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Investigation of the Remineralization Effect Tnrough Scanning Electron Microscopy

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ABSTRACT

Background: Local fluoride varnishes have been widely used as a method of non-operative treatment and for caries preventive interventions for more than three decades.

Purpose: Evaluation of the remineralization effect by means of electron microscopy of mineralization varnish - Clinpro ™ White Varnish with TCP (Tri-Calcium phosphate) (3M).

Materials and Methods: The material used is from 20 temporary intact teeth, extracted due to physiological change with permanent teeth, with a completely preserved structure and anatomy of crowns and fully physiologically resorbed roots. For the purposes of the study a scanning electron microscope JEOL JSM 6390 is used with an attachment for element analysis (EDS INCA of Oxford). Prepared samples are pre-coated with gold (cathode sputtering with apparatus JEOL JFC – 1200) to obtain a better contrast of the SEM image of early carious lesions on the smooth surfaces of the temporary teeth, with predilection for development of caries with a d1 threshold. For this purpose the two processes were monitored occurring continuously on the enamel surface- de- and remineralization. Performed was computer processing of the digital images.

Results: There is presence of certain minerals deposited in the embossed enamel prisms after of remineralization. The chemical analysis established the presence of calcium (Ca^{2+}), around the organic matrix. Demineralised surface has pores present of around 1%, which is visible through the enamel on the surface of the deciduous teeth looking like filled and pores looking like partially covered, filled with newly formed and growing crystals. The crystals, which are hydroxylapatite, fluorapatite or fluorhydroxiapatite gradually connect, growing and forming mineral structure filling the microscopi defects and the pores from the demineralisation in the surface enamel prismless layer.

***Keywords*:** SEM, deciduous teeth, demineralization, remineralization

I. INTRODUCTION

Healthy temporary teeth support the functions of the masticatory apparatus, the correct pronunciation of words, have aesthetic value and are important for the development of permanent teeth. Caries of temporary teeth is the most common cause of disruption of these functions [1]. There have been methods created for non-operative and operative preventive treatment requiring changes to the protocol for treatment of caries in deciduous teeth [2]. Local fluoride varnishes have been widely used as a method of non-operative treatment and for caries preventive interventions for more than three decades [3,4].

Purpose: Evaluation of the remineralization effect by means of electron microscopy of mineralization varnish - Clinpro ™ White Varnish with TCP (Tri-Calcium phosphate) (3M).

Materials and Methods: The material used is from 20 temporary intact teeth, extracted due to physiological change with permanent teeth, with a completely preserved structure and anatomy of crowns and fully physiologically resorbed roots. The teeth are from all groups of deciduous teeth – temporary first and second incisors, temporary canines, temporary first molars and second temporary molars of the upper and lower dental arch.

Preparation of the samples for SEM

For the purposes of the study a scanning electron microscope JEOL JSM 6390 is used with an attachment for element analysis (EDS INCA of Oxford). Prepared samples are pre-coated with gold (cathode sputtering with apparatus JEOL JFC – 1200) to obtain a better contrast of the SEM image of early carious lesions on the smooth surfaces of the temporary teeth, with predilection for development of caries with a d1 threshold. For this purpose the two processes were monitored occurring continuously on the enamel surface - de-

and remineralization. Performed was computer processing of the digital images.

They are first applied throughe preparation of the smooth temporary ena Mel surfaces with a 30 second demineralization with 37% phosphoric acid (i - gel - etching gel). Then the samples are washed and dried with water and airflow. Demineralized surfaces of the temporary teeth go through a remineralization for two weeks with application of varnish - Clinpro ™ White Varnish with TCP (Tri-Calcium phosphate) (3M). Drying of the varnish in in-vitro conditions lasts for one hour.

Preparation of model samples of tooth surfaces for observation.

The sample surface must be clean of dirt, its morphology should not change from the placing the sample in a vacuum. The sample should not accumulate electrostatic charge. The morphology is maintained by the "fixing" of proteins and lipids by means of physical methods (deep-freezing) or chemical methods (polymerization and dehydration).

