологических изменений состояния пульпы в зоне применения различных групп материалов в ближайшие и отдаленные сроки наблюдения показали, что сочетанное применение биоактивных материалов и фибрина (PRF) для прямого покрытия пульпы повышают эффективность лечения зубов с диагнозом гиперемия пульпы, а так же являются перспективным направлением в регенеративной стоматологии, в связи с высоким потенциалом влияния на стимуляцию репаративных процессов в пульпе зуба.

Ключевые слова: пульпа зуба, фибриновый каркас, гиперемия пульпы, регенеративная стоматология

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INTRODUCTION

Despite the introduction and widespread use of modern preventive and therapeutic strategies aimed at the prevention and management of pulp hyperemia, this dental condition remains one of the most prevalent pathologies worldwide [1]. The primary treatment approach for early (reversible) forms of pulpitis typically involves endodontic procedures accompanied by complete pulp removal. However, such interventions often have an adverse impact on the physiological properties of the tooth, including disruption of its protective, trophic, and reparative functions, which ultimately compromises the tooth's viability, increases the risk of complications in periapical tissues, and may result in tooth loss [1; 2]. For this reason, biological approaches to pulpitis management – based on the regenerative capacity of the dental pulp – are increasingly regarded as promising alternatives [3]. Currently, the main conservative method for treating pulp hyperemia involves the use of “therapeutic” liners and bioactive materials [4; 5].

The advancement of regenerative dentistry and the development of modern bioactive materials have necessitated the exploration of novel treatment modalities for pulp inflammation. In most published studies, regenerative procedures involve the application of autologous platelet-rich fibrin (PRF), which can be readily prepared in dental settings with minimal ex vivo manipulation. PRF is rich in growth factors – including transforming growth factor-beta (TGF- β), tumor necrosis factor (TNF), insulin-like growth factors, and angiogenic growth factors – that stimulate collagen synthesis, angiogenesis, and cellular differentiation. Moreover, PRF does not undergo rapid degradation and forms a stable three-dimensional fibrin matrix [6–9]. Its 3D architecture retains bioactive molecules that support stem cell proliferation and differentiation, thus enhancing wound healing. Consequently, the clinical application of PRF is increasingly viewed as a promising direction in regenerative dentistry [10; 11].

Another material used in regenerative dentistry is the collagen membrane *Geistlich Bio-Gide®*. This resorbable bilayer membrane is designed for guided bone regeneration and is composed of highly purified type I and III collagen. Among its advantages are active hemostasis and chemotaxis of fibroblasts. Its unique structure ensures excellent biocompatibility and minimizes the risk of inflammatory responses. Furthermore, its ability to be combined with various therapeutic fillers makes its use particularly attractive and promising in dental practice.

A comparative analysis of the literature on materials and methods for treating pulp hyperemia – aimed at preserving pulp vitality – highlights the need for developing precise diagnostic criteria to better understand the morphological changes in the pulp. It also underscores the importance of continued research into novel treatment modalities and their rational implementation in clinical practice.

AIM

To conduct a comparative morphological evaluation of the application of modern bioactive materials and platelet-rich fibrin (PRF) as a scaffold in the treatment of pulp hyperemia.

MATERIALS AND METHODS

The experimental part of the study was conducted on 8 Wistar-line white laboratory rats (both sexes), weighing between 350–600 g. All animals underwent a quarantine period of no less than 10–14 days and were housed under standard vivarium conditions at the Federal State Budgetary Educational Institution of Higher Education “Kuban State Medical University” of the Ministry of Health of Russia. A total of 64 molars were examined. The animals were randomly divided into four groups, each comprising 2 rats and 16 molars.

Group 1: Platelet-rich fibrin (PRF) + “TrioxyDent” (VladMiVa).

Group 2: Collagen membrane *Geistlich Bio-Gide®* + “Biodentine”.

Group 3: Platelet-rich fibrin (PRF) + “Biodentine” (Septodont).

Group 4: «Dycal» (Dentsply).

All surgical procedures were performed under general anesthesia using “Zoletil” at a dose of 20 mg/kg, a veterinary-approved anesthetic. After induction, the animals were fixed on a custom surgical table. Upon completion of the experiment, euthanasia was performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Tissue sampling for histological analysis was carried out following intravenous or intracardiac administration of sodium pentobarbital (Nembutal) at a dose of 200 mg/kg using an 18% (200 mg/mL) solution.

The experiment was conducted under acute surgical conditions and was approved by the Ethics Committee of Kuban State Medical University (protocol No. 125 dated September 12, 2023).

Due to the naturally rapid coagulation of rat blood, all materials were prepared in advance, and the centrifuge was pre-configured. The centrifuge used (CM-6M, ELMI, Latvia; rotor 6M, 12 × 15 mL) was set at 2300 rpm (400 g) for 8 minutes, in accordance with the established protocol. To maintain balance, three Vacutainer tubes filled with sterile saline were used as counterweights during PRF preparation. Blood for PRF was collected via cardiac puncture, a method chosen to obtain a sufficient volume for fibrin production. Before application, the *Geistlich Bio-Gide®* collagen membranes were pre-soaked in sterile saline.

On the occlusal surfaces of the first and second molars, cavities were prepared using high-speed diamond-tungsten carbide burs (200,000 rpm) under physiological saline cooling to expose the coronal pulp surface. The cavities were disinfected with 0.05% chlorhexidine solution and gently dried with sterile cotton pellets. The following materials were then applied:

- PRF + «TrioxyDent» (VladMiVa);
- *Geistlich Bio-Gide®* membrane + “Biodentine”;

- PRF + «Biodentine» (Septodont);
- «Dycal» (Dentsply).

All cavities were subsequently sealed with temporary filling material.

The animals were euthanized at 3-, 14- and 30-days post-procedure. Harvested tissues were fixed in 10% neutral buffered formalin, then decalcified in a 10% Trilon B solution for three days. Specimens were embedded in Histomix paraffin using a TISSUE-tek TEC5 embedding station. Serial sections (5–15 μ m thick) were prepared using a rotary microtome (Accu-Cut SRM 200). The histological slides were stained with hematoxylin and eosin, and van Gieson's stain, and examined under a Nikon Eclipse 80i microscope.

RESULTS AND DISCUSSION

Histological examination of specimens from Group 1 (PRF + TrioxyDent, VladMiVa) on Day 3 revealed the presence of a delicate loose fibrous matrix in the area of material contact. Moderate interstitial edema and dilated blood vessels were observed, accompanied by mild disorganization of the odontoblastic layer and a minimal lymphocytic inflammatory infiltrate. These findings indicate a low-grade pulpal inflammatory response at Day 3 in the group treated with PRF + TrioxyDent (Fig. 1, A).

Histological analysis of Group 1 specimens (PRF + TrioxyDent) on Day 14 revealed that, in the area of con-

tact with the applied material, the pulp tissue was composed of loose fibrous connective tissue. There was evidence of granulation tissue formation, a mild inflammatory infiltrate, and initial formation of a dentin bridge. Additionally, irregular alignment of odontoblasts was observed, suggesting only minor inflammatory changes. These findings indicate the early onset of regenerative processes, as demonstrated by granulation tissue development and dentin bridge formation (Fig. 1, B).

On Day 30, histological examination of Group 1 revealed a well-defined presence of granulation tissue, pronounced reparative dentinogenesis with formation of dentin bridges and focal areas of dentinogenesis, and a clearly organized layer of odontoblasts aligned along the walls of the root canal (Fig. 1, C). These features signify marked regenerative processes. By Day 30, an activation of reactive and reparative processes was noted in the dental pulp tissue, alongside preservation of pulp vitality under the influence of PRF + TrioxyDent. These regenerative signs were characterized by increased metabolic activity and enhanced cellular responses within the pulp tissue, including noticeable activation of pulp defense mechanisms aimed at resolving inflammation and restoring functional integrity. Evidence of this includes an active fibroblastic response and replacement of inflammatory foci with granulation tissue, serving as a scaffold for subsequent substitution by mature connective tissue.

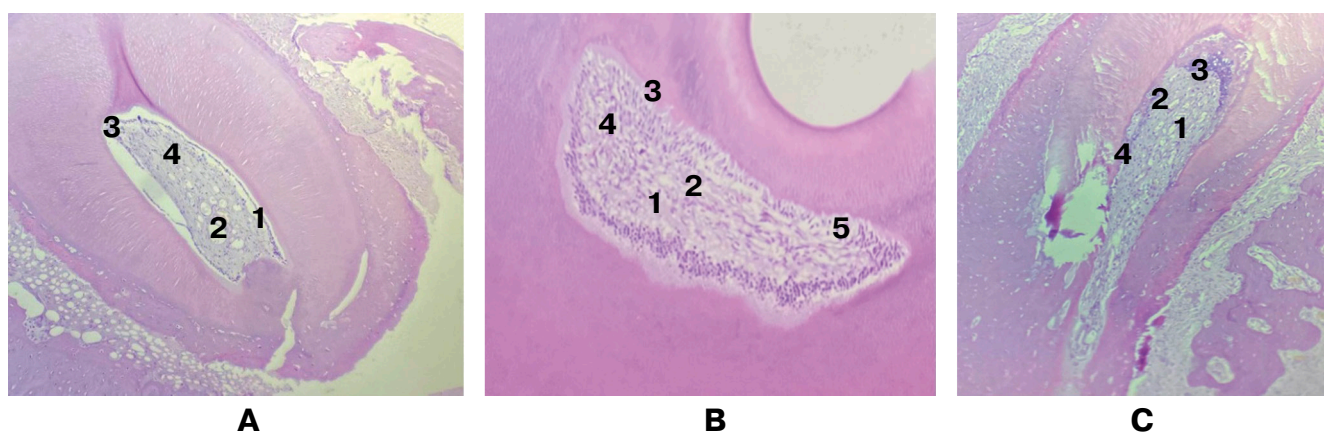


Fig. 1. Root pulp of a rat tooth treated with platelet-rich fibrin (PRF) + TrioxyDent (hematoxylin and eosin staining, $\times 100$ magnification): A – on the 3rd day from the start of the experiment: 1 – edema, 2 – dilated vessels, 3 – weak disorganization of the odontoblast layer, 4 – a delicate loose fibrous matrix and a minimally pronounced inflammatory infiltrate; B – on the 14th day after the start of the experiment: 1 – loose fibrous connective tissue, 2 – granulation tissue, 3 – formation of a dentinal bridge, 4 – mild inflammatory infiltrate, 5 – disorganization of the odontoblast layer; C – on the 30th day from the start of the experiment: 1 – granulation tissue, 2 – formation of dentinal bridges, 3 – formation of foci of dentinogenesis, 4 – the presence of a layer of odontoblasts along the walls of the root canal

Рис. 1. Корневая пульпа зуба крысы в условиях применения препарата фибрин (PRF) + «Триоксидент» (окраска гематоксилин-эозином, ув. 100х): А – на 3-и сутки от начала эксперимента: 1 – отек, 2 – расширенные сосуды, 3 – слабая дезорганизация слоя одонтобластов, 4 – «нежный» рыхлого волокнистый матрикс и минимально выраженный воспалительный инфильтрат; В – на 14-е сутки от начала эксперимента: 1 – рыхлая волокнистая соединительная ткань, 2 – грануляционная ткань, 3 – формирование дентинного мостика, 4 – слабовыраженный воспалительный инфильтрат, 5 – дезорганизация слоя одонтобластов; С – на 30-е сутки от начала эксперимента: 1 – грануляционная ткань, 2 – образование дентинных мостиков, 3 – образование очагов дентиногенеза, 4 – наличие слоя одонтобластов вдоль стенок корневого канала

Histological analysis of Group 2 specimens (Geistlich Bio-Gide® membrane + Biodentine) on Day 3 revealed, in the contact area, a large number of blood vessels with stasis within the pulp chamber, disorganization, and partial loss of the odontoblastic layer. Additionally, a dense fibrous matrix was noted, along with a moderately expressed lymphocytic inflammatory infiltrate (Fig. 2, A).

In Group 2 (Geistlich Bio-Gide® membrane + Biodentine), histological analysis on Day 14 revealed irregular alignment of odontoblasts, the presence of loose fibrous matrix, and a large number of fibroblast-like cells. A minimal lymphocytic inflammatory infiltrate was observed, which may be interpreted as moderately expressed inflammatory changes (Fig. 2, B).

By Day 30, the morphological features of the pulp tissue in Group 2 were characterized by residual signs of inflammation, pronounced reparative dentinogenesis, the presence of granulation tissue, sclerotic changes, and the formation of dentin bridges and focal areas of dentinogenesis (Fig. 2, C).

Histological examination of Group 3 specimens (PRF + Biodentine) on Day 3 showed a high number of blood vessels with stasis in the pulp chamber, moderate disorganization of the odontoblastic layer, a dense fibrous matrix, and a moderately expressed inflammatory infiltrate (Fig. 3, A).

Histological analysis of specimens from Group 3 (PRF + Biodentine) on Day 14 revealed the absence of odontoblasts in the pulp chamber, the presence of a ne-

crotic focus, and a pronounced polymorphonuclear inflammatory infiltrate with neutrophilic leukocytes in the infiltrate (Fig. 3, B).

On Day 30, the morphological features of the pulp tissue in Group 3 were characterized by more prominent signs of pulp inflammation and moderately expressed reparative dentinogenesis. A mild lymphocytic infiltrate was observed, along with the presence of granulation tissue and formation of a dentin bridge (Fig. 3, C).

Histological evaluation of Group 4 specimens (control group, Dycal) on Day 3 demonstrated pulpal tissue edema, dilated blood vessels with stasis, moderate disorganization of the odontoblastic layer, presence of loose fibrous matrix, and a mild lymphocytic inflammatory infiltrate (Fig. 4, A).

Examination of specimens from Group 4 (control group, Dycal) on Day 14 revealed the absence of odontoblasts in the pulp tissue, presence of necrotic foci, sclerotic changes, granulation tissue, and a pronounced polymorphonuclear inflammatory infiltrate with neutrophilic leukocytes (Fig. 4, B).

On Day 30, the morphological pattern of the pulp tissue in Group 4 was characterized by more pronounced signs of pulp inflammation, sclerotic alterations, and moderately expressed reparative dentinogenesis. A dense inflammatory infiltrate containing both lymphocytes and neutrophils was observed. In the area of contact with the applied material, granulation tissue, necrotic foci, sclerotic changes, and formation of dentin bridges were also present (Fig. 4, C).

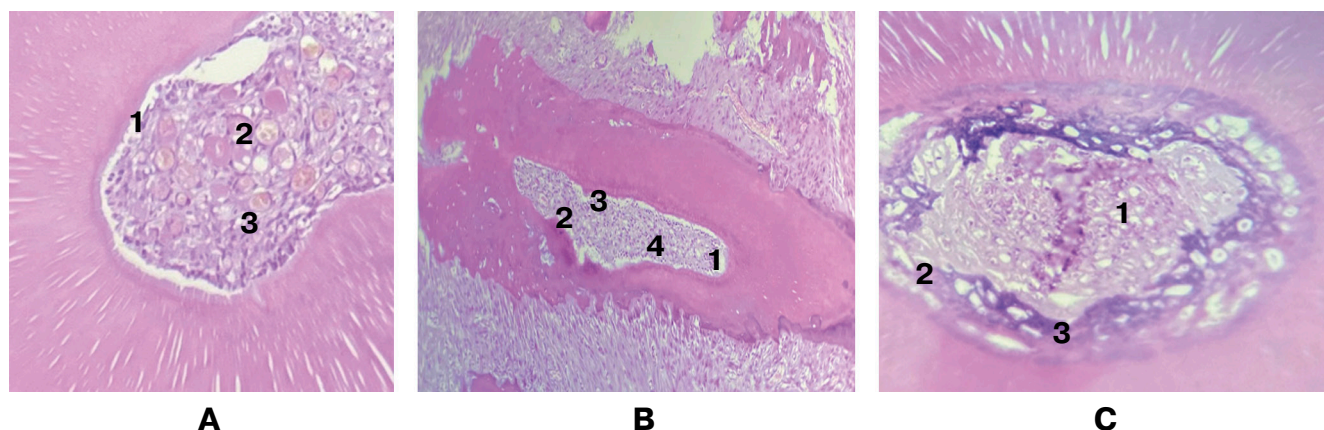


Fig. 2. Root pulp of a rat tooth under conditions of application of the drug Membrane Geistlich Bio-Gide® + “Biodentine”, experiment (hematoxylin-eosin staining): A – on the 3rd day after the start of the experiment (in V. 400x): 1 – disorganization and loss of the odontoblast layer, 2 – numerous dilated vessels with rhinestones, 3 – dense fibrous matrix, 4 – moderate inflammatory infiltrate; B – on the 14th day from the start of the experiment (for c. 100 x): 1 – disorganization of odontoblasts, 2 – dense fibrous matrix, 3 – a large number of fibroblast-like cells, 4 – minimal lymphocytic inflammatory infiltrate; C – on the 30th day from the start of the experiment (in V. 400x): 1 – granulation tissue, 2 – dentinal bridge, 3 – foci of dentinogenesis

Рис. 2. Корневая пульпа зуба крысы в условиях применения препарата Мембрана Geistlich Bio-Gide® + «Biodentine», эксперимента (окраска гематоксилин-эозином): А – на 3-и сутки от начала эксперимента (ув. 400х): 1 – дезорганизация и потеря слоя одонтобластов, 2 – многочисленные расширенные сосуды со стазами, 3 – плотный волокнистый матрикс, 4 – умеренно выраженный воспалительный инфильтрат; В – на 14-е сутки от начала эксперимента (ув. 100х): 1 – дезорганизация одонтобластов, 2 – плотный волокнистый матрикс, 3 – большое количество фибробласто-подобных клеток, 4 – минимальный лимфоцитарный воспалительный инфильтрат; С – на 30-е сутки от начала эксперимента (ув. 400х): 1 – грануляционная ткань, 2 – дентинный мостик, 3 – очаги дентиногенеза

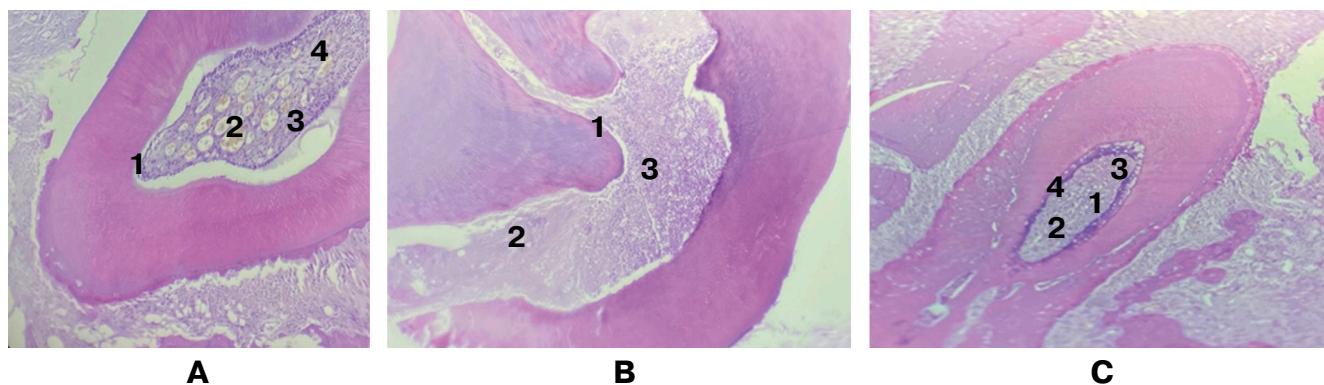


Fig. 3. Root pulp of a rat tooth under the conditions of using the drug fibrin (PRF) + “Biodentine” (hematoxylin-eosin staining, uv. 100 x): *A* – on the 3rd day after the start of the experiment: 1 – disorganization of the odontoblast layer, 2 – numerous dilated vessels with rhinestones, 3 – dense fibrous matrix, 4 – moderate inflammatory infiltrate; *B* – on the 14th day after the start of the experiment: 1 – absence of odontoblasts, 2 – foci of necrosis, 3 – pronounced polymorphic cellular inflammatory infiltrate with the presence of neutrophilic leukocytes in the infiltrate; *C* – on the 30th day from the start of the experiment: 1 – mild lymphocytic inflammatory infiltration, 2 – granulation tissue, 3 – necrosis site, 4 – the presence of dentine bridges

Рис. 3. Корневая пульпа зуба крысы в условиях применения препарата фибрин (PRF) + «Biodentine», (окраска гематоксилин-эозином, ув. 100х): *A* – на 3-и сутки от начала эксперимента: 1 – дезорганизация слоя одонтобластов, 2 – многочисленные расширенные сосуды со стазами, 3 – плотный волокнистый матрикс, 4 – умеренно выраженный воспалительный инфильтрат; *B* – на 14-и сутки от начала эксперимента: 1 – отсутствие одонтобластов, 2 – очаги некроза, 3 – выраженный полиморфный клеточный воспалительный инфильтрат с наличием в инфильтрате нейтрофильных лейкоцитов; *C* – на 30-е сутки от начала эксперимента: 1 – слабовыраженная лимфоцитарная воспалительная инфильтрация, 2 – грануляционная ткань, 3 – очаг некроза, 4 – наличие дентинных мостиков

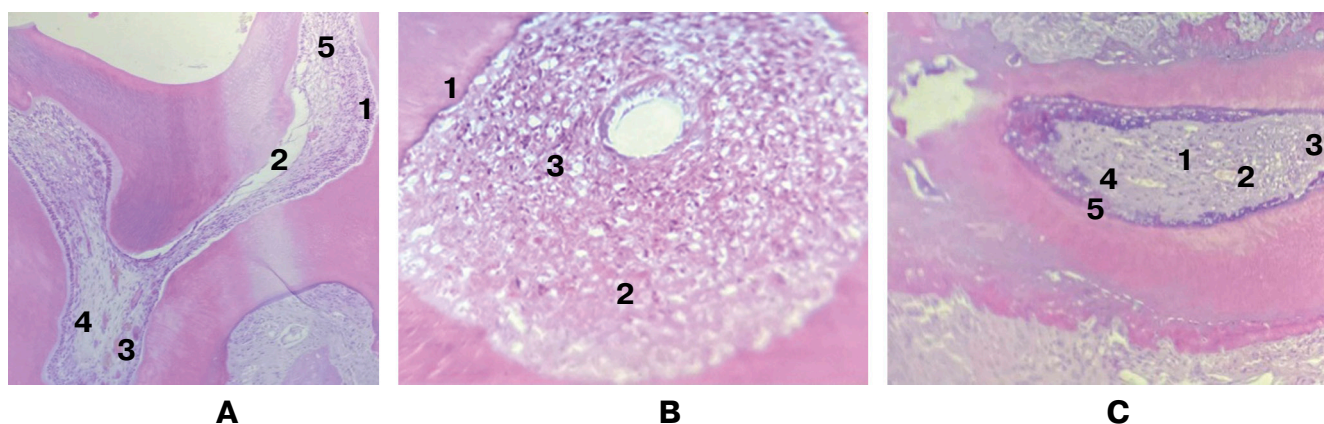


Fig. 4. Crown and root pulp of a rat tooth under the conditions of using the drug “Dycal” (hematoxylin-eosin staining): *A* – on the 3rd day from the start of the experiment, (for B. 100 x): 1 – disorganization of the odontoblast layer, 2 – edema of the pulp, 3 – dilated vessels with stasis, 4 – loose fibrous matrix, 5 – weakly expressed lymphocytic inflammatory infiltrate; *B* – on the 14th day from the start of the experiment, (in B. 400x): 1 – absence of odontoblasts, 2 – foci of necrosis, 3 – pronounced polymorphic cellular inflammatory infiltrate with the presence of neutrophil leukocytes in the infiltrate; *C* – on the 30th day from the start of the experiment (in V. 400x): 1 – Inflammatory infiltration with the presence of lymphocytes and neutrophils in the infiltrate, 2 – granulation tissue, 3 – necrosis site, 4 – sclerotic changes, 5 – the presence of dentine bridges

Рис. 4. Коронковая и корневая пульпа зуба крысы в условиях применения препарата «Dycal», (окраска гематоксилин-эозином): *A* – на 3-и сутки от начала эксперимента, (ув. 100х): 1 – дезорганизация слоя одонтобластов, 2 – отек пульпы, 3 – расширенные сосуды со стазами, 4 – рыхлый волокнистый матрикс, 5 – слабовыраженный лимфоцитарный воспалительный инфильтрат; *B* – на 14-е сутки от начала эксперимента, (ув. 400х): 1 – отсутствие одонтобластов, 2 – очаги некроза, 3 – выраженный полиморфный клеточный воспалительный инфильтрат с наличием в инфильтрате нейтрофильных лейкоцитов; *C* – на 30-е сутки от начала эксперимента, (ув. 400х): 1 – воспалительная инфильтрация с наличием в инфильтрате лимфоцитов и нейтрофилов, 2 – грануляционная ткань, 3 – очаг некроза, 4 – склеротические изменения, 5 – наличие дентинных мостиков

CONCLUSION

It is evident that the search vector for the “ideal” material and application technique for improving the effectiveness of treatment for pulp hyperemia is directly dependent on advances in understanding the morphological features of dental pulp and their clinical application. Based on the findings of the present experimental study aimed at a comparative histological evaluation of pulp tissue response to different material groups and the use of platelet-rich fibrin (PRF) as a scaffold, the following conclusions can be drawn:

The combination of modern bioactive materials for direct pulp capping significantly enhances the effectiveness of treatment in cases diagnosed with pulp hyperemia. One of the most promising directions in stimulating reparative processes in the dental pulp is the combined use of bioactive materials with PRF. The experimental morphological evaluation of pulpal responses demonstrated that the combination of PRF with the bioactive materials “TrioxyDent” and “Biodentine” resulted in the lowest inflammatory response and the highest biocompatibility with pulp tissue, while also promoting reparative activity at the site of direct pulp contact.

Among the two bioactive materials tested in combination with PRF, “TrioxyDent” produced the most favorable outcomes, possibly due to its unique formulation – specifically, the inclusion of calcium-copper hydroxide as an active bacteriostatic additive, which may enhance its biological properties.

In Group 2, where Geistlich Bio-Gide® membrane was used in combination with Biodentine, the histological pattern showed residual signs of inflammation and active reparative dentinogenesis. However, these changes were less consistent and more variable compared to those observed in Groups 1 and 3, where PRF was used as a scaffold. It can be assumed that a different combination or modified application protocol might be needed for more effective use of the Geistlich membrane.

Histological assessment of pulp tissue in Group 4, treated with Dycal, revealed significant irreversible changes by Day 30, indicating the progression of a chronic inflammatory process with a high likelihood of exacerbation. These findings suggest that Dycal does not provide adequate conditions for resolving inflammation and promoting pulp regeneration.

Based on these experimental data, one of the key challenges in regenerative dentistry remains the successful re-establishment of functionally active odontoblasts, which are essential for dentin regeneration. The materials evaluated in this study demonstrated limited capacity to stimulate full pulpal tissue regeneration. From a biological standpoint, the ideal biomaterial should provide an optimal microenvironment for pulp cells, promoting their adhesion, survival, and differentiation into mature cells capable of replacing the damaged extracellular matrix, thereby restoring both the structure and function of the pulp-dentin complex.

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Anatoly A. Adamchik – designing an experiment, analyzing the results of experiments, writing a manuscript.

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Experimental evaluation of the effectiveness of non-pigmented laser photoablation in the treatment of periodontal diseases

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Abstract

INTRODUCTION. High-quality treatment of periodontitis and prevention of further progression of periodontal diseases can be achieved with complex and systematic adherence to algorithms for performing manipulations at all stages of therapy using physical factors. This is possible with the use of laser radiation with the effects of non-pigmented laser photoablation.

AIM. Improving the effectiveness of periodontal disease treatment using pigment-free laser photoablation.

MATERIALS AND METHODS. An experimental comparative study of 70 animals with model periodontitis under the influence of laser radiation with non-pigmented photoablation, photodynamic laser therapy and standard drug therapy was conducted in 3 groups. Morphological studies were performed for group I (main) and group II (comparison) were withdrawn from the experiment on the 7th, 14th and 21st days of treatment. Animals of group III (control) were withdrawn from the experiment on the 21st day.

RESULTS. Morphological analysis showed that the use of non-pigmented laser photoablation helps to reduce inflammation in periodontal tissues, accelerate regeneration due to stimulation of fibroblast production and stimulation of neoangiogenesis. On the 21st day of the experiment, in the group using non-pigmented laser photoablation, based on morphological analysis, acceleration of the processes of formation of the periodontal ligament with even bundles of collagen and elastic fibers perpendicular to the tooth root was confirmed.

CONCLUSIONS. Our experimental studies confirm the effectiveness of non-pigmented laser photoablation in the treatment of periodontitis.

Keywords: periodontitis, non-pigmented laser photoablation, diode laser

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Экспериментальная оценка эффективности применения беспи́гментной лазерной фотоабляции при лечении заболеваний пародонта

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Резюме

ВВЕДЕНИЕ. Качественное лечение пародонтита и предупреждение дальнейшего прогрессирования заболеваний пародонта возможно осуществить при комплексном и планомерном соблюдении алгоритмов выполнения манипуляций на всех этапах терапии с использованием физических факторов. Такое возможно при использовании лазерного излучения с эффектами беспи́гментной лазерной фотоабляции.

ЦЕЛЬ. Повышение эффективности лечения заболеваний пародонта с применением беспи́гментной лазерной фотоабляции.

МАТЕРИАЛЫ И МЕТОДЫ. Экспериментальное сравнительное исследование у 70 животных с модельным пародонтитом при воздействии лазерного излучения с беспи́гментной фотоабляцией, фотодинамической лазерной терапией и стандартной медикаментозной терапией проводили в трех группах. Морфологические исследования проводили для группы I (основной) и группы II (сравнения) выводили из эксперимента на 7-е, 14-е и 21-е сутки лечения. Животных группы III (контрольной) выводили из эксперимента на 21-е сутки.

РЕЗУЛЬТАТЫ. Морфологический анализ показал, что применение беспиговой лазерной фотоабляции способствует снижению воспаления в тканях пародонта, ускорению регенерации за счет стимуляции выработки фибробластов и стимуляции неоангиогенеза. На 21-е сутки эксперимента в группе с применением беспиговой лазерной фотоабляции на основании морфологического анализа подтверждено ускорение процессов формирования периодонтальной связки с ровными пучками коллагеновых и эластических волокон, перпендикулярных корню зуба.

ВЫВОДЫ. Проведенные нами экспериментальные исследования подтверждают эффективность применения беспиговой лазерной фотоабляции при лечении пародонтита.

Ключевые слова: пародонтит, беспиговая лазерная фотоабляция, диодный лазер

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INTRODUCTION

Periodontal diseases are one of the most common pathologies that occur in all age groups of the population with a sharp further progression. In the treatment of periodontal diseases, a comprehensive approach is used, consisting of high-quality removal of subgingival dental plaque, antiseptic treatment, as well as surgical interventions aimed at eliminating gum recessions and filling bone defects and stimulating the regeneration of periodontal tissues [1; 2].

In recent years, photodynamic therapy techniques have become quite widespread in the treatment of periodontal diseases. To achieve a therapeutic effect when using PDT, photosensitizers are used that are activated with the release of singlet oxygen, low-intensity laser radiation with a wavelength corresponding to the absorption peak of the sensitizer [3; 4].

To date, a new diode laser has been created, which has passed laboratory and clinical tests, capable of generating unique harmonics of laser radiation in a nanosecond pulsed radiation mode. The radiation of such a laser has a bacteriostatic effect, is capable of performing non-pigmented photoablation without the use of photosensitizers, and also has a biostimulating effect to accelerate the regeneration of periodontal tissues after treatment [5; 6].

AIM

Improving the effectiveness of periodontal disease treatment using pigment-free laser photoablation.

MATERIALS AND METHODS

In the experimental part of the study, the effectiveness of non-pigmented laser photoablation and traditional photodynamic therapy of periodontitis was assessed on a model of experimental animals – sexually mature male Wistar rats – using morphological analysis. The studies were conducted in accordance with the rules for working with animals based on the provisions of the Helsinki Declaration and the recommendations contained in EU Directive 86/609/ECC and the Council of Europe Convention for the Protection of Vertebrate

Animals used for Experimental and Other Scientific Purposes. The study included 70 animals, which were divided into 3 groups: I – the main ($n = 30$), II – comparison ($n = 30$) and III – control ($n = 10$). All animals were modeled periodontitis on the central incisors using the technique developed by V.G. Atrushkevich et al., in which prednisolone was administered intramuscularly at a rate of 12 mg/kg of animal weight on the 1st, 3rd and 5th days of the experiment in order to provide an immunosuppressive effect [7]. On the 5th day, the animals were injured by the circular ligament (dentogingival junction) of the lower incisors using a metal iron by peeling off the gums from the vestibular and lingual sides, then a silk ligature was placed deep under the gum in the resulting dentogingival pocket, covering the teeth in the shape of a figure eight. The ligature was installed under premedication and intravenous anesthesia and additionally secured with phosphate cement, left for 14 days, the animals were fed soft food (Fig. 1).

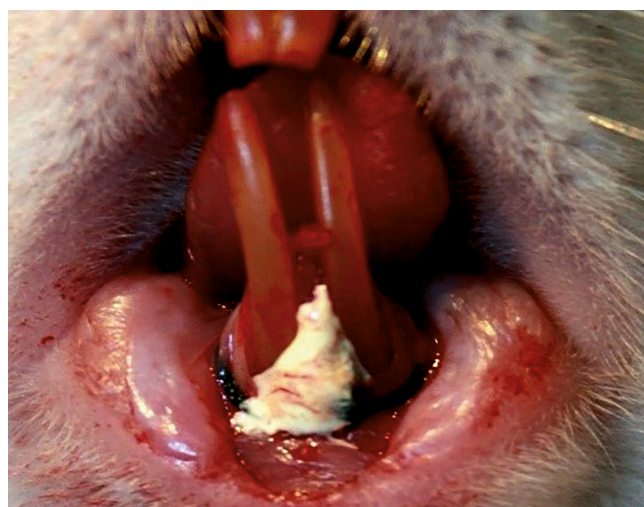


Fig. 1. Photograph of an experimental animal – fixation of the ligature with cement

Рис. 1. Фотография лабораторного животного – фиксация лигатуры с помощью цемента

After 14 days, the ligature was removed, the animals were divided into groups, and manipulations were carried out in accordance with the objectives of the study. After this, the stage of exposure to the simulated periodontitis zone in each group was started using various factors. In Group I, laser radiation of a diode laser with a wavelength of 1265 nm in a nanosecond pulsed radiation mode with the effect of pigment-free laser photoablation was used for exposure. The pockets were processed using a fiber optic light guide with a diameter of 400 μm in a contact manner. The treatment was carried out with circular movements around the lower incisors for 3 minutes. The radiation parameters were set as follows: the average radiation power, set on the control panel of the device, was 1.8 W, which corresponded to the average exposure power at 180 s of radiation – 360 J/cm², the pulsed nanosecond radiation mode was selected with a pulse duration of 100 ns and a pause duration of 200 ns. The radiation parameters were selected optimally based on previously conducted studies [8]. Non-pigmented laser photoablation was performed daily for 7 days in a row at the same fixed time for each animal in Group I (main).

In Group II, a standard photodynamic therapy procedure was performed using an exogenous photosensitizer based on chlorin E6, which was applied to the periodontal pocket for 10 minutes, then the residue was washed off using a saline solution, then laser irradiation was carried out with a wavelength of 660 nm in a continuous radiation mode with an exposure of 300 s. The photodynamic therapy procedure in this group was repeated in the same way as in Group I (main), for 7 days at the same fixed time. In Group III (control), antiseptic treatment of inflamed periodontal tissues was carried out as drug therapy. The course of treatment was 7 days. To obtain representative statistically reliable data, 10 animals were withdrawn from the experiment in

the main group and the comparison group for morphological examination on the 7th, 14th and 21st days after the start of the sessions of non-pigment laser photoablation and traditional photodynamic therapy; in the control group, animals were withdrawn from the experiment only on the 21st day. The obtained digital data were processed using the variation statistics method using the Student's T-test. The critical level of significance was considered reliable at $p < 0.05$.

RESULTS

On the 21st day in group I (main), morphological examination showed that periodontal pockets were not detected. A regenerated periodontal ligament, rich in fibroblasts, with parallel bundles of collagen and elastic fibers perpendicular to the tooth root, with single dilated vessels only at the border with the bone beams of the alveolar bone is noted. Preserved small foci of granulation tissue with full-blooded vessels without edema and inflammatory infiltration are detected (Fig. 2).

In group II (comparison), on the 21st day of observation, moderately pronounced edema of the regenerated periodontal ligament tissue, rich in fibroblasts, with dilated full-blooded vessels at the border with the bone trabeculae of the alveolar bone is noted. Preserved small foci of granulation tissue with pronounced edema, full-blooded vessels and single leukocytes are encountered (Fig. 3).

In group III (control) on the 21st day of observation, morphological analysis revealed pronounced periodontal pockets with a high content of detritus penetrated by leukocytes, partially epithelialized, with acanthosis of the epithelium and the formation of its strands growing into the granulation tissue. The periodontal ligament was significantly destroyed and replaced by granulation tissue with diffuse infiltration by leukocytes with an admixture of macrophages. Among the growths

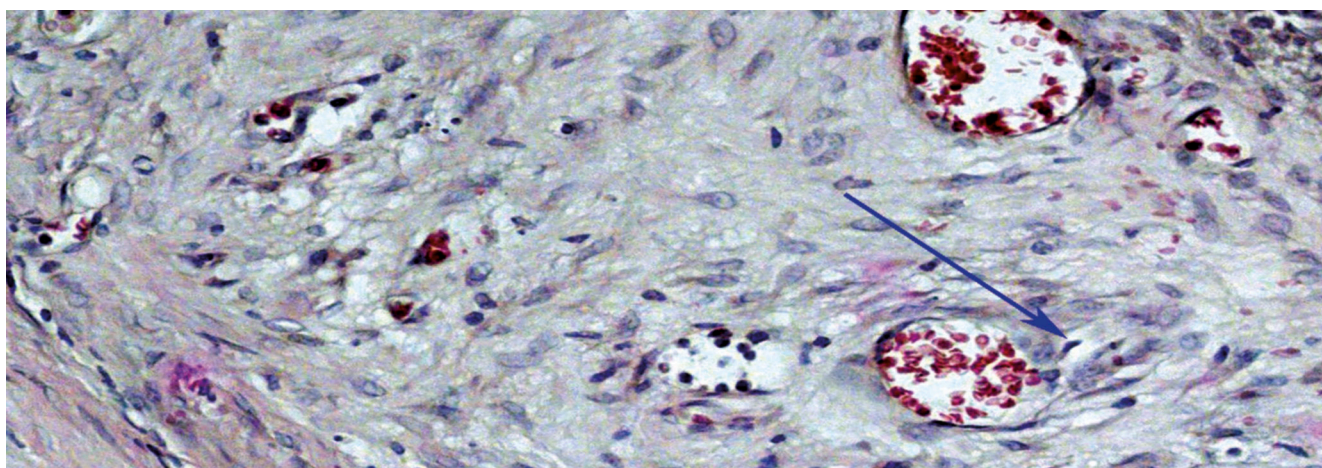


Fig. 2. Morphogram of periodontal tissues in group I on the 21st day of observation: arrow – preserved small foci of granulation tissue with full-blooded vessels without edema and inflammatory infiltration, with single macrophages and lymphocytes (hematoxylin-eosin, Zeiss, $\times 120$)

Рис. 2. Морфограмма тканей пародонта в группе I на 21-е сутки наблюдения: стрелкой обозначены сохраненные мелкие очаги грануляционной ткани с полнокровными сосудами, без отека и признаков воспалительной инфильтрации, с единичными макрофагами и лимфоцитами (гематоксилин-эозин, Zeiss, $\times 120$)

of granulation tissue with full-blooded vessels, diffuse infiltration by leukocytes with an admixture of macrophages, at the site of the periodontal ligament, single microabscesses were found. Moderately pronounced edema of the periodontal ligament tissue with partially organized remnants of cellular detritus was revealed. Restored periodontal ligament with multidirectional bundles of collagen and elastic fibers, large fibroblasts, and newly formed bone beams of the alveolar bone (Fig. 4).

Thus, based on the results of morphological analysis, a qualitative assessment of the impact of various types of laser radiation in the complex therapy of model periodontitis in experimental animals was carried out. The results of the study showed that the use of a new laser technology of pigment-free laser photoablation with a wavelength of 1265 nm promotes stimulation of vascular growth in periodontal tissues compared to traditional photodynamic laser therapy.

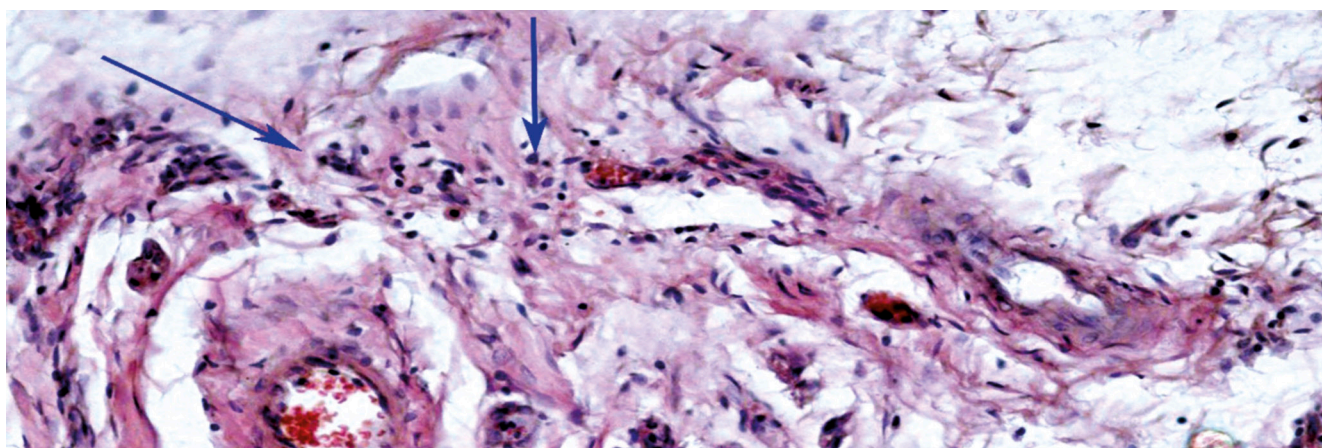


Fig. 3. Morphogram of periodontal tissues in group II on the 21st day of observation: arrows – preserved foci of granulation tissue with pronounced edema, full-blooded vessels and single leukocytes with an admixture of macrophages. (hematoxylin-eosin, Zeiss, $\times 120$)

Рис. 3. Морфограмма тканей пародонта во II группе на 21-е сутки наблюдения: стрелками обозначены сохраняющиеся очаги грануляционной ткани с выраженным отеком, полнокровными сосудами и единичными лейкоцитами с примесью макрофагов (гематоксилин-эозин, Zeiss, $\times 120$)

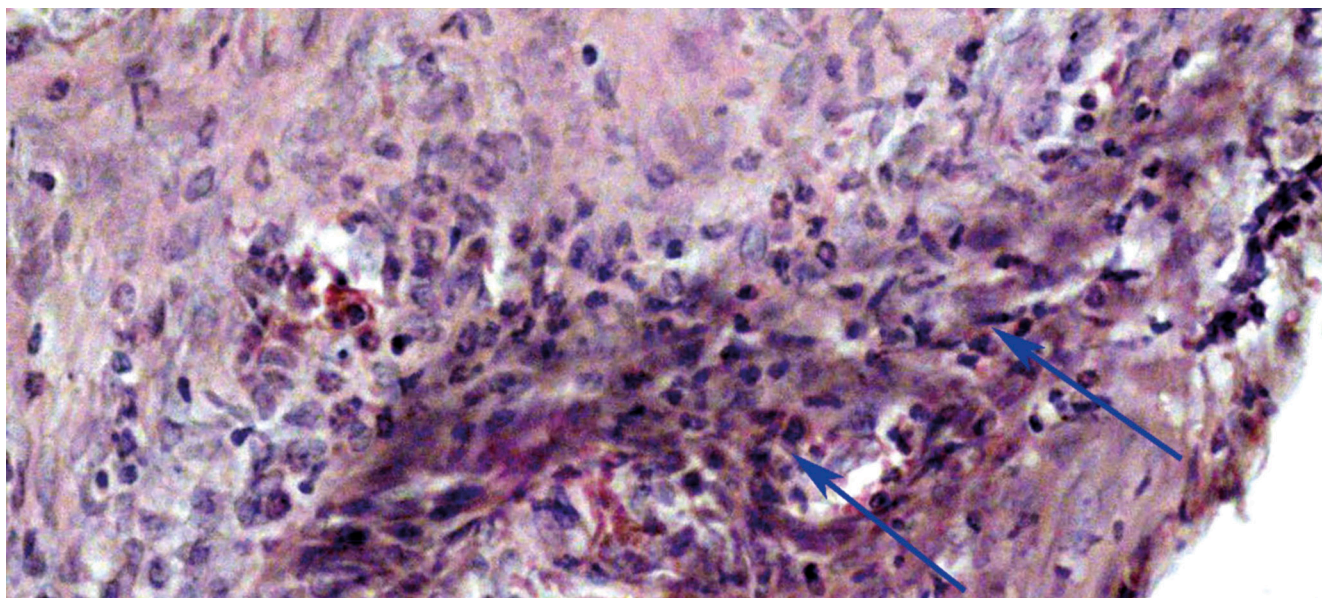


Fig. 4. Morphogram of periodontal tissues in group III on the 21st day of observation: arrows – periodontal ligament destroyed and replaced by granulation tissue with diffuse infiltration of leukocytes with an admixture of macrophages, acanthosis of the epithelium – its strands grow into the granulation tissue. (hematoxylin-eosin, Zeiss, $\times 120$)

Рис. 4. Морфограмма тканей пародонта в группе III на 21-е сутки наблюдения: стрелками указано разрушение периодонтальной связки с ее замещением грануляционной тканью, диффузной инфильтрацией лейкоцитами с примесью макрофагов, акантозом эпителия – его тяжи прорастают в грануляционную ткань (гематоксилин-эозин, Zeiss, $\times 120$)

DISCUSSION

Thus, based on the results of morphological analysis, a qualitative assessment of the impact of various types of laser radiation in the complex therapy of model periodontitis in experimental animals was carried out. The results of the study showed that the use of a new laser technology of pigment-free laser photoablation with a wavelength of 1265 nm promotes stimulation of vascular growth in periodontal tissues compared to traditional photodynamic laser therapy.

The conducted experimental morphological study allowed to prove that pigment-free laser photoablation helps to reduce inflammation, accelerate regeneration by stimulating the production of fibroblasts and stimulating neoangiogenesis. On the 21st day of the experi-

ment in the group using pigment-free laser photoablation, based on morphological analysis, acceleration of the processes of formation of the periodontal ligament with even bundles of collagen and elastic fibers perpendicular to the root of the tooth was confirmed.

CONCLUSIONS

A comparative assessment in an experimental in vivo study on animals (rats) using morphological and morphometric analysis made it possible to prove the effectiveness of non-pigmented laser photoablation by reducing inflammation, accelerating tissue regeneration, and stimulating neoangiogenesis by increasing the number of full-blooded vessels by 3.5 times compared to traditional photodynamic therapy ($p < 0.05$).

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Changes in the dentin-pulp complex of teeth through the prism of CBCT-studies: A retrospective analysis

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Abstract

INTRODUCTION. Calcifying metamorphosis and obliteration of the pulp chamber often complicate endodontic treatment, requiring the use of modern visualization and monitoring techniques during therapy.

The application of CBCT diagnostics allows for a better assessment of the condition of the dentin-pulp complex, which helps reduce risks and increase the effectiveness of complex endodontic treatment.

AIM. To conduct a statistical assessment of the prevalence of degenerative changes in the dentin-pulp complex of teeth based on data obtained from CBCT studies, as well as to identify possible correlational relationships between potential factors contributing to the development of calcifying metamorphosis in the pulp space of vital teeth.

MATERIALS AND METHODS. For the retrospective analysis, 187 archival CBCT study data were selected. The obtained images were examined in all planes for the presence of discrete radiopaque masses in the radiolucent pulp space of the teeth, while the obliteration of the root pulp was determined based on the narrowing or complete closure of the root canal space visible on the radiograph. Statistical analysis was performed using SPSS software with a significance level set at $p < 0.05$.

RESULTS. During the statistical analysis, the prevalence of pulp calcifications was identified in 56.7% of cases, and pulp space obliteration was observed in 24%. Teeth with pulp stones were 1.8 times more likely to exhibit radiographic and clinical signs of caries compared to teeth with root canal obliteration. Among all examined teeth, nearly half in each calcification group had restorations. According to the obtained results, a positive correlation was found between pulp calcification and the dental status of the teeth.

CONCLUSIONS. According to the obtained data, a higher frequency of calcifications is associated with pulp inflammation caused by local prolonged irritants, such as the presence of deep restorations, carious processes, previously performed biological treatments, prior orthodontic treatment, trauma, and wear, as well as other systemic aspects.

Keywords: endodontic treatment, degenerative changes in the dentin-pulp complex, root canal obliteration, cone-beam computed tomography

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Изменения дентинно-пульпарного комплекса зубов через призму КЛКТ-исследований: ретроспективный анализ

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Резюме

ВВЕДЕНИЕ. Кальцифицирующий метаморфоз и облитерация пульпарного пространства зачастую усложняют эндодонтическое лечение, что требует применения современных методов визуализации и контроля проводимой терапии. Использование конусно-лучевой компьютерной томограммы в качестве диагностического инструмента позволяет лучше оценивать состояние дентинно-пульпарного комплекса, что способствует снижению рисков и повышению эффективности сложного эндодонтического лечения.

ЦЕЛЬ ИССЛЕДОВАНИЯ. Провести статистическую оценку распространенности дегенеративных изменений в дентинно-пульпарном комплексе зубов на основании данных, полученных в ходе КЛКТ-исследований, а также выявить возможные корреляционные связи между потенциальными факторами, способствующими развитию кальцифицирующего метаморфоза в пульпарном пространстве витальных зубов.

МАТЕРИАЛЫ И МЕТОДЫ. Для ретроспективного анализа было отобрано 187 КЛКТ-исследований. Полученные изображения были исследованы во всех плоскостях на предмет наличия дискретной рентгеноконтрастной массы в пульпарном пространстве зубов, в то время как, облитерация корневого пульпы определялась на основе сужения или полного закрытия пространства корневого канала. Статистический анализ был выполнен с использованием программного обеспечения SPSS с установленным уровнем значимости $p < 0,05$.

РЕЗУЛЬТАТЫ. В ходе статистического анализа распространенность пульпарных кальцификатов была выявлена в 56,7% случаев, облитерация пульпарного пространства наблюдалась в 24%. Зубы с пульпарными камнями в 1,8 раза чаще имели рентгенологические и клинические признаки кариеса, в отличие от зубов с облитерацией корневого канала. Среди всех обследованных почти половина зубов в каждой группе кальцификаций имела реставрации. Согласно полученным результатам, выявлена положительная взаимосвязь между кальцификацией пульпы и стоматологическим статусом зубов.

ВЫВОДЫ. Согласно полученным данным, более высокая частота возникновения кальцификаций связана с воспалением пульпы, вызванным местными длительными раздражителями, такими как наличие глубоких реставраций, кариозный процесс, раннее проведенное лечение биологическим методом, предшествующее ортодонтическое лечение, травма и истирание, а также с другими общесоматическими аспектами.

Ключевые слова: эндодонтическое лечение, дегенеративные изменения дентинно-пульпарного комплекса, облитерация корневых каналов, конусно-лучевая компьютерная томография

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INTRODUCTION

According to current data from the World Health Organization (WHO), the increase in average life expectancy contributes to a decreasing trend in early tooth loss among the majority of patients [1]. In light of this, modern scientific and clinical advancements are focused on improving endodontic treatment strategies aimed at preserving teeth within the dental arch. The primary objective of endodontic therapy is to perform thorough chemo-mechanical preparation and achieve a hermetic obturation of the root canal system to prevent further microbial contamination [2]. However, factors such as the degree of root canal infection and the anatomical and morphological complexity of the root system significantly affect the difficulty of endodontic treatment [3].

In their study, O. Essam et al. identified 19 factors that may lead to complications or unfavorable outcomes in endodontic therapy. One such factor is the presence of pulp stones within the pulp chamber, which results in a reduced chamber volume and limited visualization of root canal orifices. Another complicating factor is pulp space obliteration, which can be caused by the deposition of secondary or tertiary dentin due to pathological processes such as deep carious lesions, restorative procedures, prior trauma, as well as age-related changes and orthodontic tooth movement [4]. These conditions lead to partial or complete sclerosis of the root canal system, significantly increasing treatment duration, the risk of intraoperative complications, and compromising the prognosis of the therapy.

In 2005, the American Association of Endodontists (AAE) developed the Endodontic Case Difficulty Assessment Form, wherein teeth with pulp stones and calcified canals are classified as moderate to high difficulty cases [5]. This is due to the fact that complex endodontic treatments are associated with a number of procedural risks, including perforation of the pulp floor or furcation area, canal transportation, excessive removal of pericervical dentin, ledge formation, and instrument separation [6]. Such complications ultimately may result in the undesired loss of the tooth [7].

According to the joint position statement by the American Academy of Oral and Maxillofacial Radiology (AAOMR) and the AAE, cone-beam computed tomography (CBCT) is recommended for preoperative assessment of teeth with complex root morphology, for intraoperative evaluation of anatomical variations or additional canals, and for the diagnosis of teeth with previous endodontic treatment errors [8]. It is noteworthy that, based on radiographic assessments, the reported prevalence of degenerative changes in the pulp chamber varies widely from 2.1% to 63.3% [9; 10]. This variability is attributed to the limitations of two-dimensional imaging, which does not allow for the reliable detection of small calcified structures ($<200 \mu\text{m}$) within the pulp cavity [10–12]. Consequently, CBCT is considered a more sensitive imaging modality for detecting degenerative changes in the dentin-pulp complex compared to traditional panoramic and intraoral periapical radiographs [12].

Given the above, there is an evident need for a more comprehensive investigation into the factors influencing

the development of degenerative changes in the dentin-pulp complex. The use of modern imaging techniques, such as cone-beam computed tomography, significantly enhances the quality of preoperative diagnostics and helps minimize complications, thereby improving the overall efficacy of endodontic treatment.

AIM

To perform a statistical assessment of the prevalence of degenerative changes in the dentin-pulp complex of vital teeth based on data obtained from cone-beam computed tomography (CBCT) studies, as well as to identify possible correlations between potential factors contributing to the development of calcific metamorphosis.

MATERIALS AND METHODS

This descriptive, retrospective, cross-sectional study was conducted from September 2024 to May 2025 at the Department of Therapeutic Dentistry and Endodontics, A.I. Evdokimov Research and Educational Institute of Dentistry.

During the analysis, archival cone-beam computed tomography (CBCT) data of 317 patients aged 18 to 74 years were reviewed. A total of 187 CBCT scans meeting the inclusion criteria were selected for the study. The inclusion criteria were: patients over 18 years of age with signs of pulp calcification or root canal obliteration. The exclusion criteria included: patients under 18 years of age, absence of degenerative pulp changes, and radiographs of inadequate diagnostic quality.

CBCT images were obtained using the KaVo DEXIS ORTHOPANTOMOGRAPH™ 3D Pro system (KaVo, Germany) at 85 kV, 5 mA, and an exposure time of 14.4 seconds. The acquired scans were evaluated in coronal, sagittal, and axial planes to identify the presence of discrete radiopaque masses within the radiolucent pulp chamber, as well as root pulp obliteration, which was defined as narrowing or complete closure of the root canal space visible on the radiograph.

Statistical analysis was performed using SPSS software (IBM Corp., USA). Pearson's chi-square (χ^2) test was used to assess the prevalence of degenerative changes in the dentin-pulp complex and to evaluate potential associations with other variables. A p -value of < 0.05 was considered statistically significant.

RESULTS

The overall prevalence of pulp calcifications in the dental pulp tissues of all examined patients was 56.7%. The highest frequency of pulp stones was observed in maxillary and mandibular molars – 71.2% (Fig. 1, A, B, D). In contrast, the lowest prevalence was recorded in the incisors – 5% (Fig. 1, C). No statistically significant difference in the occurrence of pulp stones was found between genders ($p > 0.05$). Pulp canal obliteration was identified in 24% of the teeth with calcifications. The degree of calcification ranged from partial (15.1%) to complete obliteration of the root canal space in 8.9% of cases. Nevertheless, despite radiographic evidence of complete obliteration, a narrow, barely discernible

root canal lumen may still be present at the microscopic level [12; 13].

