The Impact of Nano-Hydroxyapatite Resin Infiltrant on Enamel Remineralization: An In Vitro Study

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This study assessed the effect of nano-hydroxyapatite incorporation into resin infiltrant on the mineral content, surface tomography, and resin tag penetration of demineralized enamel. Forty specimens were exposed to a demineralized solution to form subsurface caries lesions. The lesions were treated with negative control, a resin infiltrant (ICON), ICON with 5% nano-hydroxyapatite (NHA, Sigma-Aldrich), or ICON with 10% NHA. Mineral density was assessed using microcomputed tomography scans at various stages of the experiment. Specimens were scanned by scanning electron microscope (SEM) for surface analysis and resin tag penetration. Analysis of variance was used to assess the difference among groups. Specimens treated with ICON and 5% or 10% NHA showed the most favorable mineral density regarding the percent change in mineral content (32.4% and 29.7%, respectively), compared to 8.8% in teeth treated with ICON alone and –1.8% in teeth in the control group. SEM showed that teeth treated with ICON or ICON with 5% or 10% NHA had a smooth surface. The resin penetration in all tested groups showed high-quality resin tags, regardless of the treatment protocol. NHA resin infiltrant (ICON with 5% or 10% NHA) effectively enhanced the artificial enamel caries surfaces in terms of smooth surfaces, mineral density, and resin penetration.


Enamel demineralization is an initial sign of dental caries that endangers tooth health and esthetics. Remineralization of demineralized lesions can arrest and restore the lesion to healthy enamel tissue. The prevention of dental caries and remineralization of enamel subsurface lesions before restorative intervention is the goal of modern dentistry. The treatment of these lesions is usually accomplished via fluoride treatment. Nonetheless, chronic low fluoride exposure may raise concerns in some patients related to possible associations with bone problems, birth anomalies, cancer, gastrointestinal tract complications, and dental fluorosis. Therefore, it is necessary to seek effective nonfluoride alternatives to caries treatment.

Resin infiltration is an alternative methodology to arrest initial caries lesions. The infiltration technique relies on hydrophilic and low-viscosity light-cured resins that penetrate subsurface micropores to inhibit the diffusion of cariogenic bacteria and their products, thus preventing further caries progression. The American Dental Association describes resin infiltration of incipient smooth-surface lesions as a resin infiltration technology that penetrates, seals, strengthens, and stabilizes demineralized enamel to prevent the progression of incipient caries lesions and remove...
cariogenic white spots, as well as conceal visible white spots. Lately, many benefits of the resin infiltration technique have been highlighted: mechanical stabilization of demineralized enamel, permanent plug of superficial and deeply porous areas, preservation of hard substances, the arrest of lesion progress by increasing resistance to demineralization, minimized risk of secondary caries development, and increased patient approval.6-8 A previous study verified that resin infiltration leads to increased microhardness of carious enamel.5

Nano-sized particles can be dispersed in higher concentrations and polymerized into the resin system to increase filler loading of composite material. Nano-hydroxyapatite (NHA) is one of the most biocompatible and bioactive materials, and its nano-sized particles are similar to tooth enamel in morphology and crystal structure. NHA was reported to remineralize artificial caries lesions following its incorporation into toothpaste and mouth rinse.9-12

Microhardness methods are usually used to determine mineral gain and loss, but these methods are destructive to enamel. In cases of carious tooth structure with fragile surfaces, it is difficult to prepare sections without destroying the surface layer. Microcomputed tomography (micro-CT) can evaluate tooth samples nondestructively and in three dimensions,13,14 allowing the assessment of the effect of NHA on demineralized enamel.

The aim of the present study was to assess the effect of NHA incorporation into resin infiltrant on the mineral content of demineralized enamel and its surface tomography and to compare the penetrability of the tested resin infiltrant. The null hypothesis was that the incorporation of NHA into resin infiltrant will affect neither the mineral content of demineralized enamel lesions nor their surface topography, and the penetrability of NHA resin infiltrant will not be disturbed.

**Materials and Methods**

**Tooth Selection**

Before conducting the study, patient consent was secured from all subjects, and ethical approval was obtained. Extracted teeth from adult patients were collected and visually examined with ×30 magnification, and teeth were excluded if they showed any of the following: caries, defect areas, cracks, and/or enamel structure abnormality.

**Specimen Preparation**

A free online sample size calculator was used to design the study sample size calculations (http://www.sample-size.net/means-effect-size/). The sample size calculation to obtain 80% power and alpha = .05 was 10 for each group.

Twenty freshly extracted human, sound permanent premolars were stored for 10 days at 4°C in tap water with the addition of thymol crystals dissolved in 4 mL of alcohol to prevent bacterial growth. Soft tissue remnants and calculus were cleaned with an ultrasonic scaler. Teeth were polished for 3 seconds with nonfluoride pumice and a rubber cup. After pumicing, teeth were rinsed for 10 minutes in tap water and dried using tissue paper for 3 minutes at room temperature.

