

In vitro study of the preventive activity of fluoride varnish by X-ray diffraction

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ABSTRACT

In exogenous prophylaxis, fluoride is applied directly to the tooth surface through various gels, varnishes, foams, and toothpaste. According to the global burden of disease study conducted in 2017, more than 530 million children worldwide suffer from dental caries in their primary dentition. There are few developments in the selection of non-invasive methods in the application of mineralizing varnishes. The researchers investigated experimentally, in-vitro the preventive activity of the fluoride varnish Clinpro™ White Varnish with TCP 3M (CV), using a modern method of X-ray diffraction. The 20 temporary teeth were extracted due to physiological changes. Place of study was UMDC - city of Varna and Institute of Physical Chemistry "Academician Rostislav Kaishev" of the Bulgarian Academy of Sciences, Sofia. Demineralization is carried out with 37% phosphoric acid (i-gel – etching gel) and applied to the smooth temporary enamel surfaces for 30 seconds. The models were then washed and dried with a water and air jet. Remineralization was performed with CV. The formation of small globules of calcium fluoride. The coating is composed mainly of fluorapatite. With the modern method of X-ray diffraction, it was proved that exogenous fluoride prophylaxis and remineralization therapy are effective methods of prevention and treatment of initial caries lesions.

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1. INTRODUCTION

Fluorine prophylaxis is divided into endogenous and exogenous. It is important for endogenous prevention that fluoride enters the body through the digestive system and is subsequently transported to the bones and teeth through the blood. In exogenous prophylaxis, fluoride is applied directly to the tooth surface through various sources (gels, varnishes, foams, and toothpaste). The fluoride in all sources used for fluoride prophylaxis is not in the free state but is bound in chemical compounds such as NaF (sodium fluoride) and other compounds that give it stability. More in-vivo and in-vitro studies are needed to investigate this effect. According to Bonetti and Clarkson [1], many previous publications support fluoride varnish as a caries-inhibiting trace element. From our available literature, six Cochrane systematic reviews, including 200 studies and more than 80,000 participants, found the effectiveness of fluoride varnish applied in clinical settings two to four times a year to "arrest" and stop the development of dental caries and in the two functional dentitions. Success from fluoride varnish applications appears to be available regardless of high caries risk, initial dental status, fluoride exposure, prescription of fluoride toothpaste, and even prior patient prophylaxis. The efficacy of fluoride varnish is recognized in our clinical practice guidelines worldwide, but the application of these algorithms may still present some problems. Clinical use of fluoride varnish in the

US also involves relationships with dentists and community centers and strong collaboration and communication between physicians and nurses and administrators. Disadvantages are lack of time to introduce oral health services in visits for positive patients, difficulty applying fluoride varnish (lack of skills or training), and refusal among fellow practitioners.

Publications from the UK and Scotland also support clinicians in their work to implement this treatment, to motivate pediatric dentists and parents. Key to parties with an interest in this issue is new and advanced research addressing: the cost-effectiveness of when and how fluoride varnish in routine care can be used; in their political programs related for example to inequality as well as in the provision of health care [1]. According to the Global Burden of Disease Study conducted in 2017, more than 530 million children worldwide suffer from dental caries in their temporary dentition [2]. Early childhood caries or diagnosis-affects children over one year of age and preschoolers worldwide and its prevalence is up to 85% for disadvantaged groups studied. For Early childhood caries, the presence of one or more carious, missing and filled primary teeth in children aged five years or less is important [3].

The prevalence of dental caries is very high in Romania and Bulgaria [4] compared to the recommendations of the World Health Organization (WHO) for the age group of children from 6 to 8 years, depending on socio-economic factors and oral health behavior [5]. According to another study, a bilaterally symmetrical ratio of caries lesions was observed in the groups of children from 3 to 14 years. These are the temporary teeth-the canines, the first and second molars, the permanent central and lateral incisors, the first premolars, and the permanent first molars in both the upper and lower jaws. The authors suggest that the presence of a carious lesion on the tooth surface may indicate a high risk of dental caries on the opposite tooth while allowing the dentist to undertake targeted preventive procedures [6].