Among patients aged 18–44 years, pulp calcifications predominated, whereas obliteration of the root canals was more frequently observed in the older age group (60–74 years) (Table 1).

The percentage distribution of predictors associated with the development of calcific metamorphosis is presented in Fig. 2. Among teeth with pulp stones, 46% showed no signs of carious lesions, whereas 54% exhibited radiographic and clinical evidence of caries (Fig. 1, A). In cases with root canal obliteration, deep carious lesions were identified in 28.7% of cases. Statistical analysis confirmed a significant correlation between the presence of pulp calcifications and dental caries ($p = 0.01$).

The presence and depth of restorations did not demonstrate a statistically significant difference depending on the type of calcific metamorphosis ($p > 0.05$). Nearly half of the teeth in each calcification group had restorations. Notably, deep restorations were more frequently observed among teeth with root canal obliteration (Fig. 1, D).

In the group of teeth previously treated using biologically based calcium-containing materials, obliteration of the root canal space was observed twice as often as pulp stones. A relatively low prevalence of pulp calcifications (15%) was noted in teeth with a history of orthodontic treatment. Interestingly, in unerupted (retained) teeth that had not been exposed to external factors, pulp calcifications were identified in 18% of cases, which may indicate an idiopathic nature of these degenerative changes.

Table 1. Distribution of teeth with degenerative changes in the pulp cavity according to group affiliation

Таблица 1. Распределение зубов с дегенеративными изменениями в пульпарной полости в соответствии с групповой принадлежностью

Affiliation	Pulp stones, %	Root canal obliteration, %
Age group affiliation		
18–44 years	35.7	6.1
45–59 years	52.3	25.2
60–74 years	12.0	68.7
Gender affiliation		
males	42.2	46.7
females	57.8	53.3
Group affiliation		
maxilla	54.0	53.3
mandible	46.0	46.7
Tooth type		
incisors	5.0	13.3
canines	6.6	10.0
premolars	17.2	23.4
molars	71.2	53.3

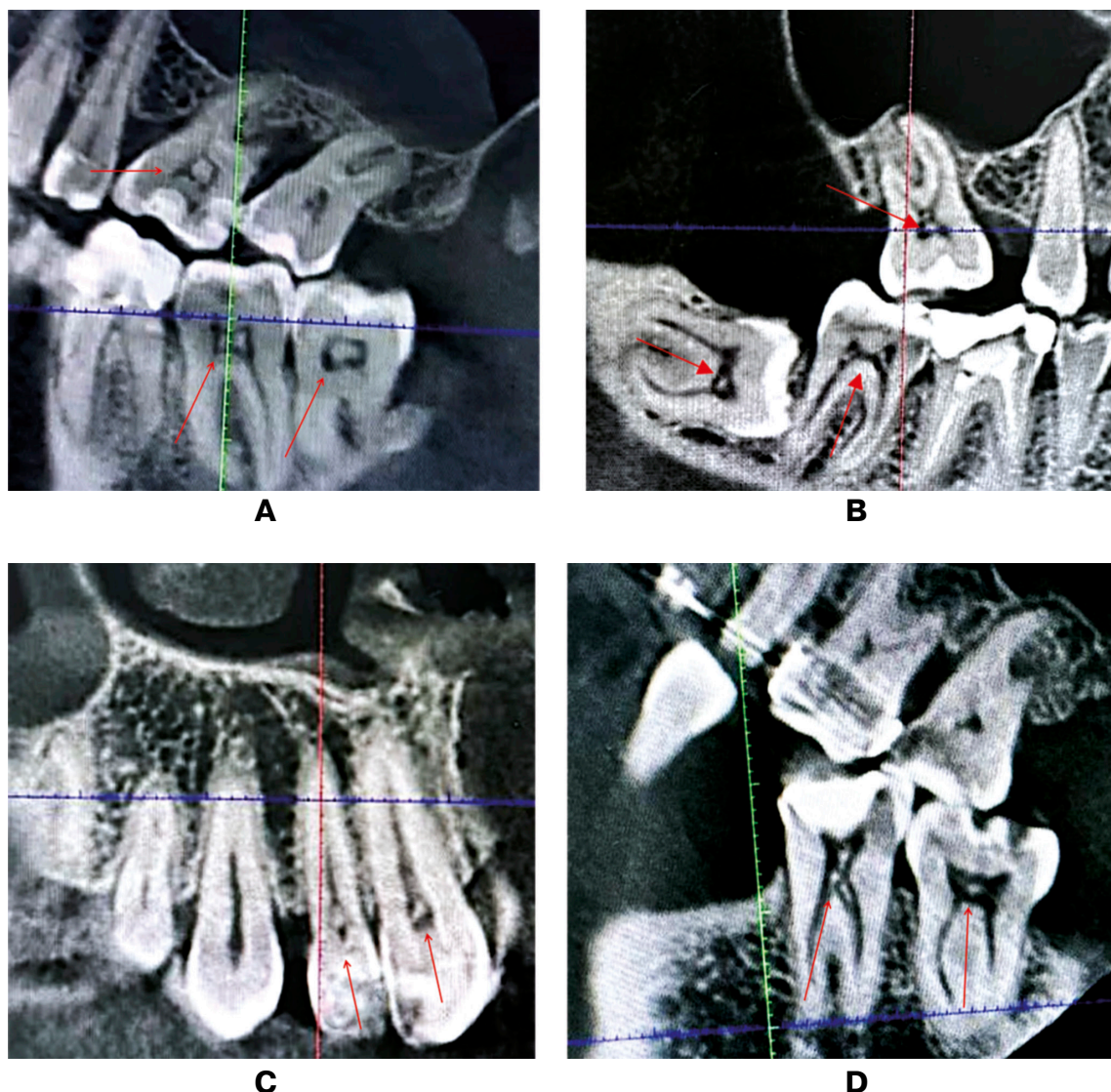


Fig. 1. Sagittal Sections of Dental CBCT Studies: A – Pulp calcifications in the cavities of teeth 2.7 (presence of secondary caries), 3.7, 3.8; B – Pulp calcifications in the cavities of teeth 1.7, 4.7, 4.8 (impacted, ectopic); C – Pulp calcifications in the cavities of teeth 1.1, 1.2; D – Pulp calcifications in the cavity of tooth 3.6 (presence of deep restoration), 3.7

Рис. 1. Сагиттальные срезы дентальных КЛКТ-исследований: А – пульпарные кальцификаты в полости зубов 2.7 (наличие вторичного кариеса), 3.7, 3.8; В – пульпарные кальцификаты в полости зуба 1.7, 4.7, 4.8 (ретинированный, дистопированный); С – пульпарные кальцификации в полости зуба 1.1, 1.2; D – пульпарные кальцификации в полости зуба 3.6 (наличие глубокой реставрации), 3.7

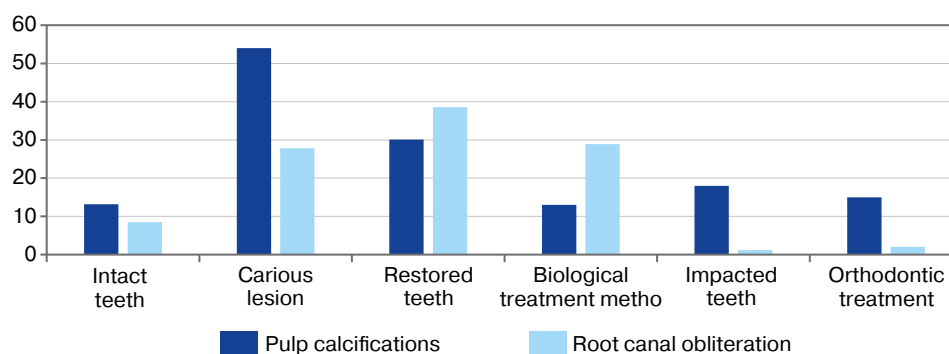


Fig. 2. Potential factors of calcifying metamorphosis development

Рис. 2. Потенциальные факторы развития кальцифицирующего метаморфоза

DISCUSSION

The aim of this study was to evaluate the radiographic characteristics of various types of dental pulp calcifications and their association with specific dental indicators.

Correlation analysis revealed a significant predisposition for the formation of pulp calcifications in molars, which is consistent with previously published studies [13–15]. The high prevalence of calcifications in this tooth group may be attributed to their anatomical features: as the largest teeth in the dental arch, molars possess a more developed pulpal blood supply, which creates favorable conditions for calcification formation [16]. Furthermore, they are the first permanent teeth to erupt and bear the greatest occlusal load throughout life, making them more susceptible to carious lesions and restorative procedures [17]. Notably, the study by A. Palatyńska-Ulatowska et al. indicated that pulp calcifications are more commonly found in the coronal pulp than in the radicular pulp space [18].

In this study, no statistically significant difference was found between gender and the presence of degenerative changes in the dentin-pulp complex. However, a number of authors report a higher prevalence of such changes among females, which is attributed to a greater incidence of bruxism and the influence of hormonal factors [15–19].

Regarding age-related indicators, an inverse correlation was identified between patient age and the presence of degenerative pulp changes ($p = 0.001$).

Pulp calcifications were predominantly observed in younger individuals (18–44 years), whereas root canal obliteration was more prevalent in patients over the age of 60. It is well established that aging promotes the formation of secondary and tertiary dentin, resulting in a gradual reduction in the size of the pulp chamber and root canal [17].

The results of the present study demonstrated a positive correlation between pulp calcification and the dental condition of the affected teeth. A higher frequency of calcifications was associated with pulpal inflammation caused by prolonged local irritants such as restorations, carious lesions, prior biologically based treatments, orthodontic therapy, trauma, and attrition, as well as idiopathic factors [20]. These findings support the hypothesis that chronic irritants may contribute to the formation of pulp stones.

CONCLUSION

The present study revealed that degenerative changes within the pulp space are a common finding, occurring in 80.7% of cases. Their formation is closely associated with both physiological and pathological conditions of the teeth and may be triggered by prolonged exposure to local irritants. These findings underscore the importance of a deeper understanding of the mechanisms underlying calcific metamorphosis in order to develop more effective strategies for the prevention and management of diseases affecting the dentin-pulp complex.

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Adaptation potential of secretory functional of the small salivary glands and gustatory analyzer besides flying composition of civil aviation

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Abstract

AIM. The feature of adaptive potential of secretory function of the small salivary glands besides flying composition of the civil aviation.

MATERIALS AND METHODS. In the article presented results of the study of secretory function of small salivary glands beside 20 employees of flying and 15 overland compositions of civil aviation at the age 20–50 years with pathology of oral cavity and with sound condition of dentistry status. For achievement delivered purposes have developed and felt the way, concluding in revealing the small salivary glands on emerged secret and fixation its paper pattern in the manner of imprint. About level of the secretions of the small salivary glands witnessed the different diameter an imprint of secret. As a matter of convenience count small salivary glands divided in four orders in depending of diameter heel secret – from the most small, referred to IV order, before the most large (I order).

RESULTS. Amongst flying composition with sound condition of dentistry status at observation of the paper pattern revealed area of the lips with concentration of the small salivary glands only 1-st and 2-nd degree. Beside they comes to light scant few of the small salivary glands II and III order, density their small, on upper lip they, as contrasted with glands IV order, concentrated to medium closer, but on lower lip – on side left and right half. Beside flying composition with dentistry pathology discovered quite other regularity in respect of concentrations of the small salivary glands on upper and lower lip: both on upper and on lower lip practically were not discovered small salivary glands III and IV orders. Together with that quantitative importance of the small salivary glands II order besides flying composition with pathology of oral cavity turned out to be similar to patient without dentistry pathology.

CONCLUSIONS. Beside flying composition with dentistry pathology on amount of the secretions in the first place answer the glands II order, then to them are connected the glands III order. Beside they practically do not exist the appearances of the secret of the small salivary glands IV order that is indicative of absence their reserve function, conditioned stress-induced influence extreme factor of aircraft flight.

Keywords: small salivary gland, flying personnel, non-flying composition, lip, civil aviation, pathology of parodont, aircraft flight

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Адаптационный потенциал выделительной функции малых слюнных желез у летного состава гражданской авиации

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Резюме

ЦЕЛЬ. Дать характеристику адаптационного потенциала выделительной функции малых слюнных желез у летного состава гражданской авиации.

МАТЕРИАЛЫ И МЕТОДЫ. В работе представлены результаты изучения выделительной функции малых слюнных желез у 20 сотрудников летного и 15 наземного состава гражданской авиации в возрасте 20–50 лет с патологией полости рта и интактным состоянием стоматологического статуса. Для достижения поставленной цели разработали и испытали способ, заключающийся в выявлении малых слюнных желез по выступившему секрету и фиксации его бумажным шаблоном в виде отпечатков. Об уровне секреции малых слюнных желез свидетельствовал различный диаметр отпечатков секрета.

Для удобства подсчета малые слюнные железы были поделены на четыре порядка в зависимости от диаметра пятен секрета – от самых мелких, отнесенных к IV порядку, до самых крупных (I порядка). РЕЗУЛЬТАТЫ. Среди летного состава с интактным состоянием стоматологического статуса при наблюдении бумажного шаблона выявлены участки губ с концентрацией малых слюнных желез только 1-й и 2-й степени. У них визуализируется незначительное количество малых слюнных желез II и III порядка, плотность их невелика, на верхней губе они, в противоположность железам IV порядка, концентрировались ближе к середине, а на нижней губе – по бокам левой и правой половин. У летного состава со стоматологической патологией обнаружена совсем иная закономерность в отношении концентрации малых слюнных желез на верхней и нижней губах: как на верхней, так и на нижней губе практически не были обнаружены малые слюнные железы III и IV порядка. Вместе с тем количественное значение малых слюнных желез II порядка у летного состава с патологией полости рта оказалось схожим с пациентами без стоматологической патологии.

ВЫВОДЫ. У летного состава со стоматологической патологией по количеству секреции в первую очередь отвечают железы II порядка, затем к ним подключаются железы III порядка. У них практически не наблюдается появления секрета малых слюнных желез IV порядка, что свидетельствует об отсутствии их резервной функции, обусловленной стресс-индуцирующим влиянием экстремальных факторов авиационного полета.

Ключевые слова: малые слюнные железы, летный персонал, нелетный состав, губа, гражданская авиация, патология пародонта, авиационный полет

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INTRODUCTION

A number of biological fluids are secreted into the oral cavity, each playing a significant role in the physiology and pathology of the oral tissues and organs. These include saliva from the ducts of the major salivary glands, as well as secretions from the numerous minor salivary glands and mucous glands distributed throughout the oral mucosa [1–3]. The full extent of saliva's importance for oral health has yet to be completely elucidated. The composition and properties of saliva largely depend on the functional state of the central nervous system, the activity of the salivary glands themselves, and the quality of ingested food [4–7].

In light of the above, particular scientific interest lies in the investigation of the functional state of the minor salivary glands under stress-inducing conditions caused by the extreme factors associated with aviation flight.

AIM

To characterize the adaptive potential of the secretory function of minor salivary glands in civil aviation flight personnel.

MATERIALS AND METHODS

The tasks aimed at assessing the adaptive potential of the secretory function of minor salivary glands in civil aviation flight personnel were addressed based on original selective observations of individuals across specific age groups. This study presents the results of an investigation into the secretory function of minor salivary glands in 20 flight crew members and 15 ground personnel of the civil aviation sector, aged between 20 and 50 years, both with oral pathology and with intact dental status.

To achieve the stated objective, we developed and tested a method involving the identification of minor salivary glands through the detection of emerging secretions and their fixation using a paper template to obtain impression marks. A square paper frame with 2 cm sides was applied to the mucosal surface of the lower lip, which had been pre-stained with a 1% solution of methylene blue or brilliant green. Secretory droplets emerging from the ducts of the minor salivary glands were then counted by identifying the decolorized spots.

In professional civil aviation personnel, the level of secretion of the minor salivary glands was reflected by the varying diameters of the secretion prints. According to I.F. Romacheva, the normal number of functioning minor salivary glands within a 2 cm square frame is 20–22. For convenience in quantification, the glands were categorized into four grades based on the diameter of the secretion spots – from the smallest (Grade IV) to the largest (Grade I).

Statistical analysis was performed using Statistica 10.0 and Microsoft Excel. The distribution type was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The following descriptive statistical parameters were determined: number of observations (n), arithmetic mean (M), standard error of the mean (m), and relative values (P , %). Statistical significance was set at $p < 0.05$.

RESULTS

The data obtained from the study of the secretory function of minor salivary glands in ground personnel of civil aviation with oral pathology indicate that the number of these glands on the lower lip averaged 71.3 ± 3.4 units, which is 1.5 times higher than on the upper lip (46.9 ± 2.7 units; $p < 0.001$). The vast majority

of minor salivary glands on the upper lip were classified as Grade IV (smallest secretion spot diameter), accounting for 27.1 ± 2.8 units (57.8%), and this number was significantly higher ($p < 0.01$) than that of Grade III glands with medium secretion spot diameters – 16.7 ± 2.3 units (35.6%). It was also highly significantly greater ($p < 0.001$) than the number of Grade II glands characterized by larger secretion spot diameters – 3.1 ± 1.1 units (6.6%) (Fig. 1, A).

It should be noted that among the examined ground personnel with dental pathology, sialometry of the minor salivary glands did not reveal any glands of the first grade (Grade I), characterized by the largest secretion spot diameters. The average secretion rate was 0.94 ± 0.46 mg/min.

As mentioned above, the average number of minor salivary glands on the lower lip was 71.3 ± 3.4 units. The grade-wise distribution of minor salivary glands on the lower lip in ground personnel of civil aviation with oral pathology demonstrated the following pattern: Grade IV glands predominated, accounting for 47.3 ± 2.2 units (66.3% of cases), which was significantly higher than the number of Grade III glands – 18.7 ± 2.6 units (26.3%; $p < 0.001$), and Grade II glands – 5.3 ± 0.7 units (7.4%) (Fig. 1, B).

In the ground personnel of civil aviation with dental pathology, the assessment of the secretory function of the labial minor salivary glands revealed that although the total number of glands on the lower lip (71.3 ± 3.4 units) exceeded that on the upper lip (46.9 ± 2.7 units), this proportional relationship was not observed across different gland grades, except for Grade IV glands, which were significantly more numerous on the lower lip (47.3 ± 2.2 units) than on the upper lip (27.1 ± 2.8 units; $p < 0.001$). In contrast, the average numbers of Grade III and Grade II glands on the lower lip (18.7 ± 2.6 and 5.3 ± 0.7 units, respectively) did not differ significantly from those on the upper lip (16.7 ± 2.3 and 3.1 ± 1.1 units, respectively).

Among ground personnel with an intact dental status, the total number of minor salivary glands on the lower and upper lips was 78.8 ± 3.7 and 52.4 ± 3.3 units, respectively. The average numbers of Grade II

and Grade III glands on the lower lip (9.5 ± 0.8 and 22.7 ± 3.2 units, respectively) were higher than on the upper lip (5.8 ± 1.5 and 17.8 ± 2.6 units, respectively).

Comparative analysis demonstrated that in ground personnel with an intact dental status, the total number of minor salivary glands on the lower and upper lips was approximately 1.1 times higher (78.8 ± 3.7 and 52.4 ± 3.3 units, respectively) than in those with oral pathology (71.3 ± 3.4 and 46.9 ± 2.7 units, respectively). These findings suggest that the presence of pathological conditions in the oral cavity is associated with a certain degree of reduction in the secretory function of minor salivary glands among ground personnel of civil aviation.

A similar study was conducted among flight personnel of civil aviation with an intact dental status. The results in this group revealed a diametrically opposite pattern in the quantitative distribution of minor salivary glands on the upper and lower lips compared to the ground personnel with healthy oral tissues. Specifically, among flight crew members with intact dental status, the number of minor salivary glands on the lower lip was 1.3 times lower (41.1 ± 1.6 units) than on the upper lip (54.7 ± 2.2 units). Moreover, the vast majority of glands on the lower lip were classified as Grade IV (32.5 ± 2.9 units), followed by Grade III (7.3 ± 1.3 units) and Grade II (1.8 ± 0.7 units), accounting for 78.1%, 17.6%, and 4.3% of the total, respectively (Fig. 2).

In flight personnel of civil aviation with an intact condition of oral tissues, structural analysis of the total secretory function of minor salivary glands on the upper lip (54.7 ± 2.2 units) revealed a predominance of Grade IV glands (64.4%; 35.2 ± 2.3 units), followed by Grade III (33.1%; 18.1 ± 1.5 units) and Grade II (2.5%; 1.4 ± 0.2 units).

A comparative evaluation of the secretory function of minor salivary glands in flight crew members without dental pathology clearly demonstrates that although the total number of glands on the lower lip (41.1 ± 1.6 units) was lower than that on the upper lip (54.7 ± 2.2 units), this ratio was also reflected in the number of Grade III (7.3 ± 1.3 vs. 18.1 ± 1.5 units) and Grade IV (32.5 ± 2.9 vs. 35.2 ± 2.3 units) glands on the lower and upper lips, respectively.

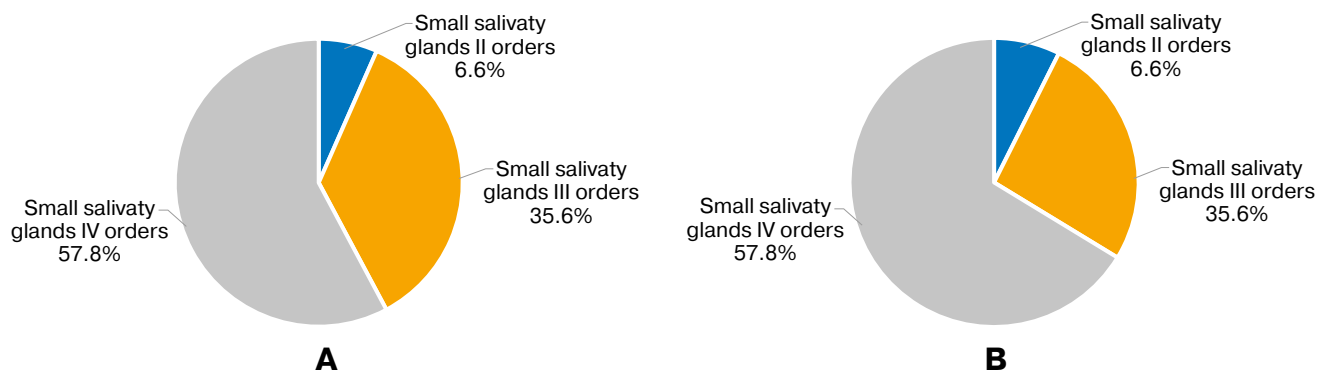


Fig. 1. Quantitative estimation of small salivary glands on upper (A) and lower (B) lip in depending of diameter heel secret beside non-flying composition of the civil aviation with dentistry pathology

Рис. 1. Количественная оценка малых слюнных желез на верхней (A) и нижней (B) губах в зависимости от диаметра пятен секрета у нелетного состава гражданской авиации со стоматологической патологией

In the examined cohort of flight personnel with an intact dental status, the secretory output of minor salivary glands was also measured. Upon recalculating the number of glands on the upper and lower lips to the corresponding amount of secreted fluid, it was found that the volume on the lower lip (14.7 ± 1.3 mg/min) was 1.5 times higher than that on the upper lip (9.8 ± 0.7 mg/min; $p < 0.01$). On the upper lip, Grade III and Grade IV glands secreted nearly equal volumes of saliva – 4.9 ± 0.6 mg/min and 4.8 ± 0.5 mg/min, respectively – with the remainder attributed to Grade II glands.

Considering the total secretory output on the lower lip (14.7 ± 1.3 mg/min), Grade IV and Grade III glands accounted for the majority of secretion, with a dominant contribution from Grade IV glands – 9.7 ± 0.8 mg/min (66.0%), which significantly exceeded the secretion from Grade III glands – 3.2 ± 0.5 mg/min (21.8%). A minimal volume of secretion was produced by Grade II glands – 1.8 ± 0.2 mg/min (12.2%). When comparing secretion volumes between the upper and lower lips, a statistically significant difference was observed only

for Grade IV glands: 4.8 ± 0.5 mg/min on the upper lip vs. 9.7 ± 0.8 mg/min on the lower lip ($p < 0.001$).

Among flight personnel with intact oral health, recalculation of the number of minor salivary glands on the upper and lower lips in terms of their secretory output revealed that secretion on the lower lip (14.7 ± 1.3 mg/min) was reduced by 1.5 times compared to that on the upper lip (9.8 ± 0.7 mg/min; $p < 0.01$). On the upper lip, Grade III and IV glands produced nearly equal amounts of saliva – 4.9 ± 0.6 mg/min (50.0%) and 4.6 ± 0.5 mg/min (46.9%), respectively – while Grade II glands accounted for the remaining 0.5 ± 0.1 mg/min (3.1%) (Fig. 3).

The average value of the secretory function of minor salivary glands on the lower lip in civil aviation flight personnel with dental pathology was 11.3 ± 0.6 mg/min, which is 1.3 times lower than the corresponding value in pilots with an intact dental status (14.7 ± 1.3 mg/min). Upon evaluating the secretory function of minor salivary glands in this area, it was found that in flight personnel with dental pathology, Grade III and IV glands also constituted the primary source of secretion, with a predominance of

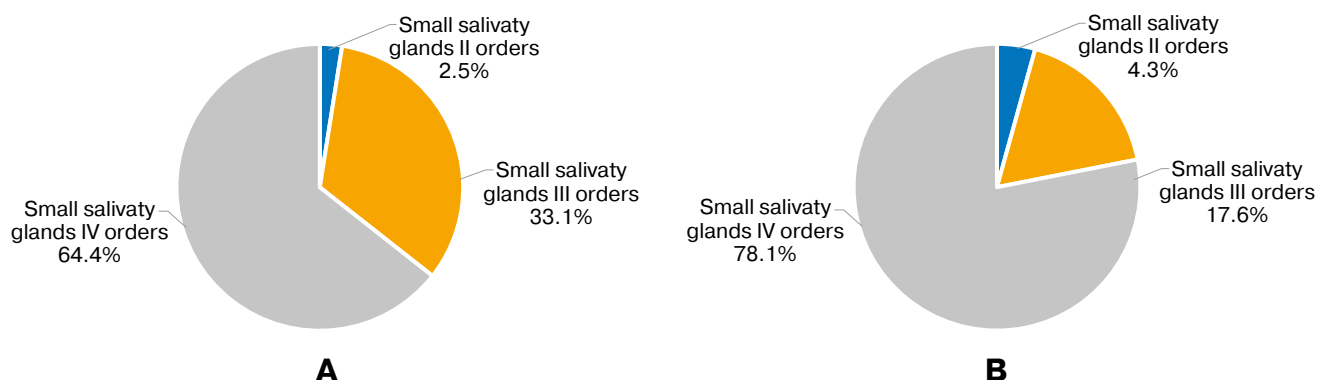


Fig. 2. Quantitative estimation of small salivary glands on upper (A) and lower (B) lip in depending on diameter heel secret beside flying composition of the civil aviation with sound condition of dentistry status

Рис. 2. Количественная оценка малых слюнных желез на верхней (A) и нижней (B) губах в зависимости от диаметра пятен секрета у летного состава гражданской авиации с интактным состоянием стоматологического статуса

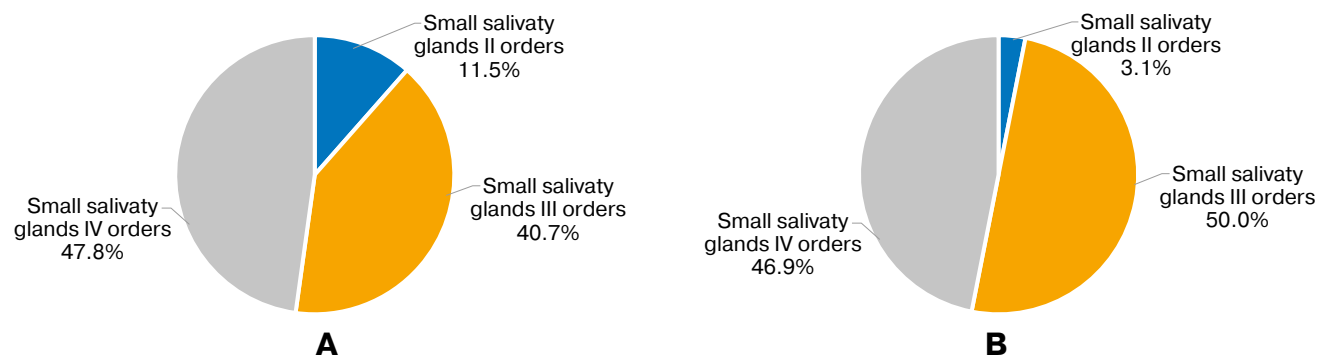


Fig. 3. Value produced secret of small salivary glands II, III and IV order on lip beside flying composition in depending of conditions dentistry status: A – patients with dentistry; B – patients without dentistry

Рис. 3. Величина продуцируемого секрета малых слюнных желез II, III и IV порядка на губах у летного состава в зависимости от состояния стоматологического статуса: A – пациенты со стоматологическим лечением; B – пациенты без стоматологического лечения

Grade IV glands – 5.4 ± 0.3 mg/min (47.8%). However, this value was significantly lower than the secretion produced by Grade III glands – 4.6 ± 0.3 mg/min (40.7%; $p < 0.05$). The remaining amount of saliva (1.3 ± 0.2 mg/min; 11.5%) was attributed to Grade II minor salivary glands.

DISCUSSION

In civil aviation pilots with pathology of the oral tissues, analysis of the overall secretory function of minor salivary glands on the upper lip (7.9 ± 0.5 units) also revealed a numerical predominance of Grade IV glands (49.4%; 3.9 ± 0.8 units), followed by Grade III (44.3%; 3.5 ± 0.5 units) and Grade II (6.3%; 0.5 ± 0.2 units). Statistically significant differences in secretion volume between the upper and lower lips were observed only for Grade II glands (11.5% vs. 6.3%) and Grade III glands (40.7% on the lower lip vs. 49.4% on the upper lip).

To investigate the existence of a consistent pattern in the topographical distribution of minor salivary glands depending on the dental status in civil aviation pilots with oral pathology, glandular imprints were transferred onto a paper template using a specialized coordinate grid, allowing for identification of zones with the highest concentration of each gland grade. The concentration density (glands per mm^2) was classified into three levels: less than one gland – Grade I; exactly one gland – Grade II; more than one gland – Grade III.

Among pilots with an intact dental status, observation of the paper templates revealed only Grade I and II concentration levels. Although glands of different orders appeared intermixed, certain areas exhibited pronounced clustering of minor salivary glands consistent across all subjects. On the upper lip, the highest concentration of Grade IV glands was found near the lateral areas of the oral vestibule arch, while on the lower lip, clustering was most prominent in the central zone. Only small numbers of Grade II and III glands were visualized, with low density. These were located more centrally on the upper lip (unlike Grade IV glands) and laterally on the left and right halves of the lower lip.

Conversely, in pilots with dental pathology, a fundamentally different pattern of gland distribution was ob-

served. The coordinate grid analysis showed an almost complete absence of Grade III and IV glands on both the upper and lower lips, indicated by the minimal and moderate secretion spot diameters on the templates. Meanwhile, the number of Grade II glands in these individuals was comparable to that of pilots without oral pathology.

Based on the data regarding the identification of minor salivary glands in flight personnel with and without dental pathology, it is essential to assess not only the total number of glands but also their distribution across functional grades. The results indicate that in pilots with an intact oral status, secretion in response to stimulation is initiated predominantly by Grade II and III glands, with Grade IV glands subsequently contributing to the response.

A fundamentally different pattern was recorded in pilots with oral pathology: secretory response to stimulation was primarily initiated by Grade II glands, followed by Grade III, whereas Grade IV glands – those with the smallest secretion diameters – were virtually absent. This observation likely reflects a loss of their reserve function due to the stress-inducing impact of extreme factors inherent to aviation flight conditions.

CONCLUSION

1. The results of the study on the secretory function of minor salivary glands in civil aviation flight personnel, depending on the condition of their dental status, demonstrated distinct patterns in the spatial distribution and secretory activity of these glands. Their topographical arrangement contributes to more effective salivary coverage of the lower teeth, which corresponds with a lower incidence of dental caries in these regions.

2. The upper teeth, particularly the central incisors, are significantly less exposed to salivary flow, with certain areas remaining entirely unmoistened. The high incidence of carious lesions in these functionally oriented anterior tooth groups, in conjunction with the presented findings, may indicate the existence of a specific interrelationship between these phenomena.

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The effect of the type of jaw growth caused by the change of baby teeth on the bioelectric activity of the chewing muscles

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Abstract

INTRODUCTION. The bioelectric activity of the masticatory muscles is influenced by many factors due to the parameters of the craniofacial complex – morphometric parameters of the head. However, there is no data in the literature on the effect of the type of jaw growth during periods of alternating bite on the functional activity of the muscles that lift the lower jaw.

AIM. To determine the impact of jaw growth type, associated with the transition from deciduous to permanent teeth, on the bioelectrical activity of the masticatory muscles.

MATERIALS AND METHODS. A survey of 150 children aged 5 to 14 years was conducted, randomized into 5 groups – group I – the final period of milk bite and preparation of the jaw for tooth replacement. Group II – the first permanent molars and incisors of the lower jaw erupted. Group III – there was a change of permanent milk incisors. Group IV – there was a complete change of all baby teeth. Group V – the second permanent molars have erupted. An analysis of the telereöntgenogram and the functional activity of the masticatory muscles is presented by determining absolute and relative indicators.

RESULTS. In patients of groups I, II and III, low bioelectric activity was detected, which increases during the complete change of all baby teeth and the eruption of the second permanent molars, which is due to the formation of chewing activity of the muscles. Also, during this period, the symmetry and synchronicity of the work of the masticatory and temporal muscles proper was noted.

CONCLUSIONS. Minimal changes during jaw growth due to the change of baby teeth lead to a change in the functional activity of the masticatory muscles, which may subsequently be a predictor factor for the development of neuromuscular imbalance in the maxillofacial region.

Keywords: removable bite, telereöntgenogram, electromyography, jaw growth

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Влияние типа роста челюстей, обусловленного сменой молочных зубов, на биоэлектрическую активность жевательных мышц

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Резюме

ВВЕДЕНИЕ. На биоэлектрическую активность жевательной мускулатуры влияет множество факторов, обусловленные параметрами кранио-фациального комплекса – морфометрические параметры головы. Однако, в литературе отсутствуют данные о влиянии типа роста челюстей в периоды сменного прикуса на функциональную активность мышц, поднимающих нижнюю челюсть.

ЦЕЛЬ ИССЛЕДОВАНИЯ. Определить влияние типа роста челюстей, обусловленного сменой молочных зубов, на биоэлектрическую активность жевательных мышц.

МАТЕРИАЛЫ И МЕТОДЫ. Проведено обследование 150 детей в возрасте от 5 до 14 лет, рандомизированные на 5 групп – I группа – завершающий период молочного прикуса и подготовка челюсти к смене зубов. II группа – прорезались первые постоянные моляры и резцы нижней челюсти. III группа – произошла смена молочных резцов постоянными. IV группа – произошла полная смена всех молочных зубов. V группа – прорезались вторые постоянные моляры. Представлен анализ телерентгенограммы и функциональной активности жевательной мускулатуры с помощью определения абсолютных и относительных показателей.

РЕЗУЛЬТАТЫ. У пациентов I, II и III групп выявлена низкая биоэлектрическая активность, которая в период полной смены всех молочных зубов и прорезывания вторых постоянных моляров увеличивается, что обусловлено формированием жевательной активности мускулатуры. Также в данный период отмечалась симметричность и синхронность работы собственно жевательной и височной мышц.

ВЫВОДЫ. Минимальные изменения в период роста челюстей, обусловленные сменой молочных зубов, приводят к изменению функциональной активности жевательных мышц, которая впоследствии может являться фактором-предиктором развития нейромышечного дисбаланса челюстно-лицевой области.

Ключевые слова: сменный прикус, телерентгенограмма, электромиография, рост челюсти

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INTRODUCTION

Morphometric parameters of the craniofacial complex determine the characteristics of the masticatory muscles, as demonstrated by previous studies [1]. However, during the mixed dentition period, structural transformations occur as a result of jaw growth associated with the eruption of primary and permanent teeth [2]. To date, the literature provides detailed data on changes in craniofacial parameters in children during the transition from primary to permanent dentition. Researchers have observed that the most significant dimensional changes occur in the facial region of the skull and are related to the phases of occlusal height increase following the eruption of key teeth [3].

At the same time, international literature reports that by the time of dental replacement, the dimensions of the neurocranium in children reach approximately 90% of adult values. It is therefore evident that the size of cranial bones contributes to the anatomical and functional features of the masticatory musculature [4]. Morphometric peculiarities of the facial skeleton in children during the early mixed dentition stage have also been documented in the presence of occlusal abnormalities, particularly in terms of vertical skeletal parameters. During this period, active growth of the mandibular ramus continues, accommodating the developing buds of permanent molars [5].

Furthermore, the type of facial skeletal growth is influenced by the gonial angle, which ranges from 118° to 122° in cases of neutral growth patterns. Consequently, the orientation of the muscle attachment site at this angle is presumed to affect the bioelectrical activity of the masticatory muscles [6]. However, no current literature provides data on the influence of jaw growth during the mixed dentition period on the functional activity of masticatory muscles.

Electromyography remains the principal method for assessing the bioelectrical activity of the masticatory muscles and is well documented in both textbook and scientific literature [7]. Surface electromyography (sEMG) is one of the primary auxiliary diagnostic tools, enabling objective assessment of the functional state of the masticatory muscles, including their bioelectrical activity [8]. While this method is described in both domestic and international publications, the majority of studies offer comparative analyses between patient groups and controls without clearly defined quantitative criteria [9; 10].

Several studies have reported asymmetry in the bioelectrical activity of the masticatory muscles in children with physiological occlusion, whereas in malocclusion cases, the temporal and masseter muscles exhibit synchronized function. However, these investigations have not addressed the typological changes in the functional state of the masticatory muscles during tooth replacement in relation to jaw growth. This gap in knowledge formed the rationale for the present study.

AIM

To examine the influence of jaw growth patterns associated with the transition from primary to permanent dentition on the bioelectrical activity of the masticatory muscles.

MATERIALS AND METHODS

The study was conducted at the Department of Dentistry and the Department of Prosthetic Dentistry and Orthodontics of the Institute of Continuing Medical and Pharmaceutical Education at Volgograd State Medical University. In accordance with the study standardization protocol, specific inclusion, non-inclusion, and exclusion criteria were developed.

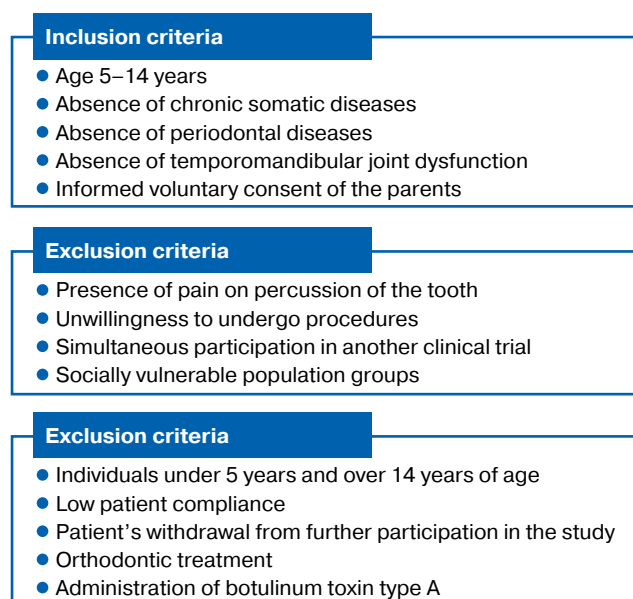


Fig. 1. Inclusion, Non-inclusion, and Exclusion Criteria for Study Participants

Рис. 1. Критерии включения / невключения / исключения пациентов в исследование

All participants were divided into five equal groups (30 individuals each), based on the eruption stage of specific tooth groups:

- Group I – final stage of primary dentition and preparation of the jaws for tooth transition.
- Group II – eruption of the first permanent molars and mandibular incisors.
- Group III – replacement of primary incisors with permanent ones.
- Group IV – complete replacement of all primary teeth.
- Group V – eruption of the second permanent molars.

Dental examinations were carried out using both basic and supplementary diagnostic methods. Clinical oral evaluation was performed using a dental mirror and probe. The dental status and presence of periodontal conditions were assessed. Lateral and posteroanterior cephalometric radiographs were obtained using the Evolution (Italy) imaging system.

Electromyographic (EMG) examination was conducted using the **Synopsis** system in accordance with standard protocols. Surface electrodes were placed on the masseter and temporalis muscles, with an inter-electrode distance of at least 1 cm. Patients were seated in a dental chair in a relaxed, resting state.

During the “rest” trial, the following parameters were recorded:

- maximum and mean amplitudes of the masseter and temporalis muscles on both sides;
- Temporalis Muscle Symmetry Index (TMSI);
- Masseter Muscle Symmetry Index (MMSI);
- Center of Muscle Coordination (CMC);
- Torsion Index (TORS).

Mean amplitude values were considered absolute parameters, measured in microvolts (μV). Relative parameters – TMSI, MMSI, CMC, and TORS – were automatically calculated by the software and expressed as percentages.

Statistical analysis was performed using Statistica 10.0 Enterprise. Descriptive and inferential statistics were applied, including the calculation of the mean (M), standard error ($\pm m$), standard deviation (σ), and inter-group comparisons using Student's t -test to assess statistical significance.

RESULTS

Analysis of the mean amplitude of the temporalis and masseter muscles revealed group-dependent differences in bioelectrical activity. It is evident that the functional activity of the examined muscles depends on the type of jaw growth and the number of erupted teeth. In Group I, the mean amplitude of the temporalis muscle was statistically significantly lower compared to other groups: 21.4% lower than Group II, 25% lower than Group III, 33.9% lower than Group IV, and 66.8% lower than Group V. No statistically significant differences were observed between Groups II, III, and IV ($p > 0.05$). However, in Group V children with erupted second permanent molars the mean amplitude was 36.7% higher than in Group II, 32.9% higher than in Group III, and 24% higher than in Group IV (children with complete primary tooth replacement).

The values of mean masseter muscle amplitude followed a similar trend to the temporalis muscle. The lowest value was recorded in Group I ($81.4 \pm 2.1 \mu V$), which was 16% lower than in Group II, 22.2% lower than in Group III, 24.6% lower than in Group IV, and 45.7% lower than in Group V ($p < 0.05$). No significant differences were noted among Groups II, III, and IV ($p > 0.05$), which is likely due to the presence of first permanent molars in all three groups. The replacement of incisors alone does not appear to affect the functional state of the masticatory muscles, as these teeth are primarily involved in incising rather than chewing. In contrast, the eruption of second permanent molars significantly enhances the bioelectrical activity of both the temporalis and masseter muscles, as confirmed by the statistical analysis ($p < 0.05$).

Table 1. Absolute values of bioelectrical activity, μV

Таблица 1. Абсолютные значения биоэлектрической активности, мкВ

Indicators	Group	Mean amplitude
Temporalis muscle	I	56.3 ± 2.4
	II	68.1 ± 3.2
	III	70.2 ± 3.1
	IV	75.1 ± 2.7
	V	93.4 ± 3.1
Masseter muscle	I	81.4 ± 2.1
	II	93.9 ± 2.4
	III	98.7 ± 2.6
	IV	101.3 ± 2.7
	V	118.4 ± 3.3

Table 2. Relative indicators of bioelectrical activity, %

Таблица 2. Относительные показатели биоэлектрической активности, в %

Group	TMSI	MMSI	TORS	CMC
I	19.7±4.6	20.3±3.1	22.4±3.6	23.1±3.5
II	15.2±3.6	16.9±2.0	15.9±1.8	14.7±2.1
III	15.6±1.4	16.7±1.7	15.2±1.9	15.6±1.5
IV	16.1±1.5	15.6±2.4	15.1±1.6	14.9±2.0
V	11.9±1.8	18.7±2.1	14.8±1.7	15.3±1.4

Relative electromyographic parameters reflecting the dynamics of neuromuscular changes are presented in Table 2.

The analysis of the relative indicators of masticatory muscle activity revealed no statistically significant differences between the comparison groups ($p > 0.05$). In children at the stage of primary dentition and in the preparatory phase for tooth transition, higher symmetry indices were observed. These values tended to decrease with age and over time. Greater symmetry and stability of muscle activity were noted during the eruption of the

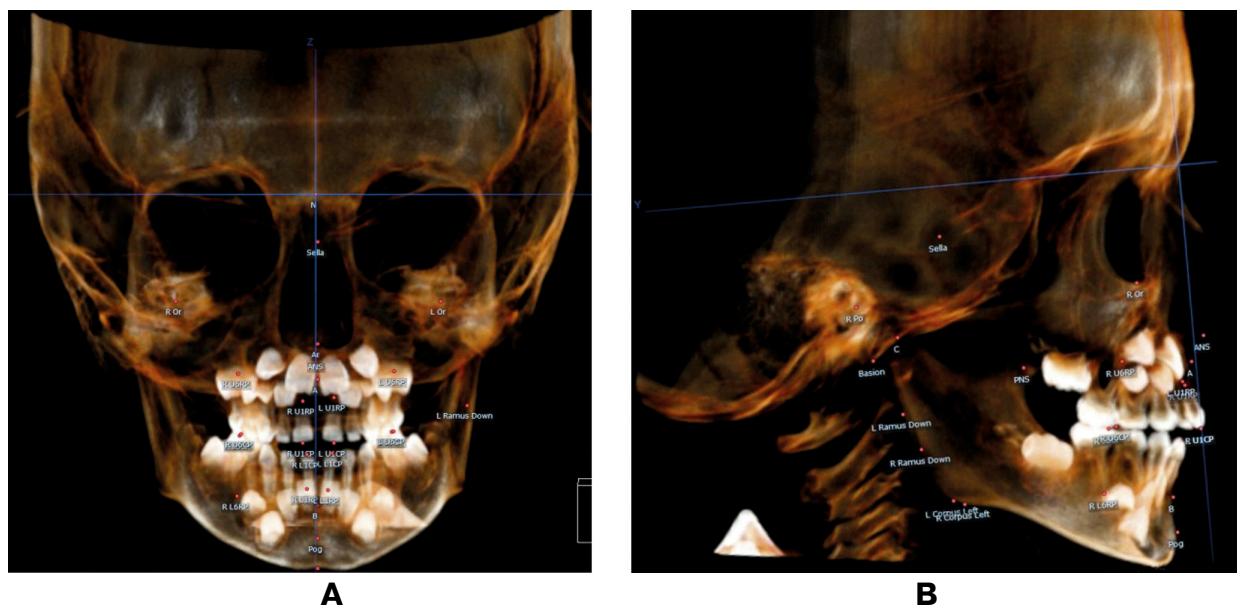
**Fig. 2.** Patient M., cephalometric radiograph: A – frontal view, B – lateral view

Рис. 2. Пациент М., телерентгенограмма: А – в прямой проекции, В – в боковой проекции

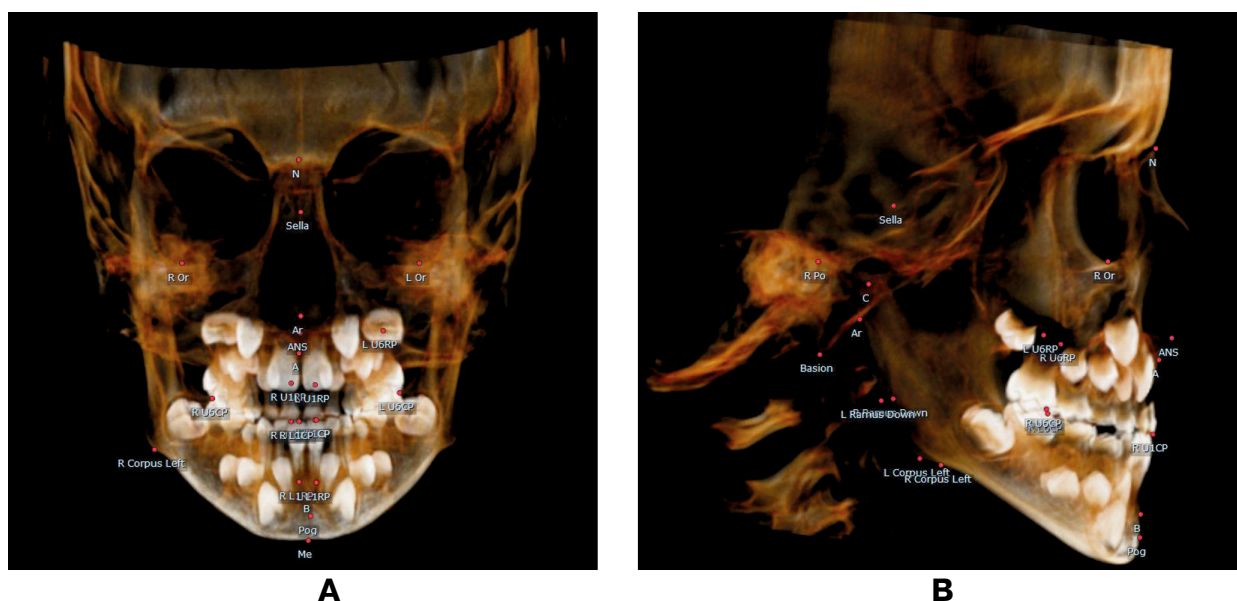
**Fig. 3.** Patient M., cephalometric radiograph in frontal (A) and lateral (B) views of a child following the eruption of the first permanent molars and mandibular incisors

Рис. 3. Пациент М., телерентгенограмма в прямой (А) и боковой (В) проекциях ребенка после прорезывания первых постоянных моляров и нижних резцов

second permanent molars, with the corresponding parameters demonstrating high reproducibility.

An increased torsion index in Group I children indicates an unbalanced activity of the masticatory muscles. In Groups II, III, and IV, the values were averaged, showing no predominance of either muscle group.

A cephalometric radiograph of patient M. from Group I, in both frontal and lateral views, is presented in Fig. 2.

Cephalometric radiograph of Patient P. from Group II, shown in both frontal and lateral projections, is presented in Fig. 3.

Cephalometric radiograph of Patient O. from Group III is presented in Fig. 4.

Cephalometric radiograph of Patient K. from Group IV is presented in Fig. 5.

Cephalometric radiograph of Patient V. from Group V is presented in Fig. 6.

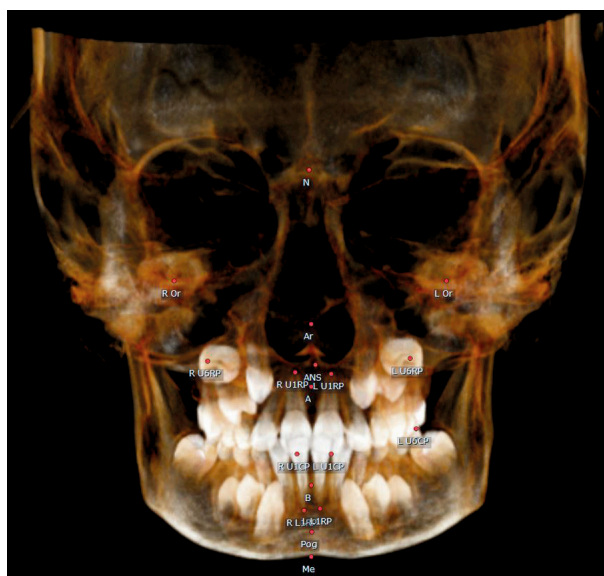
**A****B**

Fig. 4. Patient M., cephalometric radiograph in frontal (A) and lateral (B) views after the replacement of primary incisors (Fig. 5)

Рис. 4. Пациент М., телерентгенограмма в прямой (А) и боковой (В) проекциях после смены молочных резцов (рис. 5)

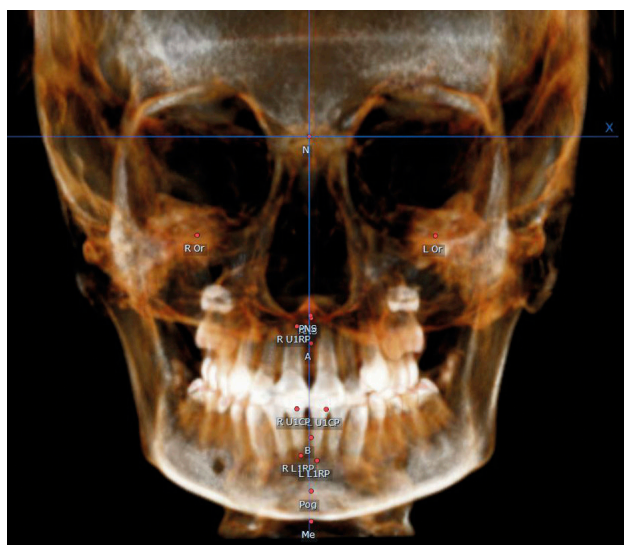
**A****B**

Fig. 5. Cephalometric radiograph in frontal (A) and lateral (B) views of a child after the complete replacement of all primary teeth

Рис. 5. Телерентгенограмма в прямой (А) и боковой (В) проекциях ребенка после смены всех молочных зубов

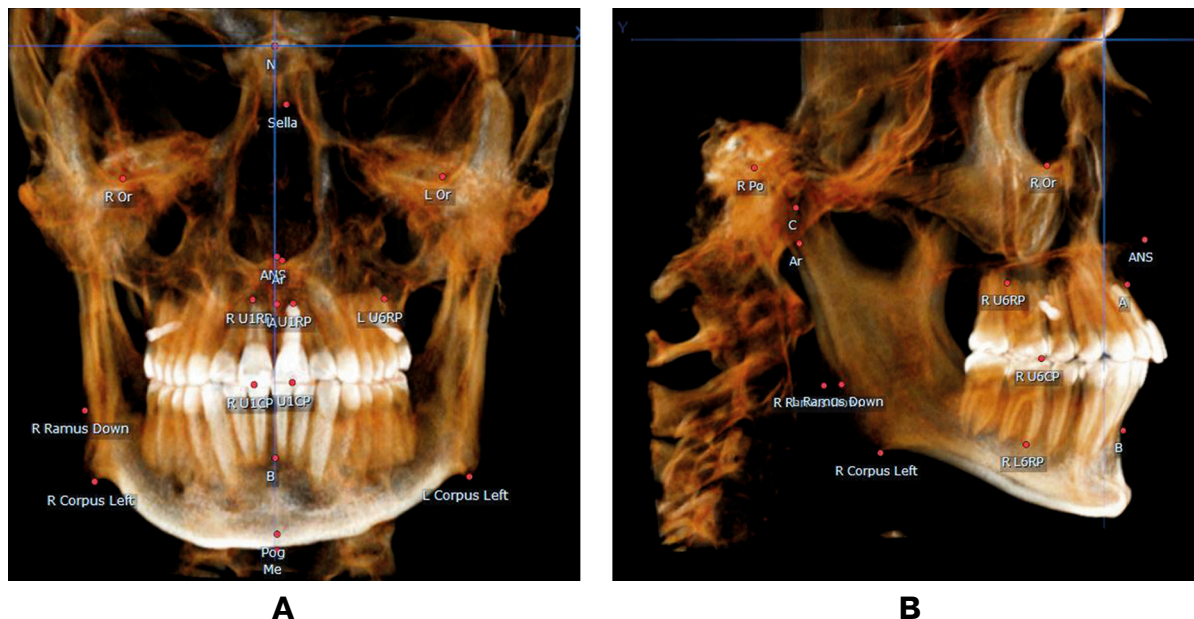


Fig. 6. Cephalometric radiograph in frontal (A) and lateral (B) views of a child after the eruption of the second permanent molars

Рис. 6. Телерентгенограмма в прямой (A) и боковой (B) проекциях ребенка после прорезывания вторых постоянных моляров

CONCLUSION

Thus, the present study demonstrated a direct correlation between jaw growth patterns during the transition from primary to permanent dentition and the bio-electrical activity of the mandibular elevator muscles. It was established that children in the final stage of primary dentition exhibited reduced functional activity of the masticatory muscles. During the eruption of the first permanent molars and mandibular incisors, as well as during the replacement of primary incisors with permanent ones, the bioelectrical activity remained slightly diminished. However, during the complete re-

placement of all primary teeth and the eruption of the second permanent molars, a clear symmetry and synchrony in the function of the masseter and temporalis muscles was observed, along with an increase in their functional activity.

It is noteworthy that even minor changes in occlusion during the mixed dentition period can alter the functional state of the masticatory muscles, potentially leading to neuromuscular imbalance. The more pronounced the changes in the dentoalveolar system and jaw structure, the more significant their impact on the neuromuscular system.

