TEM study of the effect of some regular beverages on the human dental enamel: an in vitro study

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Nineteen enamel dental specimens were prepared using nineteen healthy teeth. The specimens randomly divided in four groups: group B (six specimens) immersed in Burn Energy Drinks, group C (six specimens) immersed in Coca-Cola, group G (six specimens) immersed in Gatorade and control group (1 specimen). For specimens belonging to group B,C and D, the immersion times were: 5,15,30,60,120 and 180 minutes, respectively. Specimens were investigated using Transmission Electron Microscopy (TEM) techniques. To obtain suitable powder, the teeth were grind by means of diamond drill at 5000-10000 rpm. The powder was milled in an agate mortar and then dispersed in alcohol and put on the formvar covered Cu grid. To obtain morphological images of the specimens, conventional BFTEM investigations were performed in areas with maximum information. The same areas were used to obtain diffraction pattern (SAED), and the high-resolution (HRTEM) images. The crystallite dimension was estimated using Debye Scherrer formulas. It is found that during immersion, crystallite size changes compared with the specimen of control. We assume that this is due to the passage of hydroxyapatite in carbonate-hydroxyapatite in the presence of carbon dioxide released from drinks used. HRTEM images and SAED pattern confirms the occurrence of carbonate-hydroxyapatite crystallites in specimens immersed.

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1. Introduction

Tooth enamel is the outer surface of the tooth whose chemical composition is similar to hydroxyapatite (HAP). Due to microstructural organization [1] and excellent mechanical properties [1], [2], [3], tooth enamel can perform physiological function of the soft tissue in pulp protector and chewing.

However, tooth enamel erosion may suffer due to loss of tooth substance by chemical processes caused by a wide variety of internal and external factors [4]. External factors related to the consumption of acid foods and soft drinks, have lately become very important in the etiology of dental erosion [5], [6], [7], [8]. Thus, half of people aged 4-18 years shows signs of dental erosion and more than 46% of children aged 4-6 years shows signs of erosion of primary teeth [9], [10].

There are currently various dental erosion assessment methods such as profilometry, atomic force microscopy, scanning electron microscopy, micro-radiography, iodide permeability, micro-hardness test, transmission electron microscopy [11], [12], to measure the loss of hard tooth structure due to corrosion processes.

The purpose of this study is to evaluate the effect of regular beverages, Burn Energy Drink, Coca-Cola and Gatorade on dental enamel using TEM techniques.

2. Materials and methods

19 monoradicular healthy teeth (maxillary central incisors and canines) orthodontic and periodontal reasons extracted were collected from the Faculty of Dentistry, Ovidius University of Constanta and in private dental offices. Collection protocol was approved Teeth Ethics Committee of the Ovidius University of Constanta. Extracted teeth showed no caries, noncarioase, or dystrophic lesions. They were gently clean all debris and waste by root sealing ultrasonic bath and then were stored in the chloramine T solution (Ficher Chemical, Fair Lawn NJ, USA) consisting of 12% active chlorine diluted in distilled water at a room temperature for seven days. This solution is used for proper infection control until the start of the study.

The teeth were prepared using abrasive paper (sandpaper Wirt Buehler Dusseldorf Germany) with grit between 600 and 2000 using low speed contra piece under continuous irrigate with water, then clean with non-florid diamond paste Diamand Glass (TDV Dental Ltda, Brasil) with a circular brush with nylon wires mounted on the handpiece (Sirona Germany) and then paste into a rubber cup for 20 seconds and rinse with distilled water alternately for 30 seconds until the surface skin gets a vitreous (glass). Final glass was obtain with disc of filt (Polifix) (TDV Dental Ltda, Brasil), continuously wet (water cooling) to avoid overheating which cause changes in structure. Then the teeth were examined microscopically eliminating the teeth with cracks, fractures or other abnormalities that may interfere with the outcome. Prepared teeth were kept in distilled water, changed every 7 days until the start of the study, to avoid drying (dehydration). Before the study, teeth were examined to assess the comparative morphology before and after immersion. The time of the study, teeth

(samples) were prepared as follows: half of the facial coronal and root was once the nail polish silicon (CHRIS SDRUZENI, CZECH Republic)

2.1 Preparing of the samples (specimens). Preparation of soda

Were chosen some carbonated drinks (brand) which are popular among teenagers and young people in Romania, namely Coca-Cola (produced by Coca-Cola HBC Romania SC SRL, authorizing The Coca-Coal Company), Burn Energy Drink (EU produced and distributed by Coca-Cola HBC Romania SRL SC), Gatorade sports drinks (Pepsico Beverages Italia SRL Milan, Italy, distributed in Romania by Quadrant Amroq BEVERAGES SRL). Used beverages characteristics are shown in Tab. 1

