ABSTRACT

Introduction: Arginine is a natural component of human saliva, which has recently been incorporated in dentifrices treating teeth sensitivity and acts as a protective factor against caries. Aim of the study: This study evaluated the effect of two different concentrations of arginine; 2.5% and 8% on fluoride uptake by demineralized enamel surfaces. Methods: 80 specimens obtained from 40 human premolars were divided randomly into 4 equal groups (n=20 per group): (GI=negative control), (GII=positive control) treated by sodium fluoride (NaF 500 ppmf), group III treated by combination of sodium fluoride solution (NaF 500 ppmf)+arginine solution (2.5%) and group IV treated by combination of sodium fluoride solution (NaF 500 ppmf)+arginine solution (8%). Microhardness was tested using Vickers microhardness test, in addition to imaging using environmental scanning electron microscope (ESEM) and minerals content was assessed using energy dispersive X-ray spectroscopy (EDX). Statistical analysis was performed utilizing Kolmogorov-Smirnov and Shapiro-Wilk tests for normality of data, Levene’s tests for homogeneity and one-way ANOVA with Bonferroni post hoc tests for the evaluation of statistical significance among the groups. Results: Highest mean microhardness was in group III (370.58±12.14) and group IV (370.22±8.24). An increase in mineral density was found in group IV followed by group III, then group II and group I as revealed by ESEM. Data analysis showed increase in fluoride concentration in group III followed by group IV, group II and group I with a mean value (17.59±1.33), (14.62±1.91), (13.57±2.29) and (14.32±2.13) respectively. Conclusion: Fluoride uptake of demineralized enamel is increased when using both concentrations of arginine (2.5% and 8%). Arginine modulates areas of mineralized deposits and overall refinement of enamel ultrastructure.

INTRODUCTION

Dental caries is one of the most prevalent and costly, oral chronic infectious diseases worldwide. It is formed by the bacterial metabolism of carbohydrates, which causes acidification of dental plaque, and demineralization of dental hard tissues. Anti-caries agents inhibit the production of bacterial acid and modulate the demineralization-remineralization equilibrium of dental hard tissues\(^{(1)}\). The enamel is hardest calcified tissue in the body because of its high mineral content and crystalline structure arrangement. The main
function of enamel is to form a resistant covering of the tooth structure, rendering it suitable for masticatory purposes (1).

Fluoride is the most commonly used remineralizing agent. It raises salivary pH and changes hydroxyapatite to new and larger crystals of fluorapatite, that reduce enamel demineralization and enhance remineralization (2,3). Moreover, the application of high concentration fluoride may also seal the enamel surface before the demineralization of the subsurface zone (4). Topical fluorides encourage remineralization of enamel, inhibit bacterial metabolism and reduce the growth of plaque bacteria, and are available in many forms, like toothpastes, gels, mouth rinses, and varnish (5). However, fluoride application has its limitations like acute/chronic fluorosis at high doses, as the optimal dose of fluoride (for children & adults) is 0.05-0.07 mg F/ kg body weight, while the toxic dose of fluoride (for children & adults) is 5 mg F/kg body weight (6).

Arginine is a natural component of human saliva, and it is secreted in free form at an average concentration of 50 μM (7). Several primary bacteria, including oral streptococci, lactobacilli, and spirochetes, catabolize arginine to ornithine, ammonia, and CO2 (8). Ammonia production may alkalize the oral microenvironment and maintain the equilibrium of oral microbial ecology (9). It has been suggested that the positively charged guanidinium group of arginine, interacts with the negatively charged groups on dentine surface such as fluoride ions, promoting the deposition of calcium and phosphorus from saliva (10,11). Recent studies have also indicated that a toothpaste containing 1.5% arginine and 1450 ppm fluoride in a calcium base, is more effective in arresting and reversing early carious lesions, compared with dentifrice containing 1450 ppm fluoride alone (12-14).

The aim of this study was to evaluate and compare the effects of 2.5% concentration of arginine and 8% concentration of arginine, on uptake of fluoride on demineralized enamel surfaces.

**MATERIALS AND METHODS**

The present study was conducted after the approval of the Research Ethics Committee of Faculty of Dentistry, Suez Canal University, on 9th of September 2018, code 126/2018, as no living subjects were at any risk during this study.

**Sample collection:** Forty sound human premolars extracted for orthodontic reason were collected from the Outpatient Clinic of Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Suez Canal University.

