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Effects of citric acid modified with fluoride, nano-hydroxyapatite and casein on eroded enamel

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ABSTRACT

Objectives: The aim of this *in vitro* study was to evaluate the effects of citric acid containing fluoride, nano-hydroxyapatite, and casein on eroded enamel.
Design: The crowns of 120 extracted bovine incisors were embedded in acrylic resin. An enamel window (2 × 3 mm) was created on the surface. Before *in vitro* pellicle formation samples were eroded in 1% citric acid (pH = 3.2) for 1 h at 36 °C and were randomly classified to eight groups (n = 15) as follows: Positive control: 1% citric acid, Negative control: Distilled water, F1: 0.047 mmol/L sodium fluoride, F2: 0.071 mmol/L sodium fluoride, NHA1: %0.05 Nano-Hydroxyapatite, NHA2: %0.1 Nano-Hydroxyapatite, C1: %0.02 Casein, C2: %0.2 Casein. Erosion cycling was performed three times daily for 3 days. In each cycle, the samples were immersed in 10 mL of control or modified solutions (10 min) and in 10 mL of artificial saliva (60 min). The surface roughness and enamel loss were analyzed by using profilometer, scanning electron microscopy (SEM) and atomic force microscopy techniques (AFM).
Results: Among the groups, the positive control group was found to be having the highest erosive wear. Erosive wear in the F2, NHA2, C1, and C2 groups was not significantly different from the negative control group ($p > 0.05$). The C1 and C2 groups showed that erosion terminated and minimal tissue recovery occurred on the enamel surface.
Conclusion: Although all modifications reduced further demineralization, the citric acid modification with casein was found to be having a greater impact on dental erosion than the others.

1. Introduction

Dental erosion is a loss of hard tissues due to a chemical process, such as dissolution or chelating without the involvement of microorganisms (Imfeld, 1996). As lifestyles have changed through the decades, the total amount and frequency of the consumption of acidic food and drinks have also changed (Lussi, 2006). Because a growing trend towards increased consumption of soft drinks, sports drinks, high-energy beverages, and coffee products has occurred, the prevalence of erosion has increased among children and adolescents (Fairchild, Broughton, & Morgan, 2017; Nunn, 1996; Shenkin, Heller, Warren, & Marshall, 2003). Most children and adolescents do not know that one side effect of drinking soft drinks is that they are harmful to their teeth (Fairchild et al., 2017; May & Waterhouse, 2003). Hence, it seems acceptable to search for effective methods for the prevention or repair of dental erosion, such as reducing the erosive potential of beverages (Hughes et al., 2002; Larsen & Nyvad, 1999; Sorvari, Kiviranta, & Luoma, 1988; Sorvari, 1989).One of the protective measures is the reduction of erosive potential of beverages by the addition of various additives. There are many studies to test various modifications of beverages and acids. (Aldosari et al., 2017; Attin, Meyer, Hellwig, Buchalla, & Lennon, 2003; Attin, Weiss, Becker, Buchalla, & Wiegand, 2005; Franklin, Masih, & Thomas, 2014; Franklin, Masih, & Thomas, 2015; Hemingway et al., 2010; Lee, Min, Choi, Kwon, & Kim, 2007; Min, Kwon, & Kim, 2011; Min, Kwon, & Kim, 2015; Ramalingam, Messer, & Reynolds, 2005; Xavier, Rai, Hegde, & Shetty, 2015). Unlike these studies, there were no studies that investigate the different concentrations and different types of the additives were compared and the effect of the modifications on surface roughness. Considering these shortcomings, this work was based on the examination of the effects of

Abbreviations: SEM, scanning electron microscopy; AFM, atomic force microscopy; EDX, energy-dispersive X-ray spectroscopy

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different concentrations and different types of 3 additives, such as traditional, inorganic and organic, on tooth erosion. Fluoride is a traditional material for remineralization of enamel lesions; hydroxyapatite is an inorganic component 95% weight of tooth (Attin et al., 2003) and casein is a milk protein which is an organic compound. To determine which additive is more effective in reducing the erosion potential of beverages, addition of fluoride, nano-hydroxyapatite and casein to citric acid was tested.

One of the additive material used in this study is fluoride. Research on fluoride is preferred due to its' effect on repairing of tooth enamel. It must be noted that fluoride is an additive for drinking water. In many previous studies conducted with rats, fluoride added in concentration of 50 and 1.9 ppm to grapefruit juice (Gedalia, Anaise, Westreich, & Fuks, 1975; Spencer & Ellis, 1950), 15 ppm to an experimental sports drink (Sorvari et al., 1988; Sorvari, 1989), 5 ppm to Coca-Cola and citric acid solution (Shabat, Anaise, Westreich, & Gedalia, 1975), reduced the erosive potential of solutions. In more recent studies, fluoride concentrations were lower and the addition of fluoride to soft drinks in low concentrations was shown to reduce dental erosion *in vitro* (Attin et al., 2003; Hughes, West, & Addy, 2004). In this study, we investigated the effects of fluoride-addition at lower concentrations.