Table 1. Features used beverage

Group	Beverage	Manufacturer	Composition	pH
C	Coca-Cola	S.C. Coca-Cola HBC Romania SRL	Water, sugar, carbon dioxide, caramel coloring E150d, phosphoric acid acidifier, natural flavors, caffeine	3.73
G	Gatorade	Pepsico Beverages Italia SRL, Milan, Italy, distributed in Romania by Quadrant Amroq BEVERAGES SRL	Water, sugar, glucose and fructose syrup, acidifier citric acid, sodium chloride, acidity regulators, sodium citrate, potassium phosphate, magnesium carbonate, antioxidant, ascorbic acid, natural raspberry, flavor and other natural flavors stabilizers, gum Arabic, glycerol esters of wood resins	4.06
B	Burn	Burn Energy Drinks (EU produced and distributed by Coca-Cola HBC Romania SRL S.C.)	Water, sugar, carbon dioxide, acidifier citric acid, taurine (0.4%), acidity regulators, sodium citrate, preservatives potassium sorbate and sodium benzoate, E150d caramel coloring, flavoring, caffeine (0.03%), inositol (0.015), vitamins niacin, pantothenic acid, B6 and B12, guarana extract, antioxidant ascorbic acid, flavors	3.26

Criterion of choice of soda was their popularity in Romania, the high consumption by young people, to have sour taste and a pH of less than 4.5 so producing dental erosion.

It was determined the pH of soda using a model made in Mauritius Hanna instruments pH meter calibrated according to the manufacturers instruction using standard calibration solution between 4 and 7. The pH was measured immediately after opening the box, and end the cycle of immersion. For each cycle of immersion was used another box.

The teeth were randomly divided into groups for each solution, and a control group. Were selected three soft drinks, thus samples were divided in group Coca-Cola, Burn, Gatorade and control group in distilled water. In total there were 18 plastic containers with lids to prevent evaporation of the solutions.

Teeth prepared previously received a number, i.e. 1C, 2C, 3C, 4C, 5C, 6C Coca-Cola group, and 1G, 2G, 3G, 4G, 5G, 6G Gatorade group, and 1B, 2B, 3B, 4B, 5B, 6B Burn energy Drink group.

Small holes were drilled in the apical third of each root, through which passed a thread to be suspend in said carbonated beverages.

Immersion of the sample was done in small opaque plastic container with lid that has been filled with 50ml of beverage. All samples belonging to the same group were immersed together. The teeth were immersed in containers with lids for 5, 15, 30, 60, 120, 180 minutes respectively in a continuous gentle vibration using a vibrating table (VIBRA 20 Modelo V20-P-CIA Haedo, Buenos Aires, Argentina) at room temperature. After this immersion, teeth were washed with running water for 10 seconds and kept in distilled water. pH was then measured at the end of opening the box and immersion cycle. The control group was stored in distilled water only.

2.2 Preparing for microscopy. Materials and method

Remove from the facial coronal and root, the middle third, varish your nail with acetone, and allowed a small window of about 4 mm square. It compares the affected area with the unaffected.

Samples were investigated by TEM techniques using a CM120ST microscope. To obtain suitable powder, the teeth were grind by means of diamond drill at 5000-10000 rpm. The powder was further milled in an agate mortar. The powder was dispersed in alcohol and put on the formvar covered Cu grid.

To obtain morphological future of the samples, conventional BFTEM investigation was performed. The images were taken in areas with maximum information about morphology, especially the areas with varied content in terms of shapes and sizes of particles. The same area was used to obtain diffraction pattern and the high-resolution images.

Image acquisition was performed in iTEM platform connected with a MegaVIEW III CCD camera. The structural characterization was performed on the information extracted from diffraction profile, construct using ELD module from CRISP software.

3. Experimental data and discussions

All samples exhibit a powder like future (Fig. 1). The shape of crystalline powder is rods. Also, there is some composed particles with large dimensions (hundred nanometers) mono and polycrystalline.