In 2018, Riskesdas, shows a high prevalence of dental caries in Indonesia, while doing research with students and teenagers. The rate of dental caries in the group of 12-year-old patients was 29.8%, the group of 15-year-olds was 31.1%, and there was a tendency to increase the rate of dental caries with increasing age. The scientists concluded that there is a relationship between diet and brushing habits and the disease of dental caries in children aged 6 to 12 years at the Dental and Oral Health Hall of UPTD Sukalaksana Health Center in Tasikmalaya [7]. One of the studies was a laboratory study and assessed longitudinal surface microhardness changes in non-cavitated carious enamel lesions treated with (SDF) silver diamino-fluoride. Based on their findings, the authors report that fluoride varnish may be more appropriate than silver diamine fluoride SDF for the treatment of early, incipient, non-cavitated, white spot- or carious lesions of the enamel of children's teeth [8]. Various studies on the prevention and non-operative treatment of early caries lesions in childhood have been published in the literature [9]–[11]. Hence, this study aimed to experimentally examine in-vitro the preventive activity of the fluoride varnish Clinpro™ White Varnish with Tri-Calcium phosphate (TCP) 3M (CV), using a modern method of X-ray diffraction.

2. RESEARCH METHOD

In our study, we analyzed all the layers of the tooth enamel samples using the X-rays of the X-ray diffraction method. Based on the results obtained from the chemical analysis of the data, we gave conclusions about the effectiveness of the applied application with the fluorine varnish CV. In this study, the processes of de- and remineralization, which occur after the application of CV varnish in the surface and subsurface layer of the enamel of samples of primary teeth, the earliest changes in the enamel in carious lesions, and their remineralization or retention as a result of the CV varnish were observed. This study evaluated the effectiveness of the mineralizing varnish with tricalcium phosphate fluoride by chemical analysis in experimental conditions with modern methods of X-ray diffraction.

The Object of observation was 20 primary teeth (n=20). They were extracted due to physiological change. Observation units were divided into samples of groups. The first group was with transverse, and the second group was with a longitudinal section of the enamel surface of the primary teeth, as the direction of the enamel prisms is different, in different areas of the crown. From each group, we examined one-control group: the demineralized group, and the remineralized group with horizontal and vertical sections.

The place of study was University Medical Dental Center (UMDC) in the city of Varna and Institute of Physical Chemistry "Academician Rostislav Kaishev" of the Bulgarian Academy of Sciences, city of Sofia. It was conducted in 2015. The study was performed for the internal needs of the "High-tech laboratory for specialized X-ray methods and tomography" of the diffractometer for X-ray diffraction analysis system Empryan production of PANalytical under the following conditions: multi-channel detector (Pixel 3D); X-ray tube (Cu K α 45 kV-40mA); measuring range 20–115° 2 θ ; scanning - step 0.01° and exposure 20 s. To compare the individual sections of the tooth, the study was conducted on the basis of 3 experiments: i) Examination of the inside of the tooth under standard conditions (Gonio scan); ii) Examination of the tooth surface under standard conditions (Gonio scan); and iii) Examination of the applied coating (Grazing incidence - GIXRD).

The surfaces of the samples were dissected for de- and remineralization. The demineralization was performed with 37% phosphoric acid (i - gel - etching gel), applied to the smooth enamel temporary surfaces for 30 seconds. The models were then washed and dried with water and air jet. Remineralization was performed with a CV. Drying of the varnish in vitro was last one hour.

3. RESULTS

The results of the research in the laboratory of X-ray diffraction of the Bulgarian Academy of Sciences (BAS) were presented schematically and through graphic tables and figures. The general schemes for the two types of experiments are presented in the figure. Schematic representation of the X-ray diffraction used for the texture of the coating and determination of the angle used for polycrystalline structures is shown in Figure 1.

