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Nano-hydroxyapatite could Compensate the Adverse Effect of Soft Carbonated Drinks on Enamel

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ABSTRACT

Introduction: Since one of the most important disadvantages of soft drinks includes their adverse effect on mineral content of enamel because of their low pH, this study examined the microhardness of enamel before and after exposure to a soft drink containing different concentrations of nano-hydroxyapatite (nano-HA) as an additive.

Materials and methods: Sixty caries free human premolars were mounted in epoxy resin. After polishing, the baseline microhardness was recorded three times for each specimen using a Vickers indenter at 50 gm load. Subsequently, the samples were divided into six groups, which were treated for 5 minutes at 9°C by a cola-based drink contacting 0, 0.5, 1, 5, and 10 wt.% of nano-HA while the control group was immersed in artificial saliva. Ultimately, the final microhardness was assessed three times again for each specimen.

Results: Paired t-test showed that in groups containing 0 and 0.5 wt.% of nano-HA, the microhardness was significantly reduced after treatment protocol ($p = 0.00$ and 0.01 respectively). Whereas in the other groups the microhardness was not significantly changed after treatment ($p > 0.05$).

Conclusion: Pure cola-based drink has a pronounced adverse effect on enamel microhardness, while admixing it with nano-HA could act as a protective factor.

Clinical significance: Although soft beverages are hazardous to tooth structure, some additives could compensate their adverse effect.

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INTRODUCTION

New dietary habits in modern lifestyle lead to progressively more consumption of soft drinks.[1-7] One of the most important disadvantages of soft drinks includes their erosive effect on enamel because of their low pH since they contain citric acid, maleic acid, phosphoric acid, etc.[7,8] Moreover, these popular fluids also contain sugar as their main content that exacerbate the plaque formation on tooth surface.[8] Although erosive lesions have a multifactorial etiology, it has been documented that excessive consumption of acidic food and beverages considered as one of the most frequent extrinsic factors inducing dental erosion.[3,9,10] Actually, these acidic beverages compromise the mineral content and crystalline structure of tooth surface.[7,11] Especially, softening of enamel would be intensified by holding the drink in the mouth before swallowing it.[12] However, these early lesions could be rehardened by different remineralizing agents, such as fluoride, calcium phosphate, hydroxyapatite (HA), or even artificial saliva.[1,6,13] Therefore, various researchers admixed some remineralizing agents with acidic drinks in order to compensate their side effect on enamel.[2,4,6,11] Among these incorporated particles, HA showed quite favorable results because of its high biocompatibility and chemical homogeneity with tooth structure.[5] However, HA has the disadvantage of low solubility in oral environment.[5] Interestingly, in recent years, nanotechnology solved this limitation of HA.[5] In fact, decreasing the particle size of HA down to nano-scale altered its properties and consequently lead to its more efficacy for remineralization.[14-18]

Cola is one of the most common worldwide acidic drinks (pH < 4) that could significantly dissolve mineral content of enamel even in short time exposures.[2,6,8,19] This mineral loss was documented with obviously decrease in surface hardness.[6] Since the mineral content of enamel is directly related to its microhardness,[11] several articles have been proposed to evaluate the progression of enamel erosion by testing its microhardness.[13,17,20,21] Therefore, the aim of this study is evaluating the effect of a cola-based drink containing different amount of nano-HA on the microhardness of enamel. It was hypothesized that: (1) there is no difference between erosive effect of cola-based drink and artificial saliva. (2) Various concentration of nano-HA incorporated into cola-based drink would have similar effect on the microhardness of tooth enamel.

MATERIALS AND METHODS

Sample Preparation

Sixty healthy human premolars (extracted for orthodontic reason) were selected and stored in tap water, which were replaced twice a week during the study. After completely cleaning with water-pumice slurry using brushes in a low-speed handpiece, the teeth were visually explored to confirm that they were caries free (according to WHO guidelines). Also they were examined by stereomicroscope (40× magnification, Carton Optimal Industries Ltd., Type SCW-E, Thailand) to discard the samples with cracks, fractures, or hypocalcification that could interfere with the results. Afterwards, the teeth were embedded in epoxy resin cylinders and the exposed enamel surface was ground flat and wet polished with 5000-grit silicon carbide paper.

Microhardness Test

The baseline microhardness was assessed using a Vickers indenter (Shimadzu; M-g5037, Japan) at 50 gm load. We made three indentations on each specimen.