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Fiber post retrieval and furcal perforation repair in maxillary molar: A case report with one year follow-up

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Abstract

INTRODUCTION. Furcal perforation is a complex endodontic complication that can jeopardize treatment outcomes. Accurate diagnosis and appropriate management are essential to prevent periodontal breakdown and tooth loss.

AIM. This case report aims to present the non-surgical management of a furcal perforation in a maxillary first molar caused by fiber post-placement, emphasizing the role of CBCT, biomaterials, and magnification.

MATERIALS AND METHODS. A 33-year-old female presented with mild pain and swelling in the upper left maxillary region. Clinical examination revealed a sinus tract near the cervical area. Cone Beam Computed Tomography (CBCT) confirmed a furcal perforation with extrusion of a fiber post. The fiber post was carefully removed under magnification using ultrasonic tips. Hemostasis was achieved, and a calcium hydroxide dressing was applied to the perforation site to promote healing. After 10 days, the site was sealed with mineral trioxide aggregate (MTA). The tooth was then permanently restored following core buildup.

RESULTS. At the one-year follow-up, the tooth was asymptomatic. CBCT showed bone healing at the perforation site, and the sinus tract had resolved. Clinical and radiographic evaluation confirmed successful tissue repair and preservation of periodontal health.

CONCLUSIONS. This case highlights the importance of early detection and precise management of furcal perforations. The use of CBCT, magnification, and MTA enables predictable, non-surgical repair. Even in delayed cases, MTA provides effective sealing and promotes long-term clinical success.

Keywords: mineral trioxide aggregate, furcal perforation repair, root canal treatment, perforation repair

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Удаление стекловолоконного штифта и герметизация фуркационной перфорации у моляра верхней челюсти: клинический случай с годовым наблюдением

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Резюме

ВВЕДЕНИЕ. Фуркационная перфорация – это сложное эндодонтическое осложнение, которое может негативно повлиять на исход лечения. Точная диагностика и своевременное лечение имеют решающее значение для предотвращения разрушения пародонта и потери зуба.

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ЦЕЛЬ ИССЛЕДОВАНИЯ. Настоящий клинический случай иллюстрирует нехирургическое лечение фуркационной перфорации первого моляра верхней челюсти, возникшей в результате установки стекловолоконного штифта, с акцентом на значение КЛКТ, биоматериалов и увеличения.

МАТЕРИАЛЫ И МЕТОДЫ. 33-летняя пациентка обратилась с жалобами на умеренную боль и припухлость в области верхней левой челюсти. При клиническом осмотре был выявлен свищевой ход в пришеечной зоне. Конусно-лучевая компьютерная томография (КЛКТ) подтвердила наличие фуркационной перфорации с экструзией стекловолоконного штифта. Удаление штифта было выполнено под увеличением с использованием ультразвуковых насадок. После остановки кровотечения в область перфорации была внесена повязка с гидроксидом кальция для стимуляции репарации. Через 10 дней перфорация была герметизирована минеральным триоксидным агрегатом (МТА). Затем зуб был окончательно восстановлен с формированием культи.

РЕЗУЛЬТАТЫ. Через год после лечения зуб оставался клинически асимптоматичным. По данным КЛКТ наблюдалось восстановление костной ткани в зоне перфорации, свищевой ход был полностью закрыт. Клиническая и рентгенологическая оценка подтвердили успешное восстановление тканей и сохранение пародонтального здоровья.

ВЫВОДЫ. Данный клинический случай подчеркивает важность раннего выявления и точного нехирургического ведения фуркационных перфораций. Применение КЛКТ, оптического увеличения и МТА обеспечивает предсказуемое и эффективное восстановление даже при отсроченном вмешательстве, способствуя долгосрочному клиническому успеху.

Ключевые слова: минеральный триоксидный агрегат, герметизация фуркационной перфорации, лечение корневых каналов, восстановление перфорации

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INTRODUCTION

Furcal perforation is one of the most undesirable and frequently encountered procedural accidents in endodontics, occurring at various stages of treatment. It is often caused by using burs with inappropriate dimensions, improper angulation during pulp chamber ceiling removal, or attempts to locate root canal orifices in calcified pulp chambers. Accidental perforations account for up to 29% of all endodontic mishaps, with 87% of these occurring in the pulp chamber of molars. The management of perforations can be approached surgically or non-surgically, depending on the clinical scenario [1; 2].

The prognosis and treatment planning for this complication are influenced by key factors, including the etiology, location, size, and time elapsed before repair. Small, fresh perforations located in the coronal or apical third of the root generally have a favorable prognosis. Conversely, untreated perforations in pulp chamber floors are linked to poor outcomes, historically often necessitating tooth extraction. Although calcium hydroxide emerged as a repair material offering an alternative to extraction, its limited physical and chemical properties often resulted in suboptimal outcomes, particularly for larger perforations [2].

The introduction of mineral trioxide aggregate (MTA) in the 1990s marked a significant advancement in managing perforations. MTA, a calcium silicate-based material composed of mineral oxides such as tricalcium silicate, tricalcium aluminate, and tricalcium oxide, has been extensively studied and is recognized

for its superior performance in various clinical applications, including perforation repair, apexification, and pulp capping. With a high pH of 12.5 and the ability to set within approximately four hours in the presence of moisture, MTA offers enhanced sealing ability, biocompatibility, and long-term stability, making it a gold standard for managing complex cases of perforation [3; 4].

The objective of this case report is to describe the management of a pulpal floor perforation in a maxillary first molar, caused by the placement of a fiber post extending beyond the furcation area. The fiber post was carefully retrieved under a dental operating microscope, and the perforation was successfully repaired using mineral trioxide aggregate. Clinical and radiographic outcomes were evaluated over a one-year follow-up period.

CASE REPORT

A 33-year-old female patient visited the Department of Conservative Dentistry and Endodontics, reporting swelling in the gums and mild pain in the upper left back region of her oral cavity. Her dental history revealed that she had undergone endodontic treatment on the upper left first molar three months prior, and a post and core had been placed during the last procedure. The patient's medical history was non-contributory.

Upon initial clinical examination, a sinus tract was observed in the gingival mucosa near the radicular cervical region of the maxillary left first molar with small swelling (Fig. 1, A).

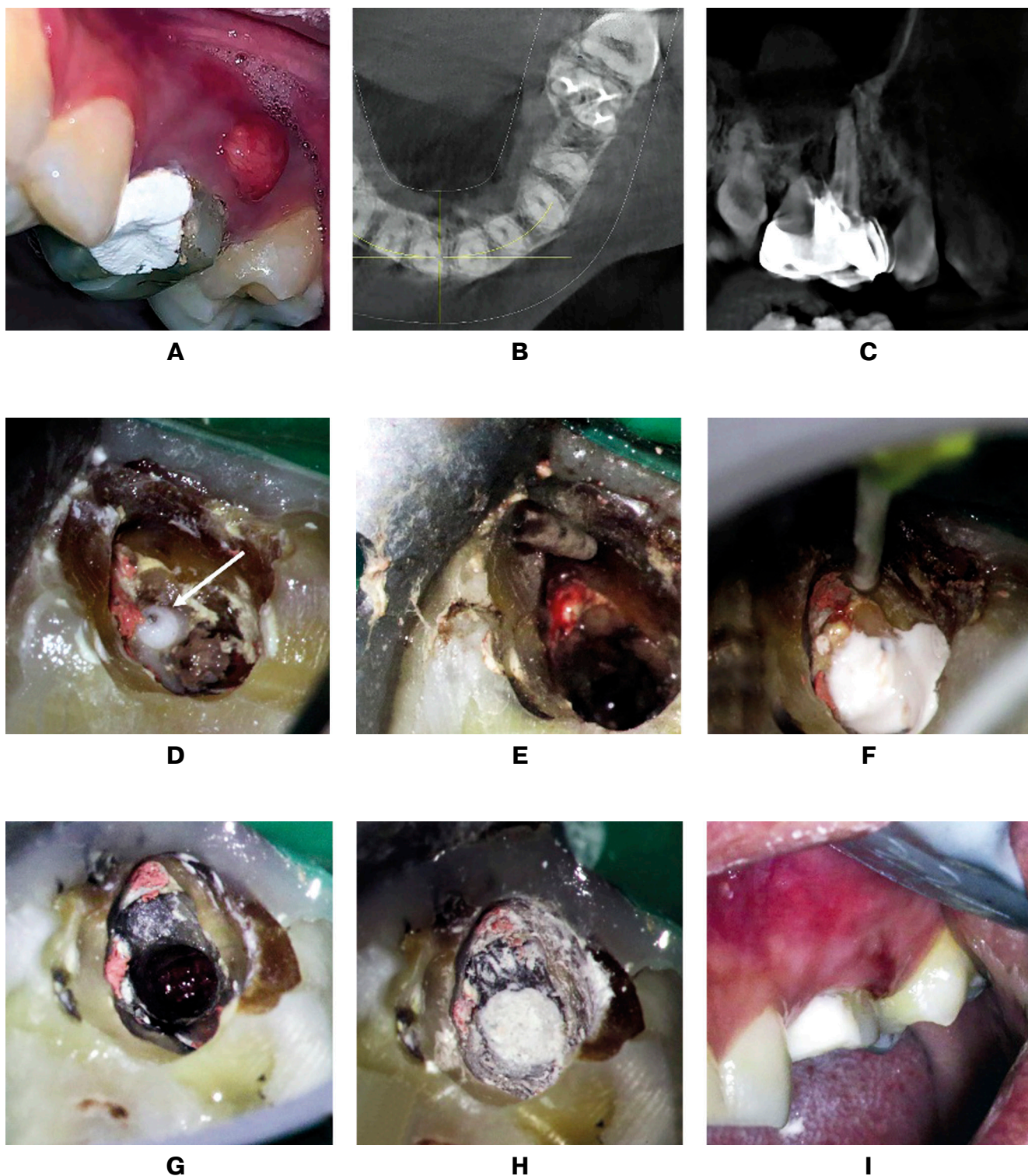


Fig. 1. Clinical examination of the patient: A – preoperative clinical image with sinus tract; B – axial CBCT showing perforation; C – coronal CBCT showing extruded fiber-post; D – pulp chamber floor with perforation (arrow); E – fiber-post retrieved; F – calcium hydroxide (CH) placement; G – follow-up after CH placement; H – MTA application; I – healed sinus

Рис. 1. Клинические обследования пациентки: А – предоперационное клиническое изображение с наличием свищевого хода; В – аксиальный срез КЛКТ, демонстрирующий область перфорации; С – корональный срез КЛКТ с визуализацией экструзии стекловолоконного штифта; D – дно пульпарной камеры с перфорацией (указано стрелкой); Е – извлеченный стекловолоконный штифт; F – внесение гидроксида кальция (CH); G – контрольное обследование после размещения CH; H – аппликация минерального триоксидного агрегата (MTA); I – заживший свищ

No pocket was present at probing. A cone-beam computed tomography (CBCT) scan of the area, revealed a radiopaque structure extending beyond the furcation, suggesting a perforation with a post in the furcation from the floor of the pulp chamber (Fig. 1, B & C). Since the previous endodontic therapy was in an acceptable state, only perforation repair was planned. The patient was informed about all available treatment options, including the prognosis for each. With the patient's consent, a non-surgical endodontic treatment was planned, to be performed under the dental operating microscope.

Absolute isolation of the operative field was achieved before proceeding. After coronal access, visual inspection at the pulp chamber floor confirmed the presence of a furcal perforation. A portion of the fiber post remained, extending beyond the furcation, as corroborated by Cone Beam Computed Tomography (CBCT) findings. Active bleeding from the perforation site indicated an unhealed and persistent defect around the fiber post (Fig. 1, D). The portion of the fiber post extending beyond the furcation was carefully removed using ultrasonic tips (ED 12 ultrasonic tips (Guilen Woodpecker, China) (Fig. 1, E) Bleeding at the furcation site was controlled with 2.5% sodium hypochlorite, and no granulation tissue was found. To aid healing, a water-based calcium hydroxide dressing (Ultracal XS, Ultradent, Jordan) was placed at the furcation site for 10 days (Fig. 1, F).

At the 10-day recall, the sinus opening had fully healed the swelling had regressed completely, and there was no bleeding at the perforation site (Fig. 1, G). The perforation was sealed with MTA Angelus, and a moist cotton pellet was placed in the pulp chamber to maintain a humid environment conducive to MTA solidification (Fig. 1, H). A CBCT showed extrusion of MTA into the periradicular tissues and closure of the defect. The

tooth was temporarily restored with Cavit until the next appointment.

The patient returned in 3 days, reporting no symptoms or discomfort and resolution of the sinus (Fig. 1, I). The temporary restoration and damp cotton pellet were carefully removed, and an explorer was used to assess the hardness of the MTA. The perforation site was covered with type II glass ionomer cement, followed by dual-cure core buildup using a total-etch protocol. The tooth was temporized with a provisional crown for one month and restored permanently.

Follow-up clinical and CBCT examinations conducted 365 days after the intervention demonstrated successful bone repair in the interradicular area, and the absence of any clinical signs or symptoms indicated the healing of the furcal perforation (Fig. 2).

DISCUSSION

Perforations can be managed using both surgical and non-surgical approaches. However, surgical interventions are often associated with complications such as loss of periodontal attachment, chronic inflammation and furcation defects. In contrast, non-surgical management has shown success rates exceeding 70% [5].

The prognosis of perforated teeth depends on several factors, including the extent of periodontal damage, perforation size, proximity to the gingival sulcus, time elapsed before repair, quality of the seal, procedural sterility, and the biocompatibility of the repair material. Optimal healing outcomes are achieved when perforations are sealed immediately. Nevertheless, in our case, a perforation that occurred three months prior also demonstrated signs of healing, highlighting that delayed repair can yield positive outcomes and should be attempted when feasible [6–8].

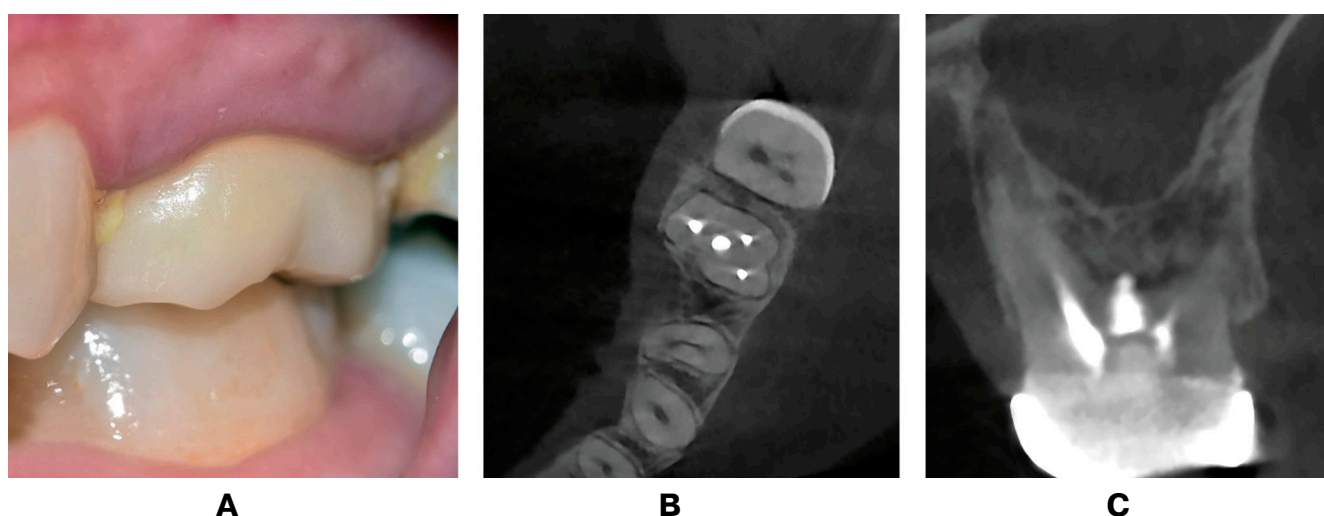


Fig. 2. One-year follow-up: A – clinical image showing absence of any sinus or swelling; B – axial CBCT showing sealed perforation area; C – coronal CBCT showing sealed perforation area

Рис. 2. Контроль через один год наблюдения: А – клиническое изображение, демонстрирующее отсутствие свища и отека; В – аксиальный срез КЛКТ, показывающий герметично закрытую область перфорации; С – корональный срез КЛКТ, демонстрирующий герметичную obturation зоны перфорации

As emphasized, accurately identifying the location of a perforation is essential for selecting the most appropriate repair material. In this case, CBCT imaging was instrumental in precisely locating the perforation and the position of the fiber post, thereby aiding in diagnosis. Additionally, CBCT provided detailed information about the defect's size, facilitated 3D reconstructions, and enabled evaluation of the morphology of surrounding structures [9].

The choice of repair material is critical to achieving favorable clinical outcomes. Ideal materials should possess appropriate physicochemical, antimicrobial, and biocompatible properties to effectively restore periodontal and dental architecture. These materials should induce minimal inflammation while promoting the formation of a mineralized tissue barrier over time [10; 11].

MTA has been extensively researched and is regarded as the gold standard for repairing furcal perforations. Systematic reviews of the histological response of the periodontium to MTA consistently highlight its ability to meet the criteria for an ideal repair material [12; 13].

One significant challenge in non-surgical perforation repair is preventing the extrusion of material into peri-

radicular tissues. Although various barriers have been proposed, their efficacy remains limited. MTA, with its superior sealing ability, biocompatibility, and capacity to promote cementum formation – even when extruded into periradicular tissues – is considered the ideal material for repairing large perforations, even in the absence of a barrier [13].

This case report underscores the importance of clinician experience and the use of magnification in successfully managing perforations, aligning with findings from other studies [14; 15].

CONCLUSION

This case report highlights the successful clinical management of furcal perforations repaired using MTA, demonstrating positive clinical outcomes. It underscores the importance of a systematic approach to diagnosis and treatment planning for achieving favorable clinical and radiographic results. Advances in imaging technology, biomaterials, and magnification have significantly improved clinicians' ability to handle complex perforations. CBCT serves as a valuable imaging modality that can be utilized in select cases to aid in diagnosis and treatment planning.

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Ex vivo study of the efficacy of two irrigation systems in retreatment of root canals filled with bioceramic sealers

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Abstract

AIM. To evaluate the presence of residual filling material in the root canal walls after retreatment and final irrigation using scanning electron microscopy (SEM).

MATERIALS AND METHODS. Fifty-two mandibular oval premolars were instrumented with ProTaper Next X1–X3 and divided into 2 groups: Group BC, filled with Bio-C Sealer and Group AH, filled with AH Plus. The experimental groups were retreated with a ProTaper Next X4 file and received different irrigation protocols ($n = 13$): Group BC-PUI: agitation with ultrasound; Group BC-EC: agitation with Easy Clean; Group AH-PUI: agitation with ultrasound; and Group AH-EC: agitation with Easy Clean. The remaining filling material in the dentinal tubules was evaluated in scanning electron microscope images by three evaluators who scored the samples. The Kappa test was performed to check interexaminer agreement. Pearson's chi-square test with a significance level < 0.05 was performed to compare the groups.

RESULTS. It was found that the BC-EC group showed better results in the apical and middle thirds. In the coronal third, the BC-PUI group also performed well. AH Plus showed the lower results in all situations.

CONCLUSIONS. In the apical third, the BC-EC group showed better performance, in the middle third, the AH-PUI group had the worst performance, and in the coronal third, the BC-PUI group stood out in terms of cleaning. This manuscript provides the importance of thorough cleaning, especially in difficult areas such as the apical third, is emphasized to improve the overall outcome of treatment.

Keywords: retreatment, ultrasound, scanning electron microscopy

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Экспериментальное *ex vivo* исследование эффективности двух ирригационных систем при повторной обработке корневых каналов, obturированных биокерамическими силерами

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Резюме

ЦЕЛЬ. Оценить наличие остаточного пломбировочного материала на стенках корневого канала после повторной обработки и финальной ирригации с использованием сканирующей электронной микроскопии (СЭМ).

МАТЕРИАЛЫ И МЕТОДЫ. Пятьдесят два овальных нижнечелюстных премоляра были инструментированы системой ProTaper Next X1–X3 и разделены на две группы: группа BC – obturация биокерамическим силером Bio-C Sealer, группа AH – obturация AH Plus. Экспериментальные группы подвергались повторной обработке инструментом ProTaper Next X4 и различным протоколам ирригации ($n = 13$): BC-PUI – активация ирриганта ультразвуком; BC-EC – активация с помощью Easy Clean; AH-PUI – активация ультразвуком; AH-EC – активация с помощью Easy Clean.

Остаточный пломбировочный материал в дентинных канальцах оценивался в сканирующем электронном микроскопе тремя независимыми экспертами по бальной системе. Для оценки согласованности между экспертами применялся коэффициент Каппа. Для статистического сравнения между группами использовался χ^2 -критерий Пирсона при уровне значимости $<5\%$.

РЕЗУЛЬТАТЫ. Группа BC-EC продемонстрировала лучшие результаты в апикальной и средней трети. В коронковой трети наилучшие показатели были зафиксированы в группе BC-PUI. Наихудшие результаты во всех зонах показала группа с силером AH Plus.

ВЫВОДЫ. В апикальной трети группа BC-EC обеспечила наилучшую очистку, в средней трети наилучшие показатели зафиксированы в группе AH-PUI, а в коронковой трети лучшую очистку обеспечила группа BC-PUI. Настоящее исследование подчеркивает важность тщательной очистки корневого канала, особенно в труднодоступных зонах, таких как апикальная треть, для повышения эффективности эндодонтического лечения.

Ключевые слова: повторная обработка, ультразвук, сканирующая электронная микроскопия

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INTRODUCTION

The root canal system has ramifications and isthmus areas, which makes the disinfection process a real challenge. The presence of microorganisms in the root canals is the main cause of failure of endodontic treatment [1].

Conventional retreatment is the first treatment option in case of endodontic treatment failure. Effective removal of obturation material is an extremely important

step to allow better cleaning and disinfection of the root canal system, so that irrigants and instruments can act along the entire canal [2].

Irrigation solution activation [3] has been used to improve the cleaning process and promote smear layer removal, facilitating root canal system disinfection [4]. The Easy Clean (EC) plastic instrument (Bassi/Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) has the shape of an “airplane wing” and can be used in

a rotary or reciprocal motion to move the irrigation solution, which promotes a mechanical action, thus improving the cleaning of the canal walls [5; 6].

The epoxy resin-based sealer AH Plus (Dentsply/Maillefer, Ballaigues, Switzerland) is considered the gold standard sealer due to its high bond strength to dentin [7–9]. Bioceramic sealers are increasingly used in endodontic practice due to their physicochemical and biological properties [10]. They are biocompatible materials that are stable and non-toxic in the biological environment [11].

Several studies have used SEM to verify the amount of remaining filling material in the dentinal tubules [12–14]. However, few studies have investigated irrigation methods in the removal of bioceramics [3; 15].

The aim of this study was to use SEM to evaluate the efficacy of retreatment with two different rinsing systems in teeth previously filled with resin sealer or bioceramics. The null hypotheses tested were that the amount of residual filling material after retreatment would be the same between (i) the two endodontic sealers and (ii) the irrigation systems.

MATERIALS AND METHODS

The present study was submitted to and approved by the local research ethics committee under number 3.090.544. The sample was calculated based on a previous study [12] using the statistical test ANOVA, with a minimum difference between treatment means of 4, a test power of 0.80, and an alpha of 0.05. Fifty-two extracted oval human mandibular premolars (bucco-lingual diameter twice the mesio-distal diameter along the first two-thirds of the canal) were selected and kept in 0.1% thymol solution until the start of the study. Teeth with single canal roots, complete rhizogenesis, and a curvature angle between 0° and 10° according to the method of Schneider [16] were included in the study.

All roots with an angle of curvature greater than 10°, with incomplete rhizogenesis, root fractures and/or perforations, and previously treated canals or whose patency was not achieved were excluded.

Teeth were standardized to 15 mm by leveling their occlusal surfaces with a 223 Carboril disk (Dentorium, New York, USA). The working length (WL) was determined by inserting a #10 K-file into the canal using a magnifying glass at 3.5x magnification until its tip appeared at the apical end of the root, subtracting 1 mm.

Preparation of the specimen

The canals were instrumented with ProTaper Next X1–X3 rotary files (Dentsply Sirona, Charlotte, USA) driven by an X Smart Plus motor (Dentsply/Maillefer) in a rotary motion with a torque of 2.0 Ncm and a speed of 300 rpm, with in and out movements and brushing of the canal walls.

The irrigant used was 2.5% sodium hypochlorite (NaOCl) in a 5 mL luer syringe (Descarpack, São Paulo, Brazil) and a 27G Endo-Eze needle (Ultradent, Indaiatuba, Brazil) 3 mm from the apex. Irrigation was performed with 5 mL of solution for each file removal, for a total of

15 mL of irrigation fluid. The final irrigation protocol was performed with 3 cycles of 20 seconds of 17% EDTA and 3 cycles of 20 seconds of NaOCl [4], irrigated with EC and finally rinsed with saline. The canal was dried with ProTaper Next X3 absorbent paper tips (Dentsply Sirona, Ballaigues, Switzerland).

Teeth were randomly divided into 2 groups ($n = 26$): Group AH, filled with AH Plus (Dentsply Sirona, Charlotte, USA) and Group BC, filled with Bio-C Sealer Bioceramic (Angelus, Londrina, Brazil). Both sealers were manipulated according to the manufacturers' recommendations and placed in the canal through a preselected gutta-percha cone (ProTaper Next X3 (Dentsply Sirona, Charlotte, USA)). The single cone technique was used to perform the root canal filling.

The roots were radiographed in the buccal-lingual and mesio-distal directions to assess the quality of the filling. The access cavities were filled with Cavit temporary filling material (3M ESPE, Saint Paul, USA). All roots were stored in an oven at 37°C and 100% humidity for 2 weeks to allow the sealers to cure.

For canal initial retreatment Gates-Glidden (Dentsply Sirona, Ballaigues, Switzerland) drills Nos. 2 and 3 were used in the cervical third and ProTaper Next X4 (40.06) rotating files coupled to an X Smart Plus motor and rotating at a speed of 300 rpm and a torque of 2.0 Ncm were used in the middle and apical thirds. Each instrument was used on three teeth and discarded. Rinsing was performed with 15 mL of NaOCl. As a criterion for assessing clearance, the canal walls should be smooth, and the instrument used should not contain any visible obturation material under a magnifying glass with 3.5x magnification.

According to the clearance procedures, the specimens were divided into 4 subgroups ($n = 13$) according to the irrigation system used:

- AH + PUI: Teeth were filled with AH Plus and ultrasonic activation was performed with an Irrisonic tip (Helse, Santa Rosa do Viterbo, São Paulo) in a Gnatus ultrasonic device with 10% power 1 mm below the WL;

- AH + EC: Teeth were filled with AH Plus, and irrigation fluid was moved 1 mm below the WL in a reciprocal motion in WaveOne all mode using EC in an X Smart Plus motor;

- BC + PUI: Teeth filled with Bio-C Sealer + agitation of the irrigant with PUI. The procedures were the same as for AH + PUI;

- BC + EC: Teeth filled with Bio-C Sealer + agitation of irrigation solution with EC. The procedures were the same as for AH + EC.

The rinsing protocol for all groups was the same as for endodontic treatment. After the final irrigation, the specimens were dried with paper points and a gutta-percha cone was inserted to WL. Using the 1190 FF drill (KG Sorensen, Cotia, Barsil), two grooves were made to near the root canal on the buccal and lingual walls along the entire length of the root longitudinally without connection to the main canal. The specimens were washed under running water to remove debris. A No. 24 spatula was used to apply a vertical force that caused the separation of the root halves [17].

The most intact half of each root was selected. Horizontal slits were drilled with a drill 1190 FF (KG Soresen, Serra, Brazil) at 3, 6 and 9 mm from the anatomical apex to determine the thirds examined: apical, middle, and coronal.

After drying, specimens were mounted on stubs with double-sided tape, coated with gold in the metallizer (Denton Vacuum, Moorestown, USA), and assessed by SEM (JSM-6390LV, JEOL, Tokyo, Japan) for the root canal walls evaluation. Stepwise magnifications of 10, 100, and up to 2000x were performed until the delineated areas (3, 6, and 9 mm from the apex) were visible, and three photographs were taken at each mark.

Statistical analysis

The images were sent in slide format in the Microsoft Office PowerPoint program to three calibrated examiners for evaluation. A score (Fig. 1) was assigned for each image based on the study by Pirani et al. [18].

For inter-rater analysis, the Kappa test was performed. After applying the Kolmogorov-Smirnov test, association analysis was performed using Pearson's chi-square test and Student's *t* test, both with a significance level of 5%. The Minitab version 17 program was used.

RESULTS

A total of 156 images were obtained and evaluated by 3 calibrated examiners. Interexaminer agreement was calculated for each of the thirds. Then, 4 experimental groups were compared in each third. In the apical third, the BC+EC group showed better results (Table 1).

In the middle third, there was similar behavior between the AH+EC / BC+PUI and BC+EC groups compared with the AH+PUI group (Table 2). The AH+PUI group showed the worst results, with a statistical difference compared with AH+EC ($p = 0.01$). There was also a statistical difference between the BC+PUI and BC+EC groups ($p < 0.00$) (Table 2).

In the coronal third, the BC+PUI group had the best performance compared with all others, followed by AH+PUI and AH+EC, with no statistical difference between them, followed by the control group and BC+EC (Table 3).

When comparing the individual groups, a statistical difference was found between the groups AH+PUI ($p = 0.01$); AH+EC ($p < 0.00$) and BC+PUI ($p < 0.00$). Performance was similar for the other groups (Table 4).

In summary, in the apical and middle mm third, the BC+EC group performed better overall, and in the coronal third, the BC+PUI group performed better.

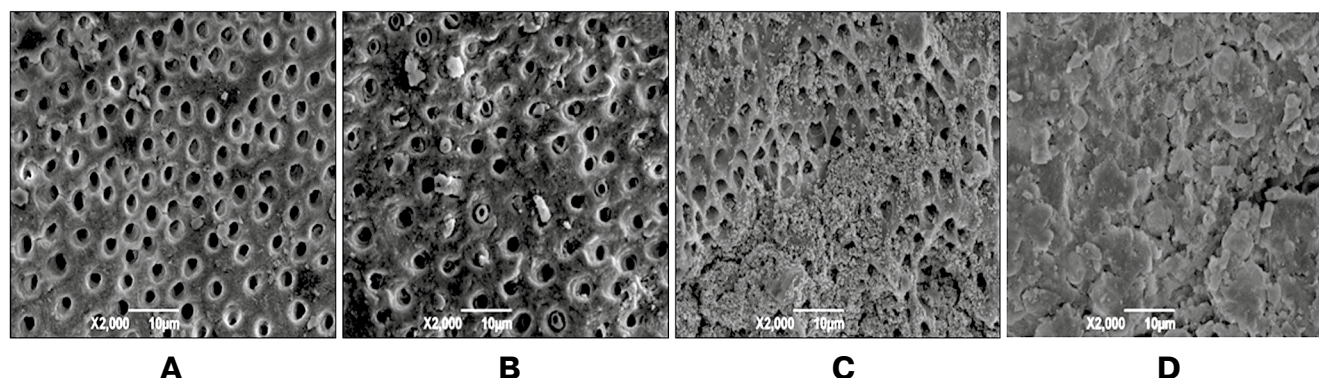


Fig. 1. SEM images of the evaluated areas: A – Score 1: smear layer/debris not present, more than 75% of tubules open; B – Score 2: less than 75% of tubules open; C – Score 3: tubules visible in limited areas, less than 50% of tubules visible; D – Score 4: homogeneous smear layer/debris, tubules not visible

Рис. 1. СЭМ-изображения оцененных участков: А – балл 1: смазанный слой/дебрис отсутствует, более 75% дентинных канальцев открыты; В – балл 2: открыто менее 75% канальцев; С – балл 3: канальцы видны лишь на отдельных участках, открыто менее 50% канальцев; D – балл 4: однородный смазанный слой/дебрис, канальцы не визуализируются

Table 1. Percentage of the score by groups in the apical third

Таблица 1. Процентное распределение баллов по группам в апикальной трети

Group / score	Controle	AH + PUI	AH + EC	BC + PUI	BC + EC*	p-value*
1	5.13	–	–	–	20.51	–
2	20.51	23.08	2.56	–	23.08	< 0.01
3	20.51	23.08	35.90	30.77	33.33	–
4	53.85	53.84	61.54	69.23	23.08	–

Note: * Difference between the BC + EC group compared to all other groups

Примечания: * Различия между группой BC + EC и всеми другими группами

Table 2. Percentage of the score by groups in the middle third**Таблица 2.** Процентное распределение баллов по группам в средней трети

Group / escore	Controle ^a	AH+PUI ^b	AH+EC ^{a, b}	BC+IUP ^{a, b}	BC+EC ^{a, b}
1	7.69	–	7.69	20.51	2.56
2	35.90	30.77	48.72	28.21	43.59
3	25.64	64.10	20.51	43.59	46.15
4	30.77	5.13	23.08	7.69	7.69

Note: a – control X BC+PUI / BC+EC – $p < 0.00$; b – AH+PUI X AH+EC / BC+EC / BC+PUI – $p = 0.037$

Примечания: a – контроль X BC+PUI / BC+EC – $p < 0.00$; b – AH+PUI X AH+EC / BC+EC / BC+PUI – $p = 0.037$

Table 3. Percentage of a score by groups in the coronal third**Таблица 3.** Процентное распределение баллов по группам в коронковой трети

Group / escore	Controle ^a	AH+PUI ^b	AH+EC ^b	BC+PUI ^c	BC+EC ^a	p-value*
1	23.08	17.95	28.21	53.85	20.51	
2	17.95	43.59	53.85	35.90	10.26	< 0.00
3	35.90	30.77	10.26	10.26	53.85	–
4	23.08	7.69	7.69	–	15.38	–

Note: * Difference between the BC+PUI group and all others; a – AH+PUI / AH+EC x control – $p = 0.023$

Примечания: * Различия между группой BC+PUI и всеми остальными группами; a – AH+PUI / AH+EC по сравнению с контролем: $p = 0.023$

Table 4. p-value of the difference in proportion between the experimental groups in each third***Таблица 4.** p-значения различий в пропорциях между экспериментальными группами в каждой трети*

Group	Apical	Middle	Coronal
AH+PUI x AH+EC	0.59	0.01	0.87
AH+PUI x BC+PUI	0.27	0.35	0.14
AH+PUI x BC+EC	0.01	0.20	0.01
AH+EC x BC+PUI	0.35	0.02	0.23
AH+EC x BC+EC	< 0.00	0.25	0.02
BC+PUI x BC+EC	< 0.00	0.03	< 0.00

Note: * Chi-square test with a significance level of 95%

Примечания: * Критерий χ^2 при уровне значимости 95%

DISCUSSION

This study investigated the presence of residual filling material in the root canal wall after retreatment and final irrigation using SEM. The null hypotheses tested were rejected because there were differences in both the sealers and the irrigant agitation methods employed.

The removal of filling material is a challenge during the endodontic treatment. To address this, several methods were investigated, including manual, rotary, and reciprocating instrumentation, as well as additional irrigant agitation methods. However, none of the methods has been shown to completely remove the filling material on the dentinal tubules [2; 5; 19].

Mandibular premolars present challenges for both treatment [20] and retreatment [21] due to their oval anatomy, which can potentially lead to a large percentage of unprepared root canal walls. In this study, the ProTaper

Next system was used for instrumentation and retreatment [19], with a larger instrument tip for retreatment compared to treatment [22].

The manufacturer of Irrisonic recommends using 2 mm short of the WL, while EC can be used at the WL, but the two instruments used to agitate the solutions were used 1 mm below the WL as a way of standardization for this study.

The evaluation of the samples was conducted using SEM, a standard technique for observing smear layer morphology, the presence of debris within the dentinal tubules, and intratubular dentin morphology [13; 14; 18; 23; 24]. A disadvantage of using SEM is the lack of standardization of the site to be evaluated. In this study, this factor was circumvented by previously delineating the areas to be studied.

In the present study, the quality of cleaning of the dentinal tubules of the coronal, middle, and apical thirds was evaluated, and it was found that there were significant differences between the methods of agitation of the irrigants with respect to these regions. EC was more effective in the apical and middle thirds, while in the coronal third PUI showed better results, which corresponds with a previous study [17] that showed that EC was more effective than PUI in removing the smear layer in the apical third of the canals. However, other studies [5; 25; 26] found no significant differences in cleaning the root canal thirds after endodontic retreatment. PUI performed well in retreatment of maxillary premolars filled with Bio-C Sealer [27]. A possible explanation for the better performance of EC in the apical third is its design: it lacks a cutting blade, is made of plastic, and therefore does not damage the canal walls, whereas ultrasound requires a larger space to achieve better results, which is not the case in the apical third.

Our results corroborate the assertion that sealers penetration depth is directly related to the diameter of tubules, which are more numerous and large in the middle and cervical thirds [28]. Both AH Plus sealer and bioceramics have sufficient fluidity to penetrate the dentin recesses and tubules of the canal, making complete removal of these materials virtually impossible [12]. The bioceramic sealer tested was easier to remove, leaving the tubules free of debris and consequently contributing to a higher rate of treatment success, possibly related to the solubility of this cement [29].

This study had some limitations, including the absence of information regarding the age and sex of the tooth donors, which could affect the interpretation of the results. In addition, the presence of tubular sclerosis poses a challenge to the standardization of the samples used in this study because they were sourced from

a dental bank [23]. Also, the method by which the teeth were sectioned may influence the outcomes and lead to debris and [30], displacement of the remaining obturator material [22].

It is important to note that the study was conducted on straight canals and further studies should investigate using canals with more complex anatomy. In addition to anatomy, different methods, and instruments as well as the type of obturator material may influence the outcomes.

CONCLUSION

It is concluded that in the apical third, the BC-EC group performed better, in the middle third, the AH-PUI group had the worst performance, and in the coronal third, the BC-PUI group excelled in terms of cleaning, however, none of the methods were able to completely remove the filling material.

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Endodontic management of C-shaped canals: A case series

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Abstract

INTRODUCTION. C-shaped root canals represent a complex anatomical variation, primarily seen in mandibular molars. Their intricate morphology poses significant challenges for diagnosis, cleaning, shaping, and obturation in endodontic practice.

AIM. To present a series of clinical cases illustrating the diagnosis and endodontic management of different types of C-shaped canals based on Fan's classification.

MATERIALS AND METHODS. Four clinical cases involving mandibular molars with C-shaped canal configurations were managed in accordance with CARE and PRICE guidelines. Each case included clinical and radiographic diagnosis, working length determination, cleaning and shaping with either the R2 Reciproc (VDW) or PlexV (Orodeka) system, irrigation with NaOCl and EDTA under ultrasonic activation, and obturation using thermocompaction or single-cone techniques with bioceramic sealers.

RESULTS. All cases demonstrated successful identification and management of C-shaped canal systems (types C1 to C4). Clinical and radiological follow-up at 1 and 3 months showed favorable healing with resolution of symptoms and periapical pathology.

CONCLUSIONS. Early and accurate diagnosis of C-shaped canals, supported by clinical and radiographic examination, is critical for successful endodontic outcomes. The use of magnification, appropriate canal preparation techniques, and bioceramic materials significantly enhances treatment success in such complex anatomical variations.

Keywords: C-shaped canal, endodontic treatment, bioceramic sealer, mandibular molar, case series

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Эндодонтическое лечение С-образных каналов: серия случаев

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Резюме

ВВЕДЕНИЕ. С-образные корневые каналы представляют собой сложный анатомический вариант, преимущественно встречающийся в нижних молярах. Их сложная морфология создает серьезные трудности при диагностике, обработке, расширении и obturации в эндодонтической практике.

ЦЕЛЬ. Представить серию клинических случаев, иллюстрирующих диагностику и эндодонтическое лечение различных типов С-образных каналов согласно классификации Фана.

МАТЕРИАЛЫ И МЕТОДЫ. Четыре клинических случая, включавших нижние моляры с С-образной конфигурацией каналов, были обработаны в соответствии с рекомендациями CARE и PRICE. В каждом случае проводилась клиническая и радиологическая диагностика, определение рабочей длины, механическая обработка системами R2 Reciproc (VDW) или PlexV (Orodeka), ирригация растворами NaOCl и ЭДТА с ультразвуковой активацией, а также obturация термокомпакцией или методом одиночного конуса с применением биокерамических силеров.

РЕЗУЛЬТАТЫ. Во всех случаях была успешно проведена идентификация и лечение С-образных каналов (типов C1–C4). Контрольные клинические и рентгенологические обследования через 1 и 3 месяца показали положительную динамику: купирование симптомов и признаки заживления периапикальных тканей.

ВЫВОДЫ. Ранняя и точная диагностика С-образных каналов на основании клинических и радиологических данных является ключевым фактором успешного эндодонтического лечения. Применение увеличения, адекватных методов обработки каналов и биокерамических материалов существенно повышает эффективность лечения при наличии сложных анатомических вариаций.

Ключевые слова: С-образный канал, эндодонтическое лечение, биокерамический силер, нижний моляр, серия клинических случаев

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INTRODUCTION

Understanding the internal anatomy of the tooth is a crucial condition in endodontic therapy. The considerable diversity in the root canal system underscores the importance of treatment strategies guided by an understanding of tooth anatomy [1]. This approach broadens the scope for accommodating and addressing these variabilities, thereby enhancing the success rate of endodontic treatments.

Among these anatomical variations, C-shaped canals are frequently observed in lower molars and premolars. These canals derive their name from the configuration formed when the root canals partially or completely merge, a characteristic visible in cross-sectional views.[2]

Early detection of canal with C-shaped configuration can assist the clinician in effective negotiation and preparation, thereby averting irreversible damage that could potentially compromise the integrity of the tooth.

This study, aimed to discuss the clinical and radiological diagnosis of C-shaped canals as well as their management through clinical cases.

CASE REPORTS

This case series was conducted in compliance with the CARE guidelines, the 2020 PRICE (Preferred Reporting Items for Case reports in Endodontics) guidelines and COPE recommendations to ensure transparent and standardized reporting. All patients were thoroughly informed about the complexity and specific fea-

tures of their cases, as well as the proposed endodontic procedures. Written informed consent was obtained from each patient for the treatment and for the use of their clinical images and radiographs in this publication.

CASE 1: C-SHAPED CANAL TYPE C1

A female patient in her 20's consulted our department for continuous spontaneous pain that did not respond to analgesics in regard with the tooth 46. The apical diagnosis was a symptomatic apical periodontitis.

The preoperative radiograph demonstrates a C-shaped root canal type 1 of Fan [3], characterized by a conical structure, featuring a subtle radiolucent longitudinal line dividing the root into discernible mesial and distal sections. The mesial and distal canals unite into a singular canal prior to emerging at an open apex [3] (Fig. 1).

A Type I C-shaped root canal with a fan-shaped configuration was identified.

Thus, the treatment plan involved nonsurgical root canal therapy. At the first appointment and after rubber dam isolation, access cavity was performed, revealing a C-shaped canal type I of Fan (Fig. 2).

After radiographic determination of the working length (Fig. 3), the root canal preparation was conducted using R2 file Réciproc (VDW). During instrumentation, a copious irrigation with 2.5% sodium hypochlorite was frequently renewed and associated to an ultrasonic activation. The final activation sequence was performed with an alternation of 2.5% NaOCl, 17% EDTA, and 2.5% NaOCl for 30 seconds each.



Fig. 1. Preoperative radiograph preparation showing

Рис. 1. Предоперационная рентгенограмма



Fig. 2. Access cavity preparation showing C-shaped canal type I of Fan

Рис. 2. Подготовка полости доступа с визуализацией С-образного канала типа I по Фану



Fig. 3. Determination of working length

Рис. 3. Определение рабочей длины

Calcium hydroxide was given as intracanal medication and the coronal access cavities were temporarily sealed. Two weeks later, the tooth was asymptomatic. The canals were once again debrided, dried, and then filled in 2 steps.

We begin with a Biodentin apical plug and then we obturated the rest of the canals using the thermo-compaction technique (Revo-condensor, MicroMega) (Fig. 4).

During clinical and radiological follow-ups, at 1 month and 3 months, the tooth was asymptomatic demonstrated periapical healing (Fig. 5, 6).

Case 2: C-shaped canal type C2

A female patient in her 40's presented to the dental medicine department in our hospital with asymptomatic apical periodontitis. Radiographic examination showed a type I root configuration, characterized by a tapered root with a subtle radiolucent longitudinal line dividing the root into distinct mesial and distal segments, with the mesial and distal canals converging into a single canal before exiting at the apical foramen [3] (Fig. 7).

The treatment plan included access cavity preparation revealing a semicolon-shaped canal resulting from the discontinuation of the "C" line [4] (Fig. 8).

Radiographic working length determination (Fig. 9, A), cleaning and shaping with the PlexV (Orodeka) system, irrigation with 5.25% sodium hypochlorite associated with ultrasonic activation, and obturation using the single-cone technique and a bioceramic sealer One-fil (MediClus) (Fig. 9, B, C).

Case 3: C-shaped canal type C3

A female patient in her 50's presented for endodontic treatment of tooth 47, exhibiting asymptomatic apical periodontitis and a clinical diagnosis of two separate canals, classified as a Fan type C3 C-shaped canal [4]. Radiographic examination revealed a type I root configuration, characterized by a conical structure with a subtle radiolucent longitudinal line dividing the root into distinct mesial and distal segments, where the canals converge into a single canal before exiting at the apical foramen (Fig. 10) [3].

The treatment plan consisted of access cavity preparation (Fig. 11), working length determination, cleaning and shaping using the PlexV (Orodeka) system, irrigation with 5.25% sodium hypochlorite associated to an ultrasonic activation, and obturation using the single-cone technique with bioceramic sealer One-fil (MediClus) (Fig. 12, 13).



Fig. 4. Periapical radiograph showing the apical plug

Рис. 4. Периапикальная рентгенограмма, показывающая апикальную пробку

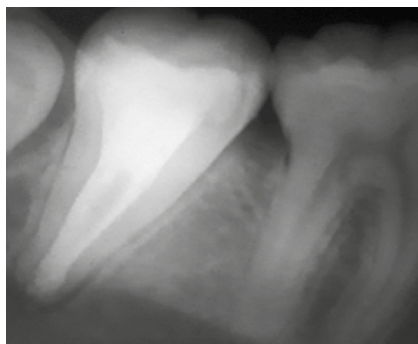


Fig. 5. One month Follow-up radiograph

Рис. 5. Контрольная рентгенограмма через месяц

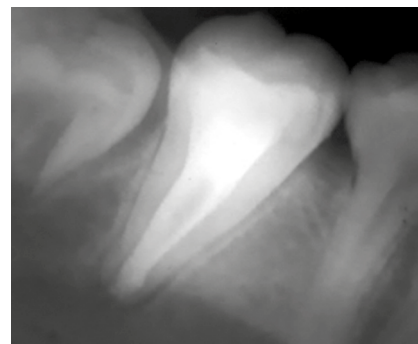


Fig. 6. Three months Follow-up radiograph

Рис. 6. Контрольная рентгенограмма через 3 месяца



Fig. 7. Preoperative radiograph

Рис. 7. Предоперационная рентгенограмма

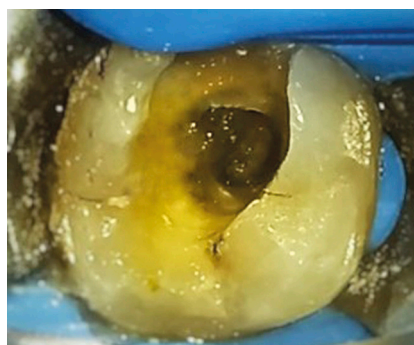


Fig. 8. Clinical examination showing C-shaped canal type C2

Рис. 8. Клиническое обследование, показывающее С-образный канал типа C2

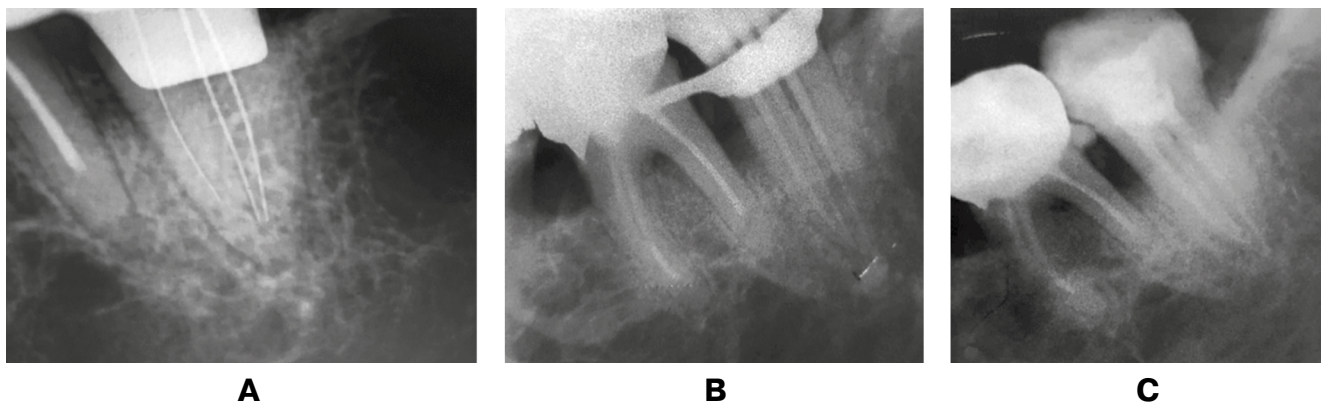


Fig. 9. Perioperative radiographs: A – working length determination; B – cone-fit radiograph; C – post operative radiograph

Рис. 9. Интраоперационные рентгенограммы: A – определение рабочей длины; B – рентгенограмма с конусовидной формой; C – послеоперационная рентгенограмма



Fig. 10. Preoperative radiograph

Рис. 10. Предоперационная рентгенограмма

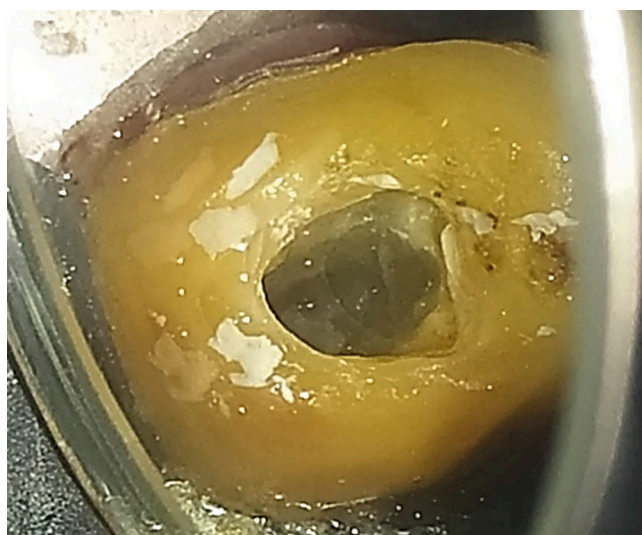


Fig. 11. Clinical examination showing A C-shaped canal type C3

Рис. 11. Клиническое обследование, показывающее С-образный канал типа C3

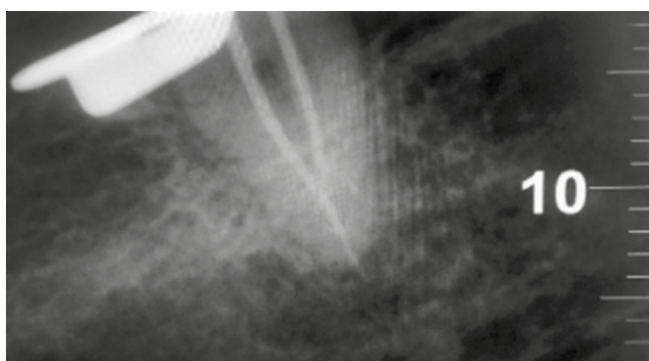


Fig. 12. Cone-fit radiograph

Рис. 12. Рентгенограмма с конусовидным изображением

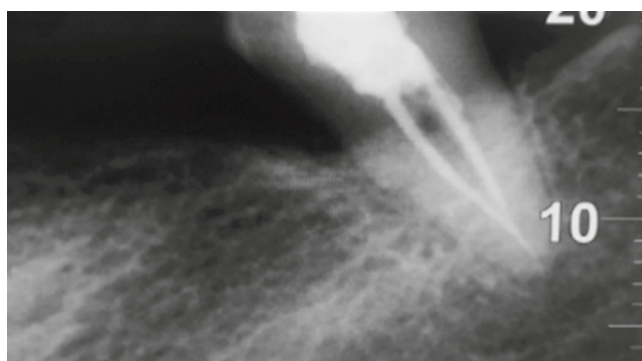


Fig. 13. Post-operative radiograph

Рис.13. Послеоперационная рентгенограмма

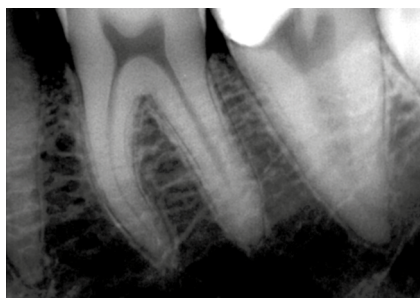


Fig. 14. Preoperative radiograph

Рис. 14. Предоперационная рентгенограмма

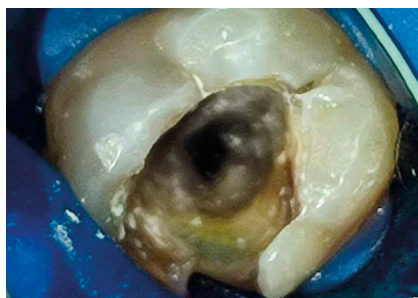


Fig. 15. Clinical examination showing C-shaped canal type C4

Рис. 15. Клиническое обследование, показывающее С-образный канал типа C4

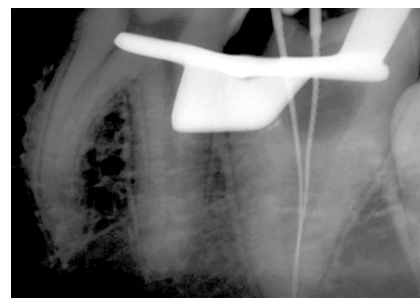


Fig. 16. Determination of working length

Рис. 16. Определение рабочей длины

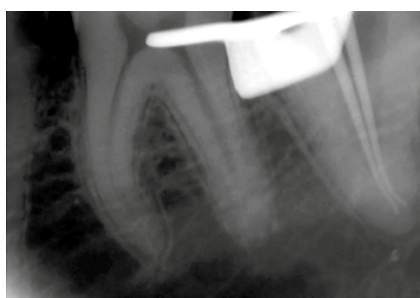


Fig. 17. Cone-fit radiograph

Рис. 17. Конусная рентгенограмма

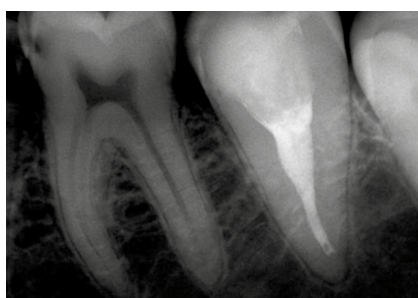


Fig. 18. Post-operative radiograph

Рис. 18. Послеоперационная рентгенограмма



Fig. 19. One-month Follow-up radiograph

Рис. 19. Контрольная рентгенограмма через месяц

Case 4: C-shaped canal type C4

A male patient in his 20's presented to our dental medicine department with continuous spontaneous pain, diagnosed with symptomatic irreversible pulpitis in tooth 47. Radiographic examination showed a type I root configuration, characterized by a conical shape with a subtle radiolucent longitudinal line dividing the root into distinct mesial and distal sections, with the canals converging into a single apical foramen (Fig. 14) [3].

Clinical examination revealed a single, round canal, classified as a Fan type C4 C-shaped canal [4] (Fig. 15).

The treatment plan included working length determination using an apex locator and radiography (Fig. 16), cleaning and shaping with the PlexV (Ordeka) system, ultrasonic activation of the irrigant, and obturation using the single-cone technique with One-fil bioceramic sealer (Fig. 17, 18).

DISCUSSION

Through a series of clinical cases involving the management of various types of C-shaped canals, this study highlights the critical role of accurate clinical and radiographic diagnosis in ensuring effective endodontic treatment of these complex anatomical configurations.