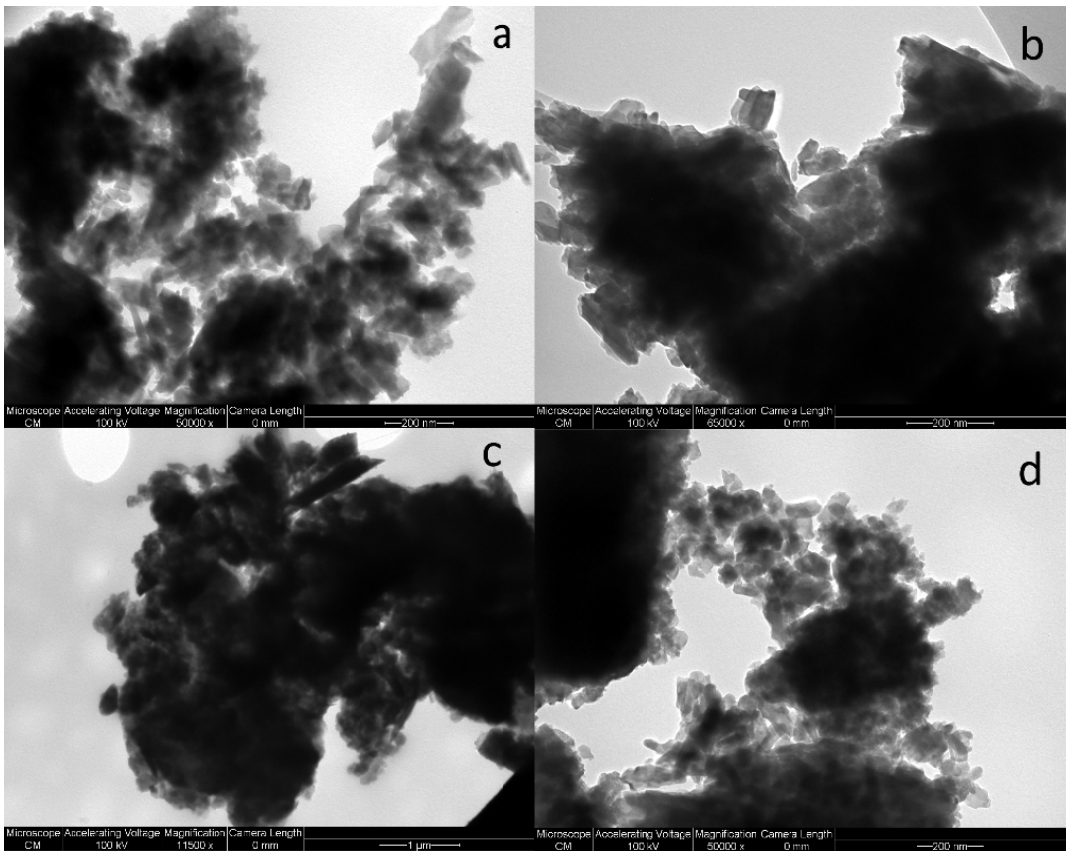

Fig. 1. Morphological images for control sample (a) and samples 4C, 4G,, 4B for 60 minutes immersion time (b), (c), (d) respectively.

The crystallite dimension was estimated using Debye-Scherrer formulas [13] applied to FWHM determined from SAED profile (Fig. 2). HAP crystallites were also studied using high-resolution images by measuring the distance between interference fringes (Fig. 3).

The result confirms the presence of hydroxyapatite and carbonate-hydroxyapatite phases.

The Fig. 4 present evolution of crystallite dimensions vs immersion time for investigated samples compared with control group.

It is found that during immersion, crystallite size changes compared with the control specimen. We suppose this is due to the passage of hydroxyapatite $Ca_5(PO_4)_3OH$ in carbonate-hydroxyapatite $Ca_5(PO_4CO_3)_3OH$ in the presence of carbon dioxide released from drinks used [14].

Increasing the crystallite size because of immersion for 15 minutes is followed by a decrease in crystallite size probably due of breaking it into pieces to immersion for 60 minutes. Immersion of 120 minutes and 180 minutes respectively, also lead to an increase in crystallite size.

