The Effect of Hydroxyapatite from Various Toothpastes on Tooth Enamel

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The process of re-demineralisation is governed by the degree of mineral saturation of oral fluids. Due to positive changes in conditions, remineralisation can become the predominant process leading to the healing of injuries. To improve remineralisation, it is necessary to increase the concentration of calcium and fluoride in oral fluids. For this purpose, fluorides have traditionally been used in varied forms and concurrently, the cariostatic mechanism can be explained by increasing the force of fluorapatites. The aim of this paper was to demonstrate the importance of using toothpastes containing hydroxyapatite on tooth enamel and how to operate at microscopic level by sealing the enamel and enamel prism defects etched by acid. The specimens obtained from extracted teeth were treated with different types of toothpastes containing hydroxyapatite: Biorepair, Sensodyne Repair & Protect and Lacalut White & Repair. We treated the teeth with the aforementioned toothpastes, followed the study under SEM microscope. We compared the control sample with the treated sample, and then the treated samples were compared to each other. All three toothpastes used had the expected result, making a protective layer on the surface of the etched enamel, but in this study, the Sensodyne toothpaste seems to be the most effective.

Keywords: remineralisation, SEM analysis, hydroxyapatite, etched enamel, toothpaste

The health of the oral cavity is not only due to a balanced nutrition but also to oral hygiene. Teeth cleaning should begin before the first milk teeth, each age requiring certain tooth care features to support their structural and functional development [1,2].

The main factor in maintaining oral hygiene is first and foremost the correct dental brushing and the use of proper sanitising means: toothbrush, toothpaste, dental floss, mouthwash. Hydroxyapatite toothpastes have an increased efficiency due to the ability to remineralize tooth enamel [3-5].

Hydroxyapatite is one of the biomaterials representative for the resorbable material category, with a calcium phosphate composition. The use of calcium phosphate biomaterials for dental applications is due to the absence of toxic compounds and their resemblance to the mineral component of the human skeleton. HA is the main crystalline component of the human skeleton that was first synthetically produced around 1970 and used since 1980 as bioactive material [6,7].

HA is considered to be a biomaterial with a chemical structure very similar to that of the human bone, due to the fact that the main form of calcium in this biomaterial is found in the bone tissue, the adherence to it being relieved by this chemical composition resemblance. Hydroxyapatite (HA), is a natural occurring mineral form of calcium apatite with the formula Ca5(PO4)3(OH), but is usually written Ca10(PO4)6(OH)2 to denote that the crystal unit cell comprises two entities[8]. The process of re-demineralisation is governed by the degree of mineral saturation of oral fluids (saliva and plaque). Due to positive changes in conditions, remineralisation can become the predominant process leading to the healing of injuries [9-11].

To improve remineralisation, it is necessary to increase the concentration of calcium and fluoride in oral fluids. For this purpose, fluorides have traditionally been used in varied forms and concurrently, the cariostatic mechanism can be explained by increasing the force of fluorapatites [12,13].

A significant decrease of carious processes in highly industrialised countries can be attributed to the widespread use of fluoride. This preventive effect is mainly due to the formation of calcium fluorides as precipitation limiting demineralisation, while the level of fluoride required for remineralisation is assumed to be higher than that required to prevent the formation of lesions.

Nano-hydroxyapatite is considered one of the most biocompatible and bioactive materials that has gained acceptance in recent years in both general medicine and dental medicine [14]. While previous attempts to clinically use hydroxyapatite failed, the synthesis of hydroxyapatite with zinc carbonate proved to be an important and high affinity process.

Nano-particles are similar in morphology and structure with the tooth enamel crystals. Recently, some studies have shown that nano-hydroxyapatites have the potential to establish dentin lesions. Currently, for the remineralisation of underlying lesions by products containing nano-hydroxyapatite, various formulas have been developed, and the first records suggest remineralisation properties.

Tooth enamel is the tissue with the highest degree of mineralisation in the body, being at the same time the only tissue of ectodermal origin that mineralizes. The hardness of this layer, estimated on the Mohs scale, varies between 5 and 8. Generally, the highest hardness is found in the deep enamel layers on the lateral surfaces of the dental crown, providing the resilience of the enamel surface to mechanical stresses.