Preparation of Solution

In this study, we made five erosive solutions containing 0, 0.5, 1, 5, and 10 wt.% of remineralizing agent and one control group consisting of artificial saliva (Kin Hidrat spray; Spain). In order to produce erosive solutions, we mixed 50 gm of cola-based drink (ZamZam, Iran) with nano-HA (diameter <100 nm, aspect ratio 2-3, Nanoshel Co., Panchkula, India) powder.

Erosion Process

We randomly divided the samples into six groups (n = 10 per group), among which five were experimental groups (containing 0, 0.5, 1, 5, and 10 wt.% of nano-HA in cola respectively) while the last group was served as control (artificial saliva).

Each specimen was soaked in the solution for 5 minutes at 9ºC and then rinsed with water for 10 minutes.

Prior to immersion of the samples, the pH and temperature of each solution were obtained by means of a pH meter (Metrohm® Ltd,CH-9101 Herisau, Swiss) and a thermometer.

Finally, the microhardness of all treated samples was assessed again using a Vickers indenter at 50 gm load while three indentations were made on each specimen.

Statistical Analysis

The data were analyzed using IBM SPSS software (version 20). After exploring the normality of the distribution (Kolmogorov–Smirnov), the average hardness of each group before and after treatment were compared using paired t-test. The level of significance was determined as p = 0.05.

RESULTS

The recorded pH for each solution is demonstrated in Table 1. As it is presented, the least and the highest pH was related to the pure cola-based drink and artificial saliva respectively. Among other groups, as the concentration of nano-HA increase, the pH increased too.

The mean microhardness value ± standard deviation related to each group before and after treatment by experimental solution has been shown in Graph 1.

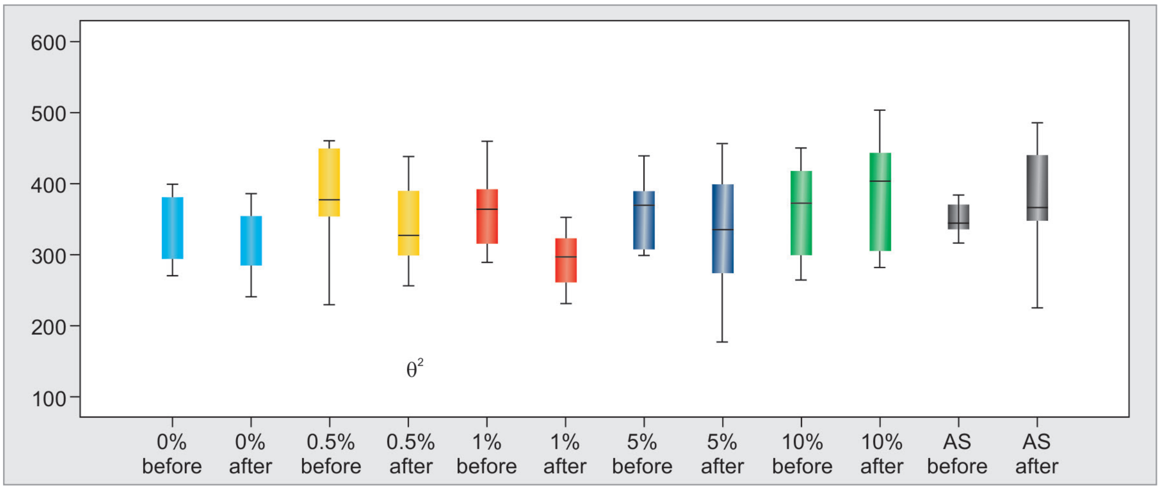
Paired t-test revealed that only the 1st two groups significantly changed after treatment (p = 0.00, 0.01, 0.23, 0.47, 0.84, and 0.25 for the 1st to 6th group respectively). It means that the Vickers hardness of the enamel exposing to pure cola drink or the cola drink containing 0.5 wt.% nano-HA was significantly decreased while the microhardness of the other groups did not significantly change after exposure to experimental solutions. In other words, the cola-based drink containing 1, 5, or 10 wt.% nano-HA affects the tooth enamel similar to artificial saliva.