The other additive material, hydroxyapatite is the primary mineral component of teeth and bones and is a biocompatible material, but it is difficult to dissolve hydroxyapatite in a solution and to utilize it in various applications (Min et al., 2015). This limitation of hydroxyapatite can be solved by minimizing the dimension of the particles using nanosized materials. Nano-hydroxyapatite ($Ca_5(PO_4)_3(OH)$) is a source of calcium and phosphate. It was shown that high amounts of calcium, phosphate, or fluoride were able to reduce the formation of erosive lesions in enamel (Larsen & Nyvad, 1999; Sorvari, 1989). In a recent study in which rats were used as subjects, it was observed that calcium containing beverages reduced the erosive tooth wear (Aldosari et al., 2017). In addition, it has been reported that nano-hydroxyapatite has a potential to remineralize initial enamel lesions under dynamic pH-cycling conditions (Huang, Gao, Cheng, & Yu, 2011; Huang, Gao, & Yu, 2009; Rezvani, Rouhollahi, Andalib, & Hamze, 2016). Calcium and phosphate supplementation of a beverages might change the taste of drink (Min et al., 2015). In this study, nano-hydroxyapatite was used as a source of calcium and phosphate in a citric acid solution at a lower concentration than the other studies.

In order to decrease the erosive potential of the beverages it may be thought that organic substances may also be added to beverages in addition to inorganic additives. One of these organic substances is casein which known as milk protein. It was reported that the milk protein, casein, reduces hydroxyapatite dissolution in simple acid solutions *in vitro* (Barbour, Shellis, Parker, Allen, & Addy, 2008), which is thought to be due to the adsorption of protein through the hydroxyapatite surface (Kawasaki, Kambara, Matsumura, & Norde, 1999; Reynolds & Wong, 1983). Similar to the study of Hemingway et al. (2010), a salivary pellicle was incorporated into the experimental model in this study. Since the protective effect of the casein against erosion is likely to be associated with protein adsorption at the hydroxyapatite surface, the presence of a salivary pellicle may reduce or eliminate this effect (Hemingway et al., 2010).

Dental erosion depends on the consumption of acidic beverages and is quite common around the world. One could expect to decrease dental erosion by reducing the erosive potential of these drinks. To remedy the current problems, this study shows the effects of a citric acid solution, which was modified with two different concentrations of fluoride, nano-hydroxyapatite, and casein on the eroded enamel surface using bovine enamel specimens in the presence of a simple *in vitro*-formed pellicle.

2. Materials and methods

2.1. Sample preparation

We cleaned the soft tissue debris of 120 bovine incisors and inspected them for cracks, hypoplasia, and white spot lesions. The selected bovine teeth were stored in a 0.5% thymol solution prior to usage in the study. The crowns of the samples were sectioned and embedded in a self-cure acrylic resin leaving the vestibular surfaces exposed. The specimens were polished using 600-, 800-, 1000-, and 1200-grit silicon carbide abrasive paper lubricated with water to flatten the outer enamel surface. A window (6 × 3 mm) was drawn using a pencil on the flattened tooth surface. Each sample was covered with acid-resistant nail varnish leaving an exposed window (3 mm × 4 mm). The specimens were stored in artificial saliva for 24 h (pH 7.0) prior to the erosion cycles.

2.2. Preparation of nano-hydroxyapatite powder

Nano-hydroxyapatite powder was prepared using a sol-gel technique (Kus et al., 2006; Sanosh, Chu, Balakrishnan, Kim, & Cho, 2009). Primarily, a sol solution was prepared. Solution A was 8.8 g of calcium phosphate monobasic dissolved in 13.17 mL water and stirred for 10 min. To prepare solution B, ammonia was added to phosphoric acid and stirred until a constant pH = 10 was obtained. We added 25 mL of solution B by dropper to solution A. This sol solution was stirred for 30 min. We added 10 g of calcium hydroxide into the stirring sol solution, which was sonicated for 1 h and kept for aging for 24 h at room temperature. The gel obtained after aging was dried at 65 °C for 24 h in a dry oven.

The powders from the dried gel were washed repeatedly using distilled water to remove ammonium phosphate. After washing, the powder was calcined in air at different temperatures ranging from 200 °C to 800 °C for 30 min using an electrical furnace and employing a heating rate of 10 °C/min.

The elemental phase composition of the hydroxyapatite powder was analyzed using the energy dispersive X-ray (EDX) (Bruker, Germany). The X-ray diffraction pattern of the final hydroxyapatite nano particles was obtained with Cu (copper) α (alpha) source (λ (wavelength) = 1.5406 Å (angstrom)) on a D8 model X-Ray diffractometer (Bruker, Germany).

The reactions involved in the formation of hydroxyapatite during the sol–gel preparation and drying are expressed as follows:

$$H_3PO_4 + 3NH_4OH \rightarrow (NH_4)_3PO_4 + 3H_20$$

$CaH_4(PO_4)_2$ (solid) = $Ca(H_2PO_4)_2$ (solid) calcium phosphate monobasic

$$3Ca(H_2PO_4)_2 \text{ (solid)} + 2(NH_4)_3PO_4 \rightarrow Ca_3(PO_4)_2 + 6NH_4H_2PO_4$$

$$3Ca_3(PO_4)_2 + Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2$$

The average crystalline size was calculated from the diffraction peaks by using Scherrer's equation as shown (Hammond, 1997):

$$Dc = 0.9.\lambda/(L.\cos\theta)$$

Where Dc is the crystalline diameter, L is the half-intensity width of the diffraction peak, λ is the X-ray wavelength, and θ is the angle of the diffraction. The crystal size obtained from this equation is 30 nm (Fig. 1).

2.3. Modifications of citric acid

The control citric acid solution (1% w/v) was prepared by adding citric acid to distilled water. To raise the pH of the citric acid solution from 2.5 to 3.2, 1 M sodium hydroxide was added.

To create 0.047 mmol/L (≅0.9 ppm) and 0.071 mmol/L