C-shaped root canals represent a significant anatomical variation, predominantly observed in man-

dibular second molars [5]. Their complex morphology poses challenges in endodontic treatment, requiring a thorough understanding of their etiology and clinical implications. Despite extensive research, the precise mechanisms underlying their formation remain unclear. Several factors have been proposed, including developmental anomalies, genetic predisposition, environmental influences, and ethnic or geographic variations. Developmentally, disruptions in epithelial-mesenchymal interactions during root formation may contribute to this atypical configuration. Genetic studies suggest that specific genes or mutations could influence root canal morphology. Additionally, environmental factors such as trauma, infections, or vascular disruptions during odontogenesis have been implicated. Epidemiological data indicate a higher prevalence of C-shaped canals in certain ethnic populations, particularly among individuals of Asian or Native American descent, suggesting a potential genetic and regional predisposition. Given these multifactorial origins, further research is essential to elucidate the underlying mechanisms and improve treatment strategies for teeth with C-shaped canals [6].

The influence of racial and geographic factors on root canal anatomy further underscores the complexity of C-shaped canals. Variations in prevalence have been documented across different populations, highlighting the role of genetic and environmental determinants.

Notably, C-shaped canals are often found bilaterally, with studies reporting a prevalence of 75.3% for bilateral occurrences and 24.7% for unilateral cases [7]. This distribution appears consistent across genders and tooth locations, suggesting an inherent anatomical pattern rather than a random occurrence. Understanding these racial and regional variations is crucial for improving diagnostic accuracy and refining endodontic treatment approaches [8].

Classification of C shaped canals

Melton et al. classified C-shaped canals based on their cross-sectional morphology, recognizing that their shape may change along the root length. This classification includes 3 classes [9].

However, given the limitations of Melton's system in describing variations along the entire root length, Fan's classification [4] was adopted in our study. In fact, Fan proposed a more comprehensive system with five categories, all illustrated in our study, considering both continuity and segmentation of the canal along the root. This classification includes:

- C1: a continuous C-shaped canal;
- C2: a discontinuous C-shape with at least one arc $\geq 60^\circ$;
- C3: two to three separate canals with no arc exceeding 60° ;
- C4: a single round or oval canal;
- C5: a root with no canal lumen, typically near the apex. By using Fan's system, our study ensures a more detailed and clinically relevant classification of C-shaped canals.

Fan et al. have also supplemented their work with a radiological classification, which classified C-shaped roots into three types based on their radiographic appearance [3]:

- **Type I:** A conical or square root with a faint radiolucent longitudinal line separating mesial and distal segments, which merge into a single canal before reaching the apex;

- **Type II:** Similar root shape with a radiolucent line, but the mesial and distal canals remain separate and follow independent paths to the apex;

- **Type III:** A conical or square root with a radiolucent line; one canal follows a curved path, overlapping with the line, while the other remains separate.

Anatomical Characterization of C-Shaped Canals in Molars

The crown structure of teeth exhibiting C-shaped anatomy does not display distinctive characteristics that can assist in diagnosis.

The clinical confirmation of C-shaped canals becomes apparent upon accessing the pulp chamber. In this case, the pulp chamber might extend considerably in the occluso-apical length, often with a shallow bifurcation [4]. As an alternative, the canal might be calcified, concealing its C-shaped configuration. Initially, multiple orifices might be explored. In a genuine C-shaped canal, smooth passage of an instrument from the mesial to distal aspect is achievable without obstructions [7].

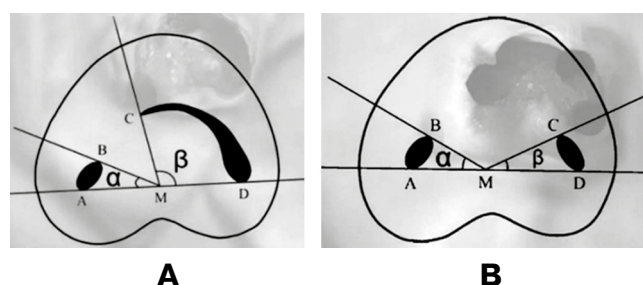


Fig. 21. Measurement of angles for the C2 (A) and C3 (B) canal. Both angle α and β are more than 60°
Source: [10]

Рис. 21. Измерение углов для канала C2 (A) и C3 (B). Оба угла α и β составляют более 60°
Источник: [10]

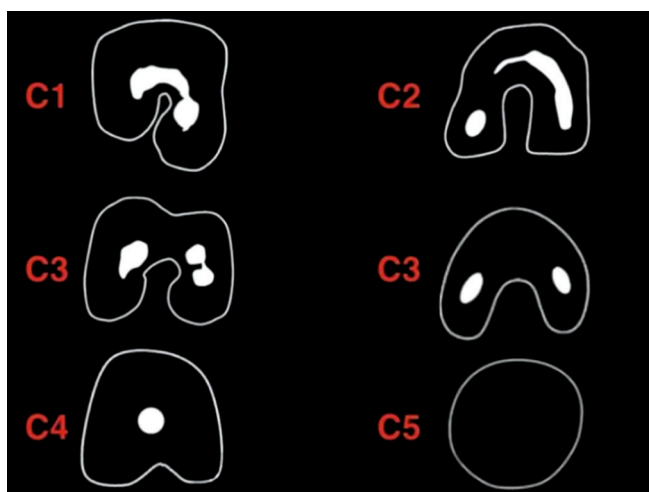


Fig. 20. Fan et al. (2004) classification
Source: [10]

Рис. 20. Классификация Фана и др. (2004)
Источник: [10]

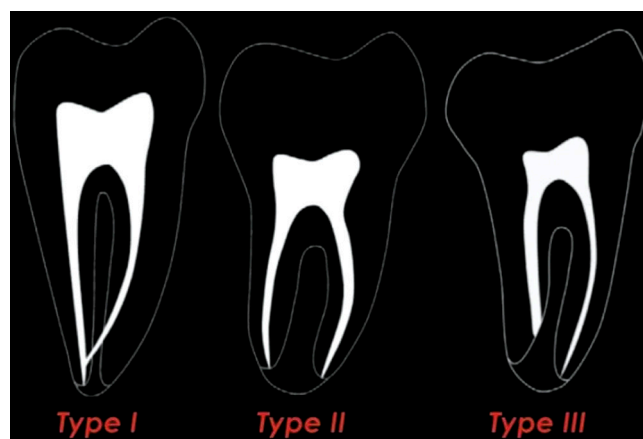


Fig. 22. Radiographic type of C-shaped Canal
Source: [10]

Рис. 23. Рентгенологический тип С-образного канала
Источник: [10]

C-shaped canals exhibit an isthmus that may or may not allow instrument passage. If an additional canal exists alongside a buccal or lingual C-shaped canal, inserting an instrument from either side of the “C” may reach the distal foramen. If the instrument cannot traverse the isthmus, the canal is considered separate [2]. According to Fan et al., a mandibular second molar is classified as having a C-shaped canal system if it meets three criteria: fused roots, a longitudinal groove on the lingual or buccal root surface, and a canal configuration falling under C1, C2, or C3 in Fan’s classification [3; 4].

Studies indicate that C-shaped canals often divide into multiple canals in the apical region, particularly when they exhibit a semicolon or continuous shape at the orifice level [11].

In another hand, Aricioğlu et al. were the first to identify a significant association between taurodontism and C-shaped canal configurations. They suggested that this correlation may arise from a common developmental anomaly affecting the dental epithelium. These findings highlight the need for clinicians to anticipate a higher prevalence of C-shaped canals when performing endodontic treatments on taurodont teeth [12].

Clinical Management

Preoperative retroalveolar radiographs taken at 20° mesial or distal angles, are essential for assessing canal morphology. However, their two-dimensional nature limits their accuracy, as they may fail to reveal all root and canal variations.

Still, Cone-Beam Computed Tomography (CBCT) has become an invaluable tool in endodontics, offering significant advantages over conventional imaging methods. In fact, it provides a comprehensive view of root canal morphology, improving diagnostic accuracy and enhances the detection of small anatomical details, including C-shaped canal configurations [13].

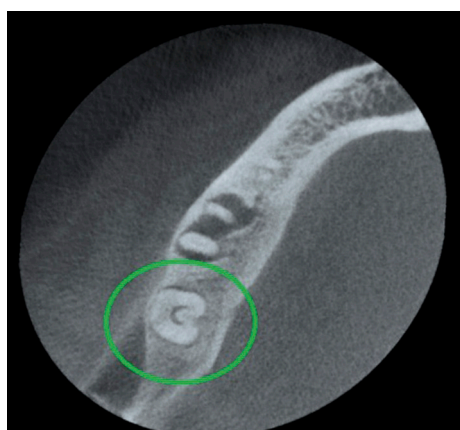


Fig. 23. A preoperative periapical radiograph of tooth 47

Source: [15]

Рис. 23. Предоперационная периапикальная рентгенограмма зуба 47

Источник: [15]

However, CBCT exposes patients to higher radiation doses compared to traditional radiography. As a result, the American Association of Endodontists recommends its use only when conventional imaging is insufficient for a definitive diagnosis [14].

In our cases, retroalveolar X-rays were used as a diagnostic and follow-up tool.

Therefore, precise access cavity preparation is essential for successful treatment, with an operating microscope improving results. For C-shaped canals, adjustments to the access outline help identify and negotiate the entire system. The number of canals varies by orifice shape, from 1 to 3 canals [4].

To navigate these variations, initial files are placed accordingly: three for a continuous orifice, two for an oval, and one for a round orifice. Calcifications and curvatures can obscure canals, requiring careful probing with small K-files to prevent missed canals [2].

Effective cleaning and shaping of C-shaped canals require special attention to isthmuses, troughs, and fins, where debris and pulp tissue tend to accumulate [2]. Instrumentation should be carefully selected, as using files larger than diameter 0.25 in the isthmus may lead to strip perforation. Extracting affected pulp or necrotic tissues from intricate regions of this root canal system with instruments [16], proves challenging. Hence, the use of chemical solutions such as Sodium Hypochlorite (NaOCl) for cleaning and irrigating root canals becomes particularly crucial.

In fact, enhanced irrigant volume and deeper penetration achieved with small instruments through sonic or ultrasonic methods might improve cleaning in the fan-shaped regions of C-shaped canals. However, excessive use of ultrasound can lead to a perforation and breaking of the ultrasonic file [5].

For the obturation, achieving a dense and uniform fill in C-shaped canals is challenging due to their complex morphology. Vertical condensation with thermoplasticized gutta-percha is often preferred over cold lateral condensation, as the latter requires deeper spreader penetration, which is difficult in narrow isthmuses [17]. On the other hand, Bioceramic sealers offer significant advantages in the obturation of C-shaped canals due to their excellent flowability and bioactivity, which enhance the sealing of irregular canal spaces, including isthmuses and fins. Their hydrophilic nature and chemical bonding to dentin improve adaptation, reducing the risk of microleakage and ensuring long-term biocompatibility in these anatomically complex systems [18].

in our case series, we used a bioceramic sealer to fill the c-shaped canals, which guaranteed periapical healing and sedation of the clinical symptoms.

CONCLUSION

C-shaped canals represent significant challenges in diagnosis and treatment. Early detection is key to effective cleaning, shaping, and obturation. This study explored the management of various types of C-shaped canals and highlights the role of the clinical and radiological diagnosis in ensuring effective endodontic treatment of these complex anatomical configurations.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

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Apexification of traumatized anterior teeth with blunderbuss canals using MTA putty and collagen membrane: A case report

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Abstract

INTRODUCTION. Trauma to developing permanent teeth can disrupt root formation, leading to open apices and weakened tooth structure.

AIM. This report details the non-surgical management of an 8-year-old male with blunderbuss canals in anterior teeth using mineral trioxide aggregate (MTA) putty and a resorbable collagen membrane to support apical barrier formation and healing.

MATERIALS AND METHODS. Non-surgical apexification was performed using MTA putty as the primary apical barrier material, supplemented by a resorbable collagen membrane to aid in barrier formation and periapical healing.

RESULTS. Over 12 months, clinical and radiographic follow-ups showed successful healing with no periapical pathology.

Keywords: immature teeth, blunderbuss canal, apexification, MTA putty, collagen membrane

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Апексификация травмированных передних зубов с каналами BLUNDERBUSS с использованием MTA PUTTY и коллагеновой мембраны: отчет о случае

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Резюме

ВВЕДЕНИЕ. Травма формирующихся постоянных зубов может нарушать формирование корней, приводя к несформированным верхушкам (открытым апексам) и ослабленной структуре зуба.

ЦЕЛЬ. В данном клиническом случае представлен нехирургический подход к лечению 8-летнего мальчика с воронкообразными каналами во фронтальных зубах с применением минерального триоксидного агрегата (MTA) в виде пасты и рассасывающейся коллагеновой мембраны для формирования апикального барьера и стимулирования заживления.

МАТЕРИАЛЫ И МЕТОДЫ. Была проведена нехирургическая апексификация с использованием пасты MTA в качестве основного материала для апикального барьера, а также дополнительным применением рассасывающейся коллагеновой мембраны для поддержания барьера и заживления периапикальной области.

РЕЗУЛЬТАТЫ. В течение 12 месяцев клинического и рентгенологического наблюдения отмечалось успешное заживление без признаков периапикальной патологии.

Ключевые слова: незрелые зубы, воронкообразный канал, апексификация, паста MTA, коллагеновая мембрана

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INTRODUCTION

Dental trauma is a common concern in young children and a leading cause of pulpal non-vitality in immature permanent incisors. Evidence shows that 13.80% to 15.10% of individuals experience trauma to permanent anterior teeth, highlighting the need for preventive strategies and early intervention [1]. Such injuries can damage the periodontium, alveolar bone, and pulp's neurovascular supply, potentially leading to pulp necrosis and long-term complications [2].

A major consequence of necrosis in immature teeth is arrested root development, resulting in open apices, blunderbuss canals, a compromised crown-to-root ratio, and thin, fragile dentinal walls, complicating conventional endodontic treatment [3]. In such cases, achieving proper cleaning, debridement, and root canal sealing becomes challenging, increasing the risk of extrusion of filling materials into periapical tissues, which can hinder healing. To prevent this, an apical calcific barrier is necessary to facilitate proper root canal filling and strengthen weakened root structures [3; 4].

Various materials have been used for apical barrier formation in apexification procedure, including calcium hydroxide, tricalcium phosphate, and osteogenic proteins, with calcium hydroxide being the gold standard [5; 6]. However, the European Academy of Paediatric Dentistry (EAPD) and American Association of Endodontists (AAE) recommended alternative approaches to calcium hydroxide apexification for immature necrotic teeth, favoring regenerative approaches because of its unpredictable outcomes and longer appointment time¹ [3]. In recent years, biocompatible materials like Mineral Trioxide Aggregate (MTA) and Biodentin have become increasingly preferred. MTA, a tricalcium silicate-based powder, has demonstrated excellent sealing ability, promotes the formation of mineralized tissue, improves treatment outcomes, and reduces the number of required appointments [7].

In cases involving blunderbuss canals with wide, funnel-shaped open apices, there is a heightened risk of material extrusion beyond the apex, particularly when using calcium silicate-based sealers and cements even though MTA-based apical closure has shown favorable results [8]. Despite their biocompatibility, the unintended extrusion of these materials may affect the healing of periapical lesions. Research indicates that hemostatic collagen membranes can serve as effective apical barriers, minimizing extrusion and promoting more accurate MTA placement [8; 9].

This case report presents the successful endodontic management of a traumatized immature tooth using the MTA apexification procedure with a collagen membrane as an apical barrier.

¹ American Association of Endodontists. Clinical considerations for a regenerative procedure. Revised 2021 May 18. Available at: <https://www.aae.org/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf> (accessed: 27.06.2025).

CASE PRESENTATION

A 11-year-old male patient presented to the Department of Pediatric and Preventive Dentistry in the month of March, 2024 with a chief complaint of pain in the upper front tooth region for the past two days. The pain was mild to moderate, continuous, localized, and aggravated during mastication. The patient reported a history of dental trauma six months ago, which resulted in a fractured and loose tooth. Splinting was performed at a nearby dental clinic. No relevant medical history was noted.

Clinical examination revealed fracture including the enamel, dentin and pulp in relation to tooth 21 (Fig 1, A). The tooth was unresponsive to both electric and thermal pulp sensibility tests. Diagnostic intraoral periapical radiograph (IOPAR) and CBCT were advised. CBCT revealed a radiolucency involving the enamel, dental and pulp and a large blunderbuss canal with an open apex, accompanied by a periapical lesion (Fig. 1, B). Based on these findings, a diagnosis of Ellis class IV fracture with open apex and symptomatic periapical abscess involving tooth 21 was established.

The treatment plan was explained to the parents, and after thorough discussion and informed consent, it was decided to proceed with MTA apexification in conjunction with a resorbable collagen membrane placement given the presence of a blunderbuss canal with an open apex followed by esthetic rehabilitation of the tooth.

The access cavity for tooth 21 was prepared using an Endo Access bur and an Endo Z bur under rubber dam isolation. The working length was established with an apex locator (Woodpecker AI motor, Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, Guangxi, China) and verified radiographically using a 60K file (MANI, Inc. Tochigi, Japan). Cleaning and shaping were performed with hand K-files and ProTaper Universal rotary files (Dentsply Maillefer, Ballaigues, Switzerland) at 300 rpm speed and 2–3 NCm torque as per the manufacturer's instructions. Canal irrigation was done with 3% sodium hypochlorite, saline, and 17% EDTA using a side vented needle followed by drying with sterile paper points.

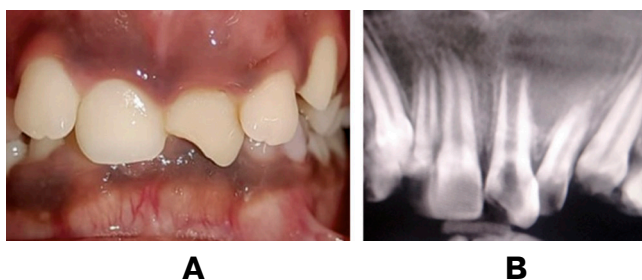


Fig. 1. Preoperative frontal view of tooth 21 in occlusion showing an Ellis Class III fracture (A) and preoperative CBCT image of 21 revealing an open apex with a wide blunderbuss canal (B)

Рис. 1. Предоперационный фронтальный вид зуба 21 в окклюзии с переломом по Эллису класса III (A) и предоперационное изображение КЛКТ зуба 21, демонстрирующее несформированную верхушку и широкий воронкообразный канал (B)

A triple antibiotic paste (TAP) (Amoxicillin, Metronidazole, and Ciprofloxacin in a 1:1:1 ratio) was applied for 21 days. The dressing was replaced with CaOH_2 applied every 21 days for 2 months until the patient became completely asymptomatic (Fig. 2, A).

After the patient became completely asymptomatic, the dressing was carefully removed, and the canal was thoroughly irrigated with a copious amount of solution to ensure complete decontamination. The canal was then dried using sterile paper points to eliminate any residual moisture. A collagen membrane (Healiguide, Advanced Biotech Products [P] Ltd., Chennai, India), precisely trimmed into a 3×3 mm square, was carefully placed at the apical region of the canal. Using a plugger (MANI, Inc., Tochigi, Japan), the membrane was gently positioned up to the predetermined working length, ensuring a proper seal and adaptation to the canal walls. To further enhance stability, an additional collagen membrane was layered above the first, creating a 1–2 mm stable base at the apical region.

Following this, MTA putty (e-MTA Putty, Kids-e-Dental LLP, Mumbai, India) was introduced into the canal using an MTA carrier. The material was incrementally placed, and each layer was carefully compacted using a plugger to form a well-adapted 5 mm apical plug, ensuring an effective seal (Fig. 2, B). Once the MTA had achieved its initial set, the canal was obturated with gutta-percha using a bioceramic sealer (BioActive RCS, SafeEndo Dental India Pvt. Ltd., Vadodara, India) to enhance the long-term sealing ability and biocompatibility (Fig. 3, A). Finally, the access cavity was restored and the crown build up was done (Fig. 3, B) with a light-cured composite (Beautifil II Dental Composite, Shofu Inc., Kyoto, Japan). The patient was followed up for upto 12 months (Fig. 4, A, B).

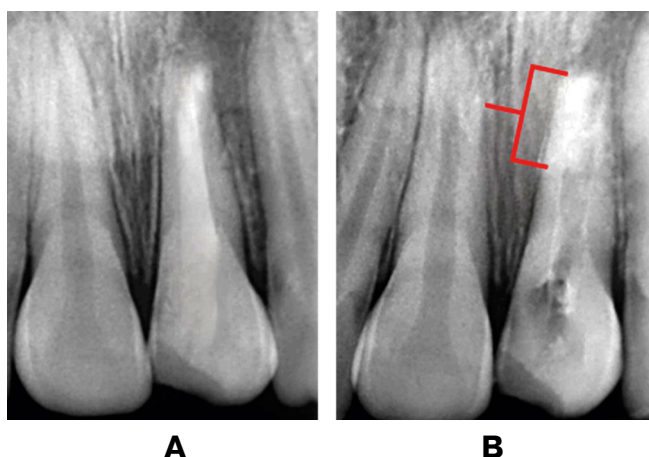


Fig. 2. Radiographic image of tooth 21: A – with calcium hydroxide dressing; B – following MTA apexification using a collagen membrane (the red mark indicates 5 mm of MTA putty placed within the canal)

Рис. 2. Рентгенологическое изображение зуба 21: А – с гидроксидом кальция в качестве временной лекарственной прокладки; В – после апексификации с применением МТА и коллагеновой мембраны (красная метка указывает на 5 мм пасты МТА, введенной в канал)



Fig. 3. Immediate postoperative radiographic image of tooth 21 following obturation with gutta-percha and bioceramic sealer (A); clinical image of tooth 21 after composite buildup (B)

Рис. 3. Рентгенологическое изображение зуба 21 сразу после пломбирования гуттаперчей и биокерамическим силером (А); клиническое изображение зуба 21 после восстановления композитным материалом (В)

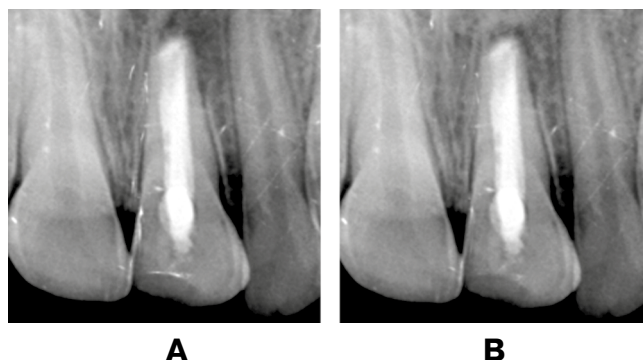


Fig. 4. Follow-up radiographic images of tooth 21: A – at 6 months; B – at 12 months post-treatment

Рис. 4. Рентгенологические изображения зуба 21 при последующем наблюдении: А – через 6 месяцев; В – через 12 месяцев после лечения

DISCUSSION

According to the AAE, apexification remains the preferred treatment approach for managing non-vital immature permanent teeth². While Calcium Hydroxide ($\text{Ca}(\text{OH})_2$) was historically the material of choice for this procedure, it presents several limitations, including the need for multiple appointments, a prolonged treatment period (typically 12–24 months) for apical closure, unpredictable outcomes, and a reliance on patient compliance throughout the process³ [3; 10]. To overcome these challenges, single-visit apexification techniques using biocompatible materials such as Mineral Trioxide Aggregate (MTA) and Biodentine have become more widely adopted. These materials offer faster, more predictable results with fewer appointments, enhancing both efficiency and patient satisfaction [7].

² American Association of Endodontists. Clinical considerations for a regenerative procedure. Revised 2021 May 18. Available at: <https://www.aae.org/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf> (accessed: 27.06.2025).

³ Ibid.

MTA is ideal for treating open apices due to its excellent sealing ability and ability to set in the presence of moisture or blood [8]. It promotes hard tissue formation, cementogenesis, and osteogenesis by creating a biocompatible environment that supports cell activity [9]. Its high pH gives it antibacterial properties, while its dense seal prevents bacterial ingress, aiding periapical healing [4; 7]. These characteristics made it the preferred material in our case.

Although newer biomimetic materials like Biodentine have gained popularity due to their improved handling properties and faster setting times, MTA was preferred in our case. This decision was based on its superior marginal adaptation [11], reduced microleakage [12], and more predictable apical barrier formation [13] when compared to Biodentine, particularly in wide or blunderbuss canals [14]. Additionally, the extended setting time of conventional MTA, along with the challenges associated with achieving the optimal consistency during manipulation, are often considered limitations. To overcome these drawbacks, a pre-mixed MTA putty was utilized in this case.

It offered a significantly faster setting time compared to traditional MTA and came with a standardized, ready-to-use consistency, facilitating easier handling. The MTA putty allowed for improved working time, enabling precise placement and condensation within the complex canal anatomy [15]. It offered enhanced adaptability in irregular spaces, and set more rapidly than conventional MTA. These properties made MTA putty the material of choice for achieving a reliable and biologically favorable outcome in the present case.

In this case, a 5 mm apical plug of MTA was placed using an MTA carrier, followed by careful condensation with hand pluggers. This thickness was selected based

on literature evidence suggesting that a 5 mm apical barrier offers superior sealing ability and resistance to microleakage compared to thinner plugs of 1 mm or 2 mm [16; 17]. Although ultrasonic condensation is an alternative technique for MTA placement, the use of hand pluggers in this case allowed for better control, adaptation, and compaction of the material with fewer voids within the wide apical region [18].

To prevent the extrusion of MTA into the periapical tissues, a resorbable collagen membrane was placed at the apex, serving as a biological scaffold against which the MTA could be compacted [8]. This not only provided a stable base for the material but also helped in maintaining the integrity of the periapical space and supporting optimal healing conditions. Acting as a scaffold, resorbable collagen sponges assist in platelet aggregation, clot stabilization, and tissue healing by attracting fibroblasts and enhancing blood vessel formation at the injury site. Additionally, studies have shown that using collagen sponges in apexification procedures may encourage alveolar bone regeneration [8; 9].

CONCLUSION

The application of pre-mixed MTA putty in this case enabled successful apexification by establishing a reliable apical barrier and creating an environment conducive to periapical tissue healing. The effective outcome was achieved through a combination of appropriate apical plug thickness, and the use of a resorbable collagen matrix to prevent material extrusion. This case reinforces the clinical effectiveness of MTA in treating non-vital immature permanent teeth and supports its continued preference as a material of choice in similar endodontic treatments.

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Tanu Nangia – Supervised the clinical case, offered professional oversight, and contributed to the final review and approval of the manuscript.

Madhulika Srivastava – Provided supervision during the clinical case, gave critical input on manuscript development, and assisted with the final review and approval.

Syed Fazil Hasan – Assisted with the literature review and preparation of clinical documentation.

Lakshmi Prasad N – Supported the literature review process and contributed to organizing clinical data and documentation.

Sakshi Shah – Participated in patient management and contributed to data interpretation.

Namrata Puri – Involved in patient management and assisted with interpreting the clinical findings.

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Modern aspects of the use of hardware methods for diagnosing pulp vitality (Part 2. Non-traditional diagnostic methods)

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Abstract

INTRODUCTION. Diagnosis of pulp diseases remains a pressing issue in dentistry, which is determined by their high prevalence and, in some cases, latent course.

AIM. To study new technologies developed for hardware testing of pulp vitality based on modern literature data. **MATERIALS AND METHODS.** A systematic search was performed in the electronic databases PubMed, Google Scholar, eLibrary, Google Patents. The search depth was 6 years – from 2019 to 2024.

RESULTS. The search in the electronic library databases initially yielded 793 results. After screening titles and abstracts and removing duplicates, 368 articles were identified, assessed by reading their full text, and analysis of whether the publication criteria were met; 65 articles were included in the systematic review. Based on the results preliminary screening and application of the eligibility criteria, 15 publications were included in the qualitative analysis and 7 publications in the quantitative analysis, 43 publications were used to write the introduction text and in the discussion of the study results. Based on the patent search, 4 patents were included in the analysis. Most of the well-conducted and documented studies were devoted to the pulse oximetry method.

CONCLUSIONS. An analysis of modern literature sources showed that the most common methods for assessing pulp vitality are laser Doppler flowmetry and pulse oximetry. Pulse oximetry is the most accurate diagnostic tool. Alternative diagnostic methods are increasingly being explored for their potential to assess pulp vitality. The most frequently mentioned methods in scientific publications for 2019–2024 are: ultrasound Doppler flowmetry, transillumination, magnetic resonance imaging, speckle imaging, tooth temperature measurements, electroodontometry and plethysmography. However, to date, none of the alternative methods for diagnosing pulp vitality have been integrated into clinical practice, indicating an ongoing challenge in creating a reliable approach to assessing pulp vitality.

Keywords: hardware methods, diagnostics, pulp vitality

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Современные аспекты использования аппаратных методов диагностики витальности пульпы (Часть 2. Нетрадиционные методы диагностики)

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Резюме

ВВЕДЕНИЕ. Одной из актуальных проблем современной стоматологии является применение диагностического арсенала изучения витальности пульпы зубов в тех или иных клинических случаях.

ЦЕЛЬ ИССЛЕДОВАНИЯ. На основании обзора литературных данных изучить инновационные технологии для определения витальности пульпы зубов.

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МАТЕРИАЛЫ И МЕТОДЫ. Систематический поиск был выполнен в электронных базах данных PubMed, Google Scholar, eLibrary, Google Patents. Глубина поиска составила 6 лет – с 2019 по 2024 г.

РЕЗУЛЬТАТЫ. Поиск в базах электронных библиотек первоначально дал 793 результата. После удаления дубликатов было идентифицировано 368 статей, на основании соответствия критериям включения публикации выделено 65 статей в систематический обзор. В дальнейшем 15 публикаций были включены в качественный анализ и 7 публикаций в количественный анализ, 43 публикаций были использованы для написания текста введения и при обсуждении результатов исследования.

ВЫВОДЫ. На основании литературных данных можно выделить следующие наиболее распространенные методиками изучения витальности пульпы зубов, к которым можно отнести метод лазерной доплеровской флоуметрии и пульсоксиметрии. Среди нетрадиционных методов диагностики витальности пульпы все чаще исследуются с помощью ультразвуковой доплеровской флоуметрии, трансиллюминации, магнитно-резонансная томографии, спекл-визуализации, измерения температуры зубов, электроодонтометрии и плетизмографии.

Ключевые слова: аппаратные методы, диагностика, витальность пульпы

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INTRODUCTION

In endodontic practice, the assessment of the dental pulp condition is a crucial and necessary diagnostic tool, as diagnosis constitutes an integral part of planning subsequent treatment strategies. The gold standard for determining pulp vitality is its direct evaluation via histological sectioning. However, since the pulp is surrounded by a calcified barrier, such assessment is not feasible prior to the initiation of endodontic treatment.

Given the inability to directly visualize the pulp, indirect methods must be employed to assess its condition by evaluating innervation – for example, pulp sensitivity tests. The most commonly used tests for assessing pulp sensitivity are thermal and electric pulp tests, which stimulate pulpal nerves either through the movement of dentinal fluid induced by temperature fluctuations, resulting in the movement of odontoblastic processes and mechanical stimulation of pulpal nerves, or through the application of an electric current to the tooth, producing electrical stimulation of the pulp nerves [1; 2].

The primary mechanism of the electric pulp test involves initiating ionic changes across the nerve membrane through electrical stimuli, which affect the action potential via rapid depolarization at the nodes of Ranvier in myelinated nerves [3]. Contemporary pulp sensitivity testing methods indirectly evaluate pulp vitality by assessing the neural response, without accounting for vascular circulation, which may result in false-positive responses in teeth that have temporarily or permanently lost sensory function and do not respond to such tests despite the presence of an intact vascular network [4].

The limitations of pulp sensitivity testing have been addressed by the development of pulp vitality testing methods such as pulse oximetry (PO), laser Doppler flowmetry (LDF), and ultrasonic Doppler flowmetry

(UDF), which assess pulpal blood flow without relying on patient responses and are considered to provide a more accurate evaluation of pulp status. PO measures oxygen saturation within the pulp chamber using a non-invasive probe with two diodes placed on the tooth, whereas LDF and UDF assess vascular flow within the dental pulp by evaluating the “concentration and velocity of blood cells,” thereby reflecting indicators of pulpal perfusion and vitality [5; 6].

The limitations of pulp sensitivity testing have been addressed through the development of pulp vitality assessment methods. However, laser Doppler flowmetry and pulse oximetry have not yet gained widespread use in routine dental practice, which has prompted increased scientific interest in alternative diagnostic approaches for evaluating pulp vitality.

Among the non-conventional methods currently under investigation are optical diagnostic techniques, ultrasonic Doppler flowmetry, magnetic resonance imaging, terahertz imaging, and dental thermography [7].

MATERIALS AND METHODS

Search Strategy

A systematic electronic search was conducted in the international databases PubMed and Google Scholar, as well as in the Russian scientific electronic library eLibrary. Patent searches were performed using the Google Patents database. The search covered a 6-year period, from 2019 to 2024. Russian literature was defined as studies conducted in Russia and published in Russian scientific journals. The search strategy included the keywords: *Diagnostic*, *Instrumental Methods*, and *Pulp Vitality*. For Russian-language sources, the following terms were used: *Диагностическая*, *Инструментальные методы*, and *Витальность пульпы*.

Inclusion Criteria

1. Articles published in Russian or English between 2019 and 2024.
2. Types of publications: scientific articles—including research and practice-oriented studies—as well as systematic reviews.
3. Full-text availability free of charge on the internet.
4. Relevance to the research topic – instrumental (device-based) methods for the diagnosis of pulp vitality.

Exclusion Criteria

1. Type of publication: conference abstracts, proceedings, and dissertations.
2. Lack of full-text availability online.
3. Absence of analysis regarding the effectiveness of the method.
4. Methods that assess only pulp sensitivity (e.g., thermal tests, electric pulp testing).

A stepwise screening process was employed for the selection of publications. After initial identification, each source underwent title and abstract screening, and duplicates were excluded. Full-text articles were then reviewed for relevance. The bibliographies of all eligible articles were examined to identify additional relevant publications. Each study was assessed for alignment with the research objective based on three criteria: evaluation of the title, abstract, and full text.

Data Extraction

Each included article was analyzed to extract information on bibliometric characteristics, study methodology, and results. The extracted variables included: the type of pulp vitality test or measurement used, patient-related variables (number, age, and sex), number of samples (teeth), type of teeth examined, as well as the technique and device employed for assessing pulp vitality.

RESULTS

Non-Conventional Approaches to Pulp Vitality Diagnosis¹

In the publications reviewed from 2019 to 2024, the following non-conventional methods for assessing pulp vitality were identified: ultrasonic Doppler flowmetry [9–11], transillumination [12], magnetic resonance imaging [13], speckle imaging [14–16], dental thermography [17; 18], and plethysmography [19].

The studies were grouped according to the type of non-conventional method used. For each method, the diagnostic objective and key findings were summarized. Where available, diagnostic accuracy indicators – such as sensitivity, specificity, positive predictive value, and negative predictive value – were also reported (Table 1).

¹ Traditional Approaches to Assessing Dental Pulp Vitality are discussed in Part 1 of the article [8].

Table 1. Characteristics of the included non-traditional studies

Таблица 1. Характеристика включенных нетрадиционных исследований

Publication, type of research	Sample size and type	Technique	Diagnostic target	Main findings	Accuracy, PPV, NPV
Optical methods					
Dar et al., 2020 [19] Clinical study (on humans)	30 incisors Mx	TLP	Pulpal blood flow	Harmless stimuli caused a reversible decrease in TLP values in human teeth. TLP was able to monitor pulpal blood circulation	Not reported
Knörzer et al., 2019 [20] In vitro (extracted human teeth)	2 mandibular molars (1 for each of the 2 models)	PPG	Simulation of pulpal blood flow	Flow data were detected using light at 625 nm and 940 nm in a model with comparable size and volumetric blood flow rates. Signal interference from the gingiva was observed at 625 nm but not at 940 nm	Not reported
Proulx et al., 2022 [12] In vivo animal model (dog)	45 dogs 45 teeth	TTI	Tooth translucency	Transillumination is less effective than cold and electric tests. In cases without discoloration, transillumination may fail to detect necrotic pulp, resulting in false-negative outcomes. Additionally, discolored teeth with enamel defects or drug-induced stains may produce false-positive results	Sensitivity: 0.59 Specificity: 0.95 PPV: 0.94 NPV: 0.67 Accuracy: 0.76
Xu et al., 2021 [14] In vitro (extracted human teeth connected to an injection pump)	3 teeth	LSI	Simulation of blood flow in the dental pulp	LSCI can detect blood flow in the pulp and is capable of measuring flow velocity. Vertically polarized LSCI demonstrated a wider dynamic detection range compared to non-polarized LSCI (np-LSCI)	The sensitivity of LSCI is sufficient to detect minor changes in blood flow
Chistyakova G.G., 2019 [15] Clinical study (on humans)	141 patients 235 teeth	LSI	Pulpal blood flow	During the analysis and screening of speckle patterns, hemomicrocirculation parameters were optimized, such as spectral power S (relative units) and mean frequency (Hz), which were interpreted as indicators of blood flow level and intensity	Not reported
Chistyakova G.G., 2020 [16] Clinical study (on humans)	190 patients 210 teeth	LSI	Pulpal blood flow	As blood flow intensity decreases, electroodontometry values increase, and the pulp's electrical excitability threshold decreases	Not reported

Table 2 (ending) / Таблица 2 (окончание)

Publication, type of research	Sample size and type	Technique	Diagnostic target	Main findings	Accuracy, PPV, NPV
Ultrasound Doppler flowmetry					
Kim et al., 2023 [9] In vivo animal model (dog)	9 dogs, 36 right and left maxillary anterior teeth	UDF	Pulpal blood flow	When using UDF, gingival blood flow affects the measurement of pulpal blood flow. Therefore, the gingiva must be isolated from the tooth being evaluated	Not reported
Magnetic resonance imaging					
Juerchott et al., 2022 [13] Clinical study (on humans)	70 individuals, 1585 teeth	MRI	Pulpal blood flow	Teeth with healthy pulp can be visualized, and pulpal tissue can be quantitatively assessed using dMRI with a specific set of parameters. This may serve as a useful diagnostic tool for identifying various pulp diseases in future patient studies	Not reported (the entire pulp is considered intact)
Tooth temperature measurement					
Ajith Kamath, Nasim, 2020 [17] Clinical study (on humans)	75 individuals	ITMI	Tooth surface temperature	There were no significant differences in baseline temperature between vital and non-vital teeth. Vital teeth may exhibit better surface heating compared to non-vital ones	Accuracy of 97.34%
Mendes et al., 2020 [18] Clinical study (on humans)	58 individuals 126 maxillary anterior teeth (including 33 pairs of vital – non-vital maxillary central incisors)	ITMI	Thermograms and tooth temperature	When using an infrared thermograph, teeth with different pulpal conditions exhibited different temperatures	The measurement error of the overall temperature is $\pm 2\%$

Note: Mx – maxillary; NPV – Negative predictive value; PPV – Positive predictive value; TLP – Transmitted light plethysmography; PPG – Photoplethysmography; TTI – tooth transillumination; LSCI – Laser speckle contrast imaging; LSI – Laser speckle imaging; UDF – Ultrasound Doppler flowmetry; MRI – Magnetic resonance imaging; ITMI – Infrared thermographic imaging

Примечание: Мх – верхняя челюсть; NPV – отрицательная прогностическая ценность; PPV – положительная прогностическая ценность; TLP – световая плетизмография; PPG – фотоплетизмография; TTI – трансиллюминация зуба; LSCI – Лазерная спекл-контрастная визуализация; LSI – лазерная спекл-визуализация; UDF – ультразвуковая доплеровская флоуметрия; MRI – магнитно-резонансная томография; ITM – инфракрасная термографическая визуализация

Table 2. Devices / systems used in studies to assess pulp vitality**Таблица 2. Устройства / системы, используемые в исследованиях для оценки витальности пульпы**

System /device (publication)	Purpose / Aim
Photoplethysmography	
The light source is a light-emitting diode (LED) with a wavelength of 525 nm. The transmitted light is delivered in pulses [19]	Pulp blood flow
The light source consists of light-emitting diodes (LEDs) with wavelengths of 625 nm and 940 nm [20]	
Laser speckle imaging	
Polarized Laser Speckle Contrast Imaging (LSCI) systems include vertically polarized LSCI (vp-LSCI) and non-polarized LSCI (np-LSCI) configurations. In vp-LSCI systems, the laser light is vertically polarized [14]	Pulp blood flow
Laser speckle-optical device “Specklemeter” [15; 16]	
Transillumination	
Intense narrow beam of light [12]	Light passing through a tooth
Ultrasound Doppler flowmetry	
Ultrasonic Doppler device and 20 MHz probe [9]	Pulp blood flow
Continuous-wave dental Doppler ultrasound system (DDUS) using 22 MHz ultrasonic probes. The DDUS comprises a dual-element transducer, a two-channel probe system (one for transmission and one for reception), and a laptop	Pulp blood flow
Magnetic resonance imaging	
A 3-Tesla system using a 16-channel coil with intravenous administration of gadolinium contrast [13]	Condition of the pulp tissue
Tooth temperature measurement	
Infrared thermometer with laser targeting [17]	Tooth temperature
FLIR E60 thermal imaging camera [18]	

Table 2 summarizes information on the non-conventional devices and systems described in publications from 2019 to 2024 that were used for the assessment of pulp vitality.

Photoplethysmography (PPG) – is a non-invasive optical method widely used to study and monitor pulsations associated with changes in blood volume in peripheral vasculature. It is characterized by low susceptibility to signal contamination from surrounding tissues. This method can be applied to assess pulp vitality, particularly in traumatized teeth, using a custom-fabricated holder.

Light sources used in PPG systems include:

- LEDs with wavelengths of 525, 625, and 940 nm [20];
- a spectrometer placed between a xenon arc lamp and a fiber optic bundle, where the transmitted light is recorded in pulses [19].

The method primarily relies on the intensity of transmitted light, which in some studies has been converted into other metrics, such as voltage and pressure signals [20]. Studies have shown that PPG can monitor pulp blood flow changes in response to harmless stimuli [19].

Knörzer et al. developed a dual-wavelength PPG system (625 and 940 nm) and successfully detected signals originating from the dental pulp without interference from surrounding tissues, demonstrating the feasibility of registering pulsed blood signals. The system revealed enhanced signal strength without requiring extensive noise suppression. At a wavelength of 625 nm, signals from both the pulpal and surrounding tissues could be detected on the tooth surface. However, at 940 nm, only signals from the pulp were recorded, free from surrounding tissue interference. These findings highlight PPG as a promising approach for future pulp vitality testing. Crucially, the ability to clearly distinguish between pulpal and non-pulpal PPG signals remains essential [20].

Transillumination (TTI). Transillumination is a diagnostic method in which visible light is directed onto a tooth, and a digital camera captures the resulting bright transmitted illumination of the dental tissues. The light source is positioned behind the tooth, and the pulp's vitality influences its translucency. A vital pulp permits light transmission, resulting in a well-transilluminated tooth, whereas a necrotic pulp appears dark and dim [12].

However, this technique may be unreliable for detecting pulp necrosis in teeth without visible discoloration, potentially leading to false-negative results [12]. Additionally, false-positive outcomes may occur in cases where tooth discoloration is caused by factors unrelated to pulp vitality [12].

Speckle imaging (LSI). LSI is based on the coherent interference of light, which generates a speckle pattern – a mix of bright and dark spots. These speckles exhibit random temporal fluctuations caused by changes in blood flow direction and particle position, allowing the measurement of blood flow velocity through frequency analysis of these fluctuations. LSI enables differentiation between necrotic and vital pulp and facilitates the evaluation of pulpal blood supply. This non-invasive technique does not require the use of holders [14].

LSI is sufficiently sensitive to detect minor changes in blood flow and is a simple, non-invasive, relatively

low-cost method that can be readily integrated into dental practice. It allows the measurement of blood flow velocity across a wide range. However, accurate assessment of pulpal circulation using speckle patterns remains challenging due to the limited penetration of light through enamel and dentin [14].

Laser Speckle Contrast Imaging (LSCI) captures a single exposure of the laser speckle pattern projected onto the tissue of interest and analyzes the resulting pattern to provide information on blood flow, including velocity, vessel diameter, and blood volume [1]. Multi-Exposure Contrast Imaging (MECI) is an enhanced extension of LSCI, offering improved resolution and sensitivity [2].

Xu et al. enhanced the detection of stimulus-induced blood flow in extracted teeth by integrating optical polarization technology with laser speckle contrast imaging (LSCI), introducing a promising tool for pulp vitality assessment in clinical settings, particularly in cases of dental trauma [14]. The results demonstrated that a vertically polarized LSCI system (vp-LSCI) could detect a broader dynamic range of pulpal blood flow contrast – 1.5 to 2 times greater – compared to non-polarized LSCI (np-LSCI). A temporal contrast algorithm was able to detect small flow velocity changes of 0.03–0.07 mm/s, with a detection index 5–10 times higher than that of a spatial contrast algorithm. The vp-LSCI system, combined with temporal contrast analysis, significantly enhances the ability of speckle-based techniques to detect pulpal blood flow, enabling the detection of a wider flow velocity range and finer hemodynamic variations [14].

G.G. Chistyakova evaluated microcirculatory parameters in dental pulp vessels using the laser speckle optical device *Speklometr* (State Registration No. Mr-7.1504-0108) in accordance with a methodology approved by the Ministry of Health of the Republic of Belarus (Instruction No. 099 0619 dated June 28, 2019) [16; 21]. During the analysis and screening of speckle patterns, key hemomicrocirculatory parameters – such as spectral power S (relative units) and mean frequency (Hz) – were optimized and interpreted as indicators of blood flow level and intensity. It was found that decreased blood flow intensity corresponded with increased electroodontometric values and a reduced threshold for pulp excitability [16].

Ultrasound Doppler Flowmetry (UDF) is a non-invasive, radiation-free technique that evaluates blood flow by transmitting ultrasonic waves through tissues. UDF operates on the same Doppler principle as laser Doppler flowmetry (LDF) but utilizes ultrasound instead of light. Parameters such as blood flow velocity, pulsatility index, and circulation index are calculated. The technique also allows real-time auditory monitoring of pulsatile sounds [22].

No human clinical studies using UDF for pulp vitality assessment were identified in the reviewed period. However, one animal study (in dogs) and one in vitro study using simulated pulp blood flow in extracted human teeth were published.

A retrospective study in dogs demonstrated that UDF had higher diagnostic effectiveness compared to electric pulp testing (EPT) when assessing pulp vitality in traumatized teeth. It also showed a correlation

between pulpal blood flow (PBF) and blood flow in the adjacent gingiva. The study concluded that effective UDF measurement requires isolation of the gingiva from the tooth structure [9].

A laboratory study on extracted human teeth indicated that UDF can detect simulated blood flow below the cemento-enamel junction, but not at the mid-root level [11].

In 2021, a continuous-wave dental Doppler ultrasound system (DDUS) was introduced, employing 22 MHz ultrasound probes. The DDUS comprises a two-element transducer, a dual-channel probe system (one for transmission and one for reception), and a laptop. The probe system performs analog front-end processing, including ultrasound transmission/reception, low-noise amplification (LNA), quadrature demodulation (QDM), low-pass filtering (LPF), and analog-to-digital conversion (ADC). The laptop conducts intermediate-level processing such as decimation, logarithmic compression, interference filtering/noise reduction, and data analysis displayed as a spectrum or spectrogram. To enhance the signal-to-noise ratio (SNR), noise reduction is implemented using Hankel Singular Value Decomposition (SVD). The results are presented in real time as Doppler spectra and spectrograms via a custom-developed graphical user interface (GUI) for the DDUS [10].

The use of Ultrasound Doppler Flowmetry (UDF) in cases of dental trauma presents a promising and potentially more sensitive approach than laser Doppler flowmetry (LDF) [23]. UDF is capable of distinguishing signals originating from the dental pulp and surrounding tissues by analyzing waveform and sound characteristics.

However, the method has several limitations. These include instability in producing consistent results over short observation periods, challenges in accurate probe positioning, potential interference from patient discomfort, and early-stage trauma-induced impairment of pulpal blood supply, which may compromise measurement reliability [23].

Currently, there is no established standard for UDF parameters that distinguish between normal and pathological pulp. Moreover, variables such as probe angle or positioning, probe holder characteristics, and gingival blood flow [9] can significantly influence the recorded values.

Magnetic resonance imaging (MRI). Teeth are difficult to visualize using conventional magnetic resonance imaging (MRI) due to their high mineral content. Moreover, the signal decays rapidly after radiofrequency excitation because of the severely restricted molecular motion of water in densely mineralized tissues. Only with the advent of later technological modifications, such as SWIFT-MRI, have image details emerged that may hold potential value for endodontic research. MRI scanners with a magnetic field strength of 1.5 Tesla have been shown to be insufficient for effective visualization of oral and maxillofacial structures [24].

In response to these limitations, Juerchott et al. conducted a prospective in vivo study aimed at optimizing the assessment of pulpal contrast enhancement (PCE) using dental MRI (dMRI) and at investigating physiologi-

cal models of PCE. Healthy teeth were examined using 3-Tesla dMRI with a 16-channel coil, and gadolinium contrast agent was administered intravenously before and after scanning. The results demonstrated that PCE in dMRI is a stable intra-individual marker with minimal differences between tooth types, forming an important basis for within-subject comparisons in the evaluation of teeth suspected of endodontic pathology. Furthermore, PCE was shown to be independent of age and sex [13].

The implementation of higher-field MRI systems operating at 3 Tesla has significantly improved dental visualization and is regarded as a promising tool for detecting pulp tissue abnormalities. Nonetheless, further improvement in spatial resolution is essential for enhancing the diagnostic utility of MRI in endodontics [24].

Tooth temperature measurement (TTM). It has been established that vital teeth emit more heat than non-vital teeth. When vital teeth are cooled, a phenomenon known as surface rewarming is observed – these teeth are able to restore their surface temperature over time. Surface rewarming is considered a potential indicator for assessing true pulpal vitality. Vital teeth successfully regained surface heat within a 3-minute period, whereas none of the non-vital teeth reached baseline temperatures within this timeframe. Thus, vital teeth demonstrate faster surface rewarming compared to non-vital teeth. This methodology demonstrated a clinical success rate of 97.34% in diagnosing pulp vitality status [17].

In another study assessing 126 maxillary anterior teeth using the FLIR E60 thermal imaging camera, it was shown that vital and non-vital anterior teeth exhibit distinct temperatures when assessed with infrared thermography [18]. Infrared thermometry with laser guidance may be employed as a routine test for pulp vitality in clinical settings and represents a simple and reliable tool. One of its main advantages is non-invasiveness [17]. However, at this stage, this test appears impractical for routine use as a standalone method of assessing dental vitality.

This review of non-conventional methods for pulp vitality assessment aims to present current research trends. However, any conclusions drawn must be interpreted with caution due to the heterogeneity of included studies and the lack of reported accuracy and predictive values in most of them.

Patents on Instrumental Methods for Assessing Pulp Vitality

The results of the patent search identified four patents published during the review period (2019–2024). One patent concerned an electronic device for intra-oral scanning [25], two patents described optical methods – a dental diagnostic probe [26] and a fiber-optic device [27] – and one patent focused on an ultrasonic probe [28] (Table 3).

Patent Overview on Instrumental Methods for Assessing Pulp Vitality Jiang X., Fu M., and Yang L. developed a cranio-maxillofacial oral cavity scanning device designed to detect necrotic pulp tissue and residual pulpal matter following operative procedures [25].

Table 3. General information on included patents**Таблица 3.** Общая информация о включенных патентах

Publication year	Patent No.	Title
Magnetic resonance imaging		
2021 (G) Jiang et al., 2020 [25]	CN-111528846-B	Oral craniomaxillofacial scanning device and scanning method and electronic device.
Optical		
2023 (G) Ertl et al., 2017 [26]	EP- 3439542- B1	Dental probe
2023 (P) Tang, Schmitt, 2023 [27]	US-2023263398-A1	Apparatus and method for tooth pulp vitality detection
Ultrasound Doppler flowmetry		
2019 (P) Kim, 2018 [28]	KR-20190089430-A	Dental pulpal vitality assessment system including ultrasound probe and method for assessment

T. Ertl, N. Karazivan, and A. Savic proposed a dental diagnostic probe that emits light within the 500–1500 nm range, which passes through part of the pulp. The transmitted signals are captured by a photodiode detector positioned on the opposite side of the tooth. The system enables detection of bacterially induced inflammatory changes in the pulp, even in teeth with crowns or restorations [26].

Tang C.-M. and Schmitt J. developed a fiber-optic device consisting of a handpiece, a rotational axis, and a caliper-like mechanism that emits light onto the tooth and receives interference signals corresponding to pulpal motion [27].

Kim H.-Y. designed an ultrasonic probe that emits ultrasound signals at 15–25 MHz and detects reflected echoes. Signal analysis allows the calculation of blood flow velocity within the pulp [28].

RESULTS

Non-conventional methods for pulp vitality assessment are increasingly being explored for their diagnostic potential. Scientific publications from 2019 to 2024 have referenced a range of such methods, including ultrasound Doppler flowmetry, transillumination, magnetic resonance imaging, speckle imaging, dental thermography, and plethysmography.

Ultrasound Doppler flowmetry has shown a more promising and sensitive approach than laser Doppler flowmetry, particularly in cases of dental trauma. The introduction of high-field 3 Tesla MRI systems has significantly improved imaging capabilities and is now considered a potential tool for detecting pulpal abnormalities. However, further enhancement of spatial resolution is necessary for successful integration into endodontic diagnostics.

The vp-LSCI system, in combination with temporal contrast algorithms, has greatly improved the ability of laser speckle imaging to detect pulpal blood flow, offering greater sensitivity to flow velocity and subtle hemodynamic changes. The transillumination technique is most effective when visible tooth discoloration is present. Photoplethysmography (PPG) shows promise for future pulp vitality testing, though accurate differentiation between pulpal and non-pulpal signals remains

a critical requirement. Meanwhile, infrared thermometry, although non-invasive and accessible, currently appears impractical for routine clinical use as a standalone diagnostic method.

To date, none of the non-conventional pulp vitality assessment methods presented in this review have been fully integrated into standard clinical practice, underscoring the persistent challenge of developing a reliable diagnostic approach. While emerging technologies aim to improve diagnostic sensitivity and specificity, further evidence-based studies and the development of clinically applicable, user-friendly equipment are needed to advance their implementation.

LIMITATIONS OF THE STUDY

The primary limitation of this review is the lack of a critical appraisal of the methodological quality of the included studies. This may have resulted in the inclusion of lower-quality research, thereby increasing heterogeneity among the studies. As the current review aims to provide a broad overview of emerging research trends, any conclusions drawn should be interpreted with caution due to the diversity of the included literature.

In several studies, data were limited exclusively to anterior teeth, which restricts the generalizability of the findings to the assessment of pulp vitality in posterior teeth. While sensitivity and specificity are commonly cited indicators of diagnostic validity, positive predictive value (PPV) and negative predictive value (NPV) may better reflect clinical utility. Pulp testing ideally requires clinical validation through histological confirmation, yet such data were absent from most of the included studies. This lack of histological evidence hampers accurate assessment of the diagnostic precision of non-conventional methods.

As a result, questions remain regarding the reliability and clinical relevance of the reviewed technologies, similar to those associated with traditional pulp sensitivity tests. Future studies should encompass both static and dynamic blood flow conditions and investigate a broader spectrum of light frequencies, which are essential for optimizing the efficacy of emerging optical pulp vitality assessment techniques.

FUTURE DIRECTIONS

Further research is necessary to refine non-conventional methods for pulp vitality assessment. Specifically, future investigations should examine their correlation with histological findings. Interdisciplinary collaboration between dental professionals and biomedical engineers will be critical for the development of standardized tooth models for diagnostic testing. Such part-

nerships will foster innovation, promote effective clinical problem-solving, and bridge the gap between clinical insights and technological advancement. Notably, many of the techniques discussed in this review have been adapted from other medical disciplines, reinforcing the need for comprehensive analysis of the medical, optical, and bioengineering literature to identify promising technologies for pulp testing.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

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Effect of calcium silicate-based repair sealers on bone healing in rat skull defects: histological and histomorphometric study

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Abstract

AIM. This study investigated the impact of calcium silicate sealers on bone healing in rat calvaria defects.

MATERIALS AND METHODS. Twenty-six rats were divided into 3 groups. Calvaria defects were prepared and treated in 3 different ways: CG ($n = 6$): Filling with clot; ES ($n = 10$): Filling with Endosequence BC RRM Putty repair sealer and BC ($n = 10$): Filling with Bio C repair sealer.

RESULTS. After 15 and 30 days, histological evaluations revealed varied levels of bone regeneration and inflammation. The group treated with a blood clot showed more bone regeneration without inflammation at 15 days. However, after 30 days, complete closure of the defect with immature tissue was observed in this group, while limited new bone formation occurred in the other groups, accompanied by mild inflammation.

CONCLUSIONS. Overall, the study suggests that calcium silicate-based sealers can induce new bone formation, but they do not offer superior bone repair compared to natural healing with a blood clot alone.

