A new model: In vitro erosion of minipig enamel cased bz fruit yogurt

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Summary

With the increasing knowledge on pig genetics and development of high resolution comparative maps of human and the porcine genome provide us minipig as new models for dental purposes. The aim of the present study was to use a new in vitro model to measure the erosive potential of a commercial fruit yogurt. The test specimens were prepared from freshly extracted minipig teeth. 48 enamel samples were divided into three groups of 16 specimens. Two groups were designated as test groups and one as the control group. For the test groups, each enamel block was immersed in 25 ml of fruit yogurt. They were then incubated for **24 h or 48 h with gentle agitation. The enamel blocks were examined with Scanning election microscope (SEM). Surface alterations were observed in the scanning electron microscopy study of minipig enamel treated with fruit yogurt. The surface topography of scanning electron micrographs of each specimen was scored visually by two investigators. No significant differences in the mean visual scores were found among the 24 h and 48 h treatment groups or control group. Fruit yogurt caused material loss of minipig enamel to some degree, however had no detectable erosive effect. Clearly, there is a need for more data from in vitro studies using minipig teeth regarding erosive potential of foodstuffs.**

Key words: dental erosion, fruit yogurt, minipig enamel, SEM.

Introduction

Dental erosion is defined as a loss of dental hard tissue caused by acid, and in contrast to caries, without bacterial involvement [1.2]. The etiology of erosion is multifactorial and not fully understood. Sources of acids that lead to erosion may be extrinsic or intrinsic [3]. Extrinsic sources of erosive acids include acidic foods and drinks, medications and environmental acids [4, 5]. It is widely believed that the prevalence of erosion is increasing as new market products are introduced to consumers everyday.

Yogurt is one of the main traditional foods in Turkish cuisine and served next to every dish. Recent research points that adding sweeteners and fruit particles into yogurt masks characteristic acetaldehyde taste and sourness, increases sweetness and favors consumer acceptability [6]. Fruit yogurt has been sold in the Turkish market since last two decades [7]. Recently, it has been shown in *vitro* that fruit yogurt had no relation with dental erosion [8, 9]. However, a new study showed a questionable prevalence of dental erosion (36%) in a sample of Istanbul children regularly consuming fruit yogurt [10].

The aim of the present study was to use a new *in vitro* model to measure the erosive effect of a commercial fruit yogurt. The model system used minipig teeth and the experimental conditions were selected to ensure that erosion would occur in order to measure the erosive effect as a function of time.

Material and method

The test specimens were prepared from freshly extracted minipig teeth (Gottingen Minipigs®, Ellegaard, Dalmose, Denmark). The teeth were cleaned with pumice, and small blocks of 3 x 5 mm were prepared from the labial surface. To ensure an even surface, a thin layer of external enamel was removed with Soflex-Pop-on discs (3M Company®, StPaui, MI, USA). Tooth blocks were stored in distilled water.

48 enamel samples were divided into three groups of 16 specimens. Two groups were designated the test groups and one the control group. For the test groups, each enamel block was immersed in 25 ml of fruit yogurt (Danone Strawberry Yogurt®, Danonesa, Istanbul, Turkey), and the vial covered with a plastic foil. Fruit yogurt used in the study had 6% strawberry fruit and 108 mg/100 ml of Ca. It had a baseline pH of 4.36 and 12 x 0.5 M NaOH was added for pH 10. They were then incubated for 24 h or 48 h with gentle agitation. After incubation, the enamel blocks were rinsed thoroughly with saline, and dried in air at 20°C.

The enamel blocks were prepared for SEM by sputter, coated with 0.2 JI palladium and gold.

The enamel blocks were then examined with Scanning electron microscope (Philips XL-20®, FEI Company, Hillsboro, Oregon, USA). Each specimen surface was observed and photographed twice, once in the center and once approximately 1-2 mm from the edge of the specimen at 400X and 1000X magnifications by two raters for surface defects. A micrograph of a non-treated specimen was selected as a reference for a score of 0 and a picture of a specimen showing extensive surface defects was selected as a reference for a score of 1.