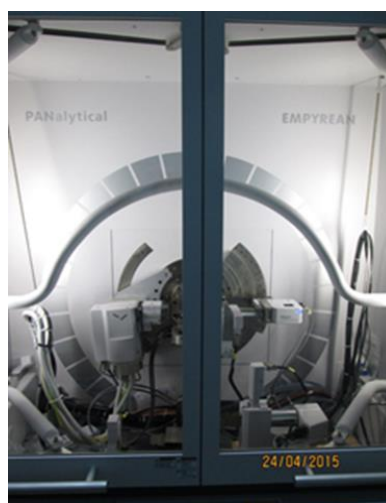


Figure 1. X-ray diffractometer for the experimental study conducted at the Bulgarian Academy of Sciences, Sofia

The inner layer of the samples is composed of: Calcite - CaCO_3 Volume fraction/% - 93.4993, Weight fraction/% - 94 (1), apatite - $\text{Ca}_5(\text{PO}_4)_2.823(\text{CO}_3).22(\text{OH})_{1.562}$. Volume fraction/% - 6.5007, Weight fraction/% - 5.6 (1). The detail of them is presented in Table 1.

Table 1. Chemical composition of the samples' inner layer

Visible	Ref. code	Score	Compound name	Displacement [$^{\circ}2\text{Th.}$]	Scale factor	Chemical formula
*	98-017-1551	42	Apatite	0.000	0.735	$\text{C}0.22 \text{H}1.562 \text{Ca}5 \text{O}13.514 \text{P}2.823$
*	98-002-0179	42	Calcite	0.000	0.604	$\text{C}1 \text{Ca}1 \text{O}3$

The incorporation of fluorine causes the growth of larger and thicker crystals, visually reflected in an increase in the size and thickness of the fluorapatite crystals densely arranged in the enamel prisms. The exposed surface coating of the enamel pattern suggests that these study patterns correspond to higher fluoride retention after washing and moistening the samples. The samples go through a period of remineralization, deposition of calcium and phosphate ions from the varnish application. The surface of the samples was composed of hydroxylapatite (Apatite-(CaOH)) with a general formula $\text{Ca}_{4.938}(\text{PO}_4)_3(\text{OH})_{0.810}$ as described in Table 2.

Table 2. Chemical composition of the samples' surface

Visible	Ref. code	Score	Compound name	Displacement [$^{\circ}2\text{Th.}$]	Scale factor	Chemical formula
*	98-015-4293	28	Apatite-(CaOH)	0.000	0.403	$\text{H}0.81 \text{Ca}4.938 \text{O}12.81 \text{P}3$

This deposition can saturate the microstructures in the enamel, making it more resistant to demineralization. When there is an acid threat, the enamel applied with CV varnish samples is able to be more resistant than the enamel of the primary teeth without the application of three-component fluoride varnish. The applied CV varnish, material was tightly adhered, dry pressed to the enamel of the temporary tooth specimen, as it releases fluoride over a long period of time. Coating analysis showed the presence of Fluorapatite (Fluorapatit, Apatit-(CaF)) with a general formula $\text{Ca}_5[\text{F}(\text{PO}_4)_3]$ as revealed in Table 3.

Table 3. Chemical composition of the coating

Visible	Ref. code	Score	Compound name	Displacement [$^{\circ}$ 2Th.]	Scale factor	Chemical formula
*	98-003-8118	15	Apatite-(CaF)	0.000	0.373	$\text{Ca}_5 \text{F}_1 \text{O}_{12} \text{P}_3$

The locations of the peaks of hydroxylapatite and fluorapatite, and it is clearly seen that the location of the peaks of the two types of apatite is different. In hydroxylapatite, they are shifted to smaller angles, and in fluorapatite to larger ones. Similar effects were observed in the experimental data. After the analysis of the surface of the samples, we found that the X-rays passed in-depth and the main phase that was observed was hydroxylapatite. In measurements of the surface thin layer of enamel, the experiment showed a shift of the peaks to larger angles corresponding to the fluorapatite present. Thus, remineralized it becomes highly resistant reconstituted and insoluble to acid demineralization teeth as shown in Figure 2.