Keywords: animal model, endodontics, dental materials.

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Влияние кальций-силикатных восстановительных цементов на заживление костной ткани при дефектах черепа у крыс: гистологическое и гистоморфометрическое исследование

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Резюме

ЦЕЛЬ. В данном исследовании изучалось влияние кальций-силикатных цементов на заживление костной ткани при дефектах свода черепа у крыс.

МАТЕРИАЛЫ И МЕТОДЫ. В исследование было включено 26 крыс, разделенных на 3 группы. Дефекты в области свода черепа создавались и обрабатывались тремя способами: CG ($n = 6$): заполнение сгустком крови (контроль); ES ($n = 10$): заполнение восстановительным цементом Endosequence BC RRM Putty; BC ($n = 10$): заполнение цементом Bio C Repair.

РЕЗУЛЬТАТЫ. Через 15 и 30 дней проводилась гистологическая оценка, которая показала различный уровень регенерации кости и выраженности воспаления. В группе, где применялся только кровяной сгусток, на 15-й день наблюдалась более активная костная регенерация и отсутствие воспаления. Однако через 30 дней в этой группе дефект был полностью закрыт незрелой тканью, тогда как в остальных группах формирование новой костной ткани было ограниченным и сопровождалось слабовыраженным воспалением.

ВЫВОДЫ. В целом исследование показало, что кальций-силикатные цементы способны индуцировать образование новой кости, однако не обеспечивают более эффективного восстановления костной ткани по сравнению с естественным заживлением при использовании одного лишь сгустка крови.

Ключевые слова: животная модель, эндодонтия, стоматологические материалы

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INTRODUCTION

Bioceramic sealers have been developed with the aim of making procedures such as pulp capping, pulpotomies, perforation repairs, root resorption treatments, apex formation treatments, and retrograde fillings more predictable [1]. However, the direct contact of these sealers with pulp and periodontal tissues may raise concerns regarding their biocompatibility [1]. The interaction of these materials with tissues is often suggested with the aim of promoting tissue regeneration/repair [2], but it is important to evaluate potential adverse reactions such as inflammation [3; 4].

MTA (mineral trioxide aggregate) is a material that revolutionized endodontics in the 1990s [5]. It is considered the biocompatible treatment option of choice in many cases of root perforations, internal and exter-

nal resorptions, and pulp capping [6; 7]. However, MTA has some disadvantages [8], such as low compressive strength, high pigmentation power of the remaining tooth structure, time-consuming setting. Due to its low flowability, it is difficult to handle when it clumps with distilled water, resulting in a sandy appearance and making it impractical to use [6; 8; 9].

To overcome the drawbacks of MTA, new calcium silicate-based materials have been developed to improve handling, setting time, and release of heavy metals [10]. These third-generation materials have been evaluated for their cytotoxicity, regenerative potential, and overall biocompatibility [4; 10–14].

Some of these sealers, such as EndoSequence Root Repair Material Putty (Brasseler USA, Savannah, GA, USA) and Bio-C Repair (Angelus, Londrina, PR, Brazil)

have been successfully applied both in vitro and in vivo [5; 7; 11; 15]. However, there is still controversy and unconvincing results compared to MTA [8; 11; 16; 17].

Although used as an adjuvant and even as the main material in various clinical situations in endodontics, several studies question their ability to form bone [18–20], an essential property within their indications.

Given the difficulty in conducting clinical trials to evaluate the efficacy of these materials, research has focused on animal models. The present study, conducted in rats, aimed to evaluate histologically and histomorphometrically the effect of calcium silicate-based repair sealers, Endosequence BC RRM Putty and Bio C Repair, on bone healing in skull defects. The null hypothesis is that there is no difference in bone healing between defects filled with clot or repair sealers.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki. Twenty-six rats of the species *Rattus norvegicus albinus*, class Mammalia, order Rodentia, of the Wistar strain, were used for this study. The research was approved by the local Ethics Committee for the Use of Animals under the number 2020/13 (Date: June 30, 2020). The selected animals were young adult animals without genetic modification, approximately 3 months old and weighing 300 g each. The animals were kept in the animal house in cages lined with autoclaved wood shavings, changed daily, with three animals per cage. The environment had controlled lighting with 12 hours of light and 12 hours of darkness, a controlled temperature of 21°C, and water and food *ad libitum*.

Sample calculations were based on a critical value of 1.96 for the 95% confidence interval, a maximum acceptable variance of 0.23 (23%) based on a preliminary experiment 21 a minimum standard error of 5% of the mean, and a significance level of $p < 0.05$. The manuscript of this laboratory study was written according to the PRIASE 2021 guidelines for reporting animal studies in Endodontology.

The 26 animals were randomly divided into 3 groups, namely CG – control group with 06 animals and experimental groups ES and BC with 10 animals each according to the intervention to be performed and, divided into two groups according to the observation period of 15 and 30 days. The division of the groups can be seen in Table 1.

For surgical interventions, the animals were previously restrained and subjected to general anesthesia. For this purpose, they were injected intraperitoneally with ketamine hydrochloride (Ketamine Agener, Agener União, Embu-Garçu, Brazil) at a dose of 75mg/Kg and xylazine hydrochloride (Dopaser, Hertape Calier S.A, Juatuba, Brazil) at a dose of 10mg/Kg. After confirming effective analgesia of the animals, they were trichotomized in the calvaria region and positioned in ventral decubitus position on a special couch on which the region to be operated was immobilized. Asepsis was performed in the region of the skullcap using iodine alcohol as a local antiseptic. Then, a semilunar incision was made in the median region of the skull of the rats, fol-

lowed by a wide lateral excision and exposure of the calvaria. A 4-mm-diameter trephine burr was used to drill a 1-mm-deep, circular, noncritical bone defect in the calvariae of the rats under constant irrigation with saline at low rotation. During surgery, sterile gauze soaked with 0.9% saline was placed over the animals' eyes to prevent corneal desiccation.

The defects were filled and treated as described in Table 1. The wound was then sutured with 4.0 silk suture (Ethicon, Johnson & Johnson, São José dos Campos, Brazil) to properly close the flap edges.

After surgery, the muscle relaxant tramadol was administered intraperitoneally at a dose of 5 mg/Kg every 24 hours for 3 days, in addition to the analgesic flunixin meglunin at a dose of 1.1 ml/Kg in a single dose. No anti-inflammatory or antibiotic drugs were administered preoperatively or postoperatively to allow the healing process to take its natural course.

The operated animals were routinely observed from the first surgical act (creation of the defect and insertion of the bioceramic cements) until euthanasia. Animals were euthanized 15 and 30 days after surgery by an overdose of anesthetic, according to the protocol of choice: 90–150 mg sodium thiopental (71-73-8) associated with 10 mg/ml lidocaine (137-58-6), intraperitoneally.

After euthanasia, histologic analysis was performed by conventional light microscopy of slides stained with hematoxylin and eosin. The analysis was performed by a single examiner. Grades from 0 to 3 were assigned to measure the intensity of the inflammatory infiltrate, with 0 representing an inflammatory infiltrate of up to 15%, 1 representing an infiltrate between 15 and 50%, 2 representing an infiltrate of more than 50% up to 75%, and 3 representing an infiltrate of more than 75%. Histomorphometry assessment was also performed by a single investigator who was blinded to the work performed. The total area observed was measured in square micrometers (μm^2) and was considered 100%, and the bone area analyzed was its percentage relative to the total area.

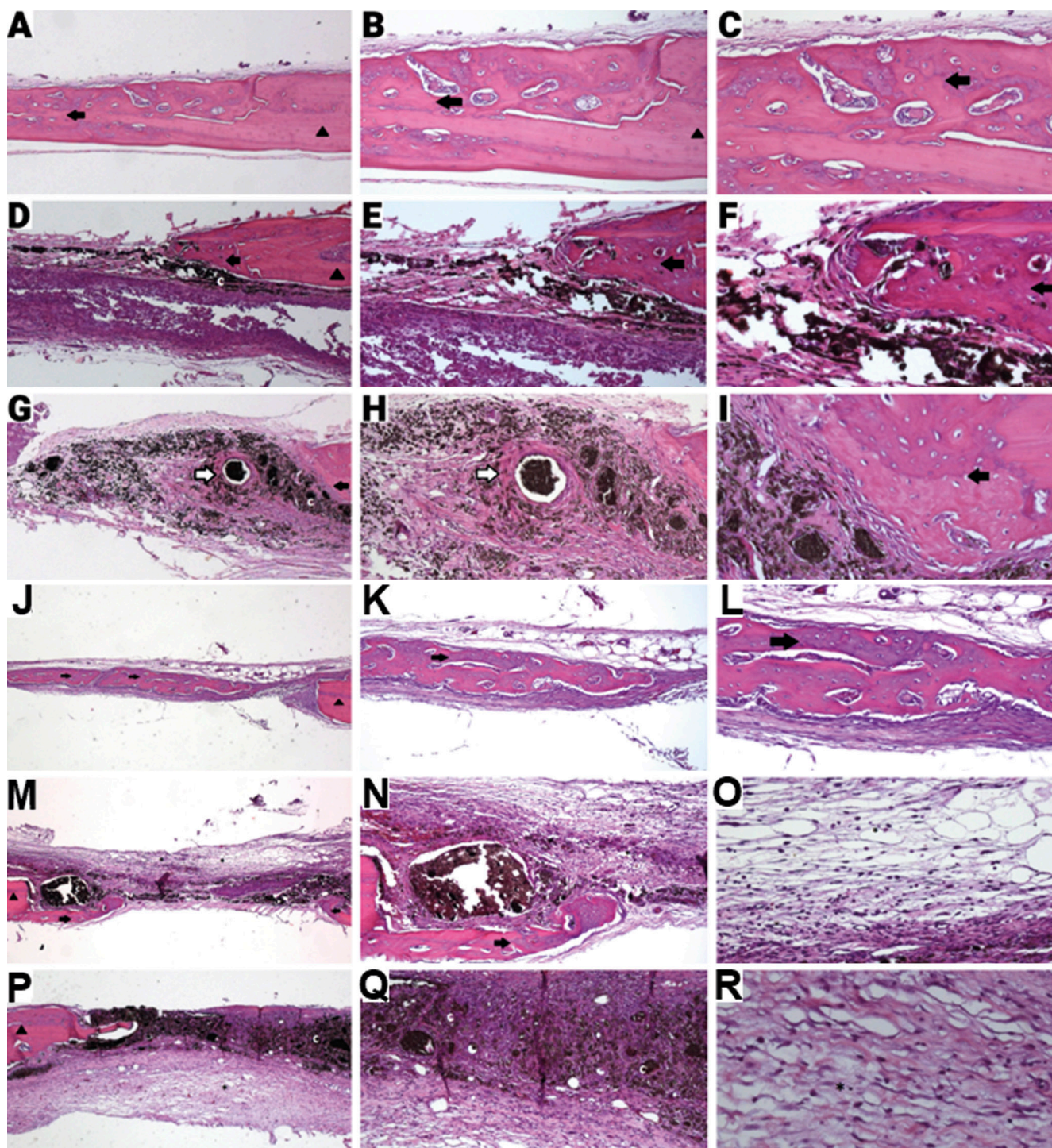
Statistical Analysis

Then, the inflammation data were statistically analyzed and subjected to Kruskal-Wallis test followed by Mann-Whitney U test with a significance level of 5%, and the histomorphometric data were subjected to descriptive statistics.

Table 1. Allocation of experimental groups

Таблица 1. Распределение экспериментальных групп

Group	N	Euthanasia	Intervention
CG	3	15 days	Animals subjected to cranial trephination without insertion of bioceramic sealer – repair with blood clot only
	3	30 days	
ES	5	15 days	Animals subject to cranial trephination using Endosequence BC RRM Putty repair sealer – Brasseler
	5	30 days	
BC	5	15 days	Animals subjected to cranial trephination using Bio C repair sealer – Angelus
	5	30 days	



Legend: ➡ area of new bone formation; ▲ mature bone; C – repair sealer; * mononuclear inflammatory infiltrate; ⇨ phagocytic inflammatory giant cell

Легенда: ➡ область новообразования кости; ▲ зрелая кость; C – репарационный цемент; * мононуклеарный воспалительный инфильтрат, ⇨ фагоцитирующая воспалительная гигантская клетка

Fig. 1. A, B and C – group GC after 15 days at 40x, 100x and 200x magnification, respectively; D, E, and F – group ES after 15 days at 40x, 100x, and 200x magnification, respectively; G, H and I – group BC after 15 days at 40x, 100x and 200x magnification, respectively; J, K, and L – group GC after 30 days at 40x, 100x, and 200x magnification, respectively; M, N, and O – group ES after 30 days at 40x, 100x, and 200x magnification, respectively; P, Q and R – group BC after 30 days at 40x, 100x and 200x magnification, respectively

Рис. 1. А, В и С – группа GC через 15 дней при увеличении 40х, 100х и 200х соответственно; D, E и F – группа ES через 15 дней при увеличении 40х, 100х и 200х соответственно; G, H и I – группа BC через 15 дней при увеличении 40х, 100х и 200х соответственно; J, K и L – группа GC через 30 дней при увеличении 40х, 100х и 200х соответственно; M, N и O – группа ES через 30 дней при увеличении 40х, 100х и 200х соответственно; P, Q и R – группа BC через 30 дней при увеличении 40х, 100х и 200х соответственно

RESULTS

The illustrative images of the histological sections stained with hematoxylin-eosin of the different groups of samples after 15 days are shown in Fig. 1. After 15 days, areas of new bone formation were observed at CG in the region near the recipient bed as well as in the central region of the defect. Osteoblasts outlining the bone spicules were also observed, and this region was well vascularized. No inflammatory process was observed. In the ES group, sealer areas distributed throughout the defect were observed with little new bone formation, strong vascularization, and a discrete, typically mononuclear inflammatory infiltrate. In the BC group, cementum and an inflammatory infiltrate were observed, also typically mononuclear, highly vascularized, and almost no area of new bone formation.

The illustrative images of the hematoxylin-eosin-stained histological sections of the different sample groups after 30 days are shown in Fig. 2. At CG, almost complete closure of the filled defect was observed with the presence of both immature bone tissue and lamellar bone. Little bone tissue was observed in the ES and BC groups. In the ES group, bone tissue was noted near the peripheral region of the defect. In the BC group, the presence of phagocytic inflammatory giant cells penetrating the biomaterial was also observed. In all groups, no lymphocytic or mononuclear infiltrate was observed in the defect region.

The control group had an inflammation value of zero during the 15-day observation period, while the ES and BC groups had a value of 1, representing a statistically significant difference between the control and experimental groups ($p = 0.014$). For the 30-day observation period, the 3 groups showed a value of 0, i.e., no statistical difference. In all groups and evaluation periods,

there was no variability in the scores evaluated. When comparing time periods, the control group showed no statistical difference, and the ES and BC groups showed a statistically significant decrease in score ($p = 0.004$) from 1 at 15 days to 0 at 30 days.

DISCUSSION

The search for restorative materials that promote bone regeneration in endodontics is not a new topic [21]. In this study, two calcium silicate-based materials indicated for cases of root resorption, root perforations, pulpotomies, revascularization, retrograde obturation, and pulp capping were evaluated. However, the results obtained showed no significant advantage in bone regeneration compared with the control group, in which the defect in the calvaria of the rats was filled only with a blood clot. These results, while contradictory to the indications of the materials, are consistent with several other studies in the literature [8; 11; 16; 17; 21–23].

The assessment of bone regeneration through calvaria defects used in this study is considered the gold standard to test the tissue response promoted by root filling materials [16]. On the other hand, it might be ideal to make two defects in the same animal, one filled with clot and the other filled with the test material, so that each test sample is a separate control [24]. Such a method was not used in this study because a separate animal served as the control group.

One aspect of these materials that has been much discussed in the literature is the setting time, and it is well known that calcium silicate-based sealers have a long setting time [17]. According to Damas et al. [17], Endosequence RRM Putty can set up to 12 hours in a 100% humidity and 37°C environment, but in their study, there was no complete set and evaluations at 24,

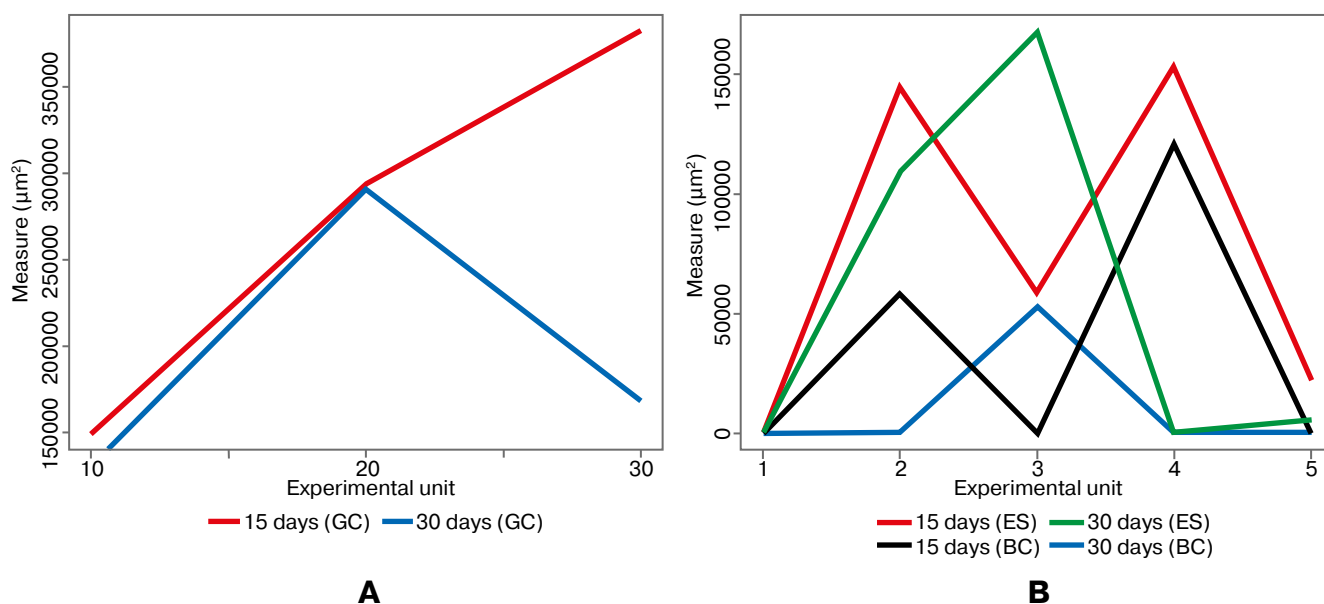


Fig. 2. Amount of newly formed bone per animal, in μm^2 , after 15 and 30 days: A – for the control group; B – for the groups ES and BC

Рис. 2. Объем новообразованной кости на животное (в $\mu\text{м}^2$) через 15 и 30 дней: A – в контрольной группе; B – в группах ES и BC

72 and 120 hours. Complete setting was observed only after 168 hours. This aspect could be one of the reasons for the greater presence of an initial inflammatory infiltrate and a delay in bone regeneration [4; 13; 14].

In a previous study [25], after a 2-week treatment with AH Plus endodontic sealer, a moderate inflammatory reaction was observed, which decreased only after 4 weeks, confirming our study, in which a decrease in the inflammatory process was also observed throughout the evaluation period. However, it is worth noting that the material tested in that study was based on epoxy resin, which is significantly different from the material used in the present study. In our study, although the inflammatory process was more persistent than in the control group at the end of the observation period, the absence of a lymphocytic or mononuclear infiltrate is indicative of the biocompatibility of the sealers tested. This result is also consistent with another study [23], in which two sealers (AH Plus and Sealer Plus) were tested on calvarial defects in rats. In this study, the sealers caused an intense inflammatory response but were considered biocompatible because they allowed bone repair. It should also be noted that endodontic defects are usually smaller than those created in this study, resulting in less contact between the exposed material and the tissue, which could also lead to a lower inflammatory response.

The assessment of inflammation in our study was semiquantitative by initial qualitative assessment followed by quantitative assessment by scores. It is known that this type of assessment may not be accurate enough to compare different materials or different time intervals [16]. On the other hand, a purely quantitative score in this type of analysis usually shows differences only when the studied groups are very different, as it may inadequately capture small differences [16].

Finally, small differences between the sealers studied can be explained by possible differences in their surface properties, which are directly related to their biological properties. The presence of calcium in the composition of these sealers may be similar but released to the medium to different extents [3]. The release of calcium from the sealer into the medium is the

main agent for cementoblast differentiation and dentin bridge formation and plays an important role in antimicrobial activity [3; 13; 14].

A recent study, also using an animal model (Wistar rats) [14], investigated eight calcium silicate-based sealers, quantifying volume change, biocompatibility, and systemic migration of sealer components using different evaluation methods. The study used sealers in powder/liquid and “ready-to-use” presentations, employing computed microtomography to assess volume variation and histological analysis to determine biocompatibility after 30 days of implantation in alveolar bones and subcutaneous tissues. Mass spectrometry was used to measure the accumulation of metals such as bismuth, tantalum, tungsten, and zirconium in the kidneys after the euthanasia of the animals. The results revealed that Biodentine, EndoSequence BC RRM Putty, and ProRoot MTA maintained better volumetric stability, although ProRoot MTA and MTA Repair HP showed metal accumulation in the kidneys. The analysis concluded that Bio-C Repair, NeoPUTTY, and MTA Repair HP lose volume in subcutaneous tissues more than in bones, with NeoPUTTY inducing more inflammation. The study suggests that the chemical composition of the sealers and the type of tissue significantly affect their clinical performance.

A limitation of this study is the use of animal model, so caution is advised during the results interpretation, due to the translation from a laboratory and in vivo study using an animal model to the patients could be distant. Further studies are needed to confirm not only the bone repair indication of calcium silicate sealers, but also the other indications that were not investigated in this study.

CONCLUSION

Within the limitations of the present study, it was found that calcium silicate-based sealers are materials that have biocompatibility properties and can enable the regeneration process in the defects in the calvaria of rats. However, compared to the control group, which was only filled with blood clots, they did not show superiority in accelerating this regeneration.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

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An ex vivo antimicrobial evaluation after the preparation with XP-Endo Shaper and Trunatomy systems

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Abstract

AIM. The aim of this study was to evaluate bacterial reduction in root canals with the XP-Endo Shaper (XP) and Trunatomy (TN) systems.

MATERIALS AND METHODS. Twenty-eight permanent human type I, oval-shaped Vertucci premolars and straight root canals were contaminated with *Enterococcus faecalis* for 30 days at 37°C. Samples were collected prior to instrumentation. The teeth were divided into two groups ($n = 14$) and processed with the tested groups at 37°C: XP group – (30/0.04) and TN group – Small (20/0.04) and Prime (26/0.04). Biological samples before and after instrumentation were collected using a sterile paper cone inserted into the canal for one minute. Bacteria were counted using colony forming units (CFU/mL) and results were subjected to Kruskal-Wallis test at 5 level of significance.

RESULTS. Both the XP and TN systems significantly reduced bacterial counts ($p < 0.0001$), but did not eliminate bacteria in the root canals.

CONCLUSIONS. Both the Trunatomy and XP-Endo Shaper systems were similar in terms of antimicrobial efficacy, but neither system was able to eliminate bacteria from the root canals.

Keywords: endodontics, *Enterococcus faecalis*, root canal treatment

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Экспериментальная *ex vivo* оценка антимикробной эффективности после препарирования с использованием систем XP-Endo Shaper и TruNatomy

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Резюме

ЦЕЛЬ. Целью данного исследования было оценить степень снижения бактериальной обсемененности в корневых каналах после обработки с использованием систем XP-Endo Shaper (XP) и TruNatomy (TN). **МАТЕРИАЛЫ И МЕТОДЫ.** Двадцать восемь постоянных человеческих премоляров типа I по Вертуччи с овальной формой и прямыми корневыми каналами были заражены *Enterococcus faecalis* в течение 30 дней при температуре 37°C. Образцы собирались до начала инструментальной обработки. Зубы были разделены на две группы ($n = 14$) и обработаны при 37°C: группа XP – инструмент (30/0.04); группа TN – инструменты Small (20/0.04) и Prime (26/0.04). Биологические образцы до и после обработки собирались стерильным бумажным штифтом, введенным в канал на одну минуту. Подсчет бактерий производился по числу колониеобразующих единиц (КОЕ/мл), результаты анализировались с использованием критерия Крускала–Уоллиса при уровне значимости 5%.

РЕЗУЛЬТАТЫ. Обе системы – XP и TN – достоверно снижали количество бактерий в корневых каналах ($p < 0.0001$), однако не обеспечивали полного их удаления.

ВЫВОДЫ. Системы TruNatomy и XP-Endo Shaper продемонстрировали сопоставимую антимикробную эффективность, однако ни одна из них не обеспечила полной элиминации бактерий из корневых каналов.

Ключевые слова: эндодонтия, *Enterococcus faecalis*, лечение корневых каналов

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INTRODUCTION

One of the main aims of endodontic treatment is to prevent or promote the healing of apical periodontitis by cleaning, shaping, disinfecting and filling the root canal system [1]. The microorganisms can organize themselves into biofilms, which increases their resistance to endodontic procedures and makes root canal disinfection a challenge [2].

One microorganism of interest in endodontics is *Enterococcus faecalis*, which has been extensively studied as it is particularly associated with persistent endodontic infections, which are a common cause of treatment failure due to their resistance in intracanal procedures [3]. This bacterium has an inherent resistance to various treatments, including irrigation solu-

tions, intracanal medications [4], antibiotics and pH changes [5]. Its ability to adhere, multiply, invade, resist host defenses and compete with other bacteria increases its virulence [6].

Mechanical preparation is the most critical phase of endodontic treatment, and great development has been made in the development of instruments [7]. Studies show that even with different techniques, complete removal of organic debris [8] and bacteria [9] is difficult to achieve. Automated systems based on a nickel-titanium alloy that work with continuous rotary motion have become widely accepted in recent decades [9]. These files, with innovative designs, manufacture, alloy composition and heat treatment [10], have redefined root canal instrumentation.

Some of these systems are XP-Endo Shaper (FKG Dentaire, La Chaux de Fonds, Switzerland) and TruNatomy (Dentsply Sirona, Ballaigues, Switzerland). These files have an innovative design and use rotary motion to enhance the root canal preparations [7], considered effective for complex anatomy that often harbor resistant microorganisms such as *Enterococcus faecalis* [5; 6].

The XP-Endo Shaper (FKG) with its serpentine design and NiTi MaxWire alloy adapts to the irregularities of the canal and minimizes stress on the dentin wall. It transitions from a straight, martensitic shape to a spoon shape at body temperature and uses its elasticity and shape memory effect to expand and contract during rotation to improve cleaning and disinfection. This dynamic ability improves root canal treatments by reaching hard-to-reach areas and effectively agitating the irrigant [7; 11]. The instrument is 30/.01 (size/taper), but can have a final canal preparation of 30/.04 [12].

Another new system is the TruNatomy (Dentsply Sirona), which ensures superior peri-cervical dentin preservation thanks to a special instrument geometry and heat treatment [13]. The TruNatomy instruments are characterized by flexibility, regressive tapering and a decentralized parallelogram cross-section and faithfully reproduce the anatomy of the canal [14]. This system comprises five instruments for cervical preparation (Orifice Modifier 20.08), the glide path (Glider 17.02v) and three sizes for canal shaping: Small (20.04v), Prime (26.04v) and Medium (36.03v) [11].

Endo Shaper (FKG Dentaire) and the TruNatomy (Dentsply Sirona) system. The null hypothesis tested was that there would be no difference between the groups in terms of bacterial reduction [15; 16].

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki. This study approved by the local ethics committee because it involved biological material (Opinion: 5.351.873, 14th April, 2022). The manuscript was written according to the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines [17] (Fig. 1).

The number of 14 samples per group was based on previous works [18; 19]. The sample was calculated using the t-test, with a minimum difference between the means of the treatments of 0.74, a standard deviation of 0.81–0.78, a number of treatments of 2, a test power of 0.80 and an alpha of 0.05.

Sample selection and standardization

The criteria included the selection of permanent human mandibular premolars with fully developed, singular roots, a straight single canal curvature $< 10^\circ$ according to Schneider [20] and a Vertucci type I and an oval shape (in which the buccolingual diameter is twice as large as the mesiodistal diameter in the first two thirds of the canal). Teeth with fractures, calcifications, dilacerations or previous endodontic treatment were excluded. Radiographs were taken in both vestibulo-lingual and mesiodistal orientations to confirm compliance with the inclusion criteria.

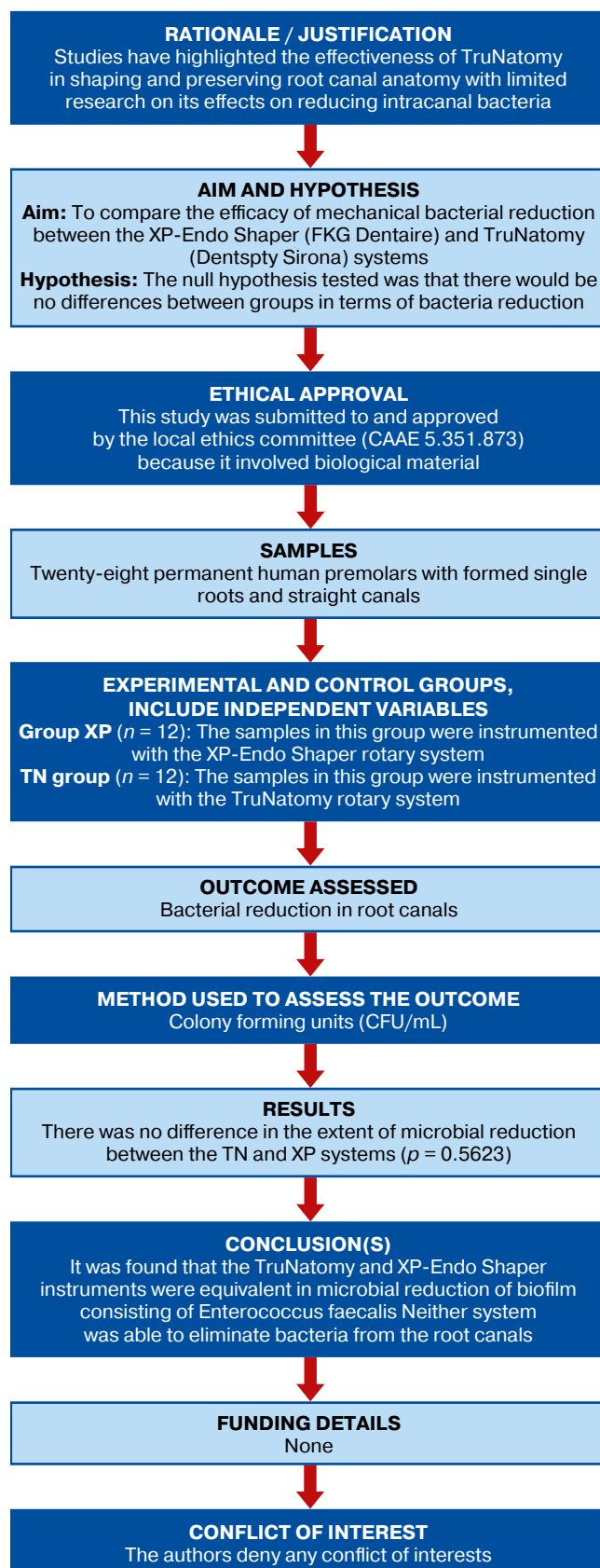


Fig. 1. Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines

Рис. 1. Рекомендуемые элементы отчета для лабораторных исследований в эндодонтии (PRILE) – рекомендации 2021 г.

The teeth were ultrasonically cleaned to remove any residual periodontal ligament or calculus. They were stored in 0.1% thymol until the start of the study. In preparation for the experiments, the roots were thoroughly rinsed under running water for one hour to remove all thymol residues. They were then dried with a stream of air and gauze. The samples were then flattened with a diamond disk attached to a ruler and measured with a conventional ruler, ensuring standardization to a length of 15 mm.

The working length (WL) was determined with a K#10 file (K-File, Dentsply Maillefer, Ballaigues, Switzerland) up to the apical foramen, whereby the WL was determined by subtracting 1 mm. The canal was then enlarged with a K#20 file (K-File, Dentsply Maillefer, Ballaigues, Switzerland) to standardize the original canal diameter and make room for bacterial contamination.

Micro-CT Scanning

All teeth were scanned in a micro-CT device (Sky-Scan 1273; Brucker micro-CT, Kontich, Belgium) at 70 kV, 114 mA, 12 μ m pixel size, 360° around the vertical axis, with 0.5 rotation step and 2 average frame and with a 1.0 mm thick aluminum filter. After scanning, the N. Recon v1.6.9.16 software (Bruker micro-CT) was used for the image's reconstruction (ring artifact correction of 5, a beam hardening correction of 50% and a smoothing of 8). The 3-dimensional volume and surface area were measured using CTAn v1.14.4.1 software (Bruker micro-CT).

The CTAn v1.14.4.1 software (Bruker micro-CT) was also used to measure the major diameter of the buccolingual root canal three millimeters from the apex. Root canals that had a buccolingual distance that was at least three times the mesiodistal distance were considered oval [21]. This procedure allowed the samples to be matched and randomly divided in two experimental groups according to *Enterococcus faecalis* contamination.

Enclosure of the roots in silicone

To prevent the irrigation solution from leaking through the apical foramen during root preparation, the root apices were first covered with utility wax. These roots were then placed in plastic cubes with 4 cm and a diameter of 3 cm, filled with silicone and finally covered with a thin layer of cyanoacrylate around the root to ensure stability. The teeth were then sterilized into an autoclave at 121°C for 15 minutes and subsequently contaminated with *E. faecalis*.

Contamination of the sample

The target microorganism for the infiltration test was *E. faecalis* (ATCC-29212), which was cultured and stored in BHI liquid medium (Brain Infusion Heart – BHI – Difco – Detroit, Michigan, USA) with 20% glycerol. To prepare the inoculum, 100 μ L of the *E. faecalis* broth was transferred to 2 mL of BHI broth and kept in the oven at 37°C for a maximum of 24 hours. After this time, the broth became turbid, which was compared with the Mac Farland 10 scale (1.0x10 CFU/mL). 37 g of BHI was dis-

solved in 1 liter of demineralized water and distributed into smaller containers. After the BHI was prepared, it was autoclaved for 40 minutes at 121 °C, pH 7.4, 0.2 at 25c with typical (g/liter).

Twenty microliters (μ L) of the suspension at the final concentration was added to the root canals using a pipette. A sterile, absorbent cotton was moistened, and placed in four wells of each cell culture plate. The lid of the plate was closed and sealed with adhesive tape and the whole was incubated in an oven at 37°C with 5% CO₂ for 30 days, when the used absorbent cotton was changed in plates wells [4]. During the contamination period, the bacteria viability was checked every three days and BHI was added daily to keep the strains viable to confirm that the contamination was effective. The container was then opened, and the contaminated teeth were used to start the experiment.

Root canal Instrumentation

Once the biofilm had matured, the individual samples were removed prior to instrumentation and randomly allocated into experimental groups ($n = 12$):

- Group TruNatomy (TN);
- Group XP – Endo Shaper (XP).

Samples were collected in a laminar flow chamber using a sterile cone of absorbent paper No. 20 (Endopoints, Manacapuru, Amazonas, Brazil) immersed in the canal for 1 minute. The samples were then transferred to an Eppendorf tube containing 5 mL of sterile 0.85% saline solution and shaken for 30 seconds (Vortex CP 600 Plus, Phoenix, Araraquara, Brazil). Serial dilutions (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) of this suspension were prepared and transferred to test tubes. Aliquots of 0.1 mL of the suspension and each dilution were seeded in Petri dishes containing BHI agar. The plates were incubated in an oven with 5% CO₂ at 37°C for 24 hours. The number of CFU/mL per culture plate was then counted before the root canals preparations.

After the antimicrobial samples prior to instrumentation, 5 mL of sterile 0.9% saline solution was used as irrigant, and the canals were instrumented. Root canal preparation was performed at 37°C in a heating cabinet (800-Heater; PlasLabs, Lansing, MI) built into the laminar flow. Instrumentation of the root canal was performed as follows:

Group XP ($n = 12$): Samples in this group were instrumented with the XP-Endo Shaper rotary system. While the canal was filled with 0.9% saline, a K#15 file (K-File, Dentsply Maillefer, Ballaigues, Switzerland) was inserted third by third up to the WL. The file reached the WL by long, even back and forth movements in the longitudinal direction. The movements were wide and uninterrupted so that the conical shape of the canal was defined. Once the WL was reached, 3 incremental movements were done. The teeth were instrumented with the X-Smart Plus motor (Dentsply Maillefer, Ballaigues, Switzerland) at 800 rpm and 1.5 Ncm. At each instrumented root third, the canal was irrigated with 5 mL of sterile 0.9% saline solution (Farmax, Brazil) using a conventional irrigation syringe (Ultradent, USA) and a 25x4 irrigation needle (Injex, São Paulo, Brazil)

and the glide path was performed with a K#20 hand file. A 30/04 gutta-percha cone was used to check the final preparation diameter.

TN group ($n = 12$): The specimens in this group were instrumented with the TruNatomy rotary system. While the canal was filled with 0.9% sterile saline, a K#15 file was inserted third by third up to the WL. Initially, the Orifice Modifier 20.08 instrument was used, followed by the 17.02v, 20.04v, 26.04v and 36/03 instruments. The torque used was 1.5 Ncm and the speed was 500 rpm with continuous rotation using the X-Smart Plus motor. Each time the instrument was used, three to four in and out movements were performed until the WL was reached.

All procedures were performed in a cabinet at 37°C with a heater (800-Heater; PlasLabs, Lansing, USA) to simulate body temperature. In both groups, each root canal was irrigated with 25 mL of saline after 3 preparation cycles (1 cycle corresponds to 3 back and forth movements).

Sample collection after instrumentation

After the final irrigation, the samples of each group were taken again using sterile paper cones, which remained in the canal for 1 minute. After 1 minute, the cones were removed and stored in a plastic container. The dilutions were prepared in test tubes according to the same protocol as the previous collection.

Statistical analysis

The results were analyzed using BioEstat 5.3 and the Shapiro-Wilk normality test was used. The sample showed non-normal behavior, then Kruskal-Wallis parametric test (Student-Newman-Keuls) was chosen, considering a significance level of 5%.

RESULTS

The previous count showed no significant difference between the groups before the preparation (0.5546). The results showed a significant intragroup microbial reduction after root canal instrumentation with the TN ($p < 0.0001$) and XP ($p < 0.0001$) systems. Also, no difference was found in an intergroup comparison between the TN and XP systems ($p = 0.5623$) (Table 1).

DISCUSSION

The primary goal of endodontic treatment involves thorough cleaning, disinfection and proper root canal obturation [22]. However, the complicated anatomy often harbors microorganisms in the bifurcations, isthmi and dentinal tubules, making them resistant to conventional mechanical and chemical treatments. This resistance could contribute to the failure of endodontic therapy [23].

In the present study, the instrumentation capacity of 2 rotary systems for the removal of *E. faecalis* was compared. Despite the different designs, cross-sections and heat treatments, there was no difference in the ability to disinfect root canals with the XP-Endo Shaper and the TruNatomy system. Both systems promoted partial bacterial reduction. The null hypothesis was therefore accepted.

Table 1. Kruskal-Wallis (Student-Newman-Keuls) statistical test of colony forming units/mL (log10) of the sample groups

Таблица 1. Результаты статистического анализа методом Крускала-Уоллиса (и критерия Стьюдента – Ньюмена – Келса) количества колониеобразующих единиц/мл (log10) в исследуемых группах

	TN		XP	
	PC	CAI	PC	CAI
MN	4.28	3.33	5.46	3.38
MX	6.30	4.98	7.05	5.40
MD (ID)	5.98(0.50) ^A	3.97(0.55) ^B	6.00(0.74) ^A	4.09(1.24) ^B
MA (SD)	5.79 (0.52)	4.00 (0.45)	6.08 (0.47)	4.29 (0.68)

Note: TN – TruNatomy system; XP – XP-Endo Shaper system; PC – prior collection; CAI – collection after instrumentation; MN – minimum values; MX – maximum values; MD – median; ID – interquartile deviations; MA – arithmetic means; SD – standard deviation; $p < 0.0001$

Примечание: TN – система TruNatomy; XP – система XP-Endo Shaper; PC – забор до инструментальной обработки; CAI – забор после инструментальной обработки; MN – минимальные значения; MX – максимальные значения; MD – медиана, ID – межквартильные размахи; средние MA – арифметические значения; SD – стандартное отклонение; $p < 0.0001$

Oval mandibular premolars were selected for this study because cleaning and shaping these teeth is challenging [12]. This is because the canal usually has a round cross-section during rotary instrumentation and the polar areas in oval canals remain unprepared.

In this study, the final preparation size was 30/0.04 for XP-Shaper and 36/0.03 for TruNatomy. Despite the differences in final apical diameter, studies have shown that both systems have similar capabilities in reducing microbial presence in root canals. This consistency of results suggests that factors such as instrument flexibility, cutting efficiency and ability to move irrigants may play a more critical role than preparation size alone [11]. The design and material composition of the instruments facilitate thorough cleaning of the canal so that even complicated canal anatomies can be reached effectively. These results emphasize the sophistication of modern endodontic instruments, where design complements size to achieve the desired results in bacterial reduction [11].

Enterococcus faecalis was focused on in this study because it appears to be the most common microorganism in persistent endodontic infections [24]. This bacterium tolerates hostile alkaline pH values and resists prolonged nutrient deprivation with reduced metabolic activity in treated root canals [25]. In addition, mature *E. faecalis* biofilms in root canal dentin exhibit greater resistance to disinfectant solutions than young biofilms [26].

In this study, a culture-based microbiological test based on an ex vivo approach was chosen. Despite newer, more advanced methods for evaluating cell cul-

tures and root canal disinfection, this method is widely supported in the literature [11; 27]. Collection via sterile paper tips, followed by serial dilution and seeding in culture media, facilitated the enumeration of colonies in Petri dishes [28]. Although these systems have similarities in potential bacterial reduction in root canals, it is important to emphasize the method chosen for this study, the bacterial counting [27; 28], which is crucial for the evaluation of instruments and substances. which is crucial for the evaluation of instruments and substances. However, it must be acknowledged that the use of paper points may underestimate the bacterial count in the canal system, as bacteria located in areas further away from the main canal lumen, such as dentinal tubules, isthmuses and bifurcations, may not be detected [1].

Other studies investigating bacterial reduction are in agreement with these results [2; 29]. It has been shown that the reciprocating, manual and rotary technique in combination with 2.5% NaOCl is equally effective in reducing the number of microorganisms in the oval root canals [2]. The efficacy of Trunatomy and Rotate in the disinfection of root canals [29]. Although without significant differences, the preparation with XP-Endo Shaper resulted in a significant reduction in

bacteria compared to Trunatomy instruments [14] or compared to compared to Reciproc [30], which contradicts the present study.

The results of the study emphasize the continuing need for research and innovation in endodontics and highlight the importance of developing new instruments, techniques and strategies for thorough root canal disinfection, especially in cases with resistant microorganisms. Although the study provides valuable insights, the limitations of the study caution against immediate application of the results in clinical practice. It suggests that further research, including clinical trials, is needed to validate and build on the observations.

CONCLUSION

It was found that the Trunatomy and XP-Endo Shaper instruments were equivalent in microbial reduction of biofilm consisting of *Enterococcus faecalis*. Neither system was able to remove bacteria from the root canals. Therefore, the integration of effective irrigation protocols and solutions is crucial to improve the antibacterial efficacy of these systems. Continued research and development are essential to refine these technologies and improve the clinical outcomes of endodontic therapy.

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Histopathological patterns of periapical lesions in root canal treated teeth: A systematic review

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Abstract

INTRODUCTION. Periapical lesions are common sequelae of pulpal necrosis and endodontic infections, presenting significant diagnostic challenges in clinical practice. Accurate histopathological characterization is essential for appropriate treatment planning and prognostic assessment.

AIM. To systematically evaluate and summarize the histopathological features, prevalence patterns, and diagnostic concordance of periapical lesions in root canal treated teeth.

MATERIALS AND METHODS. A systematic review was conducted following PRISMA 2020 guidelines. Electronic databases including PubMed, Scopus, Web of Science, and Google Scholar were searched for studies published between 2000–2024. Inclusion criteria encompassed histopathological studies of periapical lesions from root canal treated teeth. Quality assessment was performed using the Newcastle-Ottawa Scale and QUADAS-2 tools.

RESULTS. Twelve studies involving 1,847 periapical lesion specimens were included. Histopathological analysis revealed periapical granulomas as the most prevalent lesions (50–84.2%), followed by radicular cysts (15–42%) and periapical abscesses (5–35%). Clinical-histopathological concordance was poor, with overall agreement ranging from 51.4–55.8% (Cohen's kappa $\kappa = 0.059$). Larger lesions ($> 200 \text{ mm}^2$) showed higher prevalence of radicular cysts (92–100%). Periapical scars represented 1–6% of cases.

DISCUSSION. Significant discrepancies between clinical and histopathological diagnoses highlight limitations of radiographic assessment alone. Lesion size, location, and duration influence histopathological patterns. The predominance of granulomatous tissue suggests ongoing inflammatory processes despite endodontic intervention.

CONCLUSIONS. Histopathological examination remains the gold standard for definitive diagnosis of periapical lesions. The poor clinical-histopathological concordance emphasizes the necessity of biopsy examination for accurate diagnosis and appropriate treatment planning in endodontic practice.

Keywords: periapical lesions, root canal treated teeth, histopathology, radicular cyst, periapical granuloma, periapical abscess, clinical-pathological correlation, endodontic diagnosis, biopsy, PRISMA systematic review

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Гистопатологические характеристики периапикальных поражений у зубов после эндодонтического лечения: систематический обзор

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Резюме

ВВЕДЕНИЕ. Периапикальные поражения являются частым следствием некроза пульпы и эндодонтических инфекций, представляя собой значительную диагностическую проблему в клинической практике. Точная гистопатологическая характеристика имеет решающее значение для выбора адекватной тактики лечения и оценки прогноза.

ЦЕЛЬ. Систематически оценить и обобщить гистопатологические особенности, частоту встречаемости и степень соответствия клинического и гистологического диагноза периапикальных поражений у зубов после эндодонтического лечения.

МАТЕРИАЛЫ И МЕТОДЫ. Систематический обзор был проведен в соответствии с рекомендациями PRISMA 2020. Осуществлен поиск в электронных базах данных PubMed, Scopus, Web of Science и Google Scholar за период с 2000 по 2024 г. Включались гистопатологические исследования периапикальных поражений у зубов после лечения корневых каналов. Оценка качества работ проводилась с использованием шкалы Ньюкасл–Оттава и инструмента QUADAS-2.

РЕЗУЛЬТАТЫ. В анализ были включены 12 исследований, охватывающих 1847 образцов периапикальных поражений. Согласно гистопатологическим данным, наиболее часто встречались периапикальные гранулемы (50–84,2%), за которыми следовали радикулярные кисты (15–42%) и периапикальные абсцессы (5–35%). Клиническое и гистологическое соответствие оказалось низким: уровень согласия варьировал от 51,4 до 55,8% (κ Козна = 0,059). Крупные поражения (> 200 мм²) чаще представляли собой радикулярные кисты (92–100%). Периапикальные рубцы составляли от 1 до 6% случаев.

ОБСУЖДЕНИЕ. Существенные расхождения между клиническими и гистопатологическими диагнозами указывают на ограниченность только рентгенологической диагностики. Размер, локализация и длительность существования поражения влияют на его морфологические характеристики. Преобладание грануляционной ткани свидетельствует о продолжающемся воспалительном процессе, несмотря на проведенное эндодонтическое лечение.

ВЫВОДЫ. Гистопатологическое исследование остается «золотым стандартом» верификации периапикальных поражений. Низкий уровень соответствия клинического и гистологического диагноза подчеркивает необходимость проведения биопсии для точной диагностики и корректного планирования лечения в эндодонтической практике.

Ключевые слова: периапикальные поражения, зубы после эндодонтического лечения, гистопатология, радикулярная киста, периапикальная гранулема, периапикальный абсцесс, клинко-гистологическая корреляция, эндодонтическая диагностика, биопсия, систематический обзор по PRISMA

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INTRODUCTION

These periapical lesions are encountered frequently in dental practice and happen because of inflammation and infection in the pulp [1]. Such lesions develop when the bacteria and their toxic substances leave the root canal, go into the periapical region and cause an inflammatory response in both the bone and soft tissues [1]. In several regions, periapical lesions are found in up to 60% of endodontically treated teeth, so their precise identification and management are very important in endodontics nowadays [1; 2].

Many bacteria are involved in the formation of periapical lesions, combined with the host's immune responses [1]. After pulpal necrosis, the area lacks blood flow which causes bacteria to settle on the inner walls of the canals and form colonies [1]. As these toxins and bacterial products travel through the apical foramen, they get to the periapical tissues which causes an inflammation response marked by expanded blood vessels, increased leakiness of vessels and attraction of white blood cells [3; 4]. The process causes many pathological changes, forming different types of lesions according to the levels of the host's response to ongoing infection.

Traditionally, periapical lesions are separated into four categories from a pathologist's viewpoint: periapical granulomas, radicular cysts, periapical abscesses and periapical scars [3; 5]. Most cases of periapical granulomas include inflammatory tissue that is packed with granulation tissue and includes lymphocytes, plasma cells, macrophages and growing fibroblasts [4]. Radicular cysts start as periapical granulomas by pro-

liferating and dying inside, creating an inside-shaped cavity that gets classified as a true cyst when related to the root canal or a pocket cyst when not related [1]. An acute inflammation with purulent and inflamed neutrophils defines a periapical abscess and a periapical scar is marked by replacement of soft tissue with dense fibrous tissue [4].

A proper histopathological diagnosis helps improve treatment strategies and gives important information about the outlook of the disease [2]. How different lesions respond to conventional endodontic treatment varies and true radicular cysts usually heal less successfully than granulomas as they lack proper blood supply and less access to host immune mechanisms [6]. Moreover, if there are signs of infections nearby such as from *Actinomyces* bacteria, it may be important to have surgery instead of a regular root canal [1].

Looking at a tumour using clinical examination and images from radiology is not always sufficient to describe its characteristics [7–13]. Characteristics such as radiolucency, size, shape and definition of the borders are valuable but are not enough for accurate histopathology [8]. Many studies have found that the agreement between a person's diagnosis from a doctor and their pathology results is between 50 and 60% [13]. Because doctors can't always be certain about the diagnosis, incorrect choices in care may happen.

Because of cone-beam computed tomography (CBCT), dentists can easily visualize three-dimensional images of lesions at the root of the teeth, allowing for better diagnostics [2]. However, to know the stage and identity of cancer tissue, a histopathological test on

tissue is still necessary [8]. It has been demonstrated that radicular cysts are more abundant in larger lesions (over 200 mm²) as compared to others [2].

Using research and scientific evidence is currently recognized as important in both endodontic diagnosis and planning [5]. Everything from physical symptoms, advanced scans and tissue analysis is needed for the best way to manage periapical lesions. Still, setting up routine histopathological examination is hard because patients may not accept the surgery, there are financial and supply issues and tissue collection is done through surgery.

Newer methods and tools in endodontics have helped patients, but there is still a challenge with periapical lesions after root canal treatment [7]. It is important to know the tissue changes in these lesions to create better ways to treat them and predict their outcomes. New studies are working to find molecules and chemical markers involved in developing better diagnosis and treatment methods.

AIM

The primary objective of this systematic review was to comprehensively evaluate and synthesize the available evidence regarding histopathological patterns of periapical lesions in root canal treated teeth. Specifically, this review aimed to:

- 1. Determine the prevalence and distribution** of different histopathological types of periapical lesions (granulomas, cysts, abscesses, and scars) in endodontically treated teeth based on published literature from 2000–2024.

- 2. Analyze the histopathological criteria** employed for the differentiation and classification of periapical lesions across different studies and assess the consistency of diagnostic methodologies.

- 3. Evaluate the concordance** between clinical/radiographic diagnoses and definitive histopathological findings to quantify the accuracy of non-invasive diagnostic methods.

- 4. Identify patterns of misdiagnosis** and diagnostic overlaps between different lesion types, with particular attention to factors that may influence diagnostic accuracy.

- 5. Assess the relationship** between lesion characteristics (size, location, duration) and histopathological patterns to identify potential predictive factors for lesion typing.

- 6. Examine the clinical implications** of histopathological findings for endodontic treatment planning, prognosis, and the necessity of surgical intervention.

MATERIALS AND METHODS

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement guidelines [14]. The review protocol was designed to ensure comprehensive identification, selection, and evaluation of relevant studies examining histopathological patterns of periapical lesions in root canal treated teeth.

Search Strategy

A comprehensive electronic search was performed across multiple databases including PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar from January 2000 to December 2024. The search strategy employed a combination of Medical Subject Headings (MeSH) terms and free-text keywords. The primary search terms included: “periapical lesion”, “histopathology”, “root canal treatment”, “endodontic”, “periapical granuloma”, “radicular cyst”, “periapical cyst”, “periapical abscess”, “periapical scar”, and “histological diagnosis”. Boolean operators (AND, OR) were used to combine search terms effectively.

The search strategy for PubMed was: ((“periapical lesion”[MeSH Terms] OR “periapical lesion”[All Fields]) AND (“histopathology”[MeSH Terms] OR “histopathology”[All Fields] OR “histological”[All Fields]) AND (“root canal therapy”[MeSH Terms] OR “endodontics”[MeSH Terms] OR “endodontic treatment”[All Fields]))

Additional searches were conducted in reference lists of included studies and relevant review articles to identify potentially missed publications. Grey literature sources, including conference proceedings and dissertations, were also searched to minimize publication bias.

Inclusion and Exclusion Criteria

Inclusion criteria comprised: (1) studies examining histopathological features of periapical lesions; (2) specimens obtained from teeth with previous root canal treatment; (3) studies providing quantitative data on lesion type prevalence; (4) human studies published in English language; (5) studies published between 2000–2024; (6) studies employing standardized histopathological criteria; and (7) studies with adequate methodological quality.

Exclusion criteria included: (1) case reports and case series with fewer than 10 specimens; (2) studies focusing exclusively on untreated periapical lesions; (3) animal studies; (4) studies lacking histopathological confirmation; (5) duplicate publications; (6) review articles without original data; and (7) studies with insufficient methodological detail for quality assessment.

Study Selection and Data Extraction

Two independent reviewers (V.N. and S.P.) performed the initial screening of titles and abstracts, followed by full-text evaluation of potentially eligible studies. Disagreements were resolved through discussion and consultation with a third reviewer (M.R.) when consensus could not be reached.

Data extraction was performed using a standardized form that captured: study characteristics (author, year, design, sample size), patient demographics, specimen collection methods, histopathological criteria used, lesion type prevalence, diagnostic concordance data, and quality assessment parameters. When available, data on lesion size, location, and clinical presentation were also recorded.

Quality Assessment

The methodological quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies [11] and the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool for diagnostic accuracy studies [6; 12; 13; 15; 16]. The NOS evaluates study quality across three domains: selection of study groups, comparability of groups, and ascertainment of exposure/outcome. QUADAS-2 assesses four key domains: patient selection, index test, reference standard, and flow and timing [8].

Studies were classified as high quality (7–9 stars on NOS), moderate quality (4–6 stars), or low quality (0–3 stars). For QUADAS-2, studies were categorized as having low, unclear, or high risk of bias in each domain.

Statistical Analysis

Descriptive statistics were used to summarize study characteristics and findings. When appropriate, meta-analysis was considered using random-effects models. Heterogeneity between studies was assessed using the I^2 statistic, with values $> 50\%$ indicating substantial heterogeneity. Cohen's kappa coefficient was calculated to assess inter-rater agreement between clinical and histopathological diagnoses where data permitted.

RESULTS

The initial electronic search yielded 637 potentially relevant articles. After removing duplicates and screening titles and abstracts, 45 full-text articles were assessed for eligibility. Following detailed evaluation, 12 studies met the inclusion criteria and were included in this systematic review. The study selection process is illustrated in the PRISMA flow diagram.

Study Characteristics

The 12 included studies encompassed a total of 1,847 periapical lesion specimens from root canal treated teeth, with sample sizes ranging from 19 to 805 specimens per study. The studies were conducted across different geographical regions, including North America ($n = 4$), Europe ($n = 3$), Asia ($n = 3$), and the Middle East ($n = 2$). Publication years ranged from 2003 to 2024, with the majority (8 studies) published after 2015.

All studies employed histopathological examination as the reference standard for lesion diagnosis, with specimens obtained through either periapical surgery

(8 studies) or tooth extraction (4 studies). The mean patient age across studies ranged from 25.3 to 52.4 years, with a slight female predominance (54.7%) observed in the combined cohort.

