Comparative Analysis of the Remineralization Potential of Different Active Ingredients Based Toothpastes using SEM/EDX- An in vitro study

SUMMARY

Background/Aim: The principal purpose of this study was to investigate the efficacy of different active ingredients to inhibit demineralization around the margins of cervical cavities in natural teeth by scanning electron microscopy and energy dispersive X-Ray elemental analysis (SEM-EDX). Material and Methods: Thirty-two sound human molars were used. Box-shaped cavities were prepared along the cemento enamel junction (CEJ). The samples were immersed in a demineralization solution (pH=1) maintained for 72 hours and randomly divided into 4 groups. Surfaces according to the groups were treated with potassium nitrate, arginine and calciumsodiumphosphosilicate containing remineralization agents for 14 days, respectively and the samples in control group were submitted to toothbrushing with deionized water. The samples were analyzed by using SEM-EDX analysis. Data was statistically analyzed using by one way ANOVA (analysis of variance) test and LSD (least significant difference) test for comparison between means at a significance level of 0.05. Results: SEM-EDX elemental mapping was used to evaluate the degradation from depth profiles of fluoride (F), Calcium (Ca), and phosphate (P) leaching. Micromorphological and elemental analyses were done using SEM and EDX. SEM EDX Analysis on enamel showed a significant difference between the groups except the control group (p<0.05). The dentine results showed significant differences between the control group and all other groups (p<0.05). Elemental analysis showed significant differences in Ca weight percentage among the first and second observation levels in all groups (p<0.05). Conclusions: In conclusion, all tested toothpastes showed some ability to resist demineralization at the margins. The groups except control group showed better outcomes compared with the other tested samples.

Key words: Remineralization, in vitro, elemental mapping, SEM/ EDX.

Introduction

The caries process does not involve a one-way demineralization process cycle. This process is based on errors in the succession of demineralization and remineralization cycles1. Primarily, demineralization occurs when there is loss of minerals from the dental hard tissues before cavitation occurs. However, remineralization of the lesion occurs when the direction of calcium and phosphate minerals is reversed and they move inwards rather than outwards2. The treatment method, which includes a remineralization protocol for early caries lesions, has a great potential for advancement in restorative clinical treatments3. It has been claimed that products containing calcium, phosphate and fluoride in their biologically available forms increase remineralization compared to products containing only fluoride, and even that bioactive
materials containing fluoride-free calcium phosphate provide results equivalent to the remineralization potential of fluoride-containing materials.  

Calcium-based toothpaste systems are prepared by reacting minerals using a mechanochemical ball milling process to create a functionalized remineralization agent. With the latest developments in nanotechnology, the size of the particles has generally decreased to 0.1 to 100 nm, and some changes in their shape allow to obtain high bioactive calcium, phosphate compounds with a higher potential to penetrate the pores of the demineralized area with the potential for remineralization. Scanning Electron Microscopy has been a useful tool for research in dentistry, allowing images to be viewed at high magnification (50X-10,000X and above). It is used in conjunction with Energy dispersive X-ray analysis, which is a microanalytical technique for the quantitative estimation of calcium phosphate ratio.

The aim of this study was to evaluate the remineralization capacity of different active ingredients containing toothpastes on artificial subsurface lesions.

### Material and Methods

Our study is an in vitro experimental study conducted at Marmara University Faculty of Dentistry. The research project was approved by Marmara University Faculty of Dentistry Ethics Committee, Istanbul, Turkey. Informed consent was obtained before starting the research.

To make our research more reliable and reproducible, a pilot study was conducted with 16 enamel samples to evaluate the remineralization capacity by SEM/EDX analysis. The values obtained from the enamel samples were evaluated by researchers specialized in SEM/EDX Analysis. Forty enamel samples were prepared from extracted human molar teeth using a low speed diamond disc. Teeth with fractures on crowns or roots, carious lesions including white spot lesions and initial caries, determined hypoplastic lesions by visually, the teeth with developmental damage or any deformity, and teeth with any restoration regardless of size were excluded from the study. Prepared enamel samples were analyzed for Ca mineral content (% weight) using SEM-EDX analysis.

The samples were soaked in a demineralizing solution containing 20 ml of acid buffer containing 2 mmol/L Ca²⁺, 2 mmol/L PO₄³⁻ and 0.075 mol/L acetate at pH 1 for 48 h at 37°C. All samples were analyzed about weight percentage using SEM-EDX on the third day to control of mechanism of mineral content loss. The samples were classified as two groups: Group 1 contained 10 samples (control group) and group 2 contained 30 specimens (study group). The study group was subdivided into three groups of 10 specimens per group. After demineralization procedure, the samples in control group were brushed with distilled water two times a day for two minutes as the optimum time of toothbrushing. The samples in study group were also applied by three different toothpastes twice a day for two minutes during 28 days. The samples in group 2 were brushed with the %8 arginine containing toothpaste (Colgate), the samples in group 3 were brushed with potassium nitrate containing toothpaste (Concentrate Smile), the samples in group 4 were brushed with calciumsodiumphosphosilicate containing toothpaste (Sensosdyne) by the following the same procedure with the control group. The SEM EDX measurements were done twice as after demineralization procedure and after remineralization procedure.