Results

There were no significant differences between the scoring of either rater. Inter-rater reliability was r = 0.87 (intra-class correlation coefficient) for scoring all micrographs. No abnormal surface alterations could be observed in the scanning electron microscopy study of minipig enamel treated with water.

Figure la and *Figure lb* show the enamel immersed in water at 400X magnification. *Figure 1c* and *Figure Id* showed the enamel immersed in water at 1000X magnification. Fruit yogurt caused material loss of minipig enamel to

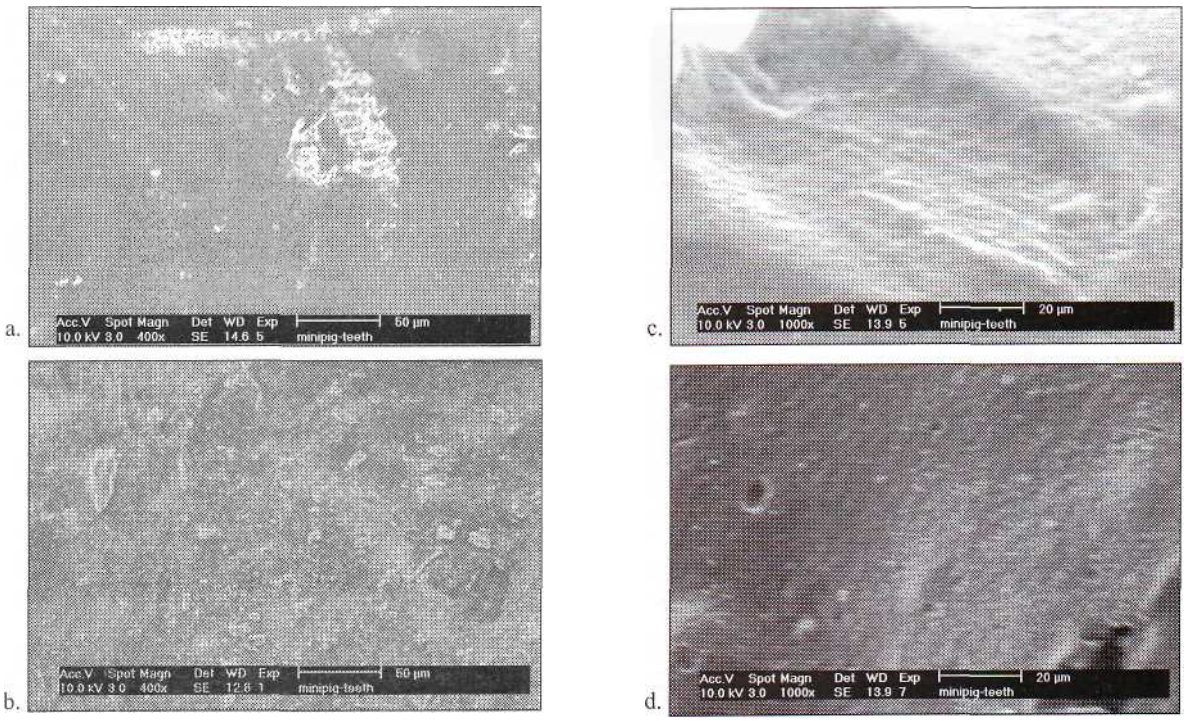

Figure 1. Scanning electron micrographs of enamel blocks immersed in water at 400X on the left (a-b). Pictures on the right are 1000X (c-d)

