UDC 616.314.13-053.2/.5
DOI https://doi.org/10.32782/2077-6594/2025.2/03

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Обґрунтування застосування екзогенних профілактичних композицій для контролю утворення біоплівки на емалі зубів у дітей

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Justification for the use of exogenous prophylactic compositions to control biofilm formation on tooth enamel in children

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Introduction

Despite the rapid development of preventive and reconstructive dentistry over the past decade, the high prevalence of caries, especially in children, is increasing [1]. The most significant factors contributing to this trend include permanent residence in regions with biogeochemical deficiencies of fluoride, iodine, and magnesium, unbalanced nutrition, and the presence of underlying medical conditions [2]. Studies on caries morbidity among children of various ethnic groups in the Transcarpathian region of Ukraine (an area with biogeochemical deficiency of iodine, fluoride, and magnesium) have highlighted the primary role of oral cavity microflora in the occurrence and progression of dental caries [3]. An essential aspect of the demineralization process, commonly associated with caries, is the study of biofilm properties on oral surfaces and methods for its control [4–6]. According to the concept of Minimal Invasive Dentistry (MID), or Microdentistry, new treatment standards have been developed based on an understanding of the processes of demineralization and remineralization in healthy tooth tissues and their correction, which helps to prevent excessive destruction of hard dental tissues in the future [8].

Purpose: to assess the effect of drugs for remineralizing caries prevention on biofilm adhesion to tooth enamel to prevent enamel demineralization in children.

Object, materials, and research methods

To evaluate the effect of remineralizing preparations on preventing biofilm formation (plaque) and subsequent prevention of demineralization of enamel in children, hygienic training for preschoolers and their parents and an exogenous medicinal caries prophylaxis using "Bifluorid 10" and "Remin Pro" remineralizing varnishes (VOCO, Germany) were performed.

To assess the effectiveness of the proposed scheme of integrated caries prevention in study groups, we included 346 children aged 3–8 years, 163 (46.9%) boys and 183 (53.1%) girls. The study included 163 preschoolers and 186 children of junior school age. The study groups comprised children first- and second-grade students from two schools in Uzhhorod and four kindergartens. The control group consisted of children of the same age who did not receive remineralizing therapy. They were schoolchildren from other schools and were not included in preventive programs (total – 175 persons).

To improve oral hygiene, we proposed group training methods (lecture, dialogue, repetition of movements), individual training (an instructor working with 5 children), and theatrical performances for groups with the involvement of favorite cartoon characters. Oral hygiene was assessed four times: the first two assessments were conducted using dental models, and the third and fourth took place individually in the dentist's office with visualization using a plaque-disclosing solution called "Finder Plaque" (Curaprox, Switzerland), which indicates deficiencies of oral hygienic and shows ways of correction.

The next step was a stage of remineralization therapy administered by a dentist. It included professional oral hygiene appropriate to the patient's age, plaque removal using Klint paste (VOCO, Germany), and the local application of Bifluorid 10 and Remin Pro. The use of these agents was justified by the harmful effect of biofilm adhesion on the enamel surface and the need for enamel saturation with fluorides, calcium, and phosphorus.

The use of "Bifluorid 10" and "Remin Pro" followed the manufacturers' instructions with a difference retained in the frequency of applications. Bifluoride-10 should be applied to a dried enamel surface isolated from the saliva followed by air-drying for 60 seconds. Therefore, this preparation was used for calm children with a greater degree of trust in the dentist and among more emotionally stable patients. Remin Pro is produced in a cream form that provides more comfort during application for any patient, regardless of their emotional state. The characteristics of this preparation include a high content of hydroxyapatite and fluorine (1450 ppm sodium fluoride), as well as the availability of flavor options such as melon, strawberry, and mint.

Among the primary mechanisms is the neutralization of the acidic environment in the oral cavity formed by cariogenic microorganisms present in saliva and plaque, as well as dietary sources (e.g., consumption of sweet or carbonated beverages). The cariesostatic effect is also enhanced by xylitol, which contributes to the formation of less soluble forms of hydroxyapatites – fluorapatites – on the surface of damaged enamel (1450 ppm), and supported by a sufficient amount of hydroxyapatites in the preparation.

Remin Pro is usually used to prevent the emergence of demineralization and hypersensitivity centers on teeth. According to the WHO, the critical pH value in the oral cavity is 6.2 [2]; a decrease below this threshold leadcreates a cariogenic environment in the oral cavity and increases the risk of enamel demineralization. Remin Pro, when applied to the tooth surface, helps normalize the pH level. Its use is recommended in clinical situations associated with somatic pathologies, such as hyperacid gastritis and gastroesophageal reflux disease.

The method of application of Remin Pro involves applying a regular layer to the tooth surface for 3 minutes to ensure the remineralization process. Patients are advised to refrain from rinsing the mouth, drinking, or eating for 30 minutes after application [10].