All teeth were sectioned centrally, parallel to their long axis, and in a buccolingual direction with an IsoMet Low Speed Saw (Buehler). Then, the crowns were separated from the roots at 1 mm cervical to the cementoenamel junction. The 40 specimens were coated with a thin coat of acid-resistant varnish, exposing a 4-mm enamel window in the center of the buccal and lingual surfaces. All specimens were immersed for 12 hours before demineralization in distilled water.

**Demineralization Procedure**

Demineralizing solution was freshly prepared, changed daily, and used to form subsurface caries lesions. The demineralizing solution consisted of 2 mM Ca (Ca[NO₃]₂), 2 mM PO₄ (KH₂PO₄), and 75 mM acetate at pH 4.3. The specimens were cycled twice daily for 21 days between freshly prepared artificial saliva (pH 6.8) and the demineralizing solution. The specimens were stored in an incubator with a constant temperature of 37°C.12,15

The demineralized lesions in all specimens were treated according to their assigned group. Then, all specimens were stored in artificial saliva for 24 hours before micro-CT scanning.
Treatment Procedure

After the pH cycle procedure, the 40 specimens were blindly and equally divided into the following four groups (n = 10 per group) using a sealed opaque envelope: a group left untreated and used as the negative control (Group I); a group treated with resin infiltrant (ICON, DMG) with 5% NHA (Group II); a group treated with ICON resin infiltrant with 10% NHA (Group III); and a group treated with ICON resin infiltrant without NHA (Group IV).

NHA resin infiltrant was prepared by adding NHA powder (Sigma-Aldrich) to the resin infiltrant (ICON) at 5% and 10% ratio by weight using an electric digital scale (AG245, Mettler). The powder and resin were mixed by a dual asymmetric centrifugal laboratory vacuum mixer system (DAC 150 Speed Mixer, FlackTek) to prevent voids.

Surface Analysis and Resin Penetration Measurements

After mineral density assessment, the specimens from each group were analyzed by scanning electron microscopy (SEM) to assess surface topography and to measure resin penetration. For SEM, the samples were mounted on metallic stubs with double-faced cohesive carbon tape. The specimens were gold-coated to minimize the charging effect and improve image quality. SEM was done at an accelerating voltage of 20 kV and working distance of approximately 10 mm. Micro-CT scans were also recorded to assess enamel surface topography. For resin penetration measurements, the length of the resin tag was recorded in micrometers (µm) at least 10 times, and the average was recorded for each specimen from the different tested groups.

Statistical Analysis

The percent change in mineral content in each group was calculated using the following formula:

\[
\text{change in mineral content} = \left(\frac{(\text{mineral content after treatment} - \text{mineral content after demineralization})}{\text{mineral content after demineralization}}\right) \times 100
\]

Normality was assessed using Kolmogorov-Smirnov tests and normality plots. The difference among the study groups in change in mineral content was assessed using a general linear univariate model, controlling for the mineral content before demineralization. The difference in mineral content per group between postdemineralization and posttreatment was assessed using paired t test. Repeated measures analysis of variance (ANOVA) was used to assess the change in mineral content and resin penetration in the four groups. Statistical analysis was performed using SPSS (version 23, IBM).

Results

Table 1 and Figure 1 show the mineral content of the four study groups at various time points. The mineral content followed a quadratic function (\(P < .0001\)), with a significant drop from baseline after demineralization and significant increase (\(P < .0001\)) after treatment.

There was a significant interaction (\(P < .0001\)) between change across time and group, but the control group did not follow this pattern. The difference in mineral content between postdemineralization and posttreatment was not statistically significant (\(P = .35\)).

The percent change in mineral density after treatment was significantly higher in the specimens treated with ICON with 5% and 10% NHA (Groups II and III, respectively) (\(P < .0001\)) than the control group specimens (Group I) and specimens treated with ICON alone (Group IV) (\(P < .0001\)). The percent change in mineral density in the two groups treated with ICON with NHA was not significantly different (\(P = .71\)). Teeth treated with ICON alone had a significantly greater percent change.
Changes in the surface properties were evident at different stages of demineralization, as shown by SEM (Fig 2) and micro-CT (Fig 3). Microphotographs taken before demineralization showed normal, smooth enamel surfaces in all groups; microphotographs taken after demineralization showed irregular surfaces with loss of some surface enamel. A few specimens also showed an etching pattern after demineralization. In the noncontrol groups, a regular surface was seen after treatment; the treated enamel had a smoother and better-sealed surface than the demineralized surfaces. Regarding penetration, means and standard deviations were computed for each group as part of descriptive statistics. Non-significant results from Kolmogorov and Shapiro-Wilk tests confirmed that the data were normally distributed; thus, parametric tests were used for analysis. ANOVA was used to assess significance in difference in means between the three groups. \( P < .05 \) was considered statistically significant. ANOVA results did not show any significant difference in the means between the three groups (\( P = .629 \)) (Tables 2 and 3, Fig 4).