DISCUSSION

Our results revealed that although the cola-based drink would significantly reduce the microhardness of enamel,

Table 1: The recorded pH for different experimental solution

Solution	*pH*
Cola-based drink	3/59
Cola-based drink containing 0.5 wt.% nano-HA	6/21
Cola-based drink containing 1 wt.% nano-HA	6/24
Cola-based drink containing 5 wt.% nano-HA	6/32
Cola-based drink containing 10 wt.% nano-HA	6/46
Artificial saliva	6/7



Graph 1: Mean Vickers hardness related to each group before and after treatment by experimental solution (As: Artificial saliva)

if it contains nano-HA with 1 wt.% concentration or more, the surface hardness would not significantly affect.

Our main result is consistent with previous studies who reported the protective effect of nano-HA against dental erosion when admixed with different acidic beverages.[5,6,22] In contrast to our results, Min et al argued that even 0.05% of nano-HA was effective while our 0.5 wt.% group could not be considered as protective. This difference in results could be explained by the difference in methodology because Min et al[5] worked on bovine enamel and they assessed the Vickers microhardness after around 7 hours exposure to experimental solutions.

Definitely, the remineralization strongly depended on the type and the concentration of the remineralizing agent.[2,5] Our results also confirmed that as the percentage of incorporated nano-HA increased, the microhardness value of the enamel increased too. Meanwhile, as it is presented in Table 1, the pH of the solution also considerably depended on the nano-HA concentration. Accordingly, even in 0.5 wt.% group the pH was around 2.6, higher than the pure cola group. This finding is in agreement with many pioneer researchers who studied different remineralizing agents supplemented to soft drinks.[2,5,22] Also, a linear correlation has been reported between the pH of the solution and the microhardness of enamel[23]; that was confirmed by our investigation since after treatment the highest surface microhardness was related to the 10 wt.% nano-HA group. However, except the pure cola group, all the observed pH values were beyond the critical pH for dissolution of tooth structure.

Since the nano-HA is chemically homogenous with tooth structure,[5] quite favorable remineralization always has been reported by HA particle. Moreover, it has been demonstrated that the nano-HA was considerably more efficient for the prevention of erosions compared to micro-HA.[5] Therefore, one of the most important causes of favorable results in our study could be assigned to the size of the particles. Furthermore, two other mechanisms have been reported for remineralization by nano-HA admixed in soft drinks. First, the nano-HA would act as a mineral reservoir because the acidity of the beverage dissolute the nano-HA into calcium, phosphate, and hydroxyl ions that lead to promotion of the remineralization process. Secondly, the nanoparticles would fill the microporosity of induced erosion.[22] In this situation also, the acidity of the environment would be probably beneficial because the clusters of calcium and phosphate would break down to smaller ion particles and the growth of high crystalline HA would be facilitated[22]; due to which, the surface would be enhanced mechanically.

The Vickers microhardness test was used in our study because it has been argued that Vickers hardness number is a better indicator of the remineralization effect compared to pH value.[22] Accordingly, the hardness tests have been frequently used in assessing the early manifestations of erosion process on tooth enamel.[24-29] Moreover, the 50 gm load was selected in our experiment because according to the available documentations, this magnitude of force would provide an appropriate size of indentation for accurate measurement.[6,27]

Our study design required us to flatten the surface of all samples in order to evaluate their microhardness. Thus, the treatment protocol was accomplished on the polished enamel which has been shown to be more susceptible to softening compared to intact original enamel[22]; because the outer enamel is hypermineralized and often contains fluoroapatite.[8] This process was a limitation in our study that could have an impact on the results and makes our experiment far from clinical condition in which the soft drinks are exposed to the uncut enamel.

In contrast, one of the other determining factors on the results of the current research, includes the duration that the specimens were exposed to the experimental solutions. We immersed each sample for 5 minutes into the related solution. This time interval was similar to the Tantbirojn et al[6] who conducted their investigation as 8 minutes exposure, while some other researchers used longer duration.[5,13] We selected our time interval and also the temperature (5 minutes at 9°C) in order to simulate clinical condition because the cola beverages are often drank cold and frequently retained for a short period in the mouth before swallowing.

Overwhelmingly, our results showed that applying the cola-based drink could reduce the indentation hardness of enamel while mixing these beverages with nano-HA could compensate their side effect on tooth. However, *in vitro* studies could not perfectly mimic the real oral environment and more clinical research is strongly suggested.

CONCLUSION

Under the limitations of this study, we may conclude that the addition of 1 wt.% of nano-HA into cola-based drink could inhibit its adverse effect on the indentation microhardness of human enamel.

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