The Fedorov-Volodkina indices were used to evaluate the condition of oral hygiene (1971). The method is determined by staining the vestibular surface of six lower front teeth with a solution of Shyllyer-Pisarev or another solution containing iodine. The criteria for scoring are as follows: 1 – absence of coloring, 2 – colored ¼ of the crown, 3 – colored crown on ½ area, 4 – ¾ of the crown is colored, 5 – colored across the entire surface of the tooth crown. The final calculation uses the formula IH = Σ / 6, where Σ is the sum of all index teeth. Quantitative evaluation is performed by a five-point scale: 1.1-1.5 points – good hygiene level; 1.6-2.0 points – satisfactory hygiene level; 2.1-2.5 points – unsatisfactory hygiene level; 2.6-3.4 points – poor hygiene level; 3.5-5.0 points – appalling hygiene level.

The control of the lack of adhesion of the biofilm to the enamel surface was performed by evaluating the oral hygiene level for all patients after 12 months. Statistical analysis of the data was performed using parametric and nonparametric statistics methods. The conformity of the empirical statistical distribution to a theoretical Gaussian (normal) distribution was tested using the Kolmogorov-Smirnov criterion and Shapiro-Wilks, a reliable indicator.

Results

During the initial assessment of hygiene levels among 346 children (163 boys and 183 girls), none demonstrated good or satisfactory hygiene. Specifically, unsatisfactory hygiene was observed in 61 children, poor hygiene in 177 children, and deplorable hygiene in 86 children.

After implementing the proposed hygienic and preventive measures, a follow-up evaluation was conducted after 12 months. At this time, good hygiene was observed in 145 children, satisfactory hygiene in 176 children, and a small group of 25 children still had unsatisfactory hygiene levels. This may be attributed to insufficient parental involvement in their children's hygiene education, which plays a significant role.

For the year, there was a significant improvement in the hygiene index among children in the leading group, as evidenced by a decrease in the number of children with nasty and appalling hygiene levels. Specifically, the percentage of children with good hygiene levels increased from 0 to 42% ($p<0.001$), while the percentage of children with satisfactory hygiene levels increased from 6 to 51% ($p<0.001$).

Furthermore, among preschoolers, these figures were significantly higher than in primary school children, which may be explained by the psychological differences in perception and response to stressful situations that arise during the beginning of schooling.

The present study aimed to evaluate the effectiveness of preventive dental care in reducing the rate of caries growth among preschool and younger school-aged children. The leading clinical group included children aged 3-5 years (5-8 years) and 6-8 years (8-10 years), while the control group comprised children of the same age groups. The results showed that the growth/reduction of caries intensity rate was significantly lower in the main clinical group than in the control group. Specifically, the rate was P = 4.6-14.2 = -9.6 for preschoolers and P = 4.8-13.3 = -8.5 for younger schoolchildren in the clinical group, while the control group exhibited rates of P = 15.1-13.9 = 1.2 and P = 14.5-13.1 = 1.4, respectively.

After a follow-up period of 24 months, the reduction of caries intensity growth rate was observed in the clinical group, with a decrease of 9.6 and 8.5 among preschoolers and younger schoolchildren, respectively. In contrast, the control group showed an increase in the rate of caries intensity, with a rise of 1.2 and 1.4 among preschoolers and younger schoolchildren, respectively.

Overall, the results suggest that preventive dental care can effectively reduce the growth rate of caries among preschool and younger school-aged children. Therefore, promoting and providing access to qualified preventive dental care should be a priority in public health strategies aimed at improving children's oral health.

The number of children with healthy teeth and no cavities can measure the quality of work of a pediatric dentist in children's groups. To improve access to preventive dental care for children living in remote areas, it is recommended that qualified dental care be provided through a general or family dentistry system.

Discussion

Biofilm is a continuous layer of bacterial cells attached and to a surface by a biopolymer matrix of different origins. In the oral cavity, this structure is formed by the adhesion of microorganisms (such as streptococci, staphylococci, and lactobacteria) to the tooth's pellicle, products of their vital functions, components of saliva, and some non-organic substances. Usually, in natural conditions, 95–99% of all microorganisms exist in the form of biofilms. A biofilm consists of a microcolony of cells surrounded by a biopolymer matrix of polysaccharides. Inside the biofilm are channels for fluid circulation, and products of metabolism are derived. Microorganisms synthesize and isolate a protective matrix through which biofilms attach to living and non-living surfaces [11].

Biofilms are distinguished by interactions between microorganisms collected in a microcolony and surrounded by a protective matrix. Inside the microcolony, there is an excellent environment for microorganisms, and they have a communication system. Microorganisms in biofilms are resistant to antibiotics, a phenomenon caused by the host's antimicrobial properties and protection factors. Bacteria in biofilms may produce other substances than when in culture, and their behavior is subject to the laws of the matrix.