**Materials:** The materials used in this study were prepared in the chemistry laboratory, in the organic Chemistry department; Faculty of Science, Suez Canal University Chemistry.

**Arginine 2.5%** (L–Arginine LOBA CHEMIE PVT.LTD, Mumbai, India) solution was prepared by dissolving 6.25 gm of Arginine solid in 250 ml in distilled water at room temperature. **Arginine 8%** (L–Arginine LOBA CHEMIE PVT.LTD, Mumbai, India) solution was prepared by dissolving 20 gm Arginine solid in 250 ml in distilled water at room temperature. **Sodium fluoride solution (500 ppmf)** (LOBA CHEMIE PVT.LTD, Mumbai, India) where 500 ppm of NaF solution was prepared by dissolving 0.5 gm of NaF solid in distilled water at room temperature. **Demineralizing solution (47)**: 2.2 mM Ca2+, 2.2 mM PO43−, 50 mM acetic acid at a pH of 4.4 and **Artificial saliva (47)**: 1.5 mM CaCl2, 0.9 mM NaH2PO4·0.15 M KCl, 20 mmol Tris buffer and had a pH of 7.49 (Adjust PH HCL).
Samples grouping:

The specimens were randomly divided into 4 equal groups (20 specimens/groups) according to different treatment modalities. Group I (GI=negative control) treated by artificial saliva, group II (GII=positive control) treated with sodium fluoride (NaF) solution (500ppmf), group III treated by sodium fluoride solution (NaF500ppmf) + arginine solution (2.5%) and group IV treated with sodium fluoride solution (NaF 500ppmf) + arginine solution (8%) as remineralizing agents. Each group was then subdivided into two subgroups, 10 specimens in each, according to outcome assessment, 10 for microhardness (A) and 10 specimens for scanning electron microscope (B) followed by EDX analysis (C).

Specimen Preparation:

Forty teeth collected were stored in saline before preparation at room temperature. They were then separated from the roots at the cemento-enamel junction and sectioned mesiodistally along the central groove of the crowns into buccal and lingual halves. 80 specimens were obtained using an ISOMET (ISOMET 4000 micro saw Buehler USA). The pulp tissue in the pulp chamber was removed with a sharp spoon excavator, and then all surfaces were disinfected with 0.05% thymol solution in water. The enamel surfaces in all specimens were polished progressively with waterproof Silicon Carbide paper (Sic) (600-800) Grit; Struers, Copenhagen, Denmark abrasive) followed by ultrasonic cleaning.

PH Cycling:

Eighty specimens were alternatively immersed daily in 10 ml of the freshly prepared demineralizing solution for 1 hour, followed by 2 hours preservation in 10 ml of artificial saliva with a pH of 7.49. This cycle was repeated 3 times a day for 7 days and stored at 37°C in an incubator.

Remineralization:

Following PH cycling, each specimen was placed in a test tube containing 10 ml of remineralizing solution according to different treatment modalities for 4 minutes 3 times a day for 7 days. After each remineralization cycle, the specimens were immersed in artificial saliva without rinsing and kept in the incubator at 37 °C. They were then incubated at 37 °C for another 7 days in artificial saliva.

Assessments Methods:

A. Microhardness

Vickers microhardness test (Tukon 1102 Wilson hardness tester Buehler Germany) was undertaken by applying 100gm load smoothly, without impact, forcing the indenter into the test specimen, and then all surfaces were averaged. Indentation points were examined under the microscope, along with the microhardness tester at 50x magnification.

B. Environmental Scanning Electron Microscopy (ESEM)

The samples were prepared for examination using an environmental scanning electron microscope (ESEM), (QUANTA FEG –250) to observe the ultra-morphology of surface enamel in all samples in the four groups.

C. Energy Dispersive X-ray Spectroscopy (EDX)

Samples were subjected to energy dispersive X-ray spectroscopy (EDX) (QUANTA FEG –250
model AMETEX), to measure the values of Calcium and Phosphate content (weight %), calcium: phosphate ratio and values of fluoride (weight %).

**Statistical Analysis**

Data was collected, compared, then statistically analyzed. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed normal (parametric) distribution. All values were presented as means ± standard deviations. First, the Levene’s tests for homogeneity of variance were performed, which is a standard assumption of one-way analysis of variance (ANOVA) (19,20). The results of the tests indicated the P values are greater than 0.05, demonstrating that the variance within each of the groups is equal (i.e., the homogeneity of variance is violated). Then, one-way ANOVA with Bonferroni post hoc tests was performed for the evaluation of statistical significance among the groups P < 0.05 was considered to be statistically significant.