The teeth are numbered and prepared for cutting of the enamel samples of models at the Medical Technical Laboratory of the University Medical and Dental Center, city of Varna. Each section of the enamel of the surface for the required study has a maximum thickness corresponding to 1 millimeter. Sections are made with a diamond turbine bur (ISO 806 314 001 534 012 for temporary teeth) and a diamond separator (Komet Dental - Germany), (ISO 806 104 401 514 220), (REF 983 104 220) and water-air cooling and a cut is done on the smooth vestibular surfaces. The depth of the cut is equal to the diameter of the bur for temporary teeth. A 1 mm maximum thickness is measured on each sample. Samples obtained for the study are then harvested. They are washed with saline, in which they remain until the direct SEM study in the laboratory of the Institute of Physical Chemistry „Acad. Rostislav Kaishev" at the Bulgarian Academy of Sciences.

Development of the SEM study. The experiment is conducted and captured by six examiners from the Institute of Physical Chemistry "Acad. Rostislav Kaishev" at the Bulgarian Academy of Sciences. Dehydration of temporary teeth was not allowed during and before the studies except for the observation itself, and for capturing images of the enamel surface. They were dried before putting samples onto the holder for electron microscopy. After placing the holder the samples were attached with the help of a duct tape with to be dusted with gold. For this purpose, the holder and the attached samples were placed in a magnetic nebulizer to be covered with a thin layer of gold. The metal layer is deposited to provide conductivity and prevent the accumulation of charge during the monitoring.

After removing the apparatus from the ready patterns wired with gold they were placed in the holder and the scanning microscope chamber. The so prepared samples were I - st group with cross and II nd group with longitudinal section of the enamel surface of the temporary teeth, since the direction of the enamel prisms is different in different areas of the crown. From each group we studied 1 – a control group; 1 – demineralized group and 1 remineralized group with horizontal and vertical sections. All samples from the groups were photographed and documented for subsequent analysis of the results of the SEM observation.

The models were examined and photographed with a zoom from 2000 to 5000x. Most often a thin (several nm) layer of gold is deposited. The aim was to establish the processes of de- and remineralization of the surface aprismatic enamel layer of temporary teeth. At a small zoom of the microscope (x 70) there were selected areas appropriate to be monitored, and images were recorded.

II. RESULTS

Performing computer processing of digital images. The test group showed no changes - homogeneous material of the control sample corresponds to the normal morphology of the surface of the enamel of deciduous teeth (Fig. 1).

Before demineralization, enamel surfaces are smooth. After demineralization, control windows showed scale appearance of the enamel prisms typical for fish scale. Presence of unevennesses, pores and irregular surface morphology of the enamel surface (Fig. 2).

There is presence of certain minerals deposited in the embossed enamel prisms after of remineralization. The chemical analysis established the presence of calcium (Ca^{2+}), around the organic matrix (Table 1, Fig. 4). Demineralised surface has pores present of around 1%, which is visible through the enamel on the surface of the deciduous teeth looking like filled and pores looking like partially covered, filled with newly formed and growing crystals.

The crystals, which are hydroxylapatite, fluorapatite or fluorhydroxiapatite gradually connect, growing and forming mineral structure filling the microscopi defects and the pores from the demineralisation in the surface enamel prismless layer (Fig. 3).

III. DISCUSSION

So remineralized, it becomes highly resistant, restored and is insoluble in the acid demineralization. The emission of fluoride ions

continues in the surrounding areas, and as a smallest and flexible it displaces the bigger hydroxyl ion forming fluorapatite, and thus caries lesion stops its development and deepening both in width and in depth of the subsurface layer of the enamel of deciduous teeth. The presence of fluorine decreases non-apatite impurities by stimulation of the crystal growth. Penetration of fluoride in the crystal lattice of the apatite depends on the contact of the mineral structure with liquids that deliver it, and our in vitro model mimics the conditions of the oral environment for the deciduous teeth enamel [3].