Now, five stages of biofilm development are recognized:

The primary attachment (adhesion, sorption) of microorganisms to the environment's surface is usually liquid.

Final (non-intrusive) attachment (fixation) – microorganisms begin to produce extracellular polymers, providing strong adhesion.

Maturing – cells attached to the surface provide attachment of other cells, the matrix holds the entire colony, nutrients accumulate, and cells proliferate (ripening-I).

Growth (ripening-II). A mature biofilm changes size and shape, and the intracellular matrix protects internal cells from external threats.

Dispersion (release of bacteria) – separate cells from the biofilm may be released and attached to surfaces, creating separate biofilms.

The matrix of the biofilm performs the following functions: structurally (due to the matrix), the colony consists of sub-colonial associations that occur in gram-positive and gram-negative bacteria; protective – the matrix acts as a protective buffer for the internal colony environment, protecting individual cells and the entire colony from adverse effects from the outside; communicative – maintaining quorum-dependent signaling (Quorum Sensing) – the unique ability of some bacteria (possibly and among other microorganisms) to communicate and coordinate their behavior through the secretion of molecular signals [12–15].

Streptococci, specifically Streptococcus mutans (J.K. Clarke, 1960), have been identified as playing an etiological role in the development of carious lesions, and Streptococcus sobrinus, the most acidophilic bacterium that persists in the oral cavity, is also a typical caries-causing species due to its biochemical properties and effects on tooth enamel and dental tissue decay. It can continue to grow and reproduce at pH 5.0 and ferment carbohydrates by glycolysis, leading to acidification of the surrounding environment. The phosphotransferase system of Streptococcus sobrinus is also more active at pH 5.0 than in a neutral medium, which can demineralize enamel, especially in the temporary teeth of children [4].

Currently, Quorum Sensing is recognized as an acid-producing strain of streptococcus. In 2002, Dr. Paul Jensen at the Karl R. Weeze Institute of Genomic Biology in Illinois, USA sequenced the genome of Streptococcus mutans with a quorum-sensing capability. In 2018, the genomes of three strains of Streptococcus sobrinus were also sequenced. The combined action of acid-producing streptococci has been proven to rapidly destroy teeth enamel hydroxyapatites, especially in temporary teeth [16–18].

Prospects for further research

Prospects for further research: inhibition of biofilm adhesion to enamel promotes the course of physiological metabolic processes of remineralization and demineralization in the surface layer of enamel, which is the key to the occurrence of lesions of hard dental tissues. Therefore, further study of the effect on adhesion is a promising direction in preventive dentistry.

Conclusions

Approximately 95–99% of all microorganisms in environmental conditions exist as biofilms. The biofilm on the enamel surface of teeth is rich in acid-producing streptococci, which, due to Quorum Sensing, can lead to the destruction of enamel hydroxyapatites, especially in temporary teeth. The most effective way to prevent biofilm formation is to prevent adhesion to the enamel surface. This can be achieved through preparations that remineralize teeth enamel and provide medical caries prophylaxis. We used *Remin Pro* and *Bifluorid 10* (VOCO, Germany) on clinical situations and indications. These drugs effectively prevent enamel demineralization in permanent and temporary teeth among children, making them an effective caries prevention method.

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Purpose: to assess the effect of drugs for remineralizing caries prevention on biofilm adhesion to tooth enamel to prevent enamel demineralization in children.

Material and methods. The study included 521 individuals (163 preschoolers and 183 younger schoolchildren in the leading group and 175 children in the control group). Patients in the leading group were given exogenous caries prophylaxis with Bifluorid 10 varnish and Remin Pro cream (VOCO, Germany). The state of the oral hygiene index was assessed using the Fedorov-Volodkina index. Descriptive statistical analysis methods and Spearman's correlation coefficient [r] were used to assess the relationship between the studied parameters. The significance level was set at 5%.

Results. The most effective method of preventing biofilm formation on the enamel surface, especially on primary teeth, which are rich in acid-forming streptococci, is to prevent bacteria from adhering to the enamel surface. Such properties are presented among the preparations used for teeth enamel remineralization, as confirmed by the significant improvement of the hygiene index in children in the leading group, particularly for the good [0; 42%; p <0.001] and satisfactory index [6%; 51%; p <0.001].

Conclusions. According to individual clinical findings, Remin Pro and Bifluorid 10 effectively prevent biofilm adhesion on enamel surfaces and stop the demineralization of enamel in permanent and temporary teeth among children. It is also an effective method of primary caries prophylaxis.

Key words: caries, temporary teeth, permanent teeth, biofilm, remineralization, Quorum Sensing, statistical correlations.

Мета наукового дослідження – оцінити вплив препаратів для ремінералізуючої профілактики карієсу на адгезію біоплівки до емалі зубів з метою профілактики демінералізації емалі у дітей.