From a chemical point of view, enamel consists of 95% mineral substances, 1% organic substances and 4% water. The high percentage of mineral substances in relation to the amount of water and organic substances contained in them is no longer found in any part of the body. Approximately 90% of the mineral substances are calcium phosphates in the form of hydroxyapatite: $Ca_{10}(PO4)_6(OH)_2$, a small part (3%) of fluorapatite: $Ca_{10}(PO4)_6(OH)_2$, and the rest is made up of carbonates, silicates, silicon.

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The mineral ions entering the chemical combinations of these salts can be in a larger amount and are called major constituents - Ca (36.6-39.4%), P (16.1-18%), CO_2 (1.95-3.66%), Na (0.25-0.9%), Mg (0.25-0.58%), Cl (0.19-0.30%) - or in a very small amount (minor constituents) such as: F, Zn, Sb, Ba, W, Cu, Mn, Au, Ag, Cr, Co, Va.

The basic unit of the enamel is the enamel prism. The number of enamel prisms is not the same for each tooth. They are numerous in teeth with bulky crowns (about 12 million in the first upper molars) and less numerous in teeth with small crowns (about 2 million at lower central incisors). Prisms have an oblique trajectory toward the surface of the tooth. The diameter of a prism is on average 4 microns, and its length is variable. Some prisms extend from the surface of the enamel to the dentin, and others disappear along the way, being continued by other prisms.

Due to the accumulation of bacterial plaque on the enamel surface and the subsequent modification of the local *p*H, it is demineralised with the exposure of the enamel prisms, which will demineralize if the low pH is maintained for a long time.

The aim of this paper was to demonstrate the importance of using toothpastes containing hydroxyapatite on tooth enamel and how to operate at microscopic level by sealing the enamel and enamel prism defects etched by acid [15-16]. Also, by using toothpastes with hydroxyapatite, the incipient lesions on the enamel surface stagnate, and over time, even a partial or total recovery of the damaged surface can be achieved [17-19]. All toothpastes are beneficial for maintaining dental hygiene, but toothpastes containing hydroxyapatite, besides their role in hygiene, also have an important role in repairing the damaged enamel surface.

Experimental part

In this study were used a total of 15 teeth extracted for orthodontic or periodontal reasons (IV grade mobility), which were randomly divided into 3 groups of 5 teeth. Each tooth was interdentally divided vertically, thus obtaining 10 specimens per group. Each group of teeth was treated with a type of toothpaste containing hydroxyapatite: Group 1 was treated with Biorepair; Group 2 was treated with Sensodyne Repair & Protect; Group 3 was treated with Lacalut White & Repair.

It was established the following working protocol:

- Tooth preparation. After extraction, the teeth were cleansed from the biological debris with a dental brush under the water jet, dried, and then subjected to UV bactericidal lamps for 30 minutes. After that, they were immersed in physiological serum until the entire batch was ready for treatment. Then the teeth were mounted in a support made of chitinous print material.

- Each tooth was etched with 37% orthophosphoric acid for 1 minute. After this step, the teeth were washed for 20 seconds under running water, then air-dried.

- Tooth Cutting: The teeth were cut using a diamond disk mounted to the elbow part and water cooling, resulting in two identical halves.

- The first half of the tooth was kept as control sample, and the second half of the tooth was brushed with the toothpaste chosen according to the group that the tooth was part of. The teeth were brushed twice a day for two minutes for two weeks. After each brushing, the teeth were rinsed under running water and then kept them in physiological serum. After 2 weeks, the control sample and the treated samples were studied under the SEM microscope.

The toothpastes chosen in this study contain nano-hydroxyapatite. Hydroxyapatite has been used as a remineralising agent in toothpaste for the past three decades, and in Japan since 1993, studies have shown a reduction in the caryogenic index among students who used this toothpaste. It is a crystalline calcium phosphate substance almost identical to natural hydroxyapatite, supplied as nanoparticles, which directly replaces minerals lost from demineralised enamel and completes microscopic cracks on the surface of the enamel [20-24]. In recent years, more oral hygiene products with hydroxyapatite have appeared on the market due to its benefits in remineralising enamel, increasing resistance to bacterial plaque adhesion and reducing dentinal hypersensitivity.