### Statistical Analysis

The suitability of numerical variables for normal distribution was tested with the Shaphiro Wilk test. Paired t test was used to compare two dependent measurements of normally distributed variables, and Wilcoxon test was used to compare non-normally distributed variables in two groups. (Table 2 and 3) ANOVA and LSD tests were used to compare normally distributed variables in four groups, and Kruskal Wallis and Dunn tests were used to compare non-normally distributed variables in four groups. (Table 1) (p<0.05) SPSS 22.0 <0.05 was considered significant.

#### Table 1. The one way analysis of variance test evaluation with four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Ca weight 1</th>
<th>Ca weight 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>8.97 ± 2.12</td>
<td>9.95 ± 0.78</td>
<td>0.289</td>
</tr>
<tr>
<td>Group 2</td>
<td>32.68 ± 4.72</td>
<td>35.69 ± 4.55</td>
<td>0.015</td>
</tr>
<tr>
<td>Group 3</td>
<td>16.02 ± 3.26</td>
<td>29.39 ± 4.55</td>
<td>0.018</td>
</tr>
<tr>
<td>Group 4</td>
<td>16.02 ± 3.26</td>
<td>32.92 ± 2.40</td>
<td>0.043</td>
</tr>
</tbody>
</table>

#### Table 2. The wilcoxon test evaluation in two groups (Ca weight detected for the first measurement)

<table>
<thead>
<tr>
<th>P</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control group-Concentrate smile</td>
<td>0.165</td>
</tr>
<tr>
<td>Control group-Sensodyneprotection</td>
<td>0.012*</td>
</tr>
<tr>
<td>Control group-Colgate Pro</td>
<td>0.001*</td>
</tr>
<tr>
<td>Concentrate smile-Sensodyneprotection</td>
<td>0.262</td>
</tr>
<tr>
<td>Concentrate smile-Colgate Pro</td>
<td>0.012*</td>
</tr>
<tr>
<td>Sensodyneprotection-Colgate Pro</td>
<td>0.165</td>
</tr>
</tbody>
</table>

#### Table 3. The wilcoxon test evaluation in two groups (Ca weight detected for the second measurement)

<table>
<thead>
<tr>
<th>P</th>
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<tbody>
<tr>
<td>Control group-Concentrate smile</td>
<td>0.001*</td>
</tr>
<tr>
<td>Control group-Sensodyneprotection</td>
<td>0.001*</td>
</tr>
<tr>
<td>Control group-Colgate Pro</td>
<td>0.001*</td>
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<tr>
<td>Concentrate smile-Sensodyneprotection</td>
<td>0.126</td>
</tr>
<tr>
<td>Concentrate smile-Colgate Pro</td>
<td>0.011*</td>
</tr>
<tr>
<td>Sensodyneprotection-Colgate Pro</td>
<td>0.223</td>
</tr>
</tbody>
</table>
Results

Our study evaluated the remineralization potential of three different toothpastes based on different remineralizing materials in artificially created carious lesions visually and qualitatively in enamel by SEM-EDX analysis. Energy-dispersive X-ray analysis was used to determine calcium content as a percentage of the weight of demineralized, and remineralized enamel in each group. It shows the remineralization capacities of the pastes and the elemental analysis based on the measurements made after the demineralization and remineralization processes. It also shows the comparison of the alteration in average Ca weight ratios of sound enamel, demineralized and remineralized enamel samples (Figures 1-8).

Figure 1. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 1)
Figure 2. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 2)
Figure 3. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 3)
Figure 4. SEM and Elemental analysis of sound enamel sample by EDX (first measurement for group 3)
Figure 5. SEM and Elemental analysis of sound enamel sample by EDX (second measurement for group 1)
Figure 6. SEM and Elemental analysis of sound enamel sample by EDX (second measurement for group 2)
Figure 7. SEM and Elemental analysis of sound enamel sample by EDX measurement group 3)
Figure 8. SEM and Elemental analysis of sound enamel sample by EDX (second measurement for group 4)
Discussion

In our study, the remineralization potential evaluated based on calcium mineral loss according to the measurements done before and after remineralization in artificially created carious lesions in sound enamel tissue, was determined using SEM-EDX. Arginine is a natural peptide found in human saliva. Arginine is secreted in free form with an average distribution of 50 μM. Arginine is also a regulator of structured ammonia metabolism. Many cellular bacteria, including oral streptococci, lactobacilli, and spirochetes, catabolize arginine to ornithine, ammonia, and CO₂. Ammonia production can also be optimal as a therapeutic home treatment to relieve sensitivity, it was shown that the use of fluorinol was more effective than calcium sodium phosphosilicate. In this study, the effectiveness of fluorinol varied over time and reached its maximum level in the 3rd and 4th weeks. According to the study performed by Freda et al., calciumsodiumphosphosilicate, Novamin as its trade name, has a great remineralization mechanism on the enamel and acquire a valuable prevention against the superficial subsurface lesions.

Conclusions

The use of micro-particles like arginine, potassium nitrate, calcium sodiumphosphosilicate in toothpastes show ability to increase the remineralization potential of the enamel and acquire a valuable prevention against the superficial subsurface lesions.

References


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**Conflict of Interests:** Nothing to declare.

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**Animal Rights Statement:** None required.

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