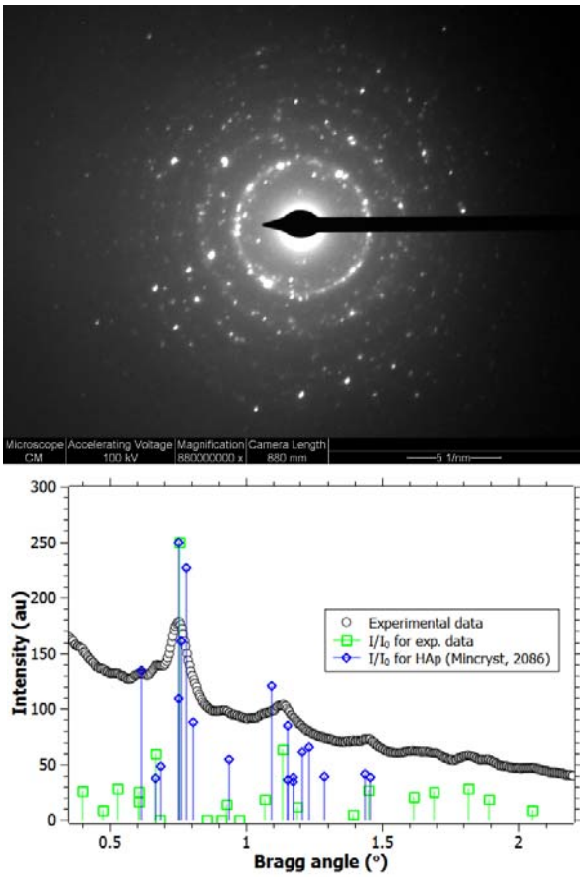

Fig. 2. Electron diffraction pattern (a) and extracted profile from diffraction figure (b) for sample 6G, 180minute immersion time (with Debye-Scherrer relationship found an average crystallite size of 7nm)

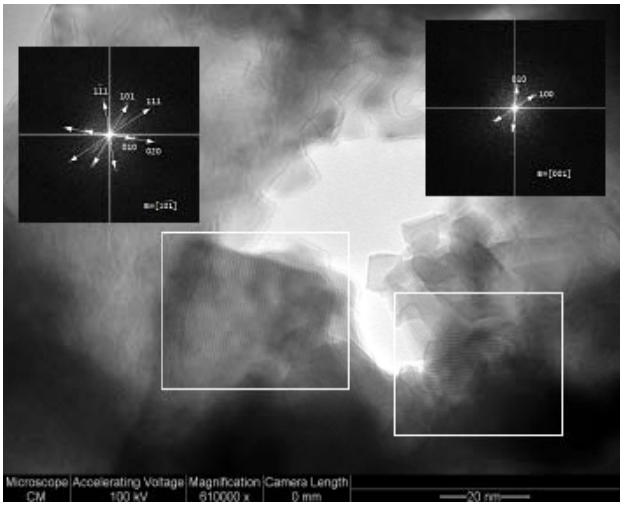

Fig. 3. HRTEM images for sample 6G, 180 min immersion time

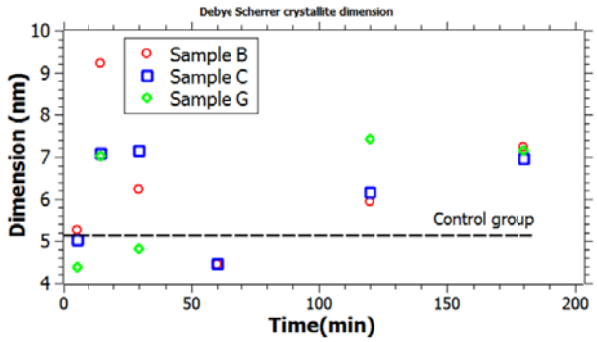

Fig.4. Crystallite dimension vs. immersion time.

4. Conclusions

Nineteen human dental enamel specimens were prepared using extracted healthy teeth to study the effect of some regular beverages by Transmission Electron Microscopy techniques. Extracted teeth showed no caries or noncarioase or dystrophic lesions. Some carbonated drinks (brand) which are popular among teenagers and young people in Romania, namely Coca-Cola, Burn Energy Drink and Gatorade sports drinks were chosen. Criterion of choice of soda was their popularity in Romania, the high consumption by young people, to have sour taste and a pH of less than 4.5, so producing dental erosion.

The teeth were randomly divided into groups for immersion in Coca-Cola, Gatorade, Burn Energy Drinks respectively, and control group in distilled water. Immersion of the teeth was done in opaque plastic containers with lids, which have been filled with 50ml of beverage. The immersion times were 5, 15, 30, 60, 120, 180 minutes respectively, in a continuous gentle vibration.

Samples were investigated by TEM techniques. To obtain suitable powder, the teeth was grind by means of diamond drill, milled in an agate mortar and then dispersed in alcohol and put on the formvar covered Cu grid.

To obtain morphological images, diffraction patterns, and high-resolution images, were used areas with varied content in terms of shapes and sizes of particles.

The crystallite dimension was estimated using Debye-Scherrer formulas applied to FWHM determined from SAED profile and using high-resolution images.

It is found that during immersion, crystallite size changes compared with the control specimen. We suppose this is due to the passage of hydroxyapatite crystallites $Ca_5(PO_4)_3OH$ in carbonate-hydroxyapatite $Ca_5(PO_4CO_3)_3OH$ in the presence of carbon dioxide released from beverages used.

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