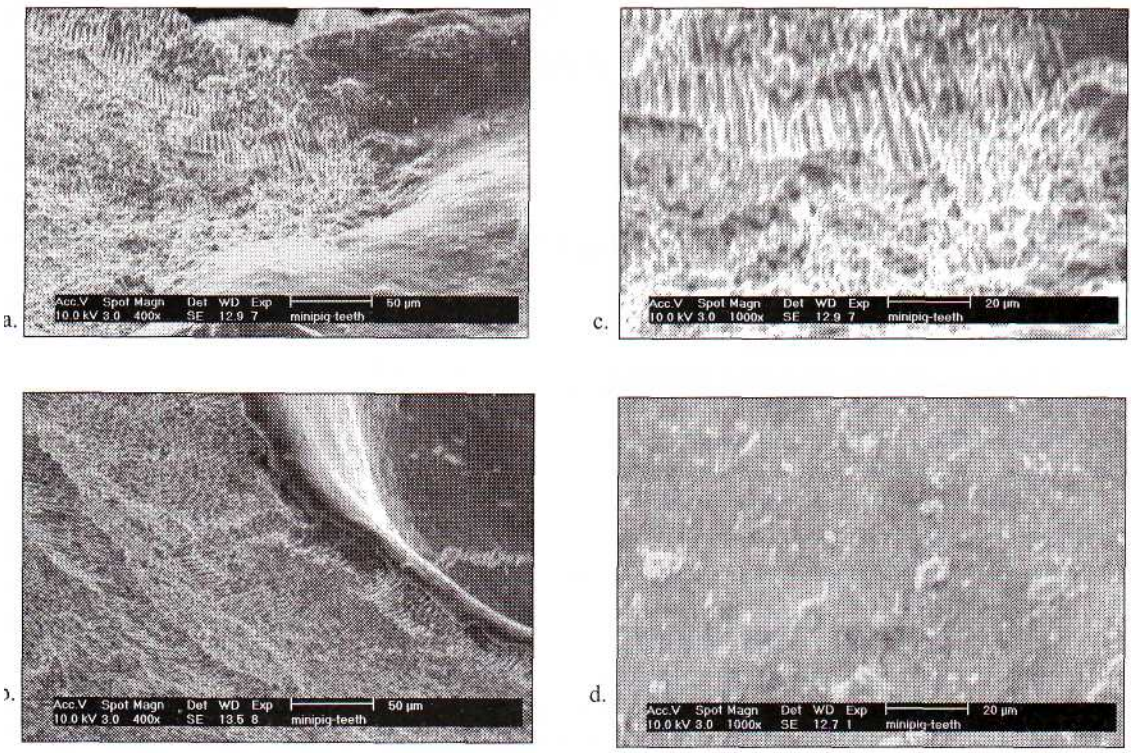

Figure 2. Scanning electron micrographs of enamel blocks treated with fruit yogurt at 400X on the left (a-b). Pictures on the right are 1000X (c-d). Exposure time is 24 h