Group 1 was treated with the Biorepair paste. The simple and innovative idea of repairing the teeth with the hydroxyapatite that is physiologically found in the structure of teeth has become possible due to high-end technology of nanoparticles. The achievements belong to the researchers at Coswell Laboratories in collaboration with the researchers from the University of Ambient and Biological Chemistry in Bologna. The innovation patented by these two laboratories, the MICROREPAIR complex, contains microparticles of bioactive hydroxyapatite (similar to the one from the tooth structure) and zinc. These hydroxyapatite microparticles have the ability to integrate into dental enamel and dentin, penetrating into the smallest tooth imperfections, repairing them. Thus, imperfections, microcracks, demineralisations will be corrected. The surface of the dental enamel will be rebuilt, brighter. The reconstructed enamel is protected from the destructive action of bacteria, acids, cavities, etc. On the surface of tooth enamel, the newly deposited microrepair layer constitutes a natural, physiological, strong barrier against external factors (food debris, bacteria, increased acidity, etc.). These unique hydroxyapatite microcrystals have an increased chemical reactivity allowing for the rapid remineralisation action of enamel and dentin, also yielding locally calcium and phosphorus.

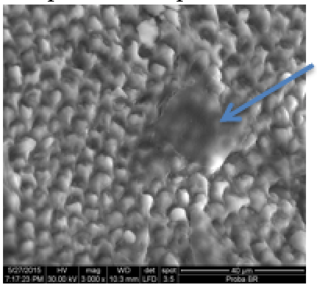
Group 2 was treated with Signal Sensitive Expert. This toothpaste combines a unique mineral formula, its main purpose being to relieve hot and cold pain in 30 seconds. Hydroxyapatite is deposited in exposed dentine channels, preventing the hot and cold thermal stimuli from reaching the nerve. Potassium citrate calms the pain experienced by the nerve endings, reducing dental discomfort. Fluoride protects against cavities and helps repair and protect the enamel.

Group 3 was treated with Lacalut White & Repair. This toothpaste is special for enamel mineralisation. It cares and smooths the teeth and prevents their demineralisation because it contains a special formula of active ingredients for dental care. Phosphate compounds whiten teeth. Sodium chloride in combination with hydroxyapatite (the main component of tooth enamel) improves the mineralisation process from the tooth surface. The tooth becomes smoother and more resistant.

Results and discussions

We treated the teeth with the aforementioned toothpastes, followed the study under SEM microscope. The SEM images are very useful to analyse the different samples [25-27]. We compared the control sample with the treated sample, and then the treated samples were compared to each other. Following the acid etching, the enamel surface was demineralised, simulating the demineralisation that can occur in the oral cavity due to poor hygiene.

Sample 1: Biorepair



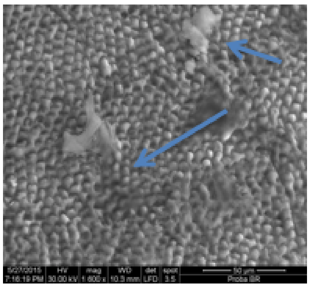


Fig. 1 and fig. 2. SEM images of the samples treated with Biorepair toothpaste taken at different magnitudes

In the SEM images taken at different magnitudes, one can notice how the Biorepair toothpaste was deposited on the surface of the etched enamel forming a protective layer. In areas with higher enamel defects, the deposition of hydroxyapatite microcrystals is noticeable. The toothpaste covers large cracks of 10-50 microns. When the surface is eroded so as to open the dentinal canals, a thin layer of Biorepair is deposited. The layer that forms is a thin and dense one that closes the dental canaliculi.