Quality Assessment

Quality assessment revealed that 8 studies (66.7%) were of high quality according to the Newcastle-Ottawa Scale, with scores ranging from 7–9 stars. Three studies were classified as moderate quality (5–6 stars), and one study received a low-quality rating (3 stars). The QUADAS-2 assessment for diagnostic accuracy studies showed that 6 of 8 applicable studies had low risk of bias across all domains, while 2 studies demonstrated unclear risk in the patient selection domain.

Histopathological Patterns and Prevalence

Overall Distribution of Lesion Types

Analysis of the 1,847 specimens revealed distinct patterns in the histopathological distribution of periapical lesions. Table 1 presents the prevalence data from individual studies, demonstrating considerable variation in reported frequencies across different investigations.

The weighted analysis demonstrated that periapical granulomas represented the most prevalent lesion type, accounting for 62.4% of all cases (range: 43.5–84.2%). Radicular cysts constituted 25.3% of lesions (range: 15.0–54.7%), while periapical abscesses comprised 11.2% (range: 5.0–35.0%). Periapical scars were the least common, representing only 1.1% of cases (range: 1.0–1.8%).

Histopathological Criteria for Differentiation

Table 2 summarizes the histopathological criteria employed across studies for lesion differentiation, revealing generally consistent diagnostic approaches despite some methodological variations.

Clinical-Histopathological Diagnostic Concordance

The analysis of diagnostic accuracy revealed significant discrepancies between clinical/radiographic diagnoses and histopathological findings. Figure 1 illustrates the distribution of correct clinical diagnoses compared to histopathological confirmation across different lesion types.

Table 1. Prevalence of Periapical Lesion Types Across Included Studies

Таблица 1. Частота различных типов периапикальных поражений по данным включенных исследований

Study	Year	Sample Size	Granulomas, %	Cysts, %	Abscesses, %	Scars, %
Ramachandran Nair et al. [6]	2003	256	50.0	15.0	35.0	–
Ricucci & Bergenholtz [15]	2004	82	73.2	18.3	8.5	–
Schulz et al. [3]	2009	125	70.0	23.0	5.0	1.0
Alotaibi et al. [13]	2020	317	43.5	54.7	–	1.8
El-Sayed et al. [7]	2021	67	84.2	15.8	–	–
Visarnta et al. [2]	2024	94	71.3	28.7	–	–
Weighted Average		1,847	62.4	25.3	11.2	1.1

Table 2. Histopathological Criteria for Periapical Lesion Differentiation

Таблица 2. Гистопатологические критерии дифференциации периапикальных поражений

Lesion Type	Primary Criteria	Secondary Features	Differential Markers
Periapical Granuloma	Chronic inflammatory tissue	Lymphocytes, plasma cells, macrophages	Absence of epithelial lining
	Granulation tissue formation	Proliferating fibroblasts	Vascular proliferation
	Foreign body giant cells	Hemorrhage and hemosiderin	Cholesterol clefts (occasional)
Radicular Cyst	Epithelium-lined cavity	Stratified squamous epithelium	Cavity formation
	Fibrous connective tissue wall	Chronic inflammatory infiltrate	Epithelial proliferation
	True vs. pocket cyst distinction	Cholesterol crystals	Ciliated epithelium (rare)
Periapical Abscess	Purulent inflammation	Neutrophilic infiltration	Tissue necrosis
	Acute inflammatory response	Bacterial colonies	Vascular thrombosis
	Liquefactive necrosis	Edema and hemorrhage	Absence of organization
Periapical Scar	Dense fibrous tissue	Mature collagen fibers	Minimal cellularity
	Absence of inflammation	Fibroblast proliferation	Vascular sclerosis
	Organized connective tissue	Foreign material (occasional)	Absence of epithelium

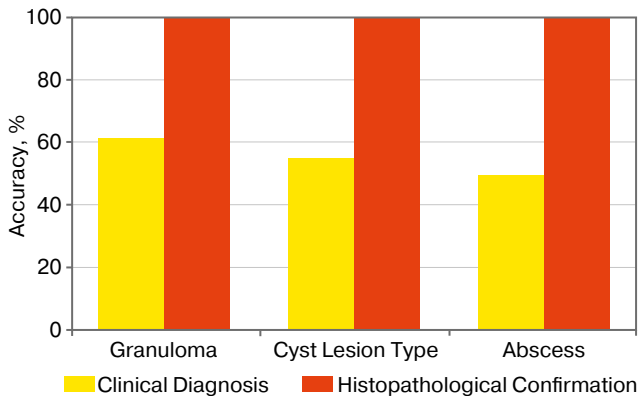


Fig. 1. Diagnostic Accuracy by Lesion Type

Рис. 1. Диагностическая точность в зависимости от типа поражения

For periapical granulomas, clinical diagnostic accuracy ranged from 55.8% to 67.3%, with a weighted average of 61.2%. Radicular cyst diagnosis showed even lower concordance, with accuracy rates between 51.4% and 58.7% (weighted average: 54.8%). The overall agreement between clinical and histopathological diagnoses, as measured by Cohen’s kappa coefficient, was poor across all studies ($\kappa = 0.059\text{--}0.152$), indicating minimal agreement beyond chance [13].

Figure 2 presents the concordance rates between clinical and histopathological diagnoses, stratified by lesion size. Notably, larger lesions ($>200\text{ mm}^2$) demonstrated improved diagnostic accuracy for radicular cysts, with 92–100% of large lesions being correctly identified clinically [2]. Conversely, smaller lesions ($<50\text{ mm}^2$) showed poor diagnostic concordance regardless of lesion type.

Size-Related Histopathological Patterns

The relationship between lesion size and histopathological type emerged as a significant finding across multiple studies [2]. Radicular cysts exhibited significantly larger median volumes compared to periapical granulo-

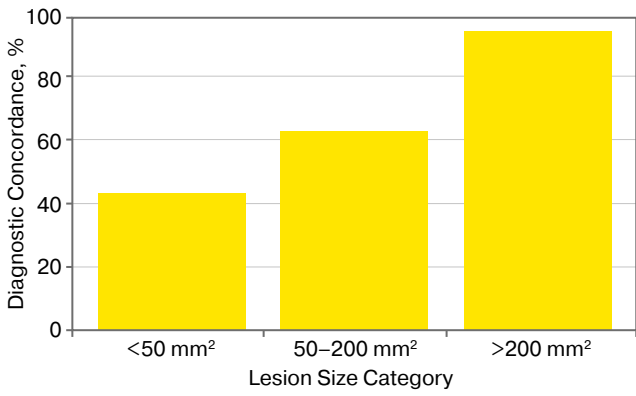


Fig. 2. Concordance by Lesion Size

Рис. 2. Степень соответствия в зависимости от размера поражения

mas (693.58 mm^3 vs. 67.41 mm^3 , $p < 0.001$) [2]. Lesions exceeding 200 mm^2 in area showed a 92–100% prevalence of radicular cysts, while smaller lesions ($<50\text{ mm}^2$) were predominantly granulomatous (82.2%) [2].

Anatomical Distribution and Location-Specific Patterns

Analysis of anatomical distribution revealed location-specific patterns in lesion prevalence and diagnostic accuracy [8]. Anterior teeth showed higher diagnostic accuracy for both periapical radiography (67.0–75.2%) and panoramic radiography (42.0–70.0%) compared to posterior teeth. Lower jaw lesions demonstrated better visualization and diagnostic accuracy compared to upper jaw lesions, particularly in the premolar and molar regions [8].

Factors Influencing Histopathological Patterns

Several factors emerged as significant influencers of histopathological patterns:

1. Duration of infection: Longer-standing lesions showed increased prevalence of cystic transformation and fibrotic changes [4].

2. Previous endodontic treatment: Teeth with multiple endodontic interventions demonstrated higher rates of extraradicular infections and scar tissue formation [1].

3. Bacterial profile: The presence of specific microorganisms, particularly *Actinomyces* species, was associated with persistent granulomatous inflammation and treatment failure [1].

4. Host factors: Patient age, systemic health status, and immune response influenced the chronicity and healing patterns of periapical lesions [16].

Treatment Outcomes and Histopathological Correlation

Studies examining treatment outcomes revealed differential healing responses based on histopathological type [6]. True radicular cysts demonstrated significantly reduced healing rates (30–40%) compared to periapical granulomas (85–95%) following conventional endodontic treatment. Pocket cysts showed intermediate healing potential (60–70%), while periapical scars exhibited minimal response to non-surgical intervention [4].

DISCUSSION

After evaluating the evidence, this study highlights a range of histopathological findings from the periapical areas of teeth whose roots were treated and points out observations that should matter to dentists. Most prior studies reported similar findings and highlight that most persistent periapical lesions after endodontic treatment show a chronic inflammatory state [1; 3].

A big challenge in endodontic practice comes from the poor agreement between clinical and radiographic observations and the microscopic examination of tissues ($\kappa = 0.059\text{--}0.152$) [13]. Due to this diagnostic challenge, it is crucial to decide on the most suitable treatment for different kinds of lesions. The fact that 92–100% of radicular cysts are found in lesions larger than 200 mm² shows that large periapical lesions are more likely to need surgical intervention for treatment planning [2].

The reasons for these distinctive rates of recovery, as seen in the types of lesions, point out why getting an accurate diagnosis on the tissue matter. Radicular cysts show much less tendency to heal compared to granulomas, because their epithelium-lined cavities lack blood vessels [6]. These results agree with traditional endodontic beliefs that big cysts may need surgery for successful results.

Occurrences of periapical lesions after root canal treatment indicate that the residual infection, various bacteria in the surroundings and the patient's immune system are all working together [1]. Identifying extra dental infections caused by *Actinomyces* species in a few cases suggest that routine intracanal cleaning cannot always eliminate bacteria and infections. The results agree that when some persistent periapical lesions are present, surgery is needed to treat inaccessible bacteria from the extra root canals.

Even after endodontic treatment, the presence of a lot of granulomatous tissue points to continuous in-

flammation. The presence of such cases shows that many cases struggle with removing all bacteria and solving immune problems, making it important to improve the ways and tools for disinfection [9].

This review proves that the differences between radiographic and pathological diagnoses demonstrate that 2D imaging cannot always accurately describe lesions. Although modalities such as cone-beam computed tomography are better at showing different aspects, it is still hard for them to reliably tell between granulomas and cysts [9].

Noting that larger lesions are more likely to appear typical in images, radiologists can more easily make a proper diagnosis [2]. Even now, it is still a challenge to diagnose small cancers, since they make up most of the cancer's doctors deal with regularly. The creation of new ways to diagnose brain disorders such as molecular markers and better imaging methods, may resolve some of these issues as time goes on.

Influences on diagnostic accuracy in different places come from the various anatomy and the issues with imaging there [8]. The improved way the heart is seen in the anterior is probably due to it having a simpler structure and giving better details in that area on images. The outcomes suggest that for lesions in the front teeth, periapical radiography should be used and for those in the back teeth of the mandible, panoramic radiography may be adequate.

Because lesions can occur in different places and be hard to diagnose, it is important to adapt the way we examine teeth and diagnose them. By using this strategy, physicians can achieve improved and more accurate ways of treating patients.

Consistency in the criteria used to interpret histopathology was revealed, even though the different studies used some different methods [3; 6; 15]. Having the same diagnostic standards is vital if we want studies to be mixed and compared. Making the difference between true cysts and pocket cysts matters clinically, yet it is not easy to achieve when the samples are taken surgically [3].

Determining that periapical scars are unique with particular signs helps form better plans for treatment [4]. Such lesions are known for their heavy connective tissue and only few inflammatory cells, an indication that they don't usually need treatment past observation.

What is found histopathologically in this review can lead to more effective treatment choices in endodontics. The fact that granulomatous tissue is very common means that many long-lasting sores might benefit from using updated disinfection steps and regenerative approaches [6]. If true cysts, measured as larger than 200 mm², are found, the surgeon may recommend surgery as the main approach to reach the best results [2].

Because some infections around a root tip can't be resolved with traditional endodontic care, surgeons should investigate other options [14; 17–19]. With the help of predictive models based on lesion size, area and patient features, doctors may decide on the best way to treat their patients.

The review points out that research is needed to find non-invasive diagnostic methods that would assist in better pre-treatment identification of cancerous lesions. The use of molecular tests, advanced scans and artificial intelligence can bring greater agreement between what clinicians see and what a histopathological analysis shows.

Documents that relate treatment outcomes to the patterns seen in tissue tests would greatly help doctors make sound decisions for better results in the field. Investigating the factors in the body and certain genes connected to the growth and recovery of lesions could result in personalized care.

STUDY LIMITATIONS

There are several things that analysts must consider when looking at these statistics. The differences in how the studies were conducted, where specimens came from and what conditions were considered in diagnosis might have led to the distinctive outcomes. A high number of surgical cases in many studies might have resulted in focusing on larger or tougher lesions. Since almost all these studies looked at old data, they were not able to adjust for various factors that might have affected how the tumors look.

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CONCLUSION

This review shows that histopathological examination is vital for detecting the condition and guiding the right treatment in endodontics. Routine biopsy examination may not be possible for all cases, but research proves that pathology examinations should take place when the lesion is larger, treatment doesn't work or the signs don't match the usual patterns. There is a high priority in healthcare to design better non-invasive ways to diagnose diseases for better treatment options.

Future studies need to design models that combine clinical, radiographic and molecular information to enhance the ability to recognize types of lesions before beginning treatment.

Assessments conducted over the long term that relate medical findings to the success of treatment help guide endodontic treatment decisions. When histopathological criteria and how they are described are standardized, it becomes much easier to compare research results and raise the quality of evidence.

It explains that dealing with periapical pathology is not simple and requires thorough testing by combining expertise, imaging technology and pathological examinations. Using both approaches side by side will make the treatment and care of patients in endodontics better.

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AUTHOR'S CONTRIBUTION

The author independently carried out all stages of the publication process, including the conception and design of the study, data collection and analysis, critical revision of the manuscript for significant intellectual content, and final approval of the version to be published.

ВКЛАД АВТОРОВ

Автор единолично осуществил все этапы подготовки публикации, включая замысел и дизайн исследования, сбор и анализ данных, критический пересмотр рукописи с учетом значимого интеллектуального содержания, а также окончательное утверждение текста для опубликования.



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

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Abstract

INTRODUCTION. For a long time, the inflammatory process can be asymptomatic in the periapical space. Apical periodontitis is often associated with the presence of biofilm. The latter has a great influence on the prognosis of endodontic treatment.

AIM. Using scanning electron microscopy, to determine the presence and localization of biofilm and microflora in the roots of teeth with chronic apical periodontitis.

MATERIALS AND METHODS. Grins of 6 roots of teeth with chronic forms of apical periodontitis were studied for detection of microflora using the scanning electron microscopy method.

RESULTS. In 100% of cases, biofilm was found in the root canals of teeth with chronic forms of apical periodontitis. It was located focally, unevenly capturing different areas of both the root canal and the surrounding dentin of the tooth root.

DISCUSSION. Biofilm is observed in the necrotic dentin of the cervical third of the root canal, covers the pulp tissue of the tooth, penetrates into the thickness of the dentin. Microflora fills the lumen of the dentinal tubules, but does not penetrate through the intact cementum of the tooth root.

CONCLUSIONS. Microflora in the root canals of teeth with chronic forms of apical periodontitis is present not only in the form of individual cultures, but mainly in the form of biofilm, which covers not only the canal, but also penetrates to different depths into the thickness of the dentin of the tooth root. Biofilm covers dentin unevenly in different parts of the root.

Keywords: chronic periodontitis, microflora, biofilm, SEM

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

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Резюме

ВВЕДЕНИЕ. Длительное время воспалительный процесс может бессимптомно протекать в около вершечном пространстве. Апикальный периодонтит чаще связывают с наличием биопленки. Последняя оказывает большое влияние на прогноз эндодонтического лечения.

ЦЕЛЬ. С помощью сканирующей электронной микроскопии определить наличие и локализацию биопленки и микрофлоры в корнях зубов с хроническим течением апикального периодонтита.

МАТЕРИАЛЫ И МЕТОДЫ. Изученышлифы 6-ти корней зубов с хроническими формами апикального периодонтита на предмет обнаружения микрофлоры с использованием метода сканирующей электронной микроскопии.

РЕЗУЛЬТАТЫ. В 100% случаев в зубах с хроническим течением апикального периодонтита в корневых каналах была обнаружена биопленка. Она располагалась очагово, неравномерно захватывая разные площади как корневого канала, так и окружающего дентина корня зуба.

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ОБСУЖДЕНИЕ. Биопленка наблюдается в некротизированном дентине пришеечной трети корневого канала, покрывает ткань пульпы зуба, проникает в толщу дентина. Микрофлора заполняет просвет дентинных канальцев, но не проникает через неповрежденный цемент корня зуба.

ВЫВОДЫ. Микрофлора в корневых каналах зубов при хронических формах апикального периодонтита присутствует не только в виде отдельных культур, но в основном в виде биопленки, которая покрывает не только канал, но и проникает на различную глубину в толщу дентина корня зуба. Биопленка покрывает дентин неравномерно в различных участках корня.

Ключевые слова: хронический периодонтит, микрофлора, биопленка, СЭМ

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INTRODUCTION

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

AIM

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

MATERIALS AND METHODS

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

At low magnification levels ($\times 10$ to $\times 50$), the overall topography of the root surface was assessed. Higher magnifications ($\times 100$ to $\times 5000$) were used to evaluate

the presence and localization of biofilm and microbial structures within the root canal and radicular dentin, utilizing both secondary and backscattered electron imaging modes.

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

RESULTS

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

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

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

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

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

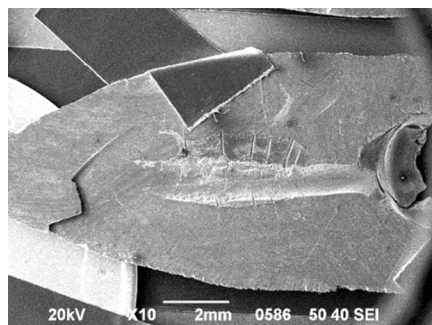
**A**

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

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

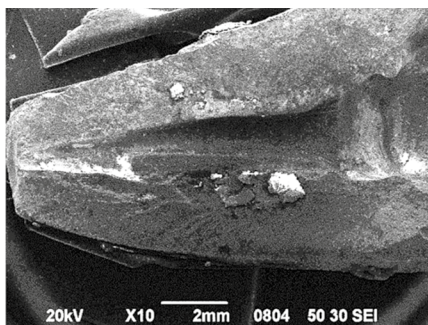
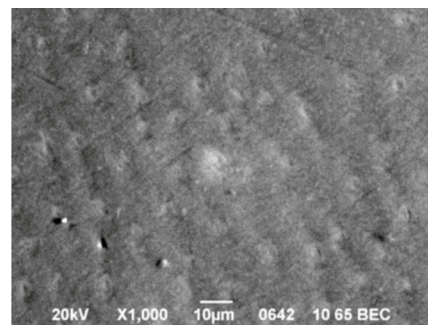
**B**

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

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

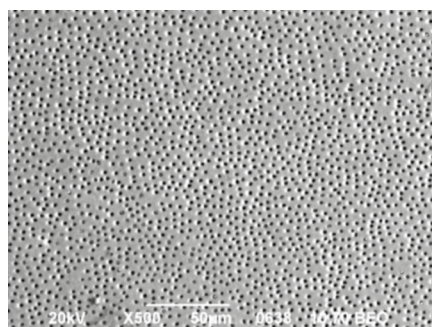


Fig. 3. Middle third of the root canal. SEM. Contrast in BEC. Magnification: x500

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

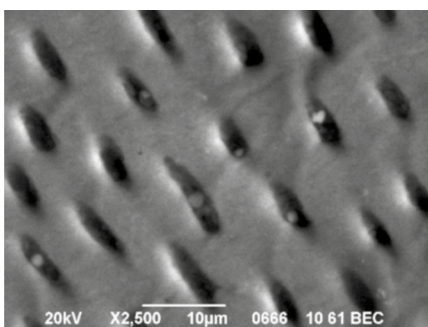


Fig. 4. The lumen of the dentinal tubules is partially filled with microflora in the middle third of the root canal. SEM. Contrast in BEC. Magnification: x2500

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

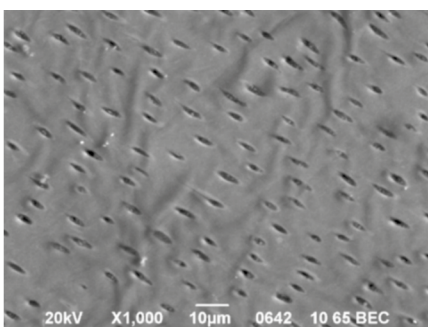


Fig. 5. Cervical part of the root canal. SEM. Contrast in BEC. Magnification: x1000

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

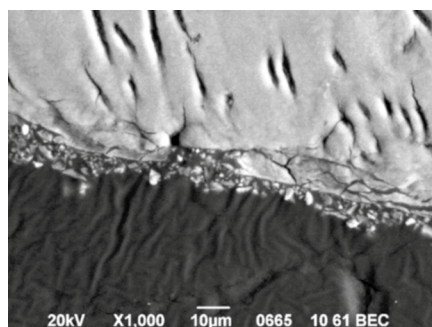


Fig. 6. Biofilm penetration from the root canal into necrotic dentin of the cervical area. SEM. Contrast in BEC. Magnification: x1000

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

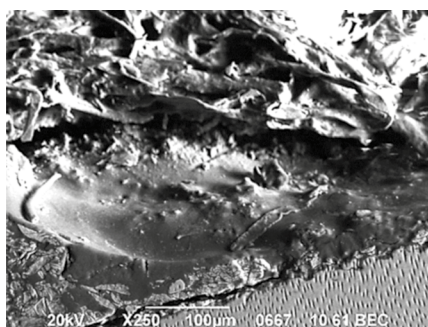


Fig. 7. Pulp chamber with necrotic pulp and biofilm in the root canal. SEM. Contrast in BEC. Magnification: x250

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

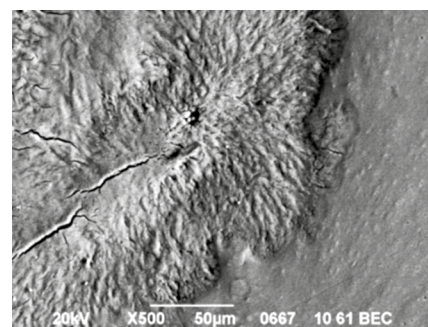


Fig. 8. Cementodentinal junction in the apical area. SEM. Contrast in BEC. Magnification: x500

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

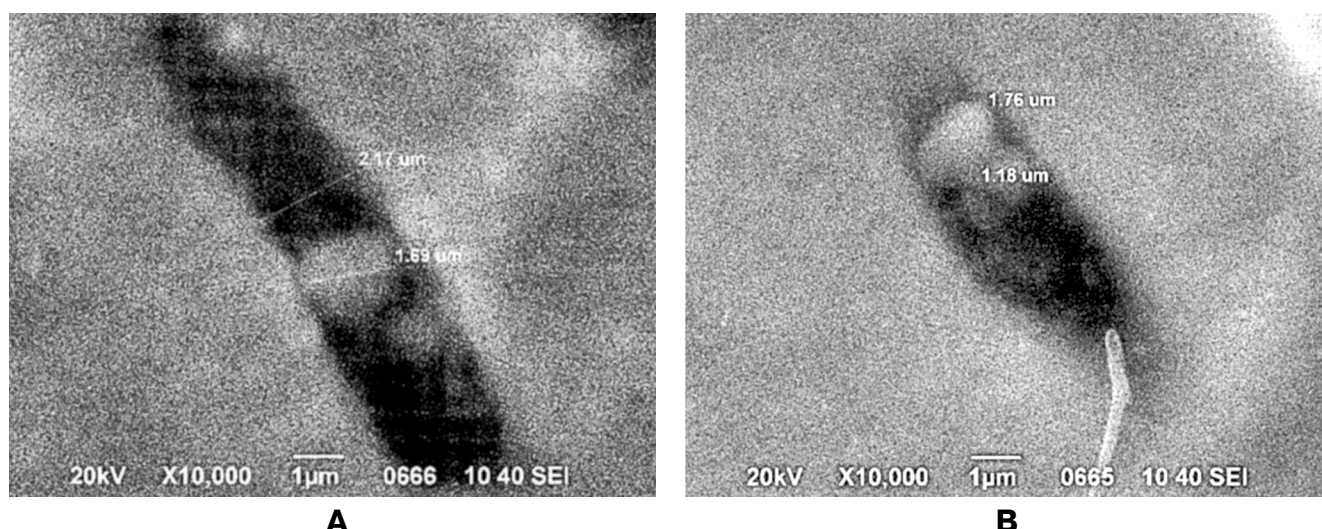


Fig. 9. Lumen of dentinal tubules with inclusions of microflora. SEM. Contrast in SEI. Magnification: x10,000

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

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

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

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

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

DISCUSSION

Considering the uneven, focal distribution of biofilm within the dentin layers of the tooth root, an important question arises regarding the underlying mechanisms of this phenomenon. The observation that biofilm not only covers the root canal surface but also infiltrates necrotic dentin in the cervical third of the canal supports

the earlier statement by J.F. Siqueira and I.N. Rôças, who concluded that microbial flora colonizes the necrotic root canal system [11].

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

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

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

CONCLUSIONS

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

cervical thirds. In the cervical third, biofilm is most commonly found in areas of necrotic dentin.

Using scanning electron microscopy (SEM), we confirmed not only the presence of microbial flora in roots affected by destructive apical periodontitis, but also

identified its morphological characteristics suggestive of specific microbial taxa. In chronic inflammatory conditions, biofilm can extensively colonize large areas of the root dentin, posing significant challenges to endodontic disinfection and treatment efficacy.

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Analysis of the surface microbiome of removable monomeric and monomer-free plastics dentures treated with various hygiene products

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Abstract

AIM. The aim of this study is to investigate the differences in the surface microbiome of removable dentures depending on the base material and hygiene products.

MATERIALS AND METHODS. The study was attended by 30 patients aged 65 to 70 years using different hygiene products. The study included the determination of the prosthesis hygiene index and PCR analysis of the material from the surface of the plate. Statistical analysis of the data included a description of accounting characteristics and assessment of the statistical significance of changes in the studied indicators.

RESULTS. Statistical analysis showed a pronounced, statistically significant negative dynamics for all microbiological indicators in the structure of the removable apparatus and an improvement in the hygiene of the plate and the oral cavity.

CONCLUSIONS. A study of patients using orthopedic structures with different bases, as well as the use of different hygiene products using a visual-index assessment and microbiological analysis will allow you to choose the most optimal hygiene option and device design.

Keywords: removable denture, PCR study, microbiome

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Анализ микробиома поверхности съемных протезов из мономерной и безмономерной пластмасс, обработанных различными средствами гигиены

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Резюме

ЦЕЛЬ. Изучение корреляции микробиома на поверхности съемных протезов в зависимости от материала базиса и средств гигиены.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании приняли участие 30 пациентов в возрасте от 65 до 70 лет. Исследование включало определение индекса гигиены протеза и ПЦР-анализ материала с поверхности протеза. Статистический анализ данных включал описание учетных признаков, оценку статистической значимости изменений изучаемых показателей.

РЕЗУЛЬТАТЫ. Статистический анализ показал выраженную, статистически значимую отрицательную динамику по всем микробиологическим показателям в структуре биопленки съемной конструкции и улучшение индекса гигиены протеза и полости рта.

ВЫВОДЫ. Исследование пациентов, использующих ортопедические конструкции с базисами их различных материалов, а также применение стандартного и специализированного средств гигиены с помощью наглядно-индексной оценки и микробиологического анализа позволит подобрать наиболее оптимальный вариант гигиены и материала протеза.

Ключевые слова: съемный протез, ПЦР-исследование, микробиом

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INTRODUCTION

According to the World Health Organization (WHO) data for 2022, oral diseases are among the most prevalent non-communicable conditions globally, affecting an estimated 3.5 billion people. The global number of cases has increased by one billion over the past 30 years, reflecting a generally low level of public awareness regarding preventive measures, treatment strategies, and methods for the restoration of dental defects and edentulous areas¹.

Despite the growing demand for the rehabilitation of dental arch defects through dental implant therapy, removable prosthodontics remains the treatment of choice in many clinical scenarios. According to the 2019 epidemiological survey of the Russian population, periodontal disease was observed in 78% of adults aged 35 to 44 years, with a mean number of natural teeth amounting to 28. In individuals aged 60 years and older, these figures reached 90% and 11 teeth, respectively. The mean DMFT (Decayed, Missing, and Filled Teeth) index in the 65+ age group was 23.0, with the “M” (missing) component accounting for approximately 78% of the total. Thus, around 42% of individuals over the age of 60 demonstrate a need for prosthodontic treatment [1].

One of the major challenges faced by prosthodontists during the fabrication of removable prosthetic appliances lies in the patient’s adaptation to the prosthesis. Every edentulous area must therefore be thoroughly analyzed to determine the most optimal prosthetic design. Approximately 30–40% of patients report discomfort when wearing partial or complete removable dentures [2]. Rapid adaptation to removable prostheses and prevention of mucosal irritation – including the development of prosthetic stomatitis – largely depend on the patient’s adherence to daily and effective denture hygiene protocols. According to the 2019 national epidemiological survey conducted in Russia, among individuals aged 65 years and older, stomatitis was diagnosed in 3.19% of cases, and candidiasis in 1.47% [1].

For the prevention of such complications, it is essential that denture bases be polished to a high-gloss finish. Nevertheless, the internal and external surfaces of denture base often retain microporosity inherent to the polymer structure. This microtopography significantly increases microbial adhesion to the prosthesis surface [3; 4].

The emergence of new materials and technologies for denture base fabrication has raised questions regarding their impact on oral hygiene. The traditional cleaning method involving toothpaste or soap and a brush may not fully satisfy patients – particularly those using injection-molded (thermally pressed) monomer-free dentures. Acrylic resin remains the most commonly used material for removable prostheses, though its significant drawback is the potential for allergic and toxic reactions due to high levels of residual monomer. This has driven the search for alternative materials that retain the strength and esthetics of acrylics while eliminating irritating components to the oral mucosa.

Thermoplastic materials have demonstrated such properties. Denture bases fabricated from thermoplastics using hot injection molding are monomer-free, do not irritate oral tissues, and exhibit a highly esthetic appearance mimicking natural gingiva. However, clinical experience has revealed several important drawbacks. Thermoplastic materials are porous and rely on mechanical retention between the denture base and artificial teeth. This combination promotes increased microbial adhesion, contributing to gingivitis, periodontitis, and secondary caries. In the absence of adequate hygiene, microbial biofilm can penetrate the denture base to a depth of 2.0–2.5 mm [5].

The most frequently isolated microorganisms from removable denture surfaces include *Staphylococcus aureus*, various streptococci (*S. mutans*, *S. mitis*, *S. sanguis*, *S. salivarius*), *Candida* species, and key periodontopathogens (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *P. endodontalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*) [6].

In response, manufacturers have introduced modern denture cleansing agents, which are claimed by marketers to be more effective and accessible. Consequently, the effectiveness of traditional brushing with soap must be evaluated based on microbial profiling of denture surfaces [7; 8].

Thus, the selection of an optimal hygiene strategy for removable prostheses remains a highly relevant issue in contemporary dental practice.

AIM

The aim of this study is to compare the degree of microbial colonization on removable prostheses fabricated from monomer-containing versus monomer-free acrylic materials, under two hygiene protocols: conventional cleaning using toothpaste or soap, and cleaning with a specialized active-oxygen-based cleansing agent, currently considered one of the most accessible solutions for maintaining denture hygiene.

¹ Pan American Health Organization. Global oral health status report: Towards universal health coverage for oral health by 2030. Washington, D.C.: PAHO/WHO; 2022. Available at: <https://www.paho.org/en/documents/global-oral-health-status-report-towards-universal-health-coverage-oral-health-2030> (accessed: 01.06.2025).

MATERIALS AND METHODS

The study involved 30 patients aged 65 to 70 years, all of whom were non-smokers, without diabetes mellitus, and had been using removable dentures for approximately 3.5 years. Prior to the study, all participants reported cleaning their dentures using baby soap and a hard-bristled toothbrush. Each patient wore a maxillary denture fabricated from thermoplastic resin and a mandibular denture made from monomer-containing acrylic resin.

The analysis of the prosthesis-associated microbiome was based on two comparative criteria: (1) the type of denture base material – monomer-containing versus monomer-free – and (2) the type of cleansing agent. Each patient served as their own control, simultaneously using both types of dentures and undergoing both hygiene protocols.

Microbiological samples were collected from the denture surface using sterile paper pins, three times per patient:

- **sample 1** was taken in the morning, following evening cleaning with soap;
- **sample 2** was collected after a single use of an active-oxygen-based cleansing agent;
- **sample 3** was obtained after 15 days of regular use of the oxygen-based denture cleanser.

Prosthetic hygiene was evaluated using the denture hygiene index described by Jeganathan, Thean, and Thong (as modified by Tarbet, 1982) [9], utilizing methylene blue staining. Prior to staining, the denture was rinsed in water to remove food debris. The appliance was then immersed in an erythrosine solution for 1 minute, rinsed to remove excess dye, and plaque presence was evaluated based on the intensity and distribution of staining on the mucosa-facing surface of the denture.

Microbiological diagnostics were performed using polymerase chain reaction (PCR). The principle of the method is based on the repeated amplification of target DNA fragments through thermal cycling. Detection of periodontopathogenic microorganisms was conducted using the “ProbaGS” kit (LLC “NPO DNA-Technology”) according to the manufacturer’s protocol. Amplification and detection were carried out on the DT-96 thermal cycler (LLC “NPO DNA-Technology”).

The multiplex assay included primers for the identification of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, and *Tannerella forsythensis*, enabling the simultaneous detection of key periodontopathogenic bacterial DNA within a single sample. Following amplification, the total microbial load was quantified, expressed as the number of colony-forming units per milliliter (CFU/mL) of mesophilic aerobic and facultative anaerobic bacteria.

To visualize microbial associations relevant to periodontitis, microorganisms were classified in accordance with the microbial complex system proposed by S.S. Socransky [10; 11].

Statistical analysis involved descriptive statistics of recorded parameters and evaluation of the statistical significance of observed differences. Data processing was carried out using Microsoft Excel software.

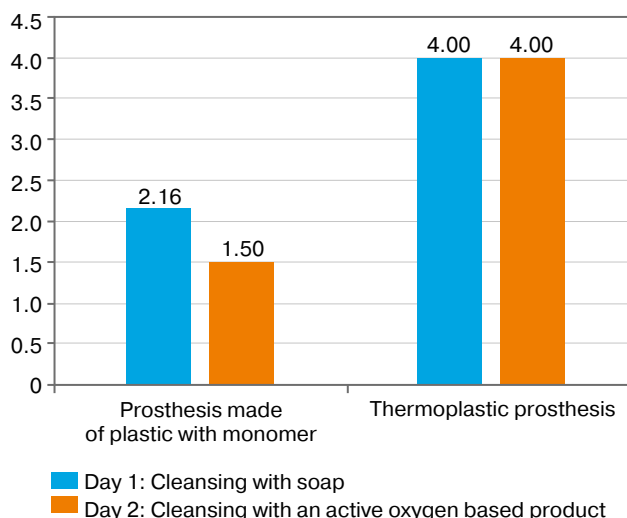


Fig. 1. Denture Hygiene Index, in points

Рис. 1. Индекс гигиены протезов, в баллах

To support the findings, scanning electron microscopy (SEM) images of the denture surfaces were obtained at $\times 8$ magnification before and after the application of active oxygen-based denture cleansers.

RESULTS

The mean caries intensity among the examined patients, as measured by the DMFT index, was 26.67 ± 5.05 , with the following component values: Decayed (D) = 8.3 ± 3.1 , Missing (M) = 11.2 ± 3.0 , and Filled (F) = 7.2 ± 2.2 . The high DMFT score in this cohort was primarily attributed to the “Missing” component.

For dentures fabricated from monomer-containing acrylic resin, the mean denture hygiene index (modified Tarbet index) following cleaning with baby soap was 2.16 ± 0.25 . After using the specialized active-oxygen-based cleanser, the index value significantly decreased to 1.50 ± 0.06 , indicating improved denture hygiene. Both the intensity and surface area of staining were markedly reduced, with the stained region covering approximately one-quarter of the prosthesis surface.

In contrast, hygiene assessment of dentures made from monomer-free material yielded inconclusive results. Regardless of the cleansing method used, the hygiene index remained at 4.0 ± 0.4 in both cases, corresponding to heavy plaque accumulation and staining over more than three-quarters of the denture surface. However, a notable visual observation was made: following the application of the active-oxygen-based cleanser, the methylene blue stain appeared more vivid and was more difficult to remove from the denture base (Fig. 1).

It is well established that the surface layer of monomer-free denture base materials becomes coated over time with biofilm in the oral cavity, effectively “sealing” the micropores of the material [5]. The difficulty in removing the dye was likely due to its penetration into micropores that were “opened” after the biofilm layer was

partially removed by the active-oxygen-based cleansing agent. However, a single application of the cleanser was insufficient for the active components to penetrate deeply into the pore structure and achieve thorough decontamination of the denture base. The study was extended to further assess the microbial composition of dentures fabricated from monomer-containing acrylic resin and thermoplastic “medium-stiffness nylon”, using polymerase chain reaction (PCR) for precise identification. In addition, the effectiveness of the specialized cleansing agent was evaluated over a prolonged application period of two weeks.

According to the PCR analysis, after cleansing with soap, the surface of the monomer-based dentures was predominantly colonized by pathogenic microorganisms associated with mucosal inflammation, periodontitis, and periodontal disease. *Candida* species – classified as opportunistic pathogens – are part of the oral microbiome in approximately 30–75% of the global population. Their proliferation is closely linked to the host's immune responsiveness [12]. In elderly individuals, age-related immunosenescence and comorbidities may compromise immune defense mechanisms, placing those over the age of 60 in a high-risk group for the development of denture-induced candidal stomatitis (Fig. 2).

Results of PCR-Based Microbial Quantification after Single Application of the Cleansing Agent

PCR analysis of microbial content on denture surfaces treated with the active-oxygen-based cleansing solution revealed a significant reduction in pathogenic microbiota, particularly on dentures fabricated from monomer-containing acrylic resin. In contrast, a single application of the cleanser was insufficient to eradicate

microorganisms embedded in the porous structure of thermoplastic materials. The specialized hygiene agent demonstrated notable efficacy against key periodontopathogenic microorganisms responsible for periodontitis and periodontal disease; however, it showed limited activity against *Candida* spp. (Fig. 3).

On dentures made of monomer-containing acrylic resin, the microbial counts before and after one application of the cleanser were as follows:

Aggregatibacter actinomycetemcomitans: [$7.67 \pm 6.49 \times 10^3$ and $1.03 \times 10^2 \pm 4.33 \times 10^1$] CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: [$8.39 \pm 8.32 \times 10^4$ and $1.57 \times 10^3 \pm 6.42 \times 10^2$] CFU/mL ($p < 0.05$);

Porphyromonas endodontalis: [$6.33 \times 10^2 \pm 4.74 \times 10^2$ and $1.02 \times 10^2 \pm 4.40 \times 10^1$] CFU/mL ($p < 0.05$);

Prevotella intermedia: [$5.70 \pm 4.88 \times 10^2$ and $5.00 \pm 2.24 \times 10^0$] CFU/mL ($p < 0.05$);

Tannerella forsythia: [$3.67 \pm 3.27 \times 10^1$ and $5.00 \pm 2.24 \times 10^0$] CFU/mL ($p < 0.05$);

Candida albicans: [$6.23 \pm 4.78 \times 10^3$ and $7.24 \pm 6.57 \times 10^3$] CFU/mL ($p = 0.47$).

In contrast, PCR results for dentures made from monomer-free (thermoplastic) material demonstrated less pronounced microbial reductions after a single cleaning cycle:

Aggregatibacter actinomycetemcomitans: [$1.39 \times 10^4 \pm 5.68 \times 10^3$ and $5.37 \pm 2.19 \times 10^2$] CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: [$5.72 \pm 2.33 \times 10^2$ and $5.33 \pm 2.18 \times 10^2$] CFU/mL ($p = 0.73$);

Porphyromonas endodontalis: [$1.35 \times 10^2 \pm 5.51 \times 10^1$ and $5.38 \pm 2.20 \times 10^2$] CFU/mL ($p = 0.67$);

Prevotella intermedia: [$1.37 \times 10^2 \pm 5.58 \times 10^1$ and $8.33 \pm 3.40 \times 10^0$] CFU/mL ($p < 0.05$);

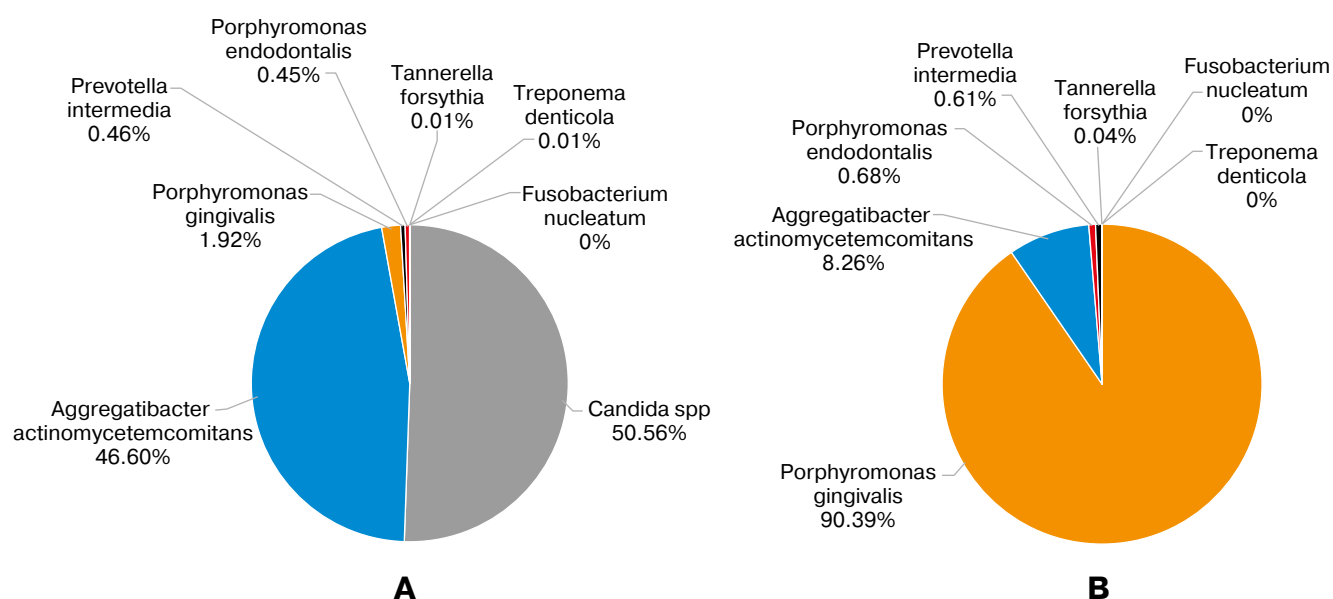


Fig. 2. PCR analysis of microbiota with prosthetic dentures cleaned before investigation: A – thermoplastic prosthesis; B – prosthesis made of plastic with monomer

Рис. 2. Результаты ПЦР-анализа микробиоты с протезов до начала исследования: A – протез из безмономерной пластмассы; B – протез из пластмассы с мономером

Tannerella forsythia: $[3.33 \pm 1.36 \times 10^0$ and $1.67 \times 10^0 \pm 6.80 \times 10^1]$ CFU/mL ($p < 0.05$);

Candida albicans: $[7.30 \pm 2.98 \times 10^3$ and $5.38 \pm 2.20 \times 10^2]$ CFU/mL ($p < 0.05$).

After 15 days of using the oxygen-based cleansing tablets, a significant improvement in denture surface cleanliness was observed for both monomer-containing acrylic and thermoplastic materials. PCR diagnostics confirmed the visual assessment, demonstrating a reduction in the quantity of pathogenic microorganisms to undetectable levels.

Comparative Efficacy of Cleansing Agents on Monomer-Based Dentures

When comparing the two hygiene protocols, it was found that dentures fabricated from monomer-containing acrylic resin were more amenable to cleansing. A positive trend in biofilm reduction was observed after the first application of the specialized active-oxygen-based cleanser. By day 15, microbiological analysis confirmed the complete eradication of periodontopathogenic microorganisms from the denture surface.

The microbial counts before and after 14 days of using the oxygen-based cleanser on monomer-based dentures were as follows:

Aggregatibacter actinomycetemcomitans: $[7.67 \pm 6.49] \times 10^3$ and $(0.00 \pm 0.00) \times 10^0$ CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: $[(8.39 \pm 8.32) \times 10^4$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Porphyromonas endodontalis: $[6.33 \times 10^2 \pm 4.74 \times 10^2$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Prevotella intermedia: $[(5.70 \pm 4.88) \times 10^2$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Tannerella forsythia: $[(3.67 \pm 3.27) \times 10^1$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$).

Although *Candida albicans* was initially present, its quantity was substantially reduced following the use of the specialized cleanser. At baseline, the total count of *Candida* spp. was $(9.81 \pm 1.65) \times 10^3$ CFU/mL. After 14 days, only *C. albicans* remained, with a significantly reduced level of $(1.67 \pm 1.67) \times 10^0$ CFU/mL (Fig. 4).

Efficacy of Long-Term Cleansing on Monomer-Free (Thermoplastic) Denture Bases

PCR analysis of biofilm samples from dentures fabricated using monomer-free thermoplastic material also demonstrated high cleansing efficacy after a 14-day hygiene protocol. Microbial loads before and after two weeks of using the active-oxygen-based cleanser were as follows:

Aggregatibacter actinomycetemcomitans: $[1.39 \times 10^4 \pm 5.68 \times 10^3$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: $[(5.72 \pm 2.33) \times 10^2$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Porphyromonas endodontalis: $[1.35 \times 10^2 \pm 5.51 \times 10^1$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Prevotella intermedia: $[1.37 \times 10^2 \pm 5.58 \times 10^1$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$);

Tannerella forsythia: $[(3.33 \pm 1.36) \times 10^0$ and $(0.00 \pm 0.00) \times 10^0]$ CFU/mL ($p < 0.05$).

These objective data demonstrate complete eradication of periodontopathogenic microorganisms. Additionally, the quantity of *Candida* spp. was reduced by nearly 60%. Following soap-based cleaning, *Candida albicans* levels were $(1.51 \pm 0.54) \times 10^4$ CFU/mL, while after two weeks of specialized cleansing, the level dropped to $(6.67 \pm 1.67) \times 10^0$ CFU/mL.

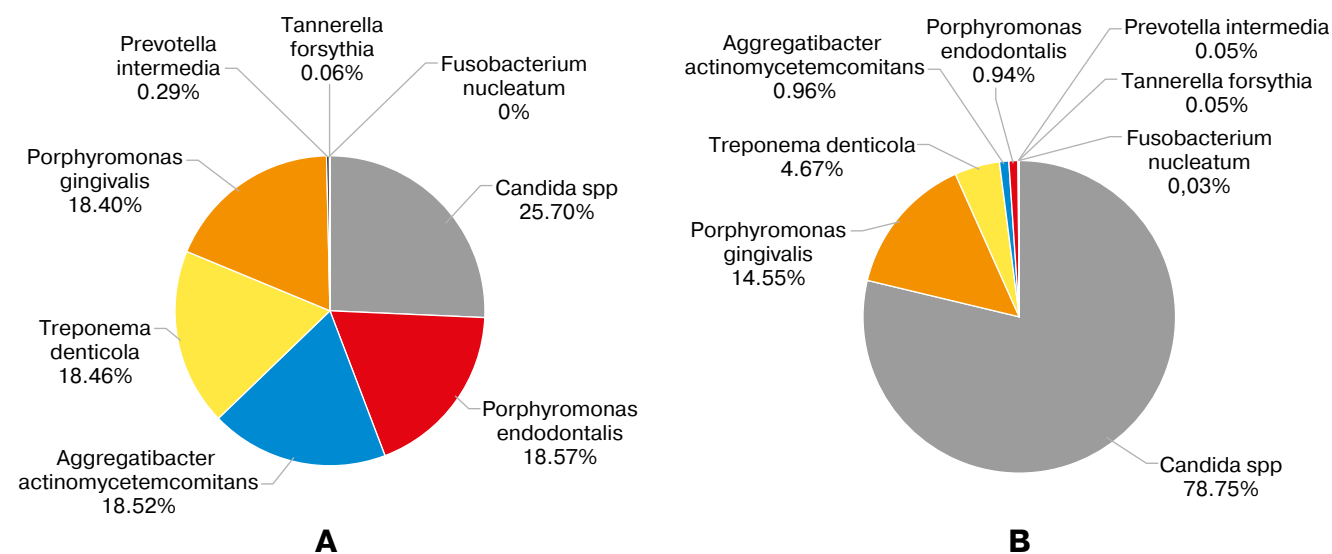


Fig. 3. PCR analysis of microorganisms from the surface of the prosthesis one-time cleaned with oxygen-containing tablets: A – thermoplastic prosthesis; B – prosthesis made of plastic with monomer

Рис. 3. Результаты ПЦР-анализа микроорганизмов с поверхности протеза, однократно очищенного кислородсодержащими таблетками:

A – протез из безмономерной пластмассы; B – протез из пластмассы с мономером

Although dentures made of thermoplastic material require a longer cleansing period due to their microporous structure, regular use of specialized active-oxygen-based cleansers ensures excellent hygiene outcomes. These agents release active oxygen upon dissolution in water, which penetrates even the smallest pores of the thermoplastic base, disrupting biofilms and removing plaque. Importantly, the cleanser is non-

abrasive and does not compromise the microstructure of the prosthesis (Fig. 5).

The photographic documentation obtained before and after treatment of removable dentures with the specialized cleansing agent – performed using scanning electron microscopy at $\times 8$ magnification – visually confirmed the results of the PCR analysis and the Tarbet denture hygiene index across all study groups (Fig. 6).

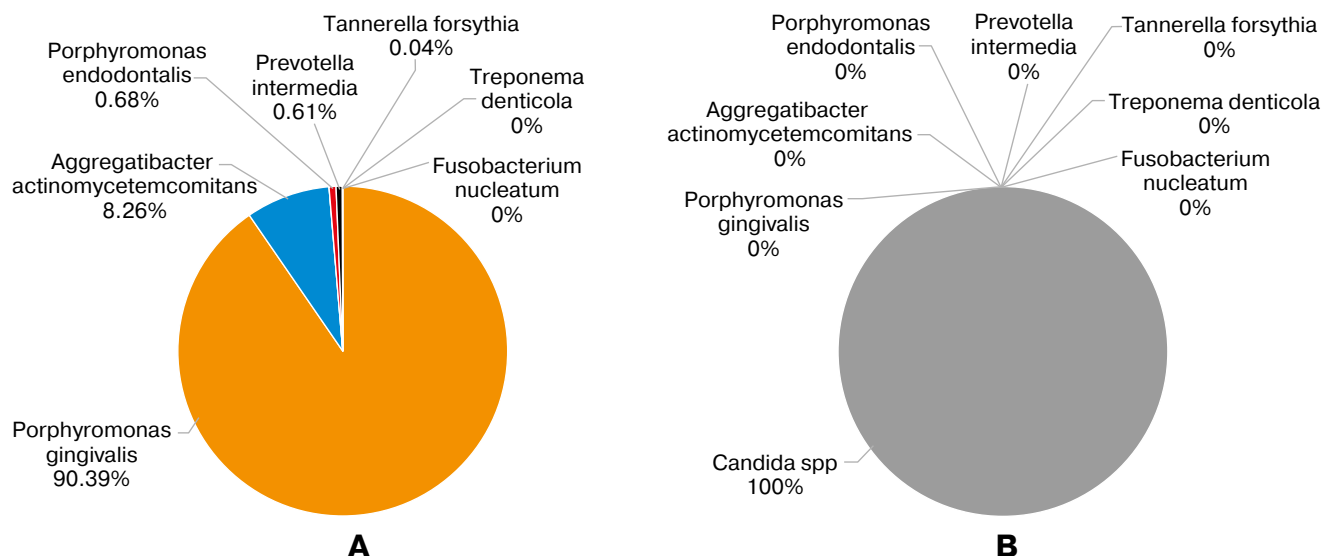


Fig. 4. Results of cleaning dentures microbial biofilm made of monomer plastic with oxygen product: A – thermoplastic prosthesis; B – prosthesis made of plastic with monomer

Рис. 4. Результаты очищения микробной биопленки средством на основе активного кислорода протезов из пластмассы с мономером: A – протез из безмономерной пластмассы; B – протез из пластмассы с мономером

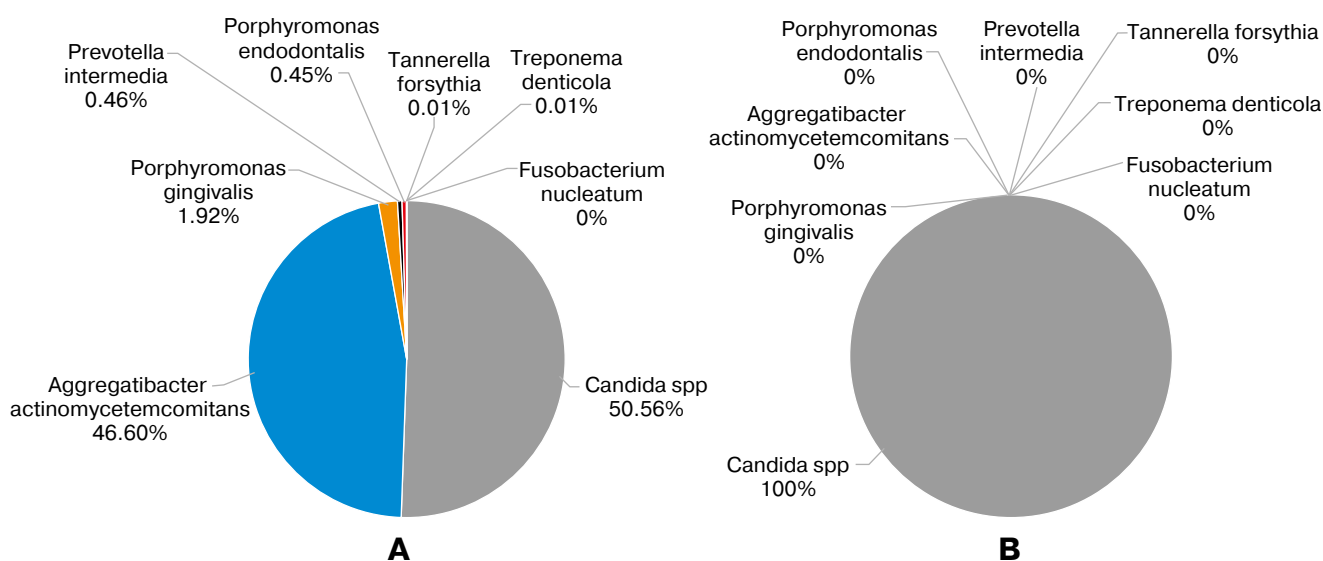


Fig. 5. Results of cleaning dentures made of thermoplastic material from biofilm with oxygen product: A – thermoplastic prosthesis; B – prosthesis made of plastic with monomer

Рис. 5. Результаты очищения биопленки средством на основе активного кислорода протезов из термопластичного (безмономерного) материала: A – протез из безмономерной пластмассы; B – протез из пластмассы с мономером

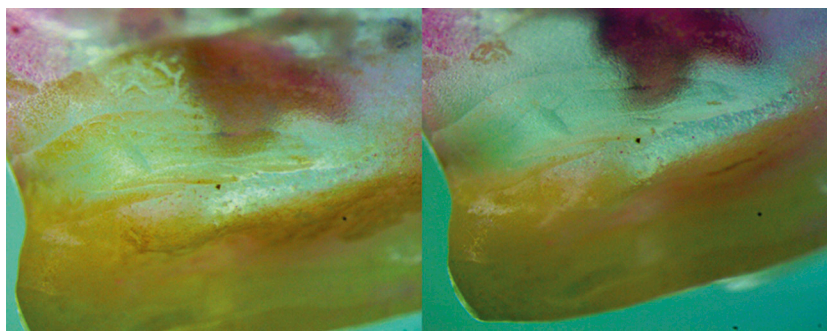


Fig. 6. Photos of an orthopedic structure taken on a scanning electron microscope (magnification x8) before and after cleaning with oxygen-containing product

Рис. 6. Фото ортопедической конструкции, сделанные на сканирующем электронном микроскопе (увеличение x8) до и после чистки кислородсодержащим средством

CONCLUSIONS

1. The prosthetic hygiene index (Tarbet index) indicates superior cleanliness of dentures fabricated from monomer-containing acrylic resin.