Statistical analysis was performed using SPSS software for Windows version 22.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels 0.01 (P-value ≤0.1) (20).

**RESULTS**

**Microhardness:**

Statistical analysis of the microhardness test data revealed that the highest mean microhardness was found in group III (370.58±12.14), group IV (370.22±8.24) respectively with no significant difference between them (P=0.0357), whereas when comparing between all treated groups, group I (353.53±9.75), group II (363.50±11.44), while group III (370.58±12.14) and in group IV (370.22±8.24), there was statically significant difference(P=0.0357), at P-value 0.05 for load.

Table (1) Comparison between microhardness of the four groups using one way ANOVA test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Micro hardness (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>353.53±9.75</td>
</tr>
<tr>
<td>Group II</td>
<td>363.50±11.44</td>
</tr>
<tr>
<td>Group III</td>
<td>370.58±12.14</td>
</tr>
<tr>
<td>Group IV</td>
<td>370.22±8.24</td>
</tr>
<tr>
<td>P- value</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*: Significant difference at P-value 0.05 and different letters means significant difference between groups at P-value 0.05

The Scanning Electron micrograph (Figure 1) of enamel of group I (artificial saliva treated) still presented with inter prismatic holes, and the structure of the enamel surface appeared porous with the loss of normal key-hole enamel pattern by a demineralizing solution. It had a very small deposit of opaque like structure when samples were treated by saliva. In group II, interprismatic substances showed areas of porosities and areas of remineralization. Calcification took place in the form of scattered deposition of globules, yet without complete coverage of the enamel surface, with the least density of deposition when compared with other groups. In group III, almost complete obstruction of inter-rod spaces in some fields could be seen. Areas of mineralized deposits were evident and were seen profusely scattered along the porous defects. While in group IV, SEM showed evenly distributed opaque colors on the micrographs. Moreover, imaging revealed improvements of the enamel ultrastructure. The specimens manifested a smoother enamel surface, with decreased number and size of previously formed pores compared to the other group’s.
1. **Dispersive x-ray spectroscopy (EDX) (Minerals Energy content):**

Data analysis showed increase in fluoride concentration in group III followed by group IV, group II and group I with a mean value (17.59±1.33), (14.62±1.91), (13.57±2.29), and (14.32±2.13) respectively. One way ANOVA test showed that there was statistically significant difference (P=0.003) when comparing the all treated groups at P<0.05, while there was no statistically significant difference P (0.003) in comparison between group I, group II & group IV. In addition, there was no statistically significant difference (P=0.2490) in calcium concentration when comparing the all treated groups. Phosphorous weight % revealed statically significant difference between all treated groups (P=0.000) at P-value 0.05 with mean values (28.88±0.32), (27.77±0.50), (27.09±0.34), and (26.82±0.73) in groups I, II, III and IV respectively. However, there was no statically significant difference P (0.000) between group III (27.09±0.34) and group IV (26.82±0.73). Neither calcium to phosphorus ratio nor calcium: Phosphorus weight % ratio showed statistically significant difference with a p-value (P=0.1543) and (p=0.1525) respectively. Table (2)

**Table (2) Comparison between elements of the four groups using one way ANOVA test**

<table>
<thead>
<tr>
<th>Groups</th>
<th>F k</th>
<th>P k</th>
<th>Ca k</th>
<th>Ca/P</th>
<th>Ca/P %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>14.32±2.13b</td>
<td>28.88±0.32e</td>
<td>57.66±2.24e</td>
<td>1.99±0.06e</td>
<td>1.99±6.37e</td>
</tr>
<tr>
<td>Group 2</td>
<td>13.57±2.29b</td>
<td>27.77±0.50b</td>
<td>58.67±2.18a</td>
<td>2.11±0.08e</td>
<td>211±8.23a</td>
</tr>
<tr>
<td>Group 3</td>
<td>17.59±1.33a</td>
<td>27.09±0.34c</td>
<td>55.31±1.30a</td>
<td>2.04±0.19e</td>
<td>204±19.04e</td>
</tr>
<tr>
<td>Group 4</td>
<td>14.62±1.91b</td>
<td>26.82±0.73c</td>
<td>56.89±6.66a</td>
<td>2.12±0.25c</td>
<td>212±25.22c</td>
</tr>
<tr>
<td>P- value &lt;0.05</td>
<td><strong>0.003</strong></td>
<td><strong>0.000</strong></td>
<td><strong>0.2490</strong> ns</td>
<td><strong>0.1543</strong> ns</td>
<td><strong>0.1525</strong> ns</td>
</tr>
</tbody>
</table>