**Discussion**

There are various direct and indirect methods for evaluating tooth mineral content. Radiographic microtomography is nondestructive and can be used to assess the mineral concentration in mineral density than teeth in the control group (\( P = .001 \)).

Changes in the surface properties were evident at different stages of demineralization, as shown by SEM (Fig 2) and micro-CT (Fig 3). Microphotographs taken before demineralization showed normal, smooth enamel surfaces in all groups; microphotographs taken after demineralization showed irregular surfaces with loss of some surface enamel. A few specimens also showed an etching pattern after demineralization. In the noncontrol groups, a regular surface was seen after treatment; the treated enamel had a smoother and better-sealed surface than the demineralized surfaces. Regarding penetration, means and standard deviations were computed for each group as part of descriptive statistics. Non-significant results from Kolmogorov and Shapiro-Wilk tests confirmed that the data were normally distributed; thus, parametric tests were used for analysis. ANOVA was used to assess significance in difference in means between the three groups. \( P < .05 \) was considered statistically significant. ANOVA results did not show any significant difference in the means between the three groups (\( P = .629 \)) (Tables 2 and 3, Fig 4).

**Table 1 Comparison of Mineral Density and Percent Change in Mineral Density Among the Study Groups**

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before demineralization</td>
<td>0.27 (0.01)</td>
<td>0.28 (0.01)</td>
<td>0.28 (0.01)</td>
<td>0.27 (0.01)</td>
</tr>
<tr>
<td>After demineralization</td>
<td>0.18 (0.01)</td>
<td>0.19 (0.02)</td>
<td>0.20 (0.01)</td>
<td>0.19 (0.02)</td>
</tr>
<tr>
<td>After treatment</td>
<td>0.17 (0.01)</td>
<td>0.28 (0.01)</td>
<td>0.28 (0.02)</td>
<td>0.21 (0.01)</td>
</tr>
<tr>
<td>( P ) (paired t test)</td>
<td>.35</td>
<td>&lt; .0001*</td>
<td>&lt; .0001*</td>
<td>.01*</td>
</tr>
<tr>
<td>Percentage change</td>
<td>–1.77 (3.73)</td>
<td>32.44 (4.70)</td>
<td>29.69 (3.34)</td>
<td>8.83 (4.54)</td>
</tr>
</tbody>
</table>

Group I = untreated negative control; Group II = ICON resin infiltrant (GMS) with 5% nano-hydroxyapatite (NHA); Group III = ICON with 10% NHA; Group IV = ICON only.

Overall \( F \) of ANOVA controlling for mineral content before demineralization = 84.91 (\( P < .0001 \)). \( F \) of ANOVA for treatment groups = 100.01 (\( P < .0001 \)).

Data are presented in percentages as mean (SD). Different superscript letters denote statistically significant differences.

*Statistically significant (\( P < .05 \)).
Fig 2  Surface morphology of the representative enamel samples from each study group, examined by SEM (×4,000 magnification): (a) artificial caries induced without treatment (Group I), (b) treated with ICON with 5% NHA (Group II), (c) treated with ICON with 10% NHA (Group III), and (d) treated with ICON only (Group IV).

Fig 3  Radiographic microtomography scans of representative enamel surface topographies in different experimental groups and stages (first scan = before demineralization; second scan = after demineralization; third scan = after treatment): (a) artificial caries induced without treatment (Group I); (b) treated with ICON with 5% NHA (Group II); (c) treated with ICON with 10% NHA (Group III); and (d) treated with ICON only (Group IV).
at the micron level in three dimensions. This approach can differentiate between sound/demineralized and remineralized enamel with high accuracy. The specimens are not destroyed and can be rescanned to sequentially evaluate and compare the mineral density of the sample throughout the different experimental stages. Accordingly, this method can assess the mineral density gain or loss with high accuracy.

The pH cycling model offers a valid stimulation of the caries process and is more reliable for the clinical condition vs demineralization and remineralization in separate studies. In the present article, the pH cycling model was used. The endogenous repair of enamel is physiologically impossible because of the lack of stem cells that are able to become enamel-forming cells (ameloblasts). For this reason, many attempts are made to induce enamel remineralization by exogenous agents such as fluoride, bioactive glass, and NHA.

NHA has been widely used in dentistry due to its biocompatibility, bioactivity, and similarity in structure to hard dental tissues. The remineralizing potential of NHA to incipient caries lesions has been validated in a previous study. Several investigations were done to determine the optimal concentration of NHA, and 10% NHA was found to be optimal for enamel remineralization. In the present study, NHA combined with resin infiltrant showed a positive impact on demineralized enamel.