Матеріали та методи. Для оцінки впливу ремінералізуючих композицій на утворення біоплівки та запобігання демінералізації емалі у дітей було проведено обстеження 521 особи. Для оцінки ефективності запропонованої схеми комплексної профілактики карієсу в досліджувані групи було включено 346 дітей віком 3–8 років, з них 163 (46,9%) хлопчики та 183 (53,1%) дівчинки. Дошкільнят було 163, а дітей молодшого шкільного віку – 186. До навчальних груп увійшли діти перших та других класів двох шкіл Ужгорода та чотирьох дитсадків. Контрольну групу склали діти того ж віку, які не отримували ремінералізуючу терапію. Це були учні інших шкіл і не були охоплені профілактичними програмами (загалом – 175 осіб).

Дітям основної досліджуваної групи проводили екзогенну медикаментозну профілактику карієсу з використанням ремінералізуючого лаку Біфторид 10 та Ремін Про (VOCO, Німеччина). Діти контрольної групи не отримували жодних профілактичних заходів. Загальновідомим ефектом при застосуванні ремінералізуючої профілактики є зміцнення кристалічної решітки емалі завдяки вмонтовуванню хімічних елементів, котрі є в іонній формі в препаратах, в структуру емалі. Стан індексу гігієни ротової порожнини оцінювали за індексом Федорова-Володкіної. Для підвищення рівня гігієни було запропонано групові методи занять (лекція, діалог, повторення рухів), індивідуальні заняття (інструктор з 5 дітьми), театралізовані ігри для груп із залученням героїв улюблених мультфільмів. Гігієну порожнини рота оцінювали чотири рази – перші два рази на моделях, третій і четвертий – індивідуально в кабінеті лікаря з візуалізацією за допомогою фарбувального розчину «Finder Plaque» (Curaprox, Швейцарія), який чітко вказує на недоліки гігієнічного догляду за порожниною рота та показує шляхи корекції. Прості описові методи та коефіцієнт кореляції Спірмена [r] використовувалися для статистичного аналізу, щоб оцінити зв'язок між досліджуваними параметрами. Рівень значущості встановили на рівні 5%.

Результати. Макроскопічно видимий наліт, що утворюється на поверхні емалі зубів між чищеннями, складається з багатьох видів мікроорганізмів, в тому числі великої кількості та різноманіння кислотоутворюючих стрептококів, які можуть спричинити руйнування гідроксиапатиту емалі, особливо в тимчасових зубах, у короткостроковій перспективі завдяки теорії відчуття кворуму. В даний час Quorum Sensing властивий кислотоутворюючим штамам стрептокока. У 2002 році д-р Пол Дженсен з Інституту геномної біології імені Карла Р. Візе в Іллінойсі, США, секвенував геном Streptococcus mutans за допомогою функції визначення кворуму. У 2018 році також секвенували геноми трьох штамів Streptococcus sobrinus. Доведено, що спільна дія кислотоутворюючих стрептококів може швидко руйнувати гідроксиапатити зубної емалі, особливо у тимчасових зубах. Найбільш ефективним методом запобігання утворенню біоплівки є запобігання прилипання бактерій до поверхні емалі. Окрім раціональної індивідуальної гігієни, такими властивостями володіють також препарати, які використовуються для ремінералізації емалі зубів, що підтверджується достовірним покращенням показників гігієни у дітей основної групи, особливо на користь [0; 42%; p<0,001] та задовільний показник [6%; 51%; p <0,001].

Висновки. Приблизно 95–99% всіх мікроорганізмів в умовах навколишнього середовища існують у вигляді біоплівок. Біоплівка, що утворюється на поверхні емалі зубів, багата на кислотоутворювальні стрептококи, які завдяки Quorum Sensing можуть спричиняти руйнування гідроксиапатитів емалі, особливо у тимчасових зубах. Найефективнішим способом запобігання утворенню біоплівки є запобігання її адгезії до поверхні емалі. Цього можна досягти за допомогою препаратів, які ремінералізують емаль зубів і забезпечують лікувальну профілактику карієсу. Використовували Remin Pro та Bifluorid 10 (VOCO, Німеччина) відповідно до індивідуальної клінічної ситуації та показань. Застосування цих препаратів ефективно запобігає демінералізації емалі постійних і тимчасових зубів у дітей, що робить їх ефективним методом профілактики карієсу.

Ключові слова: карієс, тимчасові зуби, постійні зуби, біоплівка, ремінералізація, віддуття кворому мікроорганізмів, статистичні кореляції.

Conflict of interest: absent.
Конфлікт інтересів: відсутній.

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Стаття надійшла до редакції 07.04.2025
Дата першого рішення 23.04.2025
Стаття подана до друку 05.06.2025