2. A comparative analysis of monomer-containing and monomer-free acrylic denture materials demonstrated that the surface of monomer-based dentures harbors a greater number of periodontopathogenic microorganisms.

3. Structural changes in the biofilm – characterized by a reduction in periodontopathogens and *Candida*

spp. – are observed only after two weeks of using oxygen-releasing cleansing tablets

4. Monomer-free acrylic resin appears to be a more favorable material for removable prostheses due to the absence of residual monomer, which reduces porosity and limits colonization by periodontopathogenic microorganisms.

5. All removable prosthetic appliances require thorough daily cleaning with agents based on active oxygen.

Despite the positive outcomes observed, the long-term efficacy of such cleansing agents warrants further investigation.

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Analysis of the surface microbiome of removable orthodontic appliances cleaned with various hygiene products

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Abstract

AIM. The aim of this study is to investigate the differences in the microbiota of the surface of orthodontic plates depending on the used hygiene products.

MATERIALS AND METHODS. The study was attended by 36 patients aged 6 to 12 years, undergoing treatment on removable orthodontic equipment using various hygiene products. The study was carried out using the determination of the prosthesis hygiene index and microbiological analysis of the material from the surface of the plate. Statistically, the data analysis included a description of the accounting features, an assessment of the statistical significance of changes in the indicators under study.

RESULTS. In the structure of the biofilm of a removable plate, microbiological indicators before and after the experiment showed a pronounced, statistically significant negative dynamics. And also, an improvement in the hygiene index of the plate and oral cavity.

CONCLUSIONS. The use of various hygiene products with orthodontic patients and the study of the microbiological status of the surface of the plate after cleaning them will allow you to choose the most optimal option for cleansing the removable apparatus.

Keywords: removable orthodontic plate, microbiological analysis, biofilm, hygiene products

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Анализ микробиома поверхности съемных ортодонтических пластинок, обработанных различными средствами гигиены

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Резюме

ЦЕЛЬ. Изучить различия микробиома поверхности ортодонтических пластинок в зависимости от используемых средств гигиены.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании приняли участие 36 пациентов в возрасте от 6 до 12 лет с диагнозом K07.2 Аномалии соотношения зубных дуг, получающие лечение с использованием съемной ортодонтической аппаратуры с учетом применения различных средств гигиены полости рта. Исследование проведено с помощью определения индекса гигиены протеза и microbiологического анализа материала с поверхности пластинки. Статистически анализ данных включал описание учетных признаков, оценку статистической значимости изменений изучаемых показателей.

РЕЗУЛЬТАТЫ. В структуре биопленки съемной пластинки microbiологические показатели до и после эксперимента показали выраженную, статистически значимую отрицательную динамику. А также произошло снижение индекса гигиены пластинки и полости рта.

ВЫВОДЫ. Применение различных средств гигиены ортодонтическими пациентами и изучение microbiологического статуса поверхности пластинки после их чистки позволит выбрать наиболее оптимальный вариант очищения съемного аппарата.

Ключевые слова: съемный пластиночный аппарат, microbiологическое исследование, биопленка, средства гигиены

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INTRODUCTION

According to the World Health Organization (WHO), as of 2023, oral health problems are among the most prevalent non-communicable diseases globally, accounting for nearly 3.5 billion cases. Oral diseases affect approximately 45% of the world's population, indicating a significant deficiency in preventive measures. The prevention of pathological processes in the oral cavity includes oral hygiene education, pharmacological prophylaxis, and timely treatment interventions¹.

An epidemiological survey conducted in Russia in 2019 revealed that among six-year-old children, the prevalence of caries in permanent teeth, based on the DMFT index, was 2%. The components of the index were as follows: Decayed (D) – 0.02%, Missing (M) – 0.00%, and Filled (F) – 0.00%. At the age of 12, caries prevalence reached 72%, with a mean caries experience of 2.38%, the mean number of filled teeth at 0.52%, and extracted teeth at 0.02%. Among 15-year-olds, the prevalence of carious lesions increased to 82%, with the D component at 1.58%, F – 1.38%, and M – 0.04%. Periodontal health was reported in 90% of 12-year-olds and 74% of 15-year-olds.

In the city of Kirov, according to epidemiological dental assessments conducted in 2019 and 2022, the number of 12- and 15-year-old children diagnosed with periodontal tissue pathology increased. Among 12-year-olds, the proportion of children without periodontal lesions was higher than the national average, whereas in the 15-year-old group, the figure was lower, with more frequent occurrences of bleeding on probing and the presence of dental calculus. These indicators exceed the national average and reflect insufficient routine oral hygiene practices [1; 2].

Studies conducted across various regions of Russia have shown that among all dental pathologies, dentoalveolar anomalies (DAAs) occur in 41.5% to 69.9% of cases, with approximately every second patient using removable orthodontic appliances for occlusal correction. Over the past 20 years, the prevalence of DAAs has increased by approximately 25%. According to national epidemiological data, the incidence of dentoalveolar system disorders among children varies from 37.8 to 85%, depending on the developmental stage of the jaws and the phase of occlusion formation [3].

¹ Pan American Health Organization. Global oral health status report: Towards universal health coverage for oral health by 2030. Washington, D.C.: PAHO/WHO; 2022. Available at: <https://www.paho.org/en/documents/global-oral-health-status-report-towards-universal-health-coverage-oral-health-2030> (accessed: 01.06.2025).

Research indicates that around 30–40% of patients experience discomfort when wearing removable plate-type orthodontic appliances [4]. These devices are often perceived by the body as foreign objects, leading to irritation of the oral mucosa. Improved adaptation and a reduction in inflammatory reactions can be achieved through effective cleaning of the appliance. Prolonged use of such prostheses—particularly in the absence of adequate hygiene—allows microbial biofilms originating from dental plaque to penetrate up to 2–2.5 mm into the acrylic base of the plate [5].

The microbial species most commonly found in the biofilm on orthodontic appliances include *Staphylococcus aureus*, *Streptococcus* spp. (*S. mutans*, *S. mitis*, *S. sanguis*, *S. salivarius*), *Candida* yeasts, and various periodontopathogens such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola* [6].

Therefore, the selection of optimal hygiene products for cleaning orthodontic appliances remains a relevant and important issue in contemporary orthodontic practice.

AIM

The aim of this study is to perform a comparative evaluation of the microbiota associated with removable orthodontic appliances following two different hygiene protocols: mechanical cleaning using toothpaste and a toothbrush, and chemical cleaning using a specialized active oxygen-based agent. Additionally, the study seeks to assess the impact of appliance usage on the biochemical composition of oral fluid in orthodontic patients.

MATERIALS AND METHODS

The study was conducted with the participation of 36 patients aged 6 to 12 years undergoing treatment with removable orthodontic appliances. The patients were divided into two groups of 15 individuals each. The first group included orthodontic patients who used a specialized active oxygen-based hygiene product for appliance cleaning. The second group, serving as the control, included patients who used a medium-bristled toothbrush and conventional toothpaste for cleaning.

During the study, the prosthesis hygiene index (Tarbet modification) was recorded, oral fluid was collected for biochemical analysis, and biofilm samples were taken from the surface of the orthodontic appliances for microbiological evaluation using polymerase chain reaction (PCR). These parameters were assessed on

day 1 and day 30 of appliance cleaning using either conventional toothpaste/soap or oxygen-based cleansing tablets, respectively.

To assess the modified Tarbet index, the orthodontic appliance was removed from the oral cavity and immersed in water for one minute to eliminate food debris. The tissue-facing surface of the appliance was stained with erythrosine solution for one minute, after which the dye was rinsed off. The amount of plaque was evaluated based on the stained surface area and color intensity.

Unstimulated whole saliva samples were collected in the morning on an empty stomach, prior to toothbrushing, by passive drooling into sterile disposable tubes (5 mL per patient). The biochemical analysis of calcium ions (Ca^{2+}), phosphate (PO_4^{3-}), and total protein content in the saliva was conducted using photolorimetric methods with reagent kits "Calcium-2-Olvex" and "FN-Olvex". The hydrogen ion concentration (pH) of the saliva was measured using a HI98103 Checker pH Tester (Hanna Instruments, Romania). Total antioxidant activity was determined by induced chemiluminescence, a method based on the assessment of free radical reaction activity in the sample [7]. Salivary pH was additionally measured using the "Expert-001" pH meter [8].

Microbial samples were collected from the appliance surface using sterile paper points, placed into saline solution, and transported to a microbiological laboratory. Serial tenfold dilutions were prepared using buffer solution and plated on meat-peptone agar (MPA) in Petri dishes, followed by incubation at 37°C and colony counting. DNA of periodontal pathogens was extracted using the "ProbaGS" kit (LLC "DNA-Technology"), in accordance with the manufacturer's protocol, and analyzed by real-time PCR using a DT-96 thermocycler (LLC "DNA-Technology"). The results were evaluated using the instrument's software. Based on the cycle threshold (Ct), the total microbial count was determined, expressed in colony-forming units per milliliter (CFU/mL), including the quantity of mesophilic aerobic and facultative anaerobic bacteria. Specific periodontal patho-

gens were identified, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, and *Tannerella forsythia*. The microbial composition was categorized into microbial complexes according to the classification by S.S. Socransky [7; 9].

To monitor the cleanliness of the appliance surface, a photoprotocol was performed using a light electron microscope ($\times 8$ magnification) before and after cleaning with oxygen-releasing tablets.

RESULTS

In the first and second groups, the DMFT+dmft index was 4.25 ± 0.48 and 5.25 ± 0.63 , respectively, which is considered a moderate level and falls within the relative norm for the 6–12 age group.

At the beginning of the study, the prosthesis hygiene index (modified Tarbet) in the two groups averaged 3.5 ± 0.29 and 3.75 ± 0.25 points, respectively. After 30 days of appliance cleaning, the index decreased to 1.8 ± 0.48 points in the group using toothpaste/soap and to 0.8 ± 0.25 points in the group using oxygen-releasing tablets, indicating superior efficacy of the latter. Visually, the intensity of staining on the appliances was significantly reduced, not exceeding 25% of the plate surface.

Biochemical analysis of oral fluid in the control and experimental groups did not reveal statistically significant differences. In Group 1, the salivary pH was 7.28 ± 0.24 at baseline and 7.03 ± 0.02 at the end of the experiment ($p=0.07$); in Group 2, it was 7.61 ± 0.26 and 7.59 ± 0.27 , respectively ($p=0.64$) (Fig. 1). The differences in both groups were not statistically significant. A slight shift toward acidity was observed in the control group, whereas Group 2 maintained a consistently alkaline environment in the oral cavity. Total protein levels in oral fluid decreased steadily in both groups, indicating reduced salivary viscosity and, consequently, improved oral self-cleansing, associated with regular hygiene of orthodontic appliances regardless of the cleansing method used.

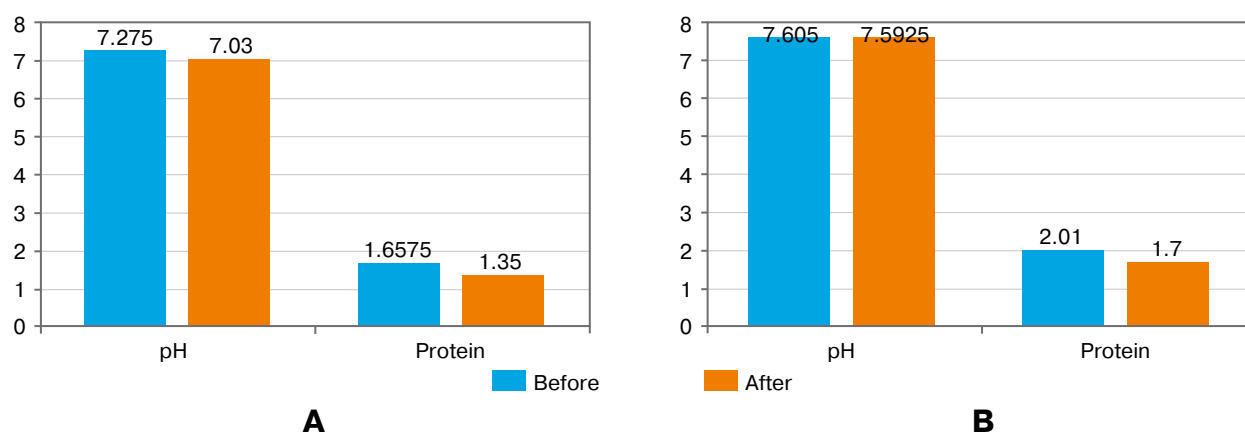


Fig. 1. Changes in biochemical parameters in the control group (A) and the group of patients using an oxygen hygiene product (B)

Рис. 1. Изменения биохимических показателей в контрольной группе (A) и группе пациентов, использующих кислородсодержащее средство гигиены (B)

The total microbial count (TMC) in the control group increased threefold after one month of cleaning with toothpaste: prior to the intervention, the microbial load was $(1.00 \pm 0.1) \times 10^7$ CFU/mL, while after the intervention it reached $(3.03 \pm 2.33) \times 10^7$ CFU/mL. In contrast, the experimental group showed a statistically significant ($p \leq 0.05$) twofold reduction in TMC. At baseline, the microbial count was $(5.50 \pm 2.6) \times 10^5$ CFU/mL, and after one month of appliance cleaning with the specialized hygiene agent, it decreased to $(3.25 \pm 2.25) \times 10^4$ CFU/mL, demonstrating the effectiveness of the active oxygen-based product (Fig. 2).

According to PCR analysis, a significant increase in the number of periodontal pathogens was observed in the control group. The microbial load on the appliances used by control group patients before and after the intervention, respectively, was as follows: *Aggregatibacter actinomycetemcomitans* $[(2.80 \pm 2.41) \times 10^3$ and $(3.25 \pm 1.00) \times 10^4$ CFU/mL, $p < 0.05$], *Porphyromonas gingivalis* $[(2.80 \pm 2.41) \times 10^3$ and $(7.75 \pm 2.25) \times 10^4$ CFU/mL, $p < 0.05$], *Porphyromonas endodontalis* $[(3.00 \pm 2.34) \times 10^3$ and $(5.25 \pm 2.75) \times 10^4$ CFU/mL, $p < 0.05$], *Prevotella intermedia* $[(2.80 \pm 2.41) \times 10^3$ and $(2.80 \pm 2.41) \times 10^4$ CFU/mL, $p < 0.05$], *Tannerella forsythia* $[(2.76 \pm 2.43) \times 10^2$ and $(2.78 \pm 2.42) \times 10^4$ CFU/mL, $p < 0.05$], and *Treponema den-*

ticola $[(2.76 \pm 2.43) \times 10^2$ and $(3.25 \pm 2.25) \times 10^2$ CFU/mL, $p = 0.65$]. An increase in *Candida albicans* colonization was also observed $[(3.03 \pm 2.33) \times 10^3$ and $(5.05 \pm 2.60) \times 10^3$ CFU/mL, $p = 0.14$], possibly due to the observed decrease in salivary pH. In parallel, a marked decline in the quantity of *Lactobacillus* spp. – representatives of the normal oral microbiota – was noted, which is considered a negative factor for oral health (Fig. 3).

In contrast, PCR analysis in the experimental group demonstrated a reduction in the levels of periodontal pathogens alongside an increase in *Lactobacillus* spp. counts. Specifically, the microbial concentrations before and after the intervention were as follows: *Aggregatibacter actinomycetemcomitans* $[(5.50 \pm 2.60) \times 10^2$ and $(3.25 \pm 2.25) \times 10^2$ CFU/mL, $p < 0.05$], *Porphyromonas gingivalis* $[(5.50 \pm 2.60) \times 10^2$ and $(7.75 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Porphyromonas endodontalis* $[(3.25 \pm 2.25) \times 10^2$ and $(7.75 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Prevotella intermedia* $[(5.50 \pm 2.60) \times 10^2$ and $(3.25 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Tannerella forsythia* $[(3.00 \pm 2.34) \times 10^2$ and $(7.75 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Treponema denticola* $[(2.75 \pm 2.43) \times 10^3$ and $(5.28 \pm 2.73) \times 10^1$ CFU/mL, $p < 0.05$], and *Candida albicans* $[(2.80 \pm 2.60) \times 10^3$ and $(5.50 \pm 2.49) \times 10^2$ CFU/mL, $p < 0.05$] (Fig. 4).

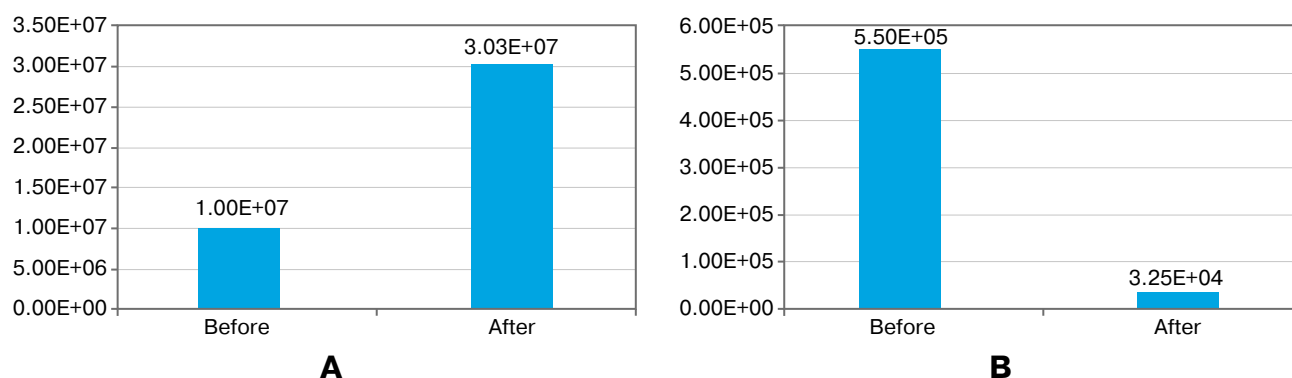


Fig. 2. Total microbial number in control (A) and experimental (B) groups before and after examination, in CFU/ml

Рис. 2. Общее микробное число в контрольной (A) и экспериментальной (B) группах до и после эксперимента, в КОЕ/мл

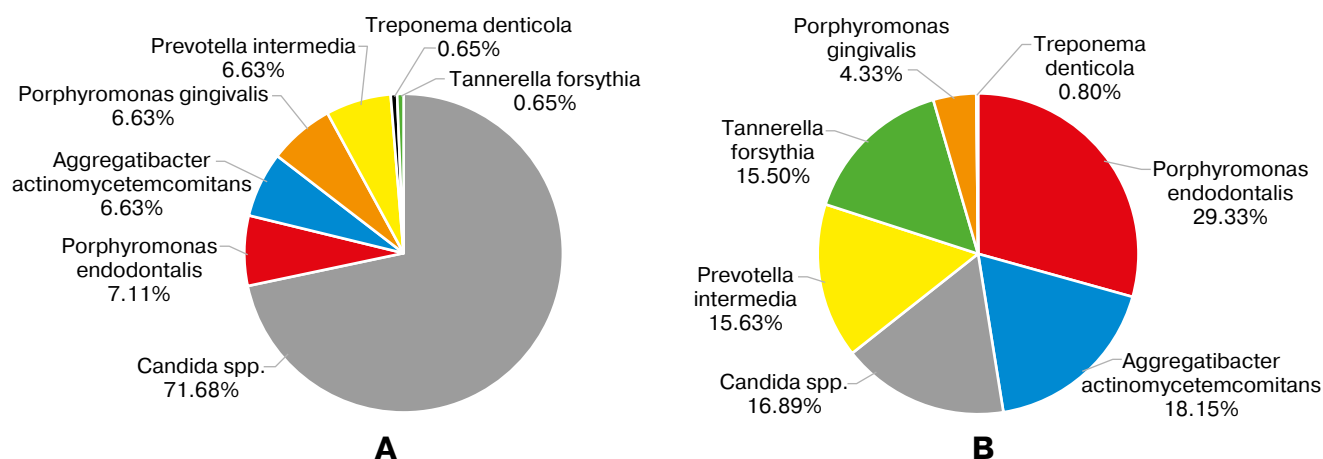


Fig. 3. PCR studies data in the control group before (A) and after (B) investigation

Рис. 3. ПЦР исследование в контрольной группе до (A) и после (B) исследования

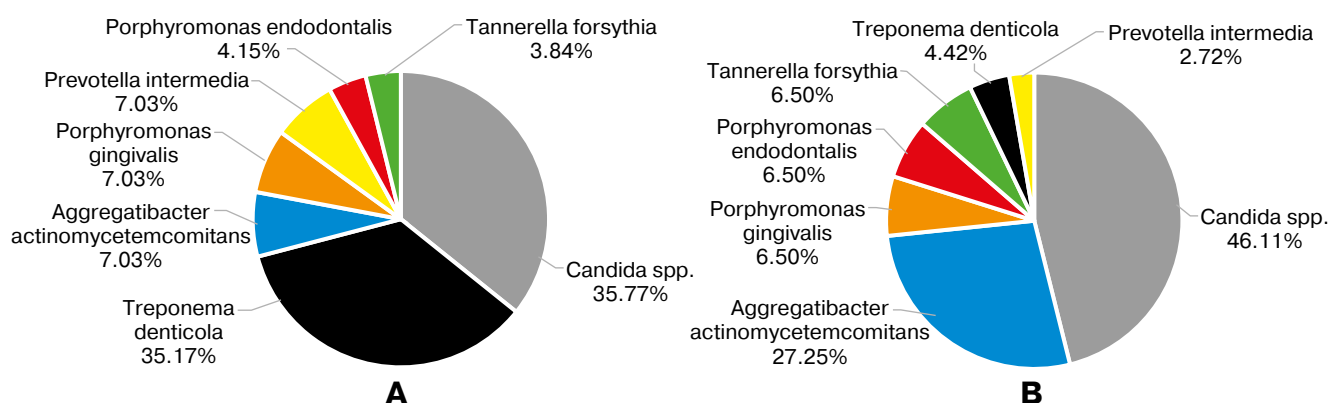


Fig. 4. PCR studies data in the experimental group before (A) and after (B) investigation

Рис. 4. ПЦР исследование в экспериментальной группе до (A) и после (B) исследования

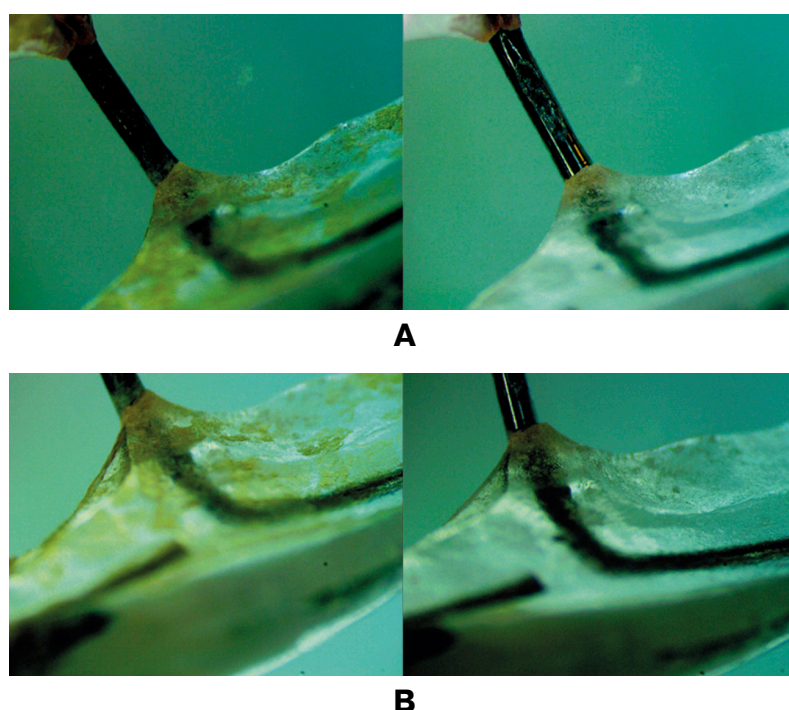


Fig. 5. Photos of an orthodontic structure taken on a scanning electron microscope (magnification x8) before (A) and after (B) cleaning with oxygen-containing tablets

Рис. 5. Фото ортодонтической конструкции, сделанные на сканирующем электронном микроскопе (увеличение x8) до (A) и после (B) чистки кислородсодержащими таблетками

The photodocumentation performed before and after treatment of the orthodontic appliances with the oxygen-releasing agent, using a scanning electron microscope at $\times 8$ magnification, visually confirmed a reduction in biofilm coverage and plaque accumulation (Fig. 5).

CONCLUSIONS

1. More effective plaque removal from removable orthodontic appliances, as assessed by the Tarbet index, was achieved using an active oxygen-based cleansing agent compared to conventional cleaning with a toothbrush and toothpaste.

2. Biochemical analysis demonstrated that the absence of significant differences in salivary pH and the reduction in total protein content in both the experimental and control groups were attributable not to the composition of the hygiene products, but rather to the overall improvement in appliance and oral hygiene practices.

3. Microbiological examination of biofilm from the appliance surface revealed that maintaining hygienic conditions is more effective with regular use of an active oxygen-based cleansing agent, resulting in a twofold reduction in total microbial count and a 75% decrease in biofilm surface area. Therefore, the use of such specialized products should be recommended as part of routine care for removable orthodontic appliances.

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А.В. Зайнутдинова – сбор данных или анализ и интерпретацию данных.

Т.В. Каравеева – сбор данных, проведение статистической обработки материалов, анализ и интерпретация данных.

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Е.А. Еликов – проведение биохимических исследований, анализ и интерпретация данных.

Е.А. Куклина – критический пересмотр статьи в части значимого интеллектуального содержания.

С.Н. Громова – окончательное одобрение варианта статьи для опубликования.

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XRF analysis of tooth enamel under conditions of experimental erosion in vitro

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Abstract

INTRODUCTION. Dental erosion is a result of chemical processes – specifically, “acid attacks” on the tooth surface that occur without bacterial involvement – leading to alterations in the mineral structure of dental tissues. In recent years, the erosive potential of fruit juices, carbonated and non-carbonated soft drinks, as well as alcoholic beverages, has been actively studied in an effort to better understand the mechanisms of demineralization and to assess the impact of drink acidity. However, there is a lack of data on experiments that provide a comprehensive profile of macro- and microelement content in erosion zones of varying severity compared to intact enamel and dentin.

AIM. To examine, using X-ray fluorescence analysis, the dynamics of changes in the calcium-to-phosphorus (Ca/P) ratio—an important indicator of tooth mineral content—under conditions of artificial erosion caused by various acidic food and beverage solutions.

MATERIALS AND METHODS. The exogenous acidic agents used included solutions of lactic, acetic, and hydrochloric acids; lemon juice; dry red wine; Dobry Cola; and a solution of Acidin-Pepsin tablets (a drug prescribed for hypoacid and anacid gastritis). Recently extracted intact teeth were immersed in the test liquids for three days. Demineralization was assessed based on observed changes in elemental composition. The chemical analysis of the solid dental tissues was performed using an M4 TORNADO X-ray fluorescence spectrometer (Bruker).

RESULTS. In all test conditions, demineralization occurred as evidenced by the active release of calcium and phosphorus – the main macroelements – from the crystal lattices of hydroxyapatite, carbonate apatite, chlorapatite, fluorapatite, and other mixed apatite forms found in enamel. Notably, the kinetics of calcium and phosphorus loss differed significantly. In all cases, the Ca/P ratio increased substantially after three days of exposure to the erosive medium, compared to baseline values in intact enamel. This finding indicates that phosphate groups are the first to be lost during erosion, dissolving into the oral environment, followed by calcium loss as a less intense secondary process.

CONCLUSIONS. Based on analysis of Ca/P ratios, enamel erosion appears to begin with dephosphorylation of the crystalline lattice, followed by decalcification.

Keywords: erosive wear, tooth erosion, artificial dental erosion, X-ray fluorescence analysis, tooth mineral structure

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Рентгенофлуоресцентный анализ (РФА) эмали зуба в условиях экспериментальной эрозии in vitro

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Резюме

ВВЕДЕНИЕ. Эрозия есть следствие химических процессов, а именно «кислотной атаки» на зуб без участия бактерий, в результате чего искажается минеральная структура тканей зуба. Эрозивный потенциал фруктовых соков, газированных и негазированных безалкогольных и алкогольных напитков

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в последние годы изучается особенно интенсивно с целью понять тонкий механизм деминерализации, оценить влияние степени кислотности напитка. Однако сведений об экспериментах, в которых была бы получена полная картина по содержанию макро- и микроэлементов в зонах эрозии разной степени в сравнении с неповрежденными участками эмали и дентина, в литературе не обнаружено.

ЦЕЛЬ. Изучить с помощью рентгенофлуоресцентного анализа динамики изменения наиболее показательного индекса Са/Р тканей зуба в условиях искусственной эрозии, вызванной различными пищевыми жидкостями с пониженным значением pH.

МАТЕРИАЛЫ И МЕТОДЫ. В качестве экзогенных источников кислотного фактора использовали растворы кислот (молочная, уксусная, соляная), сок лимона, красное сухое вино, Добрый Cola, а также раствор таблеток препарата Ацидин-пепсин, назначаемого при анацидном и гипоацидном гастритах. Интактные свежееудаленные зубы погружали в исследуемые жидкости на трое суток, после чего оценивали степень произошедшей деминерализации по наблюдаемым изменениям содержания элементов. Химический состав образцов твердых тканей зуба выполняли на рентгенофлуоресцентном спектрометре М4 TORNADO (Bruker).

РЕЗУЛЬТАТЫ. Во всех случаях наблюдался процесс деминерализации, связанный с активным выходом из кристаллических решеток гидроксиапатита, карбонатапатита, хлорапатита, фторапатита и других, смешанных форм апатитов, из которых состоит эмаль, основных макроэлементов – кальция и фосфора, причем кинетика этих двух процессов имела существенные различия. Во всех случаях индекс Са/Р через трое суток пребывания в эрозионной среде значительно отклонился в сторону увеличения по сравнению с исходными значениями, характерными для неповрежденной эмали. Это обстоятельство прямо указывает на то, что кристаллы апатитов, составляющие эмалевые призмы, при эрозии в первую очередь лишаются фосфатных групп, уходящих в ротовую жидкость, с последующей декальцинацией в статусе менее интенсивного вторичного процесса.

ВЫВОДЫ. На основе анализа значений индекса Са/Р установлено, что при эрозии дефосфорилирование кристаллической решетки эмали является первичным процессом, за которым следует декальцификация.

Ключевые слова: эрозивный износ, эрозия зубов, искусственная эрозия зубов, рентгенофлуоресцентный анализ, минеральная структура зуба

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INTRODUCTION

Erosive tooth wear (erosion, erosive attrition) is a contemporary medical issue closely related to oral health. Data from various sources indicate a growing prevalence of erosion, ranging between 30–50% of the population [1–3], while in certain geochemical provinces, substantially higher figures have been reported [4; 5]. It is generally accepted that erosion results from chemical processes triggered by an “acid attack” on the tooth surface without bacterial involvement. This process leads to the alteration of the mineral structure of dental tissues, initially affecting superficial layers and subsequently extending to deeper ones.

Erosion develops under the influence of dietary, and consequently chemical, biological, and behavioral factors, which interact and produce diverse clinical manifestations. Early detection of erosion remains a critical challenge for dental practitioners, as functional and aesthetic complications typically arise at more advanced stages. Understanding the etiological factors of erosive tooth wear is essential for preventing disease progression and for designing effective preventive strategies [6; 7].

In recent years, the erosive potential of fruit juices, carbonated and non-carbonated soft drinks, as well as alcoholic beverages, has become increasingly evi-

dent and has been the subject of intensive study [8]. These investigations aim to elucidate the mechanisms of demineralization and to assess how beverage acidity and the combined effect of dissolved substances influence the intensity of the process. To date, numerous highly cited publications have reported the results of *in vitro* experiments on extracted teeth immersed in various food-derived liquids or their analogues [9–11]. Researchers have employed advanced methods such as three-dimensional confocal laser microscopy, atomic force microscopy, laser speckle imaging, microradiography, and electron probe microanalysis.

However, we have not encountered studies that provide a comprehensive characterization of macro- and microelement composition within erosion zones of varying severity compared with intact areas of enamel and dentin. Such information would be of significant practical value, as it could contribute to a more detailed understanding of the chemical dimension of the erosive process, which comprises a complex interplay of reversible and irreversible chemical reactions. The crystalline lattice components of hard dental tissues are induced into these reactions by the surrounding liquid medium.

X-ray fluorescence analysis (XRF), which we have repeatedly applied in dental research, has proven to be an effective method for obtaining novel insights that allow

for a re-evaluation of the etiology and development of oral pathologies. This, in turn, provides clinicians with the opportunity to implement more evidence-based and thus more effective therapeutic strategies. In particular, the use of XRF has demonstrated fundamental differences in the chemical mechanisms underlying caries and erosion [12; 13].

AIM

To investigate, using X-ray fluorescence analysis (XRF), the temporal dynamics of the calcium-to-phosphorus (Ca/P) ratio in dental hard tissues under conditions of artificial erosion induced by various low-pH food and beverage solutions with differing erosive potentials.

MATERIALS AND METHODS

The study was conducted at the Departments of Therapeutic Dentistry and Endodontics, and General and Bioorganic Chemistry of the Russian University of Medicine, as well as in the laboratories of the N.S. Kurnakov Institute of General and Inorganic Chemistry of the Russian Academy of Sciences. Intact human teeth extracted for periodontal and orthodontic reasons, without signs of prior dental treatment, were used. Samples were cleaned of soft tissues, disinfected, and stored in distilled water at 4°C. The hydrogen ion concentration (pH) was measured using a portable electronic pH-meter (Hanna Instruments) at 25°C.

As exogenous sources of the acidic factor, we used solutions of lactic, acetic, and hydrochloric acids; fresh lemon juice; commercial beverages (dry red wine, Dobry Cola); and a solution of Acidin-Pepsin tablets, a preparation prescribed for anacidic and hypoaacidic gastritis. Healthy teeth were immersed in the test solutions for three days, after which the degree of demineralization was evaluated based on changes in elemental composition.

The chemical composition of dental hard tissues was analyzed using an M4 TORNADO (Bruker) X-ray diffractometer, a micro-XRF spectrometer designed for nondestructive qualitative and quantitative material characterization. The method is based on the interaction of high-energy X-rays with elements in the sample, resulting in the emission of secondary X-rays (X-ray fluorescence) characteristic for each atom. The instrument was equipped with a rhodium fine-focus X-ray tube (maximum excitation 50 kV, 30 W, 600 µA). The MultiPoint mode was applied (analysis at several points across the selected enamel surface area).

During measurements, the X-ray beam was directed to a defined point of the enamel surface, and the elemental composition was determined for a spot diameter of 20 µm. For each point, the system averaged 100 pulses and displayed the detected elements in the form of a spectrum. The resulting spectrum was converted into a table showing the percentage mass ratios of the detected atoms relative to the total elemental content, with the spectrum normalized to 100%. It is well established that XRF cannot determine with sufficient accuracy the elements of the first two periods of the Periodic Table

and sodium; however, this limitation did not affect the objectives of our study.

The spectrometer was calibrated against a standard sample of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) for calcium and phosphorus content. The measurement accuracy was 0.01%. Statistical analysis of the obtained data was performed using a personal computer and the software package *Statistica 9.0*.

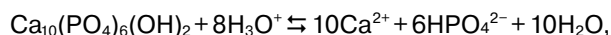
For interpretation of deviations in elemental content, the calcium-to-phosphorus (Ca/P) ratio was used as a reference index. According to the literature, enamel resistant to acidic challenge demonstrates a Ca/P ratio in the range of 1.6–2.1 (by mass). In our study, the Ca/P ratio for intact enamel samples from healthy teeth ranged between 1.9–2.1. The trace elements identified by XRF spectra corresponded to the typical elemental composition of enamel both in nomenclature and quantitative distribution.

RESULTS

The three-day immersion period of intact teeth in liquids with a predetermined erosive potential was chosen in order to avoid random fluctuations characteristic of shorter exposure intervals, while simultaneously establishing a high acid load from the outset. In our view, the obtained results largely reflect the clinical picture that develops in vivo under conditions of regular and systematic consumption of such beverages.

In all cases, demineralization was observed, associated with the active release of calcium and phosphorus – the principal macroelements – from the crystal lattices of hydroxyapatite, carbonate-apatite, chloro-apatite, fluoroapatite, and other mixed apatite forms constituting enamel. Notably, the kinetics of calcium and phosphorus release demonstrated substantial differences.

Under normal conditions, the heterogeneous equilibrium of “demineralization–remineralization” between the solid phase (enamel) and the saturated solution (oral fluid) can conventionally [14] be represented as:



whereas at a critical pH value of 5.5 or below, a cascade of ion-exchange reactions is initiated, beginning with the hydration of apatites as calcium ions are replaced by an equivalent charge of hydronium ions:



If the buffering capacity of saliva is insufficient to neutralize the exogenous acid challenge, enamel destruction ensues. In vivo, however, the kinetics of equilibrium processes is considerably more complex, being additionally influenced by factors such as the electrolyte composition and ionic strength of oral fluids and beverages, as well as gender, age, circadian rhythms, and other systemic variables.

As an illustration, Fig. 1 presents a fragment of a tooth sample immersed in “Dobry Cola”, accompanied by the corresponding XRF spectrum (Fig. 2) and the numerical values of relative elemental content at analytical points (Fig. 3).

DISCUSSION

The obtained results for the studied media, arranged in order of increasing acidity, are presented in the table. It was found that in all cases the Ca/P ratio, after three days of immersion in erosive media, showed a significant upward deviation compared to the baseline values characteristic of intact enamel. This finding directly indicates that, during erosion, the apatite crystals forming enamel prisms initially lose phosphate groups, which migrate into the oral fluid, with subsequent decalcification occurring as a less intensive secondary process.

This phenomenon has previously been noted in studies of enamel affected by erosion both *in vivo* [15] and *in vitro* [16], although without detailed consideration of the underlying chemical processes.

In our view, the cascade of processes described above should be regarded as the key mechanism in caries development, where the tooth is exposed to a complex mixture of acids produced by bacterial metabolism in the presence of numerous enzymes with affinity to phosphate groups [14]. These enzymes facilitate conjugation and thereby contribute, to some extent, to the

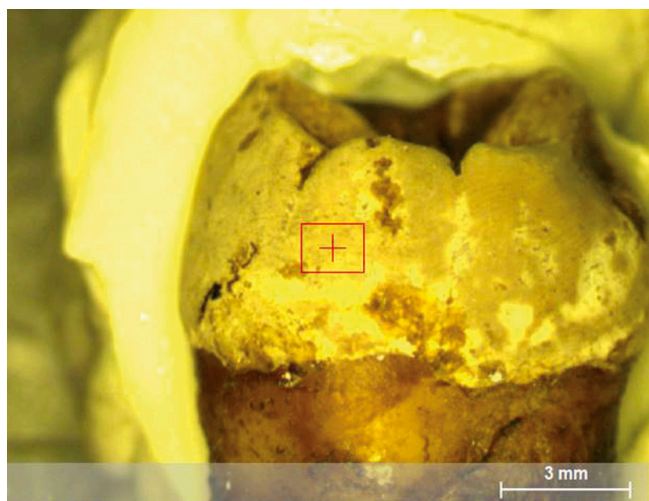


Fig. 1. Enamel area after tooth exposure to "Dobry Cola" (RFA, MultiPoint mode)

Рис. 1. Участок эмали после выдержки зуба в «Добрый Cola» (РФА, режим MultiPoint)

Mass percent (%)				
P	Cl	Ca	Zn	
8,40	0,39	90,95	0,26	
8,20	0,45	91,10	0,24	
9,04	0,32	90,37	0,27	
8,39	0,49	90,81	0,31	
7,80	0,41	91,50	0,29	
8,47	0,43	90,81	0,29	
	P	Cl	Ca	Zn
Mean value:	8,38	0,42	90,92	0,28
Sigma:	0,40	0,06	0,37	0,02
Sigma mean:	0,16	0,02	0,15	0,01

Fig. 2. X-ray diffraction spectra corresponding to the tooth after exposure to "Dobry Cola"

Рис. 2. Спектры РФА, соответствующие зубу после выдержки в «Добрый Cola»

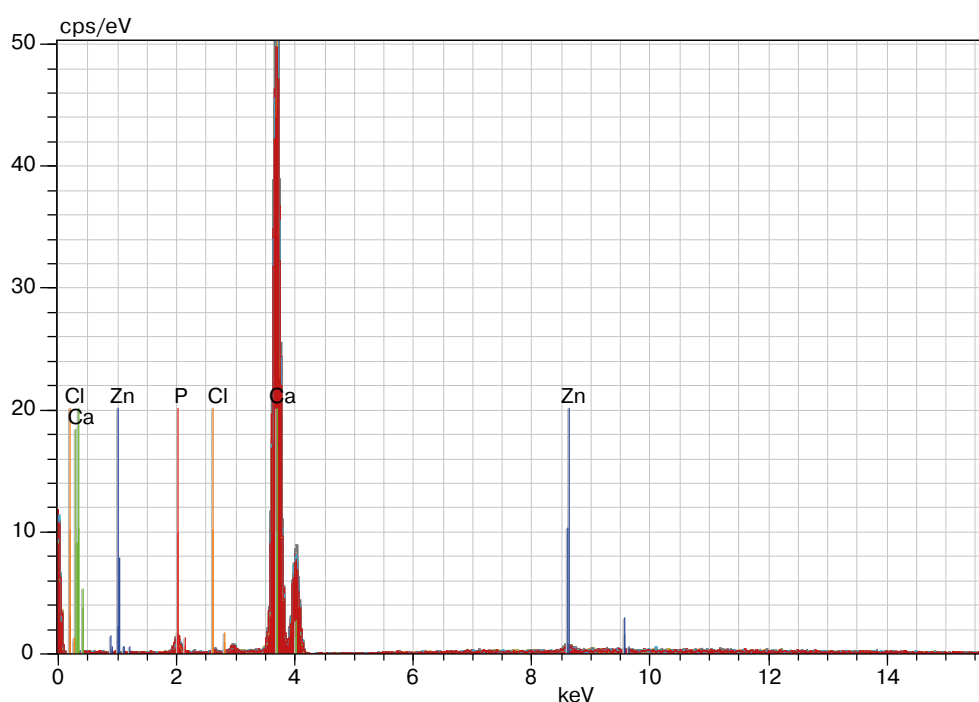
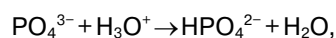


Fig. 3. Data on the analysis of the tooth surface after exposure to "Dobry Cola" (RFA, MultiPoint mode)

Рис. 3. Данные по анализу поверхности зуба после его выдержки в «Добрый Cola» (РФА, режим MultiPoint)

stabilization of phosphate groups within the crystal lattice. By contrast, in erosion, enamel is subjected to the direct action of protons with varying degrees of hydration derived from exogenous acids. In this scenario, the dominant reaction becomes:



driven by the high stability of the resulting hydrogen phosphate ion, as indicated by its dissociation constant (1.26×10^{-12}).

As shown in the table, erosion occurred in all tested solutions within the physiological pH values typical of food products. Moreover, across the broad acidity range ($\Delta\text{pH} = 3.68 - 1.86 = 1.82$, corresponding to a two-order difference in proton concentration), no correlation was observed between the degree of acidity and the severity of erosive damage. At the same time, the ratio of dephosphorylation to decalcification intensity was found to remain statistically constant, with some variability of recorded values within the examined enamel zone being consistent with its natural heterogeneity.

It should be emphasized that the present observations refer to pH values characteristic of food products. Exceeding these physiological boundaries exposes the enamel crystal lattice to extreme conditions that overwhelm compensatory mechanisms. This was confirmed in our analysis of tooth surfaces exposed to 14% hydrochloric acid, where the measured calcium and phosphorus contents and the corresponding Ca/P ratio indicated profound, irreversible fragmentation of both enamel and dentin (Table 1).

CONCLUSIONS

1. Using X-ray fluorescence analysis (*in vitro*), we demonstrated that various food and beverage solutions exhibit high erosive activity, which is virtually independent of their pH values within the range of 3.58–1.86.

2. Based on the analysis of the Ca/P ratio, it was established that during erosion, dephosphorylation of the enamel crystal lattice represents the primary process, followed by decalcification. A chemical rationale for this observation has been proposed.

Table 1. Dynamics of the content of Ca, P, and the value of the Ca/P index in the enamel of healthy teeth after three days of exposure to an erosive environment

Таблица 1. Динамика содержания Ca, P и значения индекса Ca/P в эмали здоровых зубов после трех суток пребывания в эрозионной среде

Liquid	pH	Tooth №	Calcium content, % mean (range)	Phosphorus content, % mean (range)	Ca/P ratio mean (range)
80% food-grade lactic acid solution	3.68	1	85.51 (84.66–86.18)	14.11 (13.44–14.98)	6.06 (5.65–6.41)
		2	86.16 (84.74–87.33)	13.50 (12.34–14.89)	6.38 (5.69–7.08)
Wine	2.83	1	86.54 (85.67–88.21)	12.36 (10.78–13.24)	7.01 (6.47–8.18)
		2	85.13 (83.70–85.92)	13.86 (13.06–15.19)	6.14 (5.51–6.58)
Dobry Cola	2.70	1	85.84 (84.96–86.73)	13.46 (12.57–14.45)	6.38 (5.88–6.90)
		2	85.58 (85.10–86.14)	13.96 (13.35–14.47)	6.13 (5.88–6.45)
Lemon juice	2.38	1	85.55 (85.08–86.19)	13.69 (13.10–14.16)	6.25 (6.01–6.58)
		2	85.89 (85.74–86.21)	13.28 (12.93–13.42)	6.47 (6.39–6.67)
		3	85.70 (85.03–86.73)	13.48 (12.55–14.10)	6.36 (6.03–6.91)
9% acetic acid solution	2.26	1	86.77 (85.99–87.59)	12.59 (11.74–13.41)	6.89 (6.41–7.46)
		2	85.35 (84.37–85.90)	13.63 (13.00–14.57)	6.26 (5.79–6.61)
Solution of Acidin-Pepsin tablets (15 tablets in 100 mL of water)	1.86	1	84.25 (83.40–85.53)	13.31 (12.13–14.18)	6.33 (5.88–7.05)
		2	86.08 (84.62–88.37)	12.18 (9.36–13.80)	7.07 (6.13–9.44)
14% hydrochloric acid solution	<0	1	73.85 (69.69–82.43)	21.22 (11.96–26.60)	3.98 (2.62–6.89)
		2	67.17 (57.14–75.60)	19.81 (12.86–25.62)	3.39 (2.23–5.58)

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Morphological assessment of furcal portals in human molars using scanning electron microscopy

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Abstract

AIM. To evaluate the prevalence of furcal portals in human molars using scanning electron microscopy (SEM), as well as to analyze their morphological characteristics and potential associations with sex, age, and anatomical localization.

MATERIALS AND METHODS. The study included 230 molars extracted from patients of both sexes for reasons unrelated to the research. Samples were prepared following a standard protocol: fixation, dehydration, sputter-coating with platinum, and visualization using a Vega3 TESCAN SEM. The following parameters were assessed: presence of a furcal portal, its shape, localization, and the patient's sex and age. Statistical analysis included Pearson's χ^2 test, Student's t -test, and one-way analysis of variance (ANOVA); significance level was set at $p < 0.05$.

RESULTS. Furcal portals were identified in 34 cases (14.8%). A statistically significant correlation was found between patient age and presence of a portal ($p = 0.000043$). The association with sex did not reach statistical significance, although a tendency toward higher prevalence in males was observed.

The most common portal shape was round (44.1%), followed by oval (41.2%) and slit-like (14.7%). In most cases, portals were localized in the center of the bifurcation (76.5%), less frequently in the center of the trifurcation (23.5%). No significant associations between shape or localization and age were found ($p = 0.704$ and $p = 0.681$, respectively). By anatomical groups, the highest prevalence of portals was recorded in mandibular first molars (47.1%), followed by mandibular second molars and maxillary first molars.

CONCLUSIONS. Furcal portals represent a stable morphological structure, more frequently observed in older patients. The most common shape is round, and the predominant localization is in the center of the bifurcation. Their prevalence varies across anatomical groups, with mandibular first molars showing the highest frequency. These findings have practical implications for periodontic and endodontic treatment planning, underscoring the importance of considering morphological features of the furcal region.

Keywords: furcal portals, human molars, scanning electron microscopy, tooth anatomy, root morphology, bifurcation, cementum microstructure, endodontics, periodontology, anatomical variations.

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Морфологическая оценка фуркационных порталов в молярах человека методом сканирующей электронной микроскопии

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Резюме

ЦЕЛЬ. Оценить частоту встречаемости фуркационных порталов в молярах человека с использованием сканирующей электронной микроскопии (СЭМ), а также проанализировать их морфологические характеристики и возможные зависимости от пола, возраста и анатомической локализации.

МАТЕРИАЛЫ И МЕТОДЫ. В исследование включены 230 моляров, извлеченных у пациентов обоих полов по не связанным с исследованием показаниям. Образцы были подготовлены по стандартному протоколу: фиксация, обезвоживание, нанесение проводящего слоя платины и визуализация с помощью СЭМ-микроскопа Vega3 TESCAN. Оценивались следующие параметры: наличие фуркационного портала, его форма, локализация, а также пол и возраст пациента. Статистический анализ включал:

χ^2 -критерий Пирсона, t -критерий Стьюдента и однофакторный дисперсионный анализ (ANOVA); уровень значимости – $p < 0,05$.

РЕЗУЛЬТАТЫ. Фуркационные порталы были выявлены в 34 случаях (14,8%). Установлена статистически значимая зависимость между возрастом пациента и наличием портала ($p = 0,000043$); связь с полом не достигла статистической значимости, однако отмечена тенденция к более высокой частоте у мужчин. Наиболее часто встречалась круглая форма портала (44,1%), за ней следовала овальная (41,2%) и щелевидная (14,7%). В большинстве случаев порталы были локализованы в центре бифуркации (76,5%), реже – в центре трифуркации (23,5%). Зависимости формы и локализации от возраста не выявлено ($p = 0,704$ и $p = 0,681$ соответственно). По анатомическим группам: наибольшая частота порталов зафиксирована в первых молярах нижней челюсти (47,1%), затем – во вторых молярах нижней челюсти и первых молярах верхней челюсти.

ВЫВОДЫ. Фуркационные порталы представляют собой стабильную морфологическую структуру, чаще встречающуюся у пациентов старшей возрастной группы. Наиболее распространенная форма – круглая, преобладающая локализация – центр бифуркации. Частота встречаемости варьирует в зависимости от анатомической группы, особенно высока у первых моляров нижней челюсти. Полученные данные имеют прикладное значение в планировании пародонтологического и эндодонтического лечения, подчеркивая необходимость учета морфологических особенностей фуркационной зоны.

Ключевые слова: фуркационные порталы, моляры человека, сканирующая электронная микроскопия, анатомия зубов, морфология корней, бифуркация, микроструктура цемента, эндодонтия, пародонтология, анатомические вариации

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INTRODUCTION

Anatomical features of molars, including the presence of furcal portals, are of considerable interest for both clinical dentistry and scientific research. Furcal portals may serve as pathways for the penetration of infectious agents, thereby influencing the prognosis of endodontic and periodontal treatment [1; 2]. Scanning electron microscopy (SEM) is considered the gold standard for detailed analysis of tooth microstructures. SEM enables the identification of morphological variations, including furcal portals, which often remain undetected when using conventional imaging methods [3; 4].

Previous studies have demonstrated variability in the prevalence of furcal portals in molars; however, reliable correlations with patient sex, molar type, portal shape, and localization remain limited [5; 6]. SEM provided high-contrast imaging, thereby improving diagnostic accuracy. The analysis revealed an almost equal prevalence of portals in males and females, confirming earlier findings that patient sex does not influence the formation of furcal portals [2; 7].

The shape of furcal portals varied, with round forms being the most common (64.1%). Comparable findings regarding the complexity of furcation anatomy have been reported by other authors [3,4]. In most cases (71.8%), portals were localized in the central area of the furcation, which corresponds with evidence indicating favorable vascular and metabolic conditions in the central zone that contribute to portal formation [6; 8].

The highest prevalence of portals was observed in maxillary first molars, followed by mandibular first molars and second molars. However, no statistically significant differences were found, which is consistent with the findings of other authors [9; 10].

AIM

The aim of this study is to determine the prevalence of furcal portals in human molars based on SEM data. Two null hypotheses were formulated: H_{01} – the prevalence of furcal portals is not associated with patient sex; H_{02} – the prevalence of furcal portals is not associated with molar localization or type.

MATERIALS AND METHODS

Study Design and Sample Selection

The study included extracted human molars obtained in compliance with ethical standards and with the patients' written informed consent. The sampling process was designed to ensure balanced representation of male and female specimens. All teeth were carefully cleaned of soft tissues, rinsed, and dried prior to preparation for microscopic examination.

Sample Preparation

To ensure high-quality imaging in the scanning electron microscope (SEM), the specimen surfaces were coated with a thin platinum layer (~30 nm) using magnetron sputtering. This procedure prevented charge accumulation on the non-metallic surfaces and eliminated imaging artifacts.

SEM Analysis

The microstructural examination of the furcation area was performed using a Vega3 TESCAN scanning electron microscope (TESCAN, Czech Republic) equipped with an in-chamber secondary electron detector (SE detector, TESCAN). This detector provides high topographic contrast, which is particularly impor-

tant for evaluating the microanatomical structures of the furcation region. Imaging was carried out at an electron beam energy of 30 keV, allowing for high resolution and detailed surface characterization.

Morphological Analysis

The SEM images were assessed for the presence of furcal portals, their shape (round, oval, irregular), localization (center of the bifurcation, lateral wall of the bifurcation, etc.), and number (single or multiple). The data were systematically recorded in standardized Excel tables.

Statistical Analysis

Data processing was performed using IBM SPSS Statistics 29 (IBM Corp., USA). The statistical analysis included:

- descriptive statistics (means, standard deviations, frequencies, and percentages);
- assessment of distribution normality (Shapiro–Wilk test);
- comparison of quantitative variables between groups using Student's t-test (for normally distributed data) or the Mann–Whitney U test (for non-normally distributed data);
- comparison of categorical data using Pearson's χ^2 test or Fisher's exact test (for small sample sizes);
- statistical significance level was set at $p < 0.05$.

The analysis was carried out across the following groups:

- prevalence of furcal portals by patient sex;
- distribution of portals by type (shape, number);
- localization of portals within the furcation area;
- distribution by tooth type.

RESULTS

A total of 230 molars extracted from patients of both sexes were included in the study. Furcation portals were identified in 34 cases (14.8%). Among these, 22 cases (64.7%) were recorded in men and 12 cases (35.3%) in women. The analysis performed does not allow us to assert a statistically significant association between sex and the presence of a furcation portal, as the distribution of observations rather indicates a predominance in men. However, to obtain a reliable conclusion, application of the χ^2 test on an adjusted sample is required (Table 1).

Table 1. Statistical analysis of furcation portals

Таблица 1. Статистический анализ фуркационных порталов

Comparison of groups	p-value	Comment
Sex and presence of the portal (Chi ²)	1.0072817679726312e-28	The association is significant
Age: portal vs absence (t-test)	4.335666634669491e-05	The difference is significant
Age by portal shape (ANOVA)	0.7042896821511652	The association is not significant
Age by portal localization (ANOVA)	0.6817615512893738	The association is not significant

Distribution of portals by shape

Among the 34 samples with identified furcation portals, the majority exhibited a round shape – 15 cases (44.1%). An oval shape was observed in 14 cases (41.2%), while a slit-like shape was found in 5 cases (14.7%). Thus, the most frequently recorded portal shapes were round and oval, which may be associated with the anatomical characteristics of the furcation area and the mineralization conditions in the bifurcation zone (Fig. 1).

Localization of furcation portals

Out of 34 cases with identified furcation portals:

- in the center of bifurcation – 26 cases (76.5%);
- in the center of trifurcation – 8 cases (23.5%).

Thus, the main localization of furcation portals is in the center of the bifurcation, which may be explained by the anatomical and hemodynamic characteristics of this area, including the conditions of mineralization and vascularization of the periodontium. No statistically significant association between portal localization and patient age was established ($p = 0.68$, ANOVA; see Table 1, Fig. 2).

Frequency of portal occurrence by tooth type

The analysis of the frequency of furcation portal occurrence demonstrated the following distribution:

- mandibular first molar (36) – 9 cases (26.5%);
- maxillary first molar (16) – 4 cases (11.8%);
- second molars (17, 26, 27) – 2 cases each (5.9%).