**; Significant difference at P-value 0.05 and different letters at the same column means significant difference between groups at P-value 0.05; means non-significant difference between groups**

![Fig. (1) Scanning Electron Micrograph (SEM) x12000: Group I (A), group II (B), group III (C) and group IV (D) ](image-url)
DISCUSSION

Damato et al. (24) mentioned that there is a cut-off point of 500 ppm NaF where any concentration below would not remineralize the artificially demineralized enamel lesions. Furthermore, the authors also concluded that higher fluoride concentrations did not produce any further significant increase in remineralization when compared to the 500 ppm NaF. Moreover, Lima et al. (25) suggest the dose response to fluoride concentration has no effect on its anti-caries effect, so the 500 ppm of NaF was similar to the 1100 ppm NaF when used in children.

Arginine was selected as the remineralizing agent in this study because it inhibits caries progression via inhibiting biofilm formation and facilitates enamel remineralization.

Ten Cate et al. (28) reported that the amino acid Arginine, can be incorporated into toothpaste for remineralization of early caries lesions, and for preventing the development of new lesions. This technology is based on the mechanism that when arginine is used, the amino acids will be deaminated by the arginine deaminase enzyme system in saliva. Thus producing highly alkaline ammonia, causing a rise in pH within the oral environment, creating an ideal condition for remineralization as well as modifying and reducing the pathogenicity of the cariogenic plaque.

One of the objectives of this study was to evaluate the micro-hardness of an enamel treated surface, which is considered an important mechanical characteristic of the material. The result of this study revealed that the microhardness of enamel on the samples in group I (artificial saliva) did not increase significantly. This may be due to the differences in the formula of artificial saliva, immersion time, type of teeth and study design. This finding is consistent with the results of Gedalia et al., (29) Cheng et al., (30) and Hua et al., (31) who did not show a significant rehardening effect of artificial saliva. On other hand, several studies showed that artificial saliva can reharden demineralized enamel Devlin et al., (32), and Lussi et al., (33) who reported that artificial saliva containing potassium chloride, magnesium chloride, calcium chloride and dipotassium hydrogen phosphate, can reharden demineralized enamel.

Surface microhardness of enamel was significantly higher in group II (sodium fluoride (NaF) 500ppm) when compared to group I.

These findings imply that the use of sodium fluoride NaF (500ppm) results in the deposition of a protective layer of calcium fluoride on the surface of initial caries lesions, and this layer increases surface hardness. This was in agreement with the results of Sivapriya et al. (34) who evaluated the remineralization ability of NaF on the microhardness of enamel, dentine, and dentinoenamel junction. Then they found long-term repeated application of sodium fluoride (226 ppm) can improve the microhardness of demineralized dental tissues on enamel, dentin, and DEJ-axial zone, except in the DEJ-cusp tip, and DEJ-center of fissure. Another systematic review by Bergstrand (35) found that in methods of prevention and treatment of white spot lesions (WSLs), they were able to provide evidence supporting the optimal efficacy of fluoride products for prevention of white spot lesions (WSLs).

Regarding the surface microhardness it was found that group III (treated with arginine 2.5%+sodium fluoride (NaF500ppm)) had the highest level of microhardness. This was in agreement with Cheng., et al., (30) and Oliveira, et al., (36) in which results showed that fluoride and arginine could increase the microhardness of sound and demineralized bovine enamel surfaces. It is possible that arginine-fluoride complexes could have been stored within enamel,
as an arsenal reservoir that would release fluoride upon acid challenge, in favor of remineralization.

Moreover, group IV (treated with arginine 8%+sodium fluoride (NaF 500ppm) has a high value of microhardness which was in agreement with the study done by Pengcheng et al. (37) who found that the addition of (8.0% arginine ±0.14% sodium monofluorophosphate) increased the microhardness significantly.