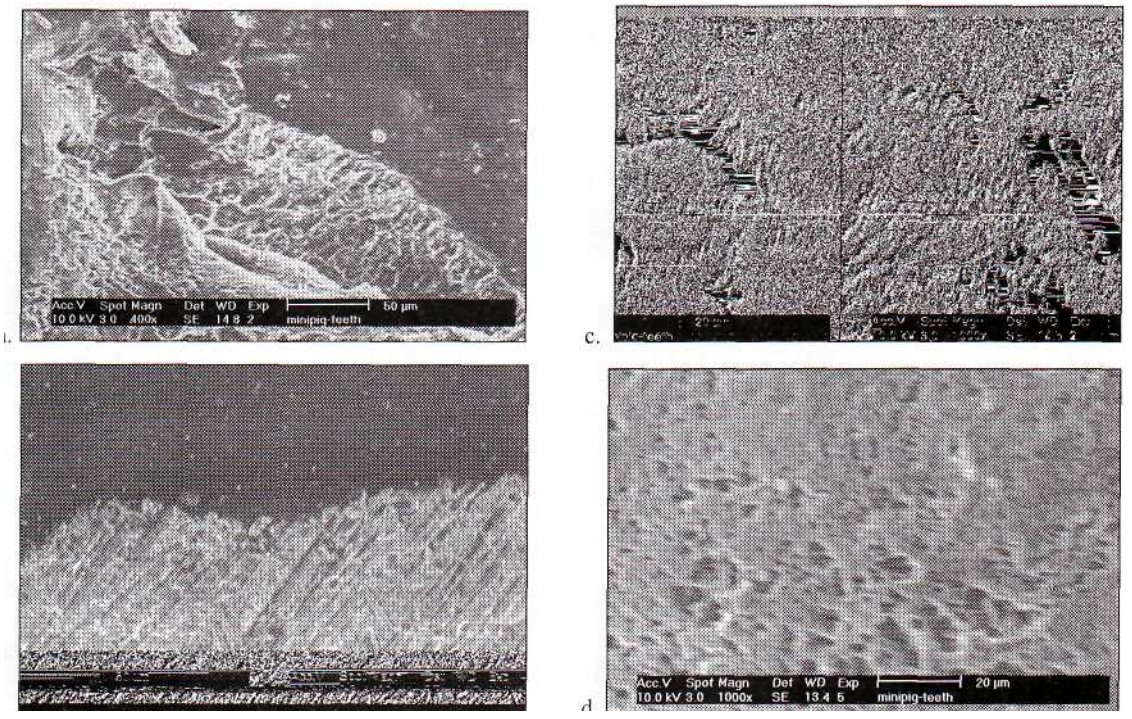

Figure 3. Scanning electron micrographs of enamel blocks treated with fruit yogurt at 400X on the left (a-b). Pictures on the right are 1000X (c-d). Exposure time is 48 h

some degree, however had no detectable erosive effect. There was no significant difference between the fruit yogurt treated enamel for 24 h and water ($p = 0.34$). The specimens had an amorphous layer on top of aprismatic structure after the erosion process. High power micrographs revealed distribution of enamel prism structures whenever erosion was observed. The SEM study revealed that the fruit yogurt treated enamel for 24 h did not differ markedly from the fruit yogurt treated enamel for 48 h. There was no significant difference between the fruit yogurt treated enamel for 24 h and 48 h ($p = 0.59$).

Figure 2a and *Figure 2b* show the enamel immersed in fruit yogurt for 24 h at 400X magnification. *Figure 2c* and *Figure 2d* show the enamel immersed in fruit yogurt for 24 h at 1000X magnification. *Figure 3a* and *Figure 3b* show the enamel immersed in fruit yogurt for 48 h at 400X magnification. *Figure 3c* and *Figure 3d* show the enamel immersed in fruit yogurt for 48 h at 1000X magnification. There was also no significant difference between the fruit yogurt treated enamel for 48 h and water ($p = 0.49$). Fruit yogurt had detectable erosive effect at some amount. In some teeth a pellicle-like material was observed, which, as already noted, did not influence the erosive effect.

Based on epidemiological studies in adults and children, a variety of acidic beverages, fruits and foodstuff are most probably associated with erosion in childhood. The increasing knowledge on pig genetics and development of comparative high-resolution maps of human and porcine genome provide us with minipig enamel, as being new models [11-13]. It is important to assume that dissolution produced by a gelatin lactic acid system progresses about threefold faster in bovine enamel than in human teeth [14], furthermore, minipig teeth may not be equivalent to human teeth. Regarding this statement, more research should be carried on minipig teeth. The erosive effect of acidic drinks and foodstuff on teeth has been studied *in vitro* by a variety of methods: micro hardness measurements [8, 15], SEM [9, 16,17], the dissolution of enamel [18], micro radiography [19, 20].

In the present study the erosive effect on minipig teeth was measured from surface profile using SEM. Erosion time (24 h, 48 h) used in the present study was certainly exaggerated. The physical and chemical (pH and buffering capacity) composition of a particular foodstuff is important in detection of erosive process. In animal experiments, foods that produce erosion have been acidic, with pH below 4.5 [21, 22]. The fruit in yogurt may also play an important role in pH changes. Adding fruit particles into yogurt increases pH, while adding fruit jam decreases pH [23]. Strawberry yogurts had been found to have lower pH values than chocolate, melon, orange, and vanilla ones [24]. Measuring the pH at the tongue surface, it was reported for strawberry yogurt (pH = 3.81) an erosive capacity greater than that of coke or orange juice [25]. Strawberry yogurt used in the present study had a pH of 4.36. However, it has also been shown that milk products do not cause demineralization because of their high calcium and phosphate content [8, 9, 15]. Absorption of calcium increases with the lactic acid that is present in fruit yogurt (0.78% - 1.06%) as most of other milk products [26, 27].

The present study showed that fruit yogurt caused certain amounts of erosion on minipig teeth enamel. However, there is a need for more data from studies *in vitro* using minipig teeth regarding erosive potential of foodstuffs.

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