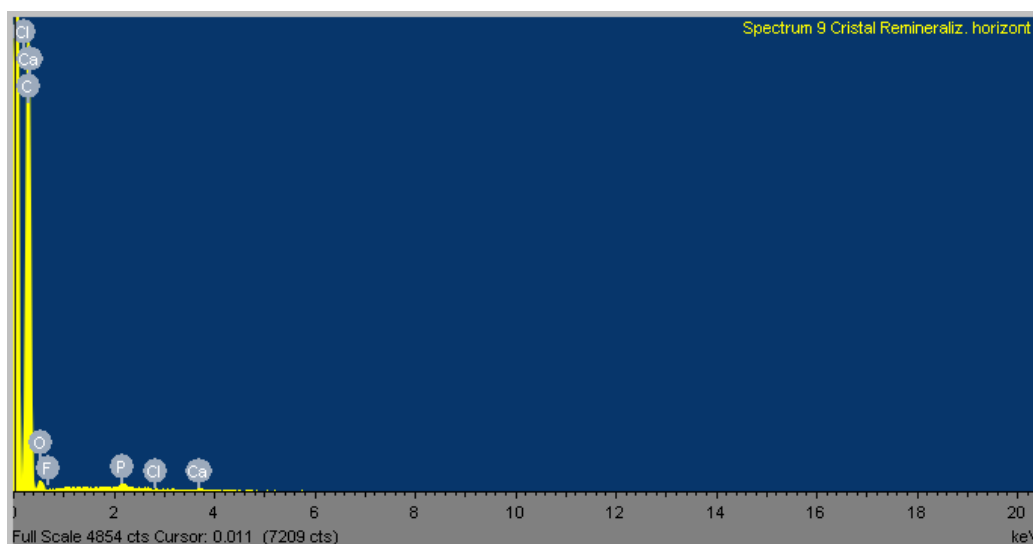


Figure 2. Graph of chemical analysis of the enamel surface layer after remineralization with Clinpro White Varnish with TCF

The compared diffractograms are shown analysis of the thin layer of hydroxylapatite, and shows analysis of the surface of the samples in primary dentition, fluorapatite layer. The emission of fluoride ions continues in the surrounding areas as the smallest and mobile one displaces the larger hydroxyl ion, forming fluorapatite. Thus, the caries lesion stops its development and deepening, both in width and in depth of the subsurface layer of the enamel of primary teeth as shown in Table 4.

Table 4. Chemical composition of the coating

Element	Weight %	Weight % Sigma	Atomic %
C K	90.68	0.80	92.97
O K	8.99	0.80	6.92
F K	0.00	0.00	0.00
P K	0.00	0.00	0.00
Cl K	0.18	0.06	0.06
Ca K	0.15	0.05	0.05
Total	100		

The entry of fluoride into the apatite crystal lattice depends on the contact of the mineral structure with the fluids that supply it. Our in vitro model mimics the conditions of the oral environment for the enamel of primary teeth.

4. DISCUSSION

This study's coating analysis showed the presence of Fluorapatite with a general formula $\text{Ca}_5[\text{F}(\text{PO}_4)_3]$. After the analysis of the surface of the samples (gonio scan), we found that the X-rays passed in-depth and the main phase that was observed was hydroxylapatite. "Minimally invasive treatment" is an extremely convenient and efficient method of treating caries in children. Authors describe that silver diamine fluoride (SDF) is commonly used for arrest or non-operative and preventive treatment of active caries lesions. But the associated black spot, as well as the possibility of soft tissue injury with the unpreferred taste, often limit its frequent use. Recently, nanosilver fluoride (NSF) has emerged as a promising topical fluoride agent with potent cariostatic and antibacterial potential. This new anti-caries agent is an alternative to overcome the shortcomings of SDF for caries "arrest" [9]. To determine the effect of applying fluoride varnish, scientists tested it in-vitro and found the following: application of the varnish before bonding the braces in combination with stimulated saliva in childhood, involving exposure to the action of acids, was beneficial in maintaining a more- high bond between bracket and enamel. Despite the higher adhesion, the adhesive remaining on the enamel did not increase [9]. Other scientists investigated the effect of the combination of chlorhexidine and fluoride on the growth and colonization of cariogenic bacteria (*Streptococcus mutans*) in children with mixed dentition aged 6–14 years. And with this study, like ours, it is again confirmed that the simultaneous use of chlorhexidine and fluoride varnish would be more effective than the individual components over a longer period of time and especially in children at high risk of caries [11]. Scientific research in this field does not stop and is also found in the field of orthodontics. The authors aimed to compare the efficacy of the three-month application of two varnishes to prevent white spot lesions (WSL) during fixed orthodontic treatment, which they performed with multiple braces. Duration of orthodontic treatment was found a higher risk of developing white spot lesions (WSL) [12].