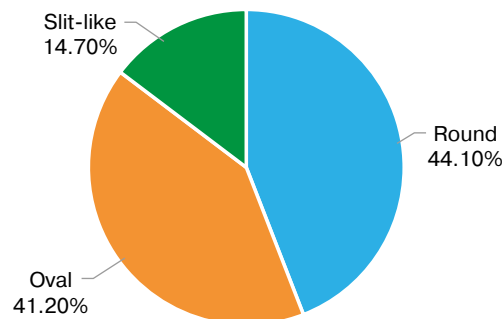


Fig. 1. Distribution of furcation portal shapes, %

Рис. 1. Распределение форм фуркационных порталов, %

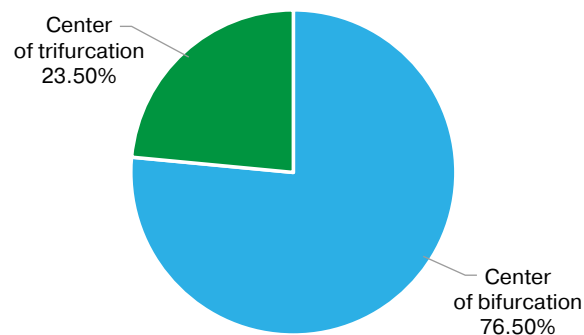


Fig. 2. Localization of furcation portals, %

Рис. 2. Локализация фуркационных порталов, %

Thus, the highest frequency of furcation portal detection was recorded in mandibular first molars, in contrast to the previously assumed predominance in maxillary molars. Visualization is presented in Fig. 3, and numerical data are summarized in Table 2.

Patient age according to portal localization

The analysis of patient age distribution depending on the localization of the furcation portal did not reveal statistically significant differences ($p = 0.68$, ANOVA; see Table 1). Nevertheless, visualization in Fig. 4 demonstrates that patients with portals localized in the center of the trifurcation tend to be in a higher age range compared with those whose portals were located in the center of the bifurcation.

Age of patients with furcation portals

The diagram in Fig. 5 demonstrates that the age of patients with furcation portals is, on average, higher than that of patients without them. The difference was statistically significant ($p = 0.000043$; Student's t -test, see Table 1), which may indicate an age-related nature of the formation or detectability of furcation portals.

Analysis of patient age according to portal shape

The analysis of age differences depending on the shape of the furcation portal did not reveal statistically significant differences ($p = 0.704$, ANOVA), which is consistent with the visual observations in Fig. 6. Nevertheless, as illustrated in Fig. 6, oval and slit-like shapes demonstrated wider age ranges compared with the round shape, which may indicate greater variability in the conditions of their formation.

Table 2. Frequency of portal occurrence according to tooth group

Таблица 2. Частота встречаемости порталов в зависимости от группы зубов

Tooth groups	Number of cases	Proportion of total (%)
Maxillary first molar (16/26)	6	17.6
Maxillary second molar (17/27)	4	11.8
Mandibular first molar (36/46)	16	47.1
Mandibular second molar (37/47)	8	23.5

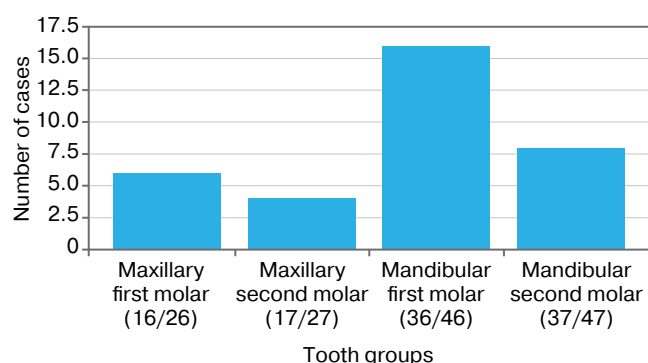


Fig. 3. Frequency of furcation portal detection by tooth groups

Рис. 3. Частота выявления фуркационных порталов по группам зубов

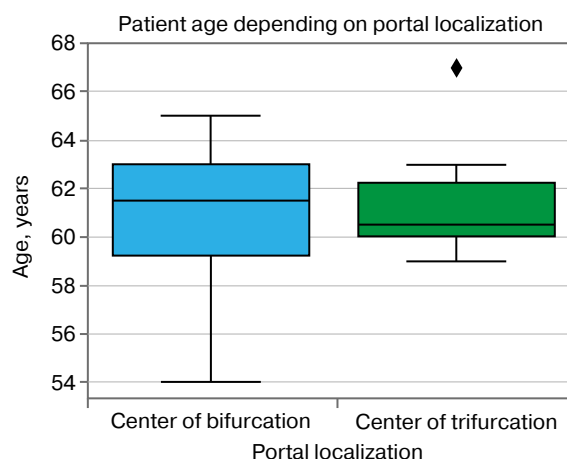


Fig. 4. Patient age distribution according to the localization of the furcation portal

Рис. 4. Возраст пациентов в зависимости от локализации фуркационного портала

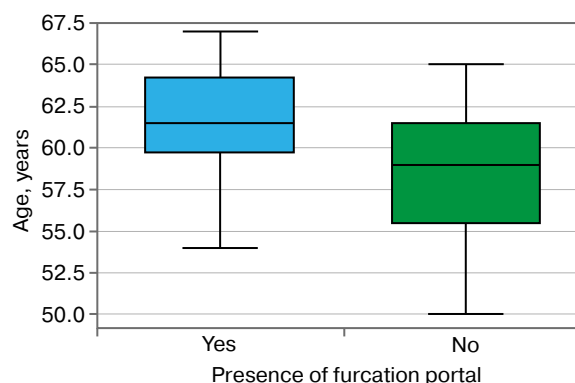


Fig. 5. Age differences between patients with and without furcation portals

Рис. 5. Различия в возрасте пациентов с фуркационными порталами и без

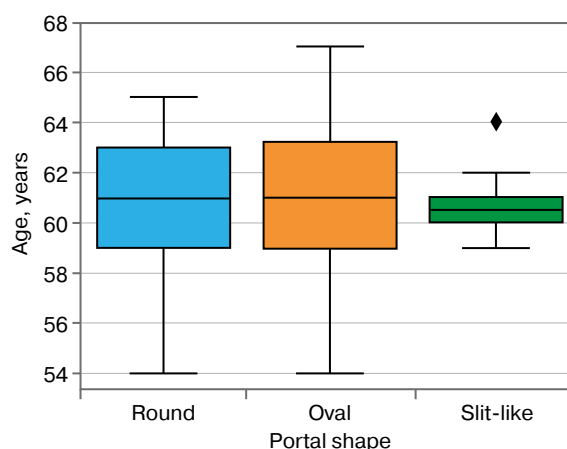


Fig. 6. Distribution of patient age according to the shape of the furcation portal

Рис. 6. Распределение возраста пациентов в зависимости от формы фуркационного портала

DISCUSSION

This study evaluated the prevalence and morphological features of furcation portals in human molars and their potential associations with sex, age, and anatomical localization. The use of scanning electron microscopy (SEM) provided high-resolution visualization of the furcation microstructure, ensuring the reliability of the findings for clinical and research applications [1–4].

Furcation portals were identified in 14.8% of examined molars, with no statistically significant association with sex ($p > 0.05$). Nonetheless, the higher proportion observed in males (64.7%) suggests that sex-related biological factors may influence cementogenesis in the furcation region [2; 11].

A clear age-related association was detected: patients with furcation portals were significantly older compared with those without ($p = 0.000043$, Student's t -test). This finding is consistent with the concept that age-related cementum remodeling and long-term functional or inflammatory stress may contribute to micro-channel formation in furcation areas [4; 6].

Regarding morphology, round and oval portals predominated, whereas slit-like portals were less frequent. No significant relationship between portal shape and age was observed ($p = 0.704$), indicating relative stability of this parameter across age groups [3; 4]. In terms of localization, portals were mainly detected in the center of bifurcations (76.5%), reflecting specific morphogenetic and hemodynamic features of this region [6; 8].

Distribution by tooth type showed the highest frequency in mandibular first molars, followed by mandibular second and maxillary first molars, with maxillary second molars least affected. This finding underlines the clinical relevance of mandibular first molars, which are commonly involved in furcation defects during periodontitis progression [9; 10].

Collectively, these results support the view that furcation portals represent a morphological risk factor that may complicate periodontal and endodontic treatment. Their association with age, sex tendencies, and tooth group emphasizes the importance of considering furcation portals in diagnostic protocols and preventive strategies [5; 12; 13].

CONCLUSION

The present SEM-based study provided a detailed characterization of the morphological features of furcation portals in human molars. A statistically significant age-related association was identified, with furcation portals occurring more frequently in older patients ($p = 0.000043$). No significant association with sex was established, although a higher prevalence in males was noted.

Most portals exhibited a round (44.1%) or oval (41.2%) shape and were predominantly localized in the center of bifurcations (76.5%). Shape and localization did not show statistically significant correlations with age, suggesting that these features may represent stable anatomical traits formed independently of patient age.

Analysis by tooth type demonstrated the highest prevalence in mandibular first molars (47.1%), followed by mandibular second and maxillary first molars. This highlights the particular clinical importance of mandibular first molars in periodontal and endodontic treatment planning.

From a clinical perspective, the presence of furcation portals may facilitate microbial penetration and negatively affect treatment outcomes. Given the observed association with age and the tendencies related to sex and tooth localization, furcation portals should be regarded as an additional morphological risk marker, warranting consideration in individualized diagnostic, preventive, and therapeutic strategies for periodontal and root-related diseases.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

ВКЛАД АВТОРОВ

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Interdisciplinary management of dental avulsion: from emergency care to long-term prognosis

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Abstract

INTRODUCTION. Dental avulsion is a traumatic dental injury defined by the total displacement of a tooth from its alveolar socket. The most effective intervention in these cases is the reimplantation of the avulsed tooth. Additionally, a long-term follow-up plays a crucial role in managing potential complications.

AIM. To present and analyze two clinical cases of dental avulsion in children, emphasizing the importance of immediate reimplantation, appropriate storage media, and strict follow-up protocols in determining long-term prognosis.

MATERIAL AND METHODS. Two pediatric patients with dental avulsion were examined and treated: Case 1: A 12-year-old patient with avulsion of two mature upper central teeth stored in milk, presented 15 minutes post-trauma. Initial reimplantation and rigid contention were performed by a general practitioner. Root canal treatment was initiated 2 weeks later at the Pediatric Dentistry and Prevention Department, Monastir Dental Clinic, followed by interceptive treatment and a 24-month follow-up. Case 2: A 10-year-old patient with avulsion of two immature upper central teeth stored in milk for 24 hours. Reimplantation and rigid contention were carried out after the appearance of inflammatory signs, followed by further management.

RESULTS. In Case 1, the rapid intervention, appropriate storage medium, and timely endodontic treatment contributed to favorable healing and tooth retention over a 24-month follow-up. In Case 2, the delayed reimplantation and prolonged extraoral time negatively affected prognosis, with clinical signs of inflammation influencing the long-term stability of the teeth.

CONCLUSION. These cases highlight that immediate reimplantation, correct choice of storage medium, and strict adherence to follow-up protocols are critical for successful management of avulsed teeth. Prompt intervention and compliant follow-up care significantly influence the long-term prognosis and survival of reimplanted teeth.

Keywords: case report, tooth Avulsion, reimplantation, complication, follow-up

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Междисциплинарное ведение авульсии зубов: от неотложной помощи до долгосрочного прогноза

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Резюме

ВВЕДЕНИЕ. Авульсия зуба – это травматическое повреждение, характеризующееся полным смещением зуба из альвеолы. Наиболее эффективным методом лечения в таких случаях является реплантация авульсированного зуба. Дополнительно важную роль играет длительное наблюдение, позволяющее контролировать и корректировать возможные осложнения.

ЦЕЛЬ. Представить и проанализировать два клинических случая авульсии зубов у детей, подчеркнув значение немедленной реплантации, выбора адекватной среды хранения и строгого соблюдения протоколов наблюдения для определения долгосрочного прогноза.

МАТЕРИАЛЫ И МЕТОДЫ. Были обследованы и пролечены два педиатрических пациента с авульсией зубов: Случай 1: 12-летний пациент с авульсией двух зрелых верхних центральных резцов, хранившихся в молоке; обращение через 15 минут после травмы. Первичная реплантация и жесткая иммобилизация были выполнены врачом общей практики. Через 2 недели в отделении детской стоматологии

логии и профилактики Стоматологической клиники Монастира проведено эндодонтическое лечение, далее – перехватывающее лечение и наблюдение в течение 24 месяцев. Случай 2: 10-летний пациент с авульсией двух незрелых верхних центральных резцов, хранившихся в молоке в течение 24 часов. Реплантация и жесткая иммобилизация были проведены после появления воспалительных признаков, с последующим лечением.

РЕЗУЛЬТАТЫ. В случае 1 быстрая медицинская помощь, использование подходящей среды хранения и своевременное эндодонтическое лечение способствовали благоприятному заживлению и сохранению зубов в течение 24 месяцев наблюдения. В случае 2 отсроченная реплантация и длительное экстраоральное время негативно сказались на прогнозе; клинические признаки воспаления повлияли на долгосрочную стабильность зубов.

ЗАКЛЮЧЕНИЕ. Представленные клинические случаи демонстрируют, что немедленная реплантация, правильный выбор среды хранения и строгая приверженность протоколам наблюдения являются ключевыми факторами успешного ведения пациентов с авульсией зубов. Своевременное вмешательство и последовательное наблюдение оказывают значительное влияние на долгосрочный прогноз и сохранность реплантационных зубов.

Ключевые слова: случай из практики, авульсия зуба, реимплантация, осложнения, динамическое наблюдение

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INTRODUCTION

Avulsions represent a major traumatic injury commonly encountered in general dental practice and entail prompt definitive treatment along with appropriate follow-up care. It represents one of the most serious dental emergencies and accounts for 0.5 to 16% of dental-alveolar traumas (DAT) [1]. The maxillary central incisors are the most frequently affected teeth, accounting for 0.5% to 3% of traumatic injuries in the permanent dentition [2]. Several predisposing factors may increase the risk of avulsion, including increased overjet, inadequate lip coverage, and anterior open bite [3].

The management of avulsed permanent teeth remains challenging, necessitating immediate intervention to optimize outcomes, and is carried out according to the guidelines of the International Association of Dental Traumatology (IADT) published in 2020 [4]. This work suggests 2 contrasting cases broaching the protocol and procedures to abide by during an emergency consultation for the avulsion of a mature and immature permanent tooth and highlighting the clinical outcomes, and complications associated with different scenarios.

In a second phase the short-, medium-, and long-term consequences.

CASE 1

Patient history

A 12-year-old patient was referred to the Pediatric Dentistry and Prevention Department at Monastir Dental Clinic two days after experiencing a domestic accident that led to the avulsion of both maxillary central incisors. The patient's medical history was unremarkable, with no systemic diseases or allergies reported. His tetanus vaccination status was current.

The avulsed teeth had been stored in milk and were reimplanted by a general doctor within 15 minutes of the traumatic incident.

Clinical findings

Clinical examination revealed a wound, swelling, and a firm nodule on the lower lip (Fig. 1, A). Intraoral assessment showed a composite resin splint stabilizing the reimplanted central incisors (11 and 21), which exhibited an enamel-dentin crown fracture without pulp exposure and associated gingival bleeding (Fig. 1, B). Radiographic assessment confirmed proper positioning of the mature reimplanted teeth, as shown on periapical X-rays (Fig. 1, C). Additionally, an occlusal X-ray revealed radiopaque fragments in the lower lip, suggesting the presence of embedded dental fragments (Fig. 1, D).

Therapeutic intervention

It involved surgical removal of the embedded dental fragments (Fig. 2, A), followed by wound disinfection with povidone-iodine, and suturing. (Fig. 2, B).

In a second phase, the rigid composite splint was replaced with a flexible twisted orthodontic wire splint extending from canine to canine for two weeks (Fig. 2, C, D) to promote physiologic mobility and reduce the risk of ankylosis.

The prescribed medication included a 7-day course of amoxicillin, pain management with paracetamol for 5 days, and ibuprofen for 5 days to control inflammation. Oral hygiene care included an antiseptic mouthwash for 10 days, a soft diet for one week.

Two weeks post-trauma, root canal treatment was initiated with calcium hydroxide medication, followed by final obturation gutta-percha (Fig. 3, A, B) and composite restoration. (Fig. 3, E).

In the last phase, due to a thumb-sucking habit causing an anterior open bite, the patient was fitted with a removable anti-thumb-sucking appliance and tongue-strengthening exercises. (Fig. 4).

Follow-up and outcomes

Clinical and radiographic evaluations were conducted at regular intervals (1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months, and 24 months).

At 6 months, a significant reduction in the anterior open bite was observed (Fig. 4, C). By 12 months, no clinical complications were noted, though but periapical radiographs revealed evidence of external root resorption in the apical third of both central incisors (Fig. 3, C). At the 24-month follow-up, the teeth remained stable without mobility or signs of inflammation, and radiographic findings confirmed that the external root resorption had ceased (Fig. 3, D).



Fig. 1. Clinical findings patient: A – exobuccal photograph showing facial trauma with swelling and nodule formation on the lower lip; B – intraoral photograph of reimplanted maxillary permanent central incisors with rigid composite splint in place. Note the enamel-dentin crown fractures without pulp exposure and associated gingival inflammation; C – preoperative periapical X-ray confirming proper positioning of the reimplanted mature central incisors; D – occlusal X-ray revealing multiple radiopaque dental fragments embedded in the lower lip

Рис. 1. Клинические данные пациента: А – внеоральная фотография, демонстрирующая лицевую травму с отеком и формированием узелка на нижней губе; В – интраоральная фотография реплантированных постоянных верхних центральных резцов с установленной жесткой композитной шиной. Отмечаются коронковые переломы эмаль-дентинного типа без вскрытия пульпы, сопровождающиеся воспалением десны; С – предоперационный прицельный рентгеновский снимок, подтверждающий правильное положение реплантированных зрелых центральных резцов; D – окклюзионный снимок, выявляющий множественные рентгеноконтрастные зубные фрагменты, внедренные в мягкие ткани нижней губы

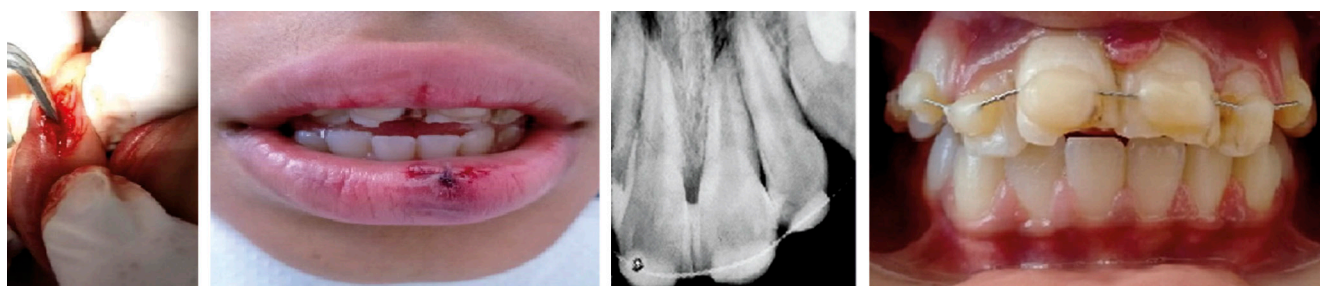


Fig. 2. Initial treatment including surgical management and suturing and splinting modification: A – horizontal incision to remove the tooth fragments; B – resorbable suture to close the wound; C – removal of the initial rigid composite and Application of the flexible twisted orthodontic wire splint (extending from canine to canine); D – postoperative periapical X-ray showing proper repositioning

Рис. 2. Первоначальное лечение, включающее хирургическую обработку, ушивание раны и модификацию шинирования: А – горизонтальный разрез для удаления зубных фрагментов; В – закрытие раны с использованием рассасывающегося шовного материала; С – снятие исходной жесткой композитной шины и наложение эластичной витой ортодонтической проволочной шины, фиксированной от клыка до клыка; D – послеоперационный прицельный рентгеновский снимок, демонстрирующий правильное репозиционирование

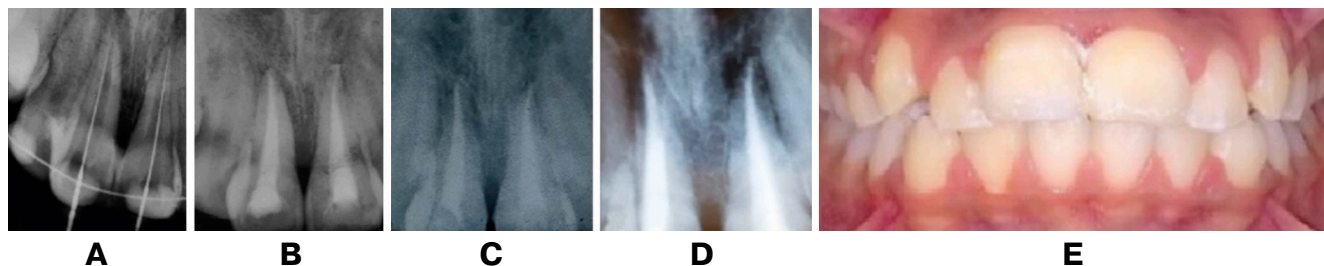


Fig. 3. Conservative treatment illustrating the endodontic treatment preceded by follow-up care at 12 months and 24 months and the coronal restoration: *A* – working length determination confirmed with periapical radiograph; *B* – radiograph confirming complete obturation of the root canals with gutta-percha and sealer; *C* – periapical X-ray at 12 months showing evidence of external root resorption in the apical third of both central incisors; *D* – periapical X-ray at 24 months demonstrating stabilization of the external root resorption process with no further progression. Note the intact periodontal ligament space and absence of periapical pathology; *E* – final composite resin restorations

Рис. 3. Консервативное лечение, включающее эндодонтическое вмешательство, последующее наблюдение через 12 и 24 месяца, а также коронковую реставрацию: *A* – определение рабочей длины, подтвержденное прицельным рентгеновским снимком; *B* – рентгенограмма, подтверждающая полную obturацию корневых каналов гуттаперчей и силером; *C* – прицельный рентгеновский снимок через 12 месяцев, демонстрирующий признаки внешней резорбции корня в апикальной трети обоих центральных резцов; *D* – прицельный рентгеновский снимок через 24 месяца, демонстрирующий стабилизацию процесса внешней резорбции без дальнейшего прогрессирования. Отмечается сохраненное пространство периодонтальной связки и отсутствие периапикальной патологии; *E* – окончательные реставрации композитной смолой

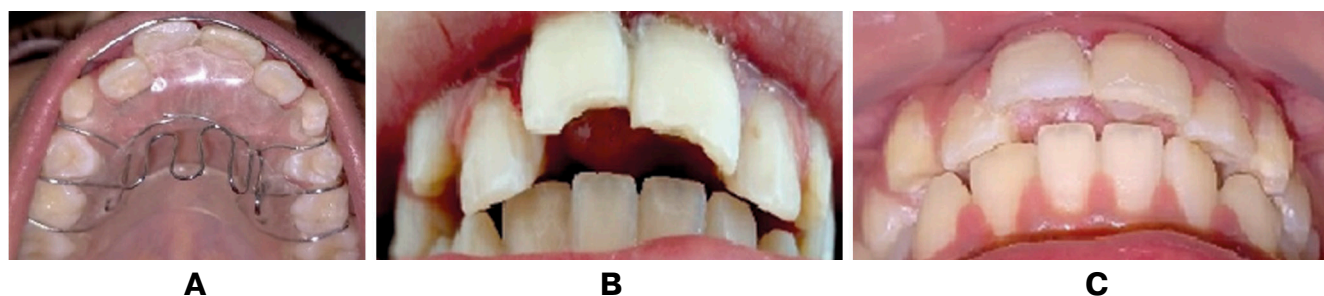


Fig. 4. Interceptive orthodontic treatment: *A* – intra-oral view of the appliance in place, positioned to prevent digit placement during thumb-sucking attempts; *B* – initial presentation of anterior open bite before interceptive treatment; *C* – day 180 with thumb guard showing a reduction in the open bite

Рис. 4. Перехватывающее ортодонтическое лечение: *A* – интраоральный вид установленного аппарата, препятствующего введению пальца в полость рта при попытках сосания; *B* – первичное состояние – передний открытый прикус до начала перехватывающего лечения; *C* – состояние на 180-й день с использованием защитного аппарата для большого пальца отмечается уменьшение открытого прикуса

CASE 2

Patient history

A 10-year-old patient consulted the Pediatric Dentistry and Prevention Department at Monastir Dental Clinic with his mother 24 hours after experiencing a traumatic incident, resulting in the complete avulsion of both maxillary central incisors.

The patient's medical history revealed no systemic diseases, and their health history was not contributory. The tetanus vaccination was up to date.

Clinical findings

Clinical examination revealed swelling of the upper and lower lips, lacerations and wounds on the nose, and scab formation on the lips (Fig. 5, A). Intraoral as-

essment showed empty alveolar sockets for teeth 11 and 21 with blood clot formation. Since the avulsed teeth had been adequately preserved in milk (Fig. 5, B), reimplantation was deemed appropriate.

Therapeutic intervention

The emergency treatment consisted in gentle alveolar curettage (Fig. 6, A), blood clot removal, followed by irrigation with sterile saline solution. The root surfaces were cleaned with sterile gauze soaked in saline to remove necrotic periodontal ligament tissue. Teeth 11 and 21 were then reimplanted into their respective sockets (Fig. 6, B), with clinical and radiographic verification ensuring proper positioning (Fig. 6, C).

Then, a flexible splint was placed from canine to canine for two weeks to stabilize the reimplanted teeth (Fig. 6, D, E).

The patient was prescribed a 7-day course of amoxicillin, paracetamol for 5 days for pain management, and ibuprofen (400 mg) for 5 days as an anti-inflammatory. Oral care guidelines emphasized using an antiseptic mouthwash for 10 days, adhering to a soft diet for one week.

Follow-up and outcomes

After missing the first follow-up, the patient returned after four weeks with signs of acute apical periodontitis,

necessitating immediate endodontic intervention with calcium hydroxide medication.

At the 8-week follow-up, inflammation had improved, but external root resorption was detected, requiring another round of medication (Fig. 7, A). The patient subsequently missed several follow-up appointments. At 4 months the patient returned, with accelerated resorption in tooth 21, while tooth 11 appeared relatively stable. Tooth 11 underwent apexification with a bioceramic plug (Fig. 7, B), followed by permanent root canal filling (Fig. 7, C). Despite the aggressive resorption observed in tooth 21, the stabilization allowed for apexification and final root canal obturation. (Fig. 7, D, E).



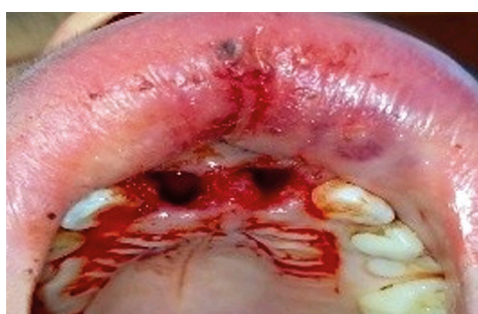
A



B

Fig. 5. Clinical findings patient: A – exobuccal photograph showing facial trauma with swelling of the upper and lower lips, lacerations and wounds on the nose, and scab formation on the lips; B – avulsed 11 and 21 stored in milk for 24 hours prior to reimplantation. Note the absence of visible root fractures

Рис. 5. Клинические данные пациента: А – внеоральная фотография, демонстрирующая лицевую травму с отеком верхней и нижней губ, наличием расщечений и ран в области носа, а также образованием корок на губах; В – авульсированные зубы 11 и 21, хранившиеся в молоке в течение 24 часов перед реплантацией. Отмечается отсутствие видимых переломов корней



A



B



C



D



E

Fig. 6. Emergency treatment involving gentle alveolar curettage, correct teeth reimplantation and x-ray verification then flexible splint application followed by x-ray confirmation: A – alveolar socket preparation showing gentle curettage to remove blood clots, followed by irrigation with sterile saline solution; B – clinical view immediately after reimplantation of teeth 11 and 21 into their respective sockets; C – post-reimplantation periapical radiograph confirming proper positioning; D – placement of flexible wire extending from primary canine to primary canine; E – post-splinting X-ray showing proper repositioning

Рис. 6. Экстренное лечение, включающее щадящий альвеолярный кюретаж, правильную реплантацию зубов и рентгенологическую верификацию, затем установку эластичной шины с последующим рентгенологическим контролем: А – подготовка альвеолярной лунки: щадящий кюретаж для удаления сгустков крови с последующим промыванием стерильным физиологическим раствором; В – клинический вид сразу после реплантации зубов 11 и 21 в их анатомические лунки; С – прицельный рентгеновский снимок после реплантации, подтверждающий правильное положение зубов; D – установка эластичной проволочной шины, фиксированной от временного клыка до временного клыка; E – рентгенограмма после шинирования, демонстрирующая корректное репозиционирование

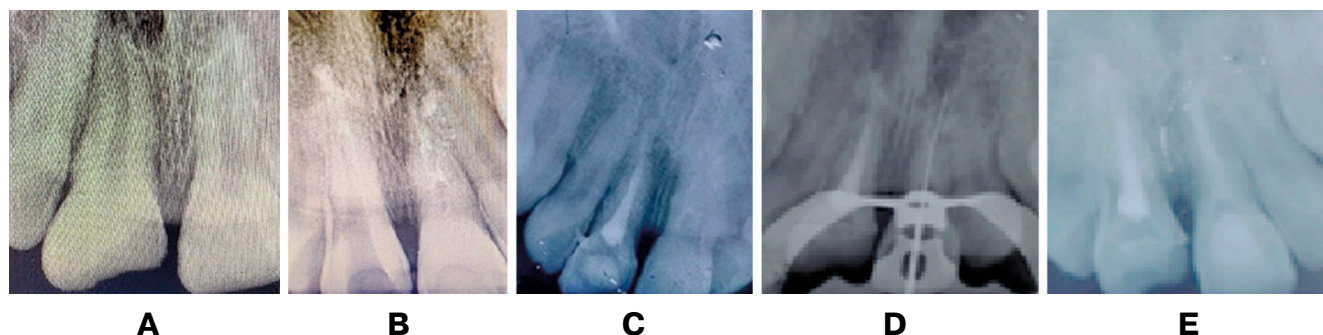


Fig. 7. Follow-up with periapical X-ray showing proof of root resorption and complication management involving root canal treatment with biodentine plug then final obturation on both incisor 11 and 21: *A* – eight-week follow-up radiograph showing early signs of external root resorption; *B* – apexification procedure for tooth 11 showing placement of biodentine apical plug (approximately 4 mm thickness) to establish an artificial barrier at the apex; *C* – final obturation of tooth 11 with gutta-percha and sealer; *D* – working length determination confirmed with periapical radiograph on tooth 21; *E* – apexification procedure for tooth 21 with biodentine apical plug placement and final obturation of tooth 21 with gutta-percha and sealer

Рис. 7. Динамическое наблюдение с прицельной рентгенографией, демонстрирующей признаки резорбции корня и проведение лечебных мероприятий, включающих эндодонтическое лечение с использованием апикальной пробки из Biodentine, а затем окончательную obturацию обоих резцов 11 и 21: *A* – рентгенограмма через 8 недель, показывающая ранние признаки внешней резорбции корня; *B* – процедура апексофикации зуба 11 с установкой апикальной пробки из Biodentine толщиной около 4 мм для формирования искусственного барьера в области верхушки; *C* – окончательная obturация зуба 11 гуттаперчей и силером; *D* – определение рабочей длины зуба 21, подтвержденное прицельной рентгенограммой; *E* – процедура апексофикации зуба 21 с установкой апикальной пробки из Biodentine и последующей окончательной obturацией гуттаперчей и силером

DISCUSSION

Traumatic dental injuries, particularly avulsion of maxillary central incisors, continue to present significant challenges in pediatric dentistry. The present cases underscore the critical role of expeditious intervention, appropriate storage media, and longitudinal follow-up in preserving tooth vitality and function.

Reimplantation and splinting protocols

The treatment approach in these cases was based on well-established principles of dental trauma management. Immediate reimplantation is the gold standard for avulsed teeth. In the first case, reimplantation was performed within 15 minutes of the traumatic incident, which is considered optimal for maintaining PDL cell viability. In the first case, reimplantation was performed within 15 minutes of the traumatic incident, which is considered optimal for maintaining PDL cell viability [5]. In contrast, the second case involved a 24-hour extra-alveolar period, which significantly compromises PDL cell survival and increases the risk of complications such as inflammatory root resorption and ankylosis [6]. The IADT guidelines recommend immediate reimplantation at the site of injury whenever possible, or otherwise, preservation in an appropriate storage medium such as milk, Hank's Balanced Salt Solution (HBSS), or saline, followed by prompt professional intervention [6].

Regarding splinting, both cases initially employed flexible wire-composite splints, which allow physio-

logical mobility of the reimplanted teeth and reduce the risk of ankylosis. Current evidence suggests that a short-term (2 weeks) flexible splint provides superior outcomes compared to rigid splinting, as it promotes functional healing of the periodontal ligament [7]. The first case initially had a rigid composite splint, which was replaced with a flexible wire splint to facilitate optimal healing. This approach aligns with evidence-based protocols advocating physiological functional fixation to mitigate the risk of ankylosis and reduce the likelihood of complications such as root resorption [8]

Endodontic management and external root resorption

Endodontic intervention is essential for reimplanted teeth, particularly when they are mature with closed apices, as observed in the first case. The timing of endodontic treatment is critical, with the IADT recommending initiation within 7–10 days post-reimplantation for mature teeth to prevent inflammatory root resorption [4]. In both cases, calcium hydroxide was used as an intracanal medicament due to its antimicrobial properties and ability to inhibit inflammatory root resorption [9].

The first case demonstrated stabilization of external root resorption by the 24-month follow-up, suggesting effective management through timely endodontic intervention and calcium hydroxide medication. In contrast, the second case exhibited progressive external root resorption, likely exacerbated by delayed follow-

up and the extended extra-alveolar period. This highlights the importance of strict adherence to follow-up protocols and the potential consequences of delayed intervention.

For immature teeth with open apices, as observed in the second case, apexification with biocompatible materials such as biodentine or mineral trioxide aggregate (MTA) is recommended to establish an apical barrier and facilitate obturation [10]. Biodentine was chosen for the second case due to its superior biocompatibility, sealing ability, and ability to promote dentinogenesis [11]. While the resorption process was aggressive, particularly in tooth 21, apexification and final obturation were successfully performed, emphasizing the resilience of immature teeth despite significant challenges.

Complications and Long-Term Prognosis: Complications, such as acute apical periodontitis observed in Case 2, underscore the necessity of timely endodontic intervention. Noncompliance with scheduled follow-ups contributed to progressive external resorption, necessitating apexification with a biodentine plug [11].

In cases of tooth avulsion followed by reimplantation, pulpal necrosis is the most common complication. This occurs in two-thirds of replanted immature teeth and systematically in mature teeth due to rupture of the neurovascular bundle during trauma, leading to pulp degeneration.

Clinical signs include negative pulp sensitivity tests, axial percussion sensitivity, greyish discoloration, and radiographic loss of the lamina dura with periapical radiolucency. Necrosis can manifest as early as three weeks post-trauma, exacerbating periodontal inflammation and worsening prognosis [12]. Endodontic treatment, including intra-canal medication, is typically needed; however, complications such as external root resorption may still occur.

Root resorption is a destructive process that can be external or internal, though trauma-related cases are mostly external. It's triggered by damage to the cementum layer and can be worsened by factors like prolonged extraoral time (over 2 hours), bacterial contamination, improper cleaning, or extended splinting [13].

Three types exist: surface resorption, inflammatory resorption, and replacement resorption (ankylosis). Inflammatory resorption is most common and results from bacterial invasion and pulpal necrosis, leading to progressive root loss. Replacement resorption (ankylosis) involves direct fusion of bone to root due to damage to periodontal ligament cells, ultimately leading to infraocclusion as the tooth becomes integrated into the bone [14].

While guidelines often emphasize immediate replantation, cases with extended dry periods (e.g., 13 hours) have shown limited success with revascularization in immature teeth, suggesting flexibility in certain scenarios.

The anterior open bite noted in Case 1 highlights the influence of parafunctional habits on occlusal development, necessitating interceptive orthodontic interventions.

Anterior open bite is a significant malocclusion that not only affects dental aesthetics and function but also

increases the susceptibility to traumatic dental injuries in children.

The increased overjet and protrusion often seen in these cases leave the upper incisors more exposed and less protected during incidents such as falls or impacts, making them more vulnerable to fractures, chipping, or even avulsion.

Additionally, the risk of dental injury in children with an anterior open bite is about twice as high as in those without this condition [15]. Even more, children with anterior open bite exhibited a 47% higher prevalence of severe dental trauma, and a 46% increase in injuries affecting multiple teeth, compared to their peers without this malocclusion [16].

Impact of Delayed Follow-Up: Patient adherence to follow-up appointments is crucial for successful long-term outcomes in dental trauma management. The second case illustrated the adverse consequences of delayed follow-up, with progressive external root resorption developing due to missed appointments and delayed endodontic treatment.

Regular follow-up evaluations, as recommended by the IADT guidelines, include clinical and radiographic assessments at 1 week, 2 weeks, 4 weeks, 3 months, 6 months, and annually for at least 5 years [4]. These evaluations allow for early detection of complications such as pulp necrosis, inflammatory root resorption, and ankylosis, facilitating timely intervention, such as intracanal medication with calcium hydroxide or mineral trioxide aggregate (MTA), which can halt the resorptive process and improve prognosis [9].

Missed follow-ups also delay adjustments to treatment plans, such as replacing splints or modifying medication regimens, which can directly impact healing outcomes. The first case demonstrated better adherence, leading to controlled resorption and a more favourable long-term prognosis. In contrast, the second case demonstrated the consequences of delayed follow-up, with accelerated external root resorption requiring more aggressive endodontic management.

This reinforces the necessity of patient education and caregiver involvement in ensuring compliance with follow-up care [17].

Prognostic Indicators and Future Considerations: These clinical cases corroborate the significance of appropriate emergency management protocols, adherence to structured follow-up evaluation, and interdisciplinary therapeutic approaches in the comprehensive management of avulsed permanent dentition. The extra-alveolar time, condition of the periodontal ligament cells, stage of root development, and patient age serve as critical prognostic indicators for reimplanted teeth. Future investigative efforts should be directed toward enhancement of regenerative endodontic methodologies and biocompatible materials to optimize long-term clinical and radiographic outcomes in traumatically avulsed permanent teeth [17].

Limitations and Clinical Implications: The primary limitation of this study is its retrospective nature, which precludes standardized assessment and intervention protocols. Additionally, the limited sample

size (two cases) restricts the generalizability of the findings. Nevertheless, these cases provide valuable insights into the management of avulsed teeth and the potential complications associated with different scenarios.

From a clinical perspective, these cases emphasize the importance of immediate reimplantation, appropriate storage media, and strict adherence to follow-up protocols in optimizing outcomes for avulsed teeth. Additionally, they highlight the need for comprehensive patient education regarding the urgency of seeking dental care following avulsion and the importance of compliance with follow-up appointments.

Notably, the first case underscores the crucial role of general medical doctors in dental trauma management. The successful outcome can be partially attributed to the general doctor's prompt intervention with reimplantation, demonstrating that non-dental healthcare providers can significantly impact prognosis when ade-

quately educated about emergency dental procedures. Since general doctors are often first responders to dental trauma, especially in remote areas or during off-hours, their training in emergency dental procedures is essential for better patient outcomes.

CONCLUSION

Dental avulsion cases emphasize the importance of quick and proper intervention for successful reimplantation, especially in children. Following established protocols, such as correct tooth storage and prompt reimplantation, helps prevent complications like infections and pulp necrosis. Post-operative follow-up is crucial for monitoring and managing potential issues. Despite improvements in treatment protocols, complications can still arise, often due to delays or improper handling of the tooth. Ongoing advancements in treatment are necessary to further improve patient outcomes and effectively manage complications.

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AUTHOR'S CONTRIBUTION

All the authors made equal contributions to the publication preparation in terms of the idea and design of the article; data collection; critical revision of the article in terms of significant intellectual content and final approval of the version of the article for publication.

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Non-surgical endodontic management of calcific metamorphosis with periapical lesion using bioactive glass: a case report

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Abstract

INTRODUCTION. Calcified root canals lead to various complications in endodontic treatment, as they complicate the locating of canal orifices, negotiating access, preparing the canals, and factoring in the time required for these procedures. Even with extensive precautions, the most serious problems can occur at any phase of the root canal treatment process. The pulpal chamber or root canal space is most frequently partially or completely obliterated in a tooth that has experienced a traumatic injury. A total or partial loss of pulp space is the most typical radiological representation of such a tooth. Pulp necrosis and radiographic signs of chronic periapical periodontitis are present in 7–27% of teeth with partial canal calcification. Small percentage of teeth (2–3%) may exhibit a complete occlusion of the pulp chamber and root canal system.

AIM. To describe the clinical management of a calcified mandibular lateral incisor with a periapical lesion using a bioactive glass-based sealer.

MATERIALS AND METHODS. A traumatized mandibular lateral incisor with radiographic evidence of canal calcification and a periapical lesion was identified. The root canal was located, negotiated, and prepared using standard endodontic techniques with special consideration for calcified anatomy. A recently developed bioactive glass-based sealer was selected for obturation.

RESULTS. The calcified canal was successfully located and treated. Radiographic follow-up demonstrated proper canal obturation with resolution of the periapical lesion.

CONCLUSIONS. Bioactive glass-based sealers show promising outcomes in the treatment of calcified root canals. Their bioactivity and sealing ability may enhance prognosis in complex endodontic cases involving traumatic canal calcification.

Keywords: calcific metamorphosis, endodontic treatment, calcified canals, bioactive glass

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Эндодонтическое лечение кальцифицирующей метаморфозы с периапикальным поражением с применением биоактивного стекла (клиническое наблюдение)

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Резюме

ВВЕДЕНИЕ. Кальцифицированные корневые каналы приводят к ряду осложнений при эндодонтическом лечении, так как затрудняют определение устьев каналов, прохождение доступа, проведение инструментальной обработки, а также увеличивают временные затраты на выполнение процедур. Даже при соблюдении всех предосторожностей серьезные осложнения могут возникнуть на любом этапе лечения корневых каналов. У зубов, подвергшихся травматическому воздействию, полость пульпы и/или пространство корневого канала чаще всего частично или полностью облитерированы. Радиологическим проявлением данного состояния обычно является полная либо частичная утрата пульпарного пространства. Некроз пульпы и рентгенологические признаки хронического периапикального периодонтита выявляются в 7–27% случаев частичной кальцификации каналов. У небольшой части зубов (2–3%) может наблюдаться полная облитерация пульпарной камеры и системы корневых каналов.

ЦЕЛЬ. Описать клиническое ведение случая кальцифицированного нижнего латерального резца с периапикальным поражением с использованием силера на основе биоактивного стекла.

МАТЕРИАЛЫ И МЕТОДЫ. Был выявлен нижний латеральный резец после травмы с рентгенологическими признаками кальцификации канала и периапикального поражения. Корневой канал был найден, пройден и подготовлен с применением стандартных эндодонтических методик с учетом кальцифицированной анатомии. Для obturation был выбран недавно разработанный силер на основе биоактивного стекла. **РЕЗУЛЬТАТЫ.** Кальцифицированный канал успешно локализован и пролечен. Рентгенологическое наблюдение показало адекватную obturation канала и разрешение периапикального очага.

ВЫВОДЫ. Силеры на основе биоактивного стекла демонстрируют перспективные результаты при лечении кальцифицированных корневых каналов. Их биоактивные свойства и высокая герметичность могут улучшить прогноз в сложных эндодонтических случаях, связанных с травматической кальцификацией каналов.

Ключевые слова: кальцифицирующая метаморфоза, эндодонтическое лечение, кальцифицированные каналы, биоактивное стекло

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INTRODUCTION

Dental trauma often results in complications like calcifications and periapical lesions, leading to tooth discoloration that frequently necessitates root canal therapy [1]. Root canal treatment is generally carried out to diminish or entirely eradicate all micro-organisms and their byproducts from the root canal system. Standard debridement, appropriate disinfection, and full 3D obturation are required to eradicate all microbes and their waste products from the root canal system. However, attaining the intended results becomes very difficult if the canal is small, blocked, or packed with any biological calcific substance or foreign particles. This makes it challenging in location of canal orifice and making entry into the root canal system. Calcific metamorphosis, Pulp Canal Obliteration, Dystrophic Calcification, Diffuse Calcification, and Calcific Degeneration are the names given to this condition.

Dental pulp tissues frequently exhibit characteristics of dystrophic mineralization or calcific metamorphosis. According to American Association of Endodontists [2] Calcific Metamorphosis is defined as “A pulpal response to trauma characterized by rapid deposition of hard tissue within the canal space”.

Patterson and Mitchell suggested that if such teeth are clinically identified, either endodontic treatment or extraction should be performed. It was suggested that the pulp tissue affected by such conditions could act as a source of infection and should therefore be removed or treated [3]. When performing endodontic therapy in calcified canals, operators or physicians face a number of difficulties. Recent developments in endodontic equipment, like as high-resolution magnification, flexible rotary files, and high-quality imaging, make it easier to precisely and quickly negotiate and handle calcified canals while reducing procedural errors.

In order to help doctors better understand this difficult clinical disease, this case report examines the causes, diagnosis, and available treatments for calcified canals.

CASE REPORT

A 24-year-old woman visited the department with discolored lower front teeth and a history of trauma that occurred 10 years earlier. She had been experiencing pain in the same tooth for the past two weeks. No abnormalities were observed during the extraoral examination; however, the intraoral examination revealed discoloration of the mandibular lateral incisor. The tooth was tender on percussion. Clinical and radiographic evaluation revealed canal obliteration in coronal 1/3rd of root and a periapical lesion associated with 42. Thermal test exhibited no response, signifying the need for root canal treatment (Fig. 1).

The case was diagnosed as calcification with a chronic apical abscess secondary to trauma concerning tooth 42, based on historical, clinical, and radiological findings.

A root canal procedure was planned using conventional techniques to address calcified canals and a periapical lesion.

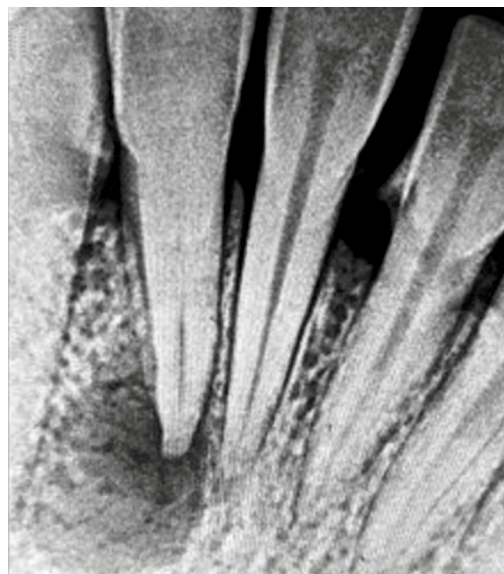


Fig. 1. Pre-operative Radiograph

Рис. 1. Предоперационный рентгеновский снимок

TREATMENT PROCEDURE

With the patient's written consent obtained, the entire treatment procedure was thoroughly explained to them. The potential advantages and disadvantages were also explained to the patient. The preoperative evaluation was carried out through meticulous examination of the preoperative radiograph. Administration of L.A (with 2% Lignocaine hydrochloride, 1:1,00,000 ADR) was done and tooth was isolated using rubber dam (Coltene). Under magnification, preparation of straight-line access cavity was done for the concerned tooth with the high speed no 4 round bur (Mani). Troughing done using ultrasonic tips (Dentsply start-X Tips EMS# 1&3) and Endodontic Explorer DG 16 was used to cautiously inspect the canal orifice and find a catch point in the middle of the tooth where a small blackish discoloration point was observed.

The line of patency of canal negotiated with the help of no 8-k file (Dentsply-Maillefer) (Fig. 2). 17% EDTA (RC Help, Prime Dental) was used to demineralise the restricted hard tissues along the canal. No 10 kfile was used to obtain the glide route (Dentsply). Using an apex locator (Root ZX, J. Morita), the working length was determined and verified radiographically.

Multiple files were used to rule out any missed canal (Fig. 3). Once the working length was established, canals were prepared using a standardized method up to the 25/04 (Herogold) file. Canals were irrigated with 3% NaOCl (Prime dental) for 5–10 minutes during each instrument change, after which normal saline was used. Irrigant was activated ultrasonically (Fig. 4). Glass ionomer cement was used to seal the orifices of root canal after $\text{Ca}(\text{OH})_2$ was combined with equal parts of glycerin and distilled water and left for seven days.

A week later, patient was recalled and intracanal medicament of calcium hydroxide was placed after irrigation and drying of the canals. Similar protocol was repeated for 2 weeks.

The canal was cleaned and dried during the next visit, and a master cone that matched ISO #25 4% size was chosen. Bioactive glass-based root canal sealer (NISHIKA

CANAL SEALER BG) was used for obturation (Fig. 5). Permanent restoration was done using composite restorative material. After treatment, the patient exhibited no subjective or objective signs and symptoms. Patient was advised to follow up at 1, 6, and 24 months (Fig. 6).

DISCUSSION

Trauma to maxillary anterior teeth, resulting in tooth discoloration is a common occurrence that often leads to pulpal necrosis and potential calcification [4]. A comprehensive clinical and radiographic evaluation is necessary for an accurate diagnosis.

Assessing the vitality status of the tooth using thermal tests is crucially important in these cases [5]. The actual pathophysiology of calcification of pulp tissue is not known yet. Several researchers claim that formation of blood clot due to bleeding or hemorrhage from a traumatic injury might play key role for calcification, if pulp remains vital or recovers after the trauma [6]. Furthermore, important variables in the calcification of pulp tissue include the nature, severity, frequency, and amount of trauma. The cellular constituents of pulp substances play a critical role in the calcification of pulp tissue, which may impact blood flow to the pulp. In general, around 4–24% of the teeth radiologically show different degrees of calcification which leads to gradual loss of pulp space and clinically characterized as yellowish discoloration [7]. According to the American Association of Endodontists Case Assessment criteria, these teeth requiring endodontic are considered as high difficulty category [8]. The Dental Operating Microscope offers superb magnification, leading to the accurate identification of orifices in cases of calcification.

In a tooth with calcific metamorphosis the pulp chamber becomes darker than that of the rest root dentine as compared to normal tooth where pulp chamber is located near the Cemento enamel junction at the center [9].

For deep troughing, which is occasionally required to locate canals, specialized tools are recommended, such as the Mueller bur and ultrasonic tips.

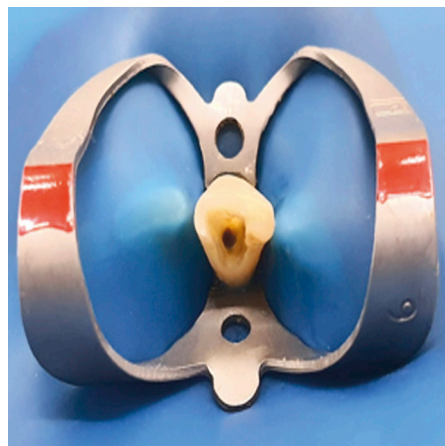


Fig. 2. Location of canal using ultrasonics and magnification

Рис. 2. Определение канала с использованием ультразвука и увеличения

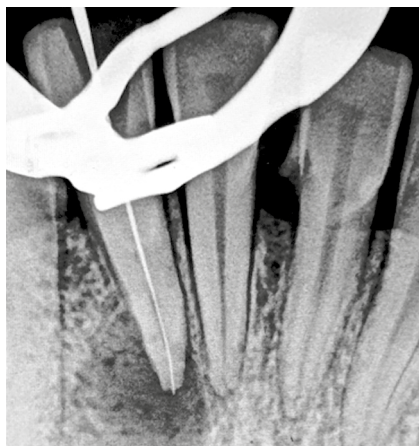


Fig. 3. Canal negotiation and working length determination

Рис. 3. Прохождение канала и определение рабочей длины

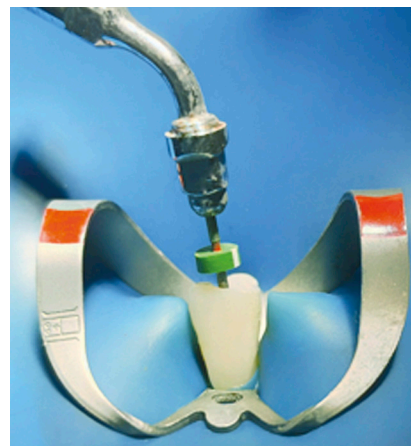


Fig. 4. Ultrasonic activation of irrigant

Рис. 4. Ультразвуковая активация ирриганта

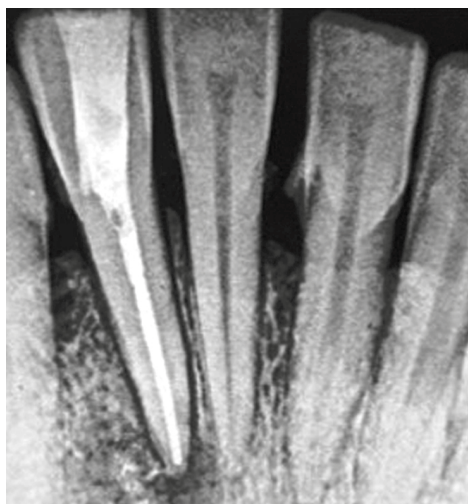
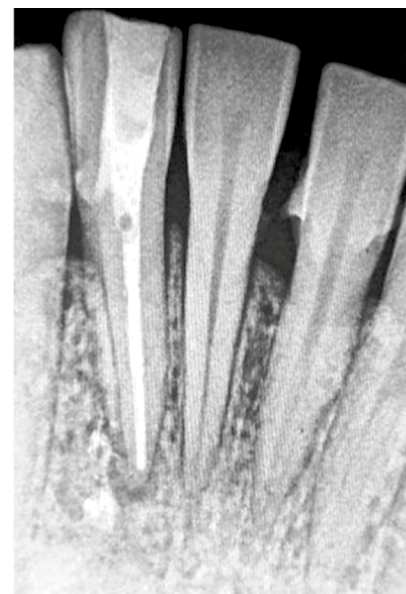


Fig. 5. Post operative, obturation using bioactive glass-based sealer followed by composite restoration

Рис. 5. Послеоперационный снимок: obturation с использованием силера на основе биоактивного стекла с последующей композитной реставрацией



A



B

Fig. 6. Follow up at 6 months (A) and 2-year (B)

Рис. 6. Контрольный осмотр через 6 месяцев (A) и 2 года (B)

CM can be radiographically divided into two groups: Total Obliteration, in which the pulp chamber and canal are hardly or not visible, and Partial Obliteration, in which the pulp chamber is not visible but the canal is narrowed and still visible.

Various types of “pathfinding” instruments have recently been introduced in root canal treatment to achieve this goal. These pathfinders or glide path instruments have unique feature that all the instruments possess a quadrangular cross-section, which has significantly enhanced the rigidity of the files compared to that of finishing files [10]. Furthermore, different chelating chemicals at varying concentrations – for example, 17% EDTA – can be used as lubricants or to facilitate instrumentation after root canal negotiation.

The use of “bioactive” materials in restorative and reconstructive dentistry is a topic of great interest nowadays. Within the field of regenerative dentistry, the term “bioactive” typically denotes a material’s capacity to generate hydroxyapatite crystals on its surface. From a biological standpoint, bioactive substances are regarded as agents that have the potential to positively interact with living cells and tissues [11]. Recently, a more reactive form of calcium-silicate-based bioactive glass, referred to as “bioactive root canal sealers”, and calcium-silicate-based sealers have been deve-

loped [12]. While bioactive glass (BG) has traditionally been employed for regenerating dental hard tissues, it has shown recent potential in treating diverse complex tissues [11]. Specifically, calcium and silicate ions, pivotal in biological processes, exhibit the capability to expedite both osteoinduction and angiogenesis, crucial for supporting periapical healing [13].

Schindler & Gullickson concluded that when acceptable cleaning and shaping cannot be achieved through conventional endodontic treatment, periapical surgery involving root-end resection and retrograde filling must be done [14]. It is widely recognized that endodontic microsurgery offers a direct approach to the root apex and serves as an alternative treatment for calcified canals when conventional root canal treatment fails.

CONCLUSION

The case study demonstrated that effective periapical healing of a big lesion can be achieved non-surgically with careful debridement, disinfection, and three-dimensional obturation of the root canal system. It has been shown that bioactive glass may regenerate hard tissue, which helps periapical lesions recover. For big preapical lesions, a non-surgical method that uses magnification, ultrasonics, and novel bioactive materials can avoid the need for invasive procedures like surgery.

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