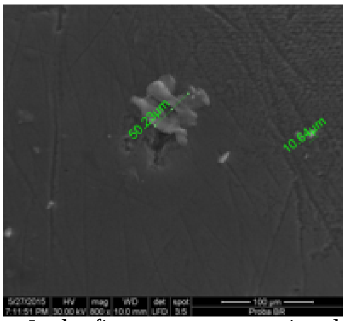


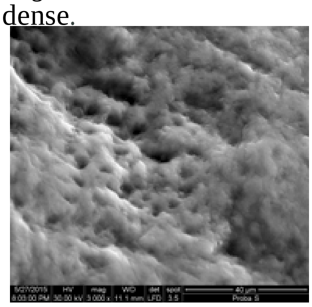
Fig.3. The size of the hidroxyapatite microcristals

In the figure one can notice the size of the hydroxyapatite microcrystals that adhered on the enamel surface. The particles in the toothpaste have the ability to penetrate even the smallest defects of the enamel resulting in a rebuilt and bright surface.

Sample 2: Sensodyne Repair & Protect

Fig. 4 and fig. 5. Hydroxyapatite microcrystals on etched enamel prisms

When using the Sensodyne Repair and Protect toothpaste, one can notice that hydroxyapatite microcrystals have been deposited on the etched enamel prisms and the enamel surface. The toothpaste covers both large and small cracks, the covering layer being thick and dense.



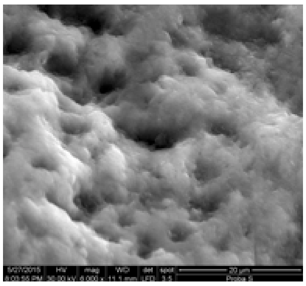
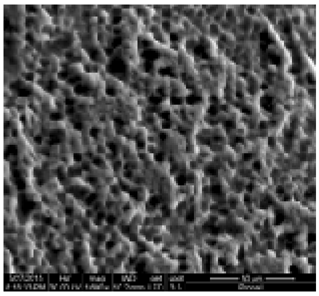
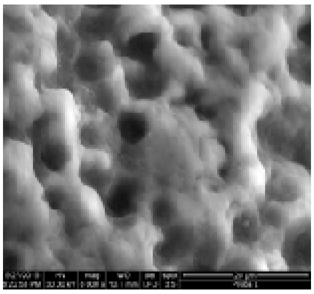


Fig. 6 and fig.7. Hydroxyapatite adhered to the surface of the exposed prisms

The enamel surface treated with acid is restored. There is a large amount of reparative substance that adhered to the surface of the exposed prisms.

Sample 3: Lacalut White & Repair





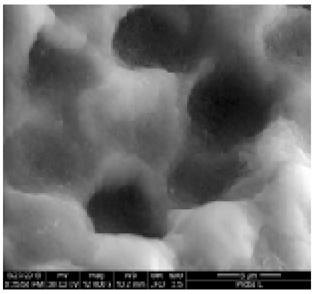


Fig.8., Fig.9. and Fig.10. Deposition of the toothpaste on the enamel surface and the tendency to form a thin protective layer

When using the Lacalut toothpaste, one can also notice the deposition of the toothpaste on the enamel surface and the tendency to form a thin protective layer. At a higher magnitude, one can notice the clear layout and shape of the exposed enamel prisms, but also the fact that the repairing of the etched surface is not as effective as the other toothpastes used. Sodium fluoride in combination with hydroxyapatite - the main component of tooth enamel improves the mineralisation process from the tooth surface. The tooth becomes smoother and more resistant, the small flaws in the enamel are repaired.

Conclusions

The use of toothpastes containing hydroxyapatite is effective in treating demineralised enamel surfaces and repairing small imperfections on the enamel surface [28].

All three toothpastes used had the expected result, making a protective layer on the surface of the etched enamel.

In the case of the Biorepair toothpaste, there were obseved deposits of hydroxyapatite microcrystals at the level of enamel defects.

The Sensodyne toothpaste was the most effective, at the studied samples It was noticed the deposition of a denser and thicker layer on the enamel surface, a layer that protects and recovers the enamel defects.

When using the Lacalut toothpaste, the deposited protective layer is thinner and one may notice the enamel prisms which are not completely covered.

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