Scientists like Lee *et al.* investigated silver diamine fluoride (SDF) as a potent medication for the control and retention of dental caries and for the remineralization of demineralized enamel lesions. The authors used 38% diamino-fluoride with potassium iodide (KI) for the enamel remineralization process and compared the varnish used with a varnish containing 5% sodium fluoride (NaF) for the treatment of specimens with in vitro dental caries lesions prepared from bovine teeth [13]. The following recommendations were followed in this study: The American Academy of Pediatric Dentistry recommends that the risk of caries be assessed for each child patient [14]–[16] as it is important for modeling the oral environment and reducing the risk factors responsible for the development of this process [17]–[22]. Based on the study, an algorithm was created for the clinical management of initial and early carious lesions in the enamel of temporary teeth. They are applied with success in clinical practice-the preventive and non-operative treatment of initial white non-cavitated and singly cavitated active enamel lesions.

In our country, the knowledge of pediatric dentists about the new treatment concepts is still insufficiently thorough in terms of diagnosis and choice of treatment method. There are few developments for the selection of non-invasive methods in the application of mineralizing varnishes [23]–[25]. There are predominant publications about their prophylactic and therapeutic effect, mainly in permanent dentition. In Bulgaria, there are no publications on the choice of non-invasive methods of treatment for primary dentition. In vitro studies have a limited number of scientists because some scientists and clinicians believe that they are not accurate enough and cannot mimic the natural oral environment and the action of natural saliva. Therefore, it is necessary to verify the obtained results in clinical settings and with the studied patients.

The search for new solutions motivated us to prove the possibility of choosing a means/method for preventive treatment of the initial carious lesions, with the local application of fluorides [26]–[28]. It is necessary: To carry out a timely and early diagnosis to cover the carious lesions in their early phase, applying preventive and non-invasive treatment, which saves time, and pain and protects the child from complicated caries. To conduct preventive programs in which methods and means are used to prevent the main risk factors for the development of caries of temporary teeth. Creating recommendations on this basis would allow the selection of the most appropriate methods and tools for prevention and treatment and would increase their effectiveness [29], [30]

Other scientists such as Ortiz *et al.* [31] consider that untreated dental caries and low socioeconomic status in the period of early adolescence are risk factors for oral diseases among young patients. Major public health concern is oral health, with dental caries being the most common global disease, followed by periodontal disease and orthodontic and maxillofacial deformities [32]–[35]. To prevent and control it requires complexity with an initial assessment of the risk of dental caries, clinical preventive care for the

child patient, training in personal oral prevention, and training and assistance of parents for their strict and daily compliance with their children [36]–[38]. Current knowledge of the etiology of dental caries and the definition of a behavioral disease, as well as the lack of early control with the possibility of development at any age, requires the constant implementation of prevention programs [39], [40].

5. CONCLUSION

Using the X-ray diffraction method, it is proved that the formation of small globules of calcium fluoride and coating on the surface of the samples was observed as a result of the diffusion of fluoride ions from the varnish to the surface and micro spaces of the tooth enamel. Fluorapatite is formed as a secondary reaction from the active fluoride ions that are released. The coating is composed mainly of fluorapatite. The precipitation of ions under clinical conditions in the surface and subsurface layer of the enamel can be obtained in the presence of fluoride ions even when the medium is acidified. The mechanism of action of fluorine will depend on its concentration in the solution. In the depth of the enamel gradually the predominant phase is that of hydroxylapatite. The analysis of the inner enamel layer of the samples of primary teeth shows that it is composed of hydroxylapatite and calcite. With the modern method of X-ray diffraction, it was proved: Exogenous fluoride prophylaxis and remineralization therapy are effective methods of prevention and treatment of initial caries lesions in the primary dentition.

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