IV. CONCLUSIONS

1. In the tooth enamel monitor an area is observed that is "sealed" with a layer of a density close to that of healthy dental enamel. In cutting of a sample such layer has also emerged on its surface.
2. The application of Clinpro ™ White Varnish with TCP is effective in preventing enamel demineralization as a method for non-invasive treatment of deciduous teeth.
3. Clinpro ™ White Varnish with TCP is effective in reducing demineralization in the subsurface layer and in improving the remineralization of both the surface and subsurface enamel layer.

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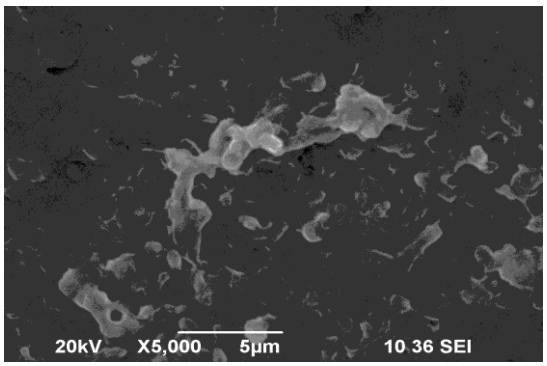

Fig.1. Temporary tooth enamel observed with scanning electron microscope (SEM)

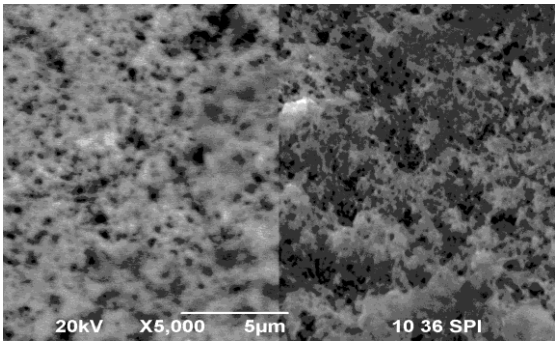

Fig.2. SEM image of the surface layer of the enamel of a temporary tooth after etching. Formation of pores in enamel

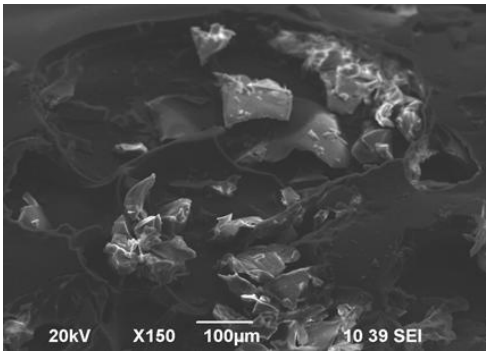

Fig.3. SEM induced microscopic crystal aggregates obtained two weeks after application of Clinpro ™ White Varnish with TCP (Tri-Calcium phosphate) (3M)

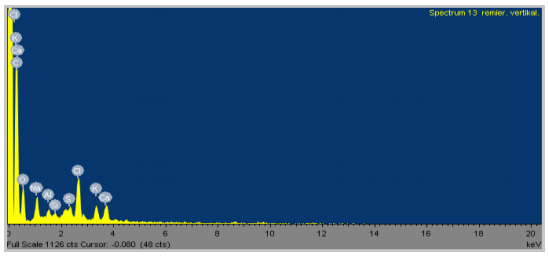

Fig.4. Graph of the chemical analysis of the surface layer of enamel after the varnish remineralization

Table 1. Chemical analysis
Standard :
C - CaCO3, O- SiO2 , Na , Al- Al2O3 , Si- SiO2, S, Cl- KCl, K, Ca

Element	App	Intensity	Weight %	Weight %	Atomic %
	Conc.	Corrn.		Sigma	
C K	4.40	0.7882	67.04	1.78	76.22
O K	0.70	0.3682	22.76	1.44	19.43
Na K	0.16	0.8913	2.15	0.20	1.28
Al K	0.03	0.8778	0.44	0.09	0.22
Si K	0.02	0.9392	0.27	0.08	0.13
S K	0.04	0.9967	0.50	0.10	0.21
Cl K	0.26	0.8348	3.76	0.24	1.45
K K	0.12	1.0327	1.39	0.13	0.49
Ca K	0.13	0.9602	1.68	0.15	0.57
Totals			100.00		