The environmental scanning electron microscope (SEM) evaluated and recorded the ultra-structural changes of the enamel surface, as it is a non-destructive characterization technique, which requires little or no sample preparation.

Only samples treated with artificial saliva showed an increase in interprismatic space, with change in normal architecture of enamel due to the effects of the demineralizing agent which increased enamel porosity. This agrees with Rirattanapong et al. (38) and Vidya et al. (39) who found very minimal remineralization of enamel surface as saliva has some remineralization potential.

Also specimens treated with sodium fluoride showed increased amounts of deposits when compared to group I (artificial saliva), which proves the remineralizing effects of NaF. This was proven by the presence of an opaque deposit on enamel surfaces that closed some interprismatic spaces as the fluoride incorporated into the hydroxyapatite structure of tooth enamel. This finding may be due to presence of fluoride in enamel in the form of fluoridated apatite, or CaF2-like minerals. Calcium fluoride is the main product formed after the application of a topical fluoride which has the tendency to be transformed into fluoridated apatite.

Moreover, samples treated with NaF (500ppm) + arginine solution 2.5% (group III) showed a smooth surface, with almost complete obtrusion of inter-rod spaces in some fields, and in group IV (8% arginine+500ppm NaF treated group) evenly distributed opaque color on the micrographs is noticed, as well as improvements of the enamel ultrastructure. So the specimens manifested a smoother enamel surface with decreased number and size of previously formed pores compared to other group’s this could indicate an increase in mineral density comparable to group III.

This issue was not discussed in any previous literature for 2.5%, 8% Arginine. However, such changes may be explained also by the fact that arginine promotes fluoride uptake to demineralized enamel surfaces, as well as formation of calcium fluoride globules, which makes a remineralization by deposition of globules in the enamel surface.

Energy dispersive x-ray spectroscopy (EDX) was used for assessing F, Ca, and P weight percentage in different groups. Since the main components of hydroxyapatite are Ca and P, these elements were the main objects of this study. Monitoring Ca/P ratio changes were done for all samples in the four groups. Also, the availability of calcium and phosphorus in the oral environment is a key requisite in the remineralization of enamel as well as microhardness.

The negative control group (artificial saliva), showed the least values of remineralization due to the low ion concentration gradient from saliva into the lesion, precipitating only superficially, and preventing the remineralization process from occurring in the body of the lesion, thus saliva fails to initiate the process of increasing the levels of calcium and phosphate delivery, compared to the other remineralizing agents (41-43).

EDX results provides a specific method to evaluate the concentration of chemical elements on substance surfaces in this study. There were significant increases in F (wt %) between control
and experimental groups, agreeing with Cheng et al.\(^{(30)}\) who proved that the interaction of arginine and fluoride on the remineralization of artificial enamel caries lesions in vitro. Arginine solution promoted remineralization compared with deionized water control. In addition, they concluded that when used in combination with fluoride, arginine significantly increased in fluoride uptake compared with fluoride alone, and the lesions treated with arginine-containing toothpaste also showed superior fluoride uptake compared with those treated with conventional fluoride toothpaste. This may be due to the increase in fluoride accelerating the absorption of calcium and phosphorus, and thereby increasing the deposition of crystals.

On the other hand, Rege et al.,\(^{(44)}\) found that arginine could promote the absorption of fluoride through the combination of arginine’s positive charge, and fluoride’s negative charge. Under this interpretation, the arginine and fluoride within the 8% arginine toothpaste acted in synergism to promote better remineralization of the enamel. Bijle et al.,\(^{(45)}\) proposed that the incorporation of 2% arginine in NaF toothpaste significantly increased the remineralization of enamel caries-like lesions when compared to NaF toothpaste; while 4% and 8% arginine in NaF toothpastes were ineffective in improving enamel remineralization, which disagreed with our results regarding the concentration of 8% particularly.

**CONCLUSION**

The present study concluded that both concentrations of arginine (2.5% & 8%) increase fluoride uptake of demineralized enamel, and show areas of mineralized deposits and improvement of enamel ultrastructure.

**RECOMMENDATIONS**

Arginine (2.5%, 8%) is recommended to be used as new efficient therapeutic for remineralization and for increasing fluoride uptake of demineralized enamel surfaces. Further in-vivo studies are needed to investigate actual effects of arginine on enamel for longer duration and further microbiological studies are required to evaluate antibacterial effect of Arginine.

**REFERENCES**