Changes in the surface properties were evident in different stages of the experiment, based on the micro-CT and SEM analyses. All

### Table 2 Mean Resin Penetration for Each Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean, µm</th>
<th>SD, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>118.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Group III</td>
<td>117.6</td>
<td>5.2</td>
</tr>
<tr>
<td>Group IV</td>
<td>116.9</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*Group II = ICON resin infiltrant (GMS) with 5% nano-hydroxyapatite (NHA); Group III = ICON with 10% NHA; Group IV = ICON only.*

### Table 3 ANOVA Results Comparing Resin Penetration Among the Study Groups

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>20.600</td>
<td>2</td>
<td>10.300</td>
<td>0.471</td>
<td>.629</td>
</tr>
<tr>
<td>Within groups</td>
<td>590.200</td>
<td>27</td>
<td>21.859</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>610.800</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA = analysis of variance.

**Fig 4** Radiographic microtomographic view of a representative Group II enamel surface and resin tag penetration in enamel lesions after treatment.
microphotographs before demineralization showed normal enamel surfaces (consistently smooth) in all groups; however, microphotographs after demineralization showed irregular rough surfaces and the loss of some surface enamel. An etching pattern was recorded in a few specimens.

A regular, smooth enamel surface was observed after treatment in all tested groups. The treated enamel had a smoother and better-sealed surface than the demineralized surfaces. The present tested treatment regimens on the demineralized enamel surface filled and sealed the porous structure of the lesions, leading to smoother surfaces (Figs 2 and 3). Several studies demonstrated the efficacy of the infiltration treatment technique using ICON for noncavitated caries lesions.4,5,25

Previous studies stated that the original aim of caries infiltration is to arrest lesion progression by replacing lost minerals with resin and occluding microporosities.26 The term “remineralization” has previously been used to describe mineral gain, including precipitation of mineral onto enamel surfaces.27 Based on the present results, ICON treatment did not inherently highly impact the mineral density of the demineralized enamel, which means that the remineralization process did not successfully occur; however, incorporation of NHA increased the mineral content, and ICON with 5% and 10% NHA (Groups II and III, respectively) showed the most satisfactory mineral density results.

The penetrability of the resin tags in the tested groups were both qualitatively and quantitatively acceptable compared to group IV, which revealed that the quality of resin penetration was good and unhampered by adding NHA.

The results of Groups II and III (with 5% and 10% NHA resin infiltrant, respectively) were very promising: Both the density and the quality of the resin tags were found to be adequate, and SEM serial photomicrography demonstrated that the morphologic characteristics of the resin tags penetrating demineralized enamel exceeded 100 µm. This result suggests that the nano-sized particles do not hinder resin penetration in the demineralized tissue and that the use of 15% hydrochloric acid gel (ICON) erodes the surface layer more effectively than 37% phosphoric acid, making it more porous and thereby increasing the penetration depth. Similar findings have been reported in studies utilizing resin infiltrants in white-spot lesions on permanent teeth, describing the considerable variation in penetration depths.28–31

In the present study, pretreating the enamel surface with 15% hydrochloric acid gel effectively degraded the highly mineralized surface layer and was deemed important to achieve high penetration depths by the resin infiltrant, which is in agreement with previous studies.32–34

The experimental group showed resin penetration comparable to the ICON group, suggesting that penetration is unhampered by structural differences. Therefore, NHA-reinforced resin infiltrant is a potential treatment method for demineralized enamel. The present study is the first evaluation of the experimental ICON-NHA resin infiltrant against enamel demineralization in an in vitro setting.

**Limitations**

The present study is limited by its in vitro design. Elements of the oral cavity may challenge the effectiveness of NHA and ICON.

**Conclusions**

Recently, noninvasive dentistry has become highly recommended. All tested groups showed enhanced artificial enamel caries surfaces with resulting smooth and regular surfaces. ICON treatment did not inherently have a significant impact on enamel mineral density. Experimental NHA resin infiltrant showed the most favorable enamel remineralization results.

Though promising, the resin infiltration technique requires more modification. The present results suggest that resin infiltration of enamel lesions should aim to arrest the progression of demineralization. Resin infiltrant combined with NHA as a substantial caries remineralizing agent may provide therapeutic benefits and may significantly reduce both long-term restorative needs and costs, thus achieving the concept of minimum intervention dentistry.
Acknowledgments

Extracted teeth from adult patients in the Oral Surgery Clinic of the Imam Abdulrahman Bin Faisal University were collected. Patient consent was secured from all subjects. Before conducting the study, an approval was gained from the Research Unit, College of Dentistry (EA 2018026). The authors do not have any financial interest in the companies whose materials are included in this article. The authors declare no conflicts of interest.

References


