Incorporation of nano-hydroxyapatite into experimental resin infiltrant and its performance on color stability and reinforcement in demineralized enamel: in vitro study

Jade Laísa Gordilio Zago (jadelgzago@gmail.com)
State University of Campinas

Gabriela Alves de Cerqueira
State University of Campinas

Robson Ferreira de Souza
State University of Campinas

Flávio Henrique Baggio Aguiar
State University of Campinas

Cínthia Pereira Machado Tabchoury
State University of Campinas

Giselle Maria Marchi
State University of Campinas

Research Article

Keywords: Dental caries, Demineralization, Nanoparticles, Resin infiltrants, Color stability

Posted Date: July 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3121663/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Objective

The aim of the study was to evaluate the influence of the incorporation of 10% nano-hydroxyapatite into an experimental resin infiltrant on color stability and cross-sectional microhardness.

Material and methods

135 blocks were divided into five groups: H: healthy; MB: white spot; I: Icon®; E: experimental; EH: experimental containing 10% nano-hydroxyapatite. For color evaluation (n = 15), CIEL*a*b* values were obtained at the following time points: T0 (before immersion), T1 (14 days after immersion), and T2 (28 days after). Data were applied to CIEDE2000 formula. Cross-sectional microhardness (n = 12) data from the MB, I, E, and EH groups were applied to the mineral loss formula (\(\Delta S\)). Polarized Light Optical Microscopy images were obtained (n = 5) at 40x magnification. Shapiro-Wilk test was used to assess data normality for color stability and mineral loss. One-way ANOVA analysis was performed, followed by Bonferroni's post hoc test (color stability) and Tukey's test (mineral loss).

Results

In color stability results, regardless of time, there was no significant difference between H and MB groups; at 14 days and at 28 days, MB differed from all infiltrated groups, and H differed from E and EH. For \(\Delta S\), MB group showed a significant difference compared to I and EH groups but did not differ from E.

Conclusion

E and EH showcased similar performance to I regarding color variation. In terms of \(\Delta S\), I and EH had less mineral loss, suggesting a reinforcement of the dental structure.

Clinical Relevance:

Predict color stability and structural reinforcement of resinous infiltrants applied to white spot lesions.

INTRODUCTION

Non-invasive methods are very important for the treatment of early-stage carious lesions, such as the white spot lesion, ideally being able to reinforce the structure weakened by demineralization caused by cariogenic biofilms [1]. Aiming to promote enamel remineralization, the most widely used and safe therapies are the topical application of fluoride and mouth rinses or tooth pastes containing stannous fluoride [2–4].

Besides the potential progression to cavitation when left untreated, early white spot lesions have a different refractive index from healthy enamel, hence being characterized by its whitish appearance that can cause aesthetic damage to the patient [5].
Considering the need to halt the lesion at an early stage and mask its coloration, resin infiltrants have
been recommended as treatment [6–8]. Their mechanism of action starts with the activity of 15% hydrochloric acid, which dilates the enamel microtubules to allow the material to penetrate [9]. As the commercially available infiltrant Icon is still patented by the manufacturer DMG its exact formulation is proprietary, but it is known to contain triethylene glycol dimethacrylate (TEGDMA) and additives.

These monomers are responsible for ensuring that the material has low viscosity and can penetrate the enamel microtubules. Through this penetration, the microtubules are occluded, leading to the interruption of the interaction between acids from the biofilm and the dental structure, thereby halting the lesion. Also, by filling the tubules, it masks the whitish coloration, providing an aesthetic result [6, 8, 10].

Icon presents a behavior similar to dental adhesives due to its composition, for this reason, Icon does not offer good color stability over time, as well as its flexural strength and physicochemical properties are inferior when compared to sealants and resins [7, 11]. However, several studies aim to improve these properties, such as with the incorporation of particles and alternative use of monomers [12–16].

Hydroxyapatite, abundantly present in the enamel and highly biocompatible, has been employed in various materials to improve their performance, as seen in sealants, resins, and adhesives [17–19]. The incorporation of hydroxyapatite into resin infiltrants has shown good results in terms of mineral density and a high degree of conversion, resulting in the good physical and mechanical performance of the material [11, 20].

However, since the infiltrant is a material that is intended to act within the dental structure, the methodology for evaluating it becomes challenging. Studies assessing their structural reinforcement are restricted to surface microhardness, confocal microscopy to verify its penetration, and quantification methods of chemical elements (EDS) [11, 20]. Frequently used in cariology studies, cross-sectional microhardness is employed to measure the internal hardness of the dental structure and therefore can be useful in evaluating the action of resin infiltrants, as demonstrated [21, 22].

Regarding color stability, there are studies that evaluate staining in several solutions and demonstrate that the material tends to become highly pigmented [23, 24]. Clinically, this would lead to the need for intervention due to its non-aesthetic appearance, as the resin would have a different coloring from the dental substrate.

Reflecting on new formulations for resin infiltrants and their proposed action, the present study aimed to evaluate the incorporation of nano-hydroxyapatite into experimental infiltrant, comparing it with a commercial infiltrant, an experimental infiltrant, and untreated surfaces. The study verified color stability against staining by an extrinsic solution, and internal reinforcement of the dental structure through cross-sectional microhardness and mineral loss calculation. The null hypotheses are: 1) the infiltrated groups will not differ in color stability from untreated surfaces, and 2) there will be no difference in mineral loss among the infiltrated groups compared to the white spot control.
MATERIALS AND METHODS

A total of 135 bovine incisors were obtained, cleaned using scalpel blades and pumice stone, and stored in a 0.1% thymol solution. Specimens following the 4x4 standard were prepared from these incisors using cutting disks in a metallographic cutter (Buehler LTD., Lake Bluff, IL, USA). The back surface was flattened using 600-grit sandpaper, and the buccal surfaces were polished with 600, 1200, and 2000-grit water sandpaper on a polisher (Arotec S/A Indústria e Comércio, Cotia-SP, Brazil) for 30 seconds each, with ultrasonic baths in between for 5 minutes. They were then manually polished with diamond paste on felt for 2 minutes. Finally, the varnish (Colorama, São Paulo-SP, Brazil) was applied to all lateral and back walls, leaving the buccal surface exposed. The groups were divided into healthy enamel, initial white spot lesion, Icon, Experimental, and Experimental containing 10% nano-hydroxyapatite (Table 1). They were allocated to the tests according to the flowchart 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (H)</td>
<td>No treatment</td>
</tr>
<tr>
<td>White spot (MB)</td>
<td>Superficial white spot lesion</td>
</tr>
<tr>
<td>Icon (I)</td>
<td>Resin infiltrant TEGDMA based, initiators and additives*</td>
</tr>
<tr>
<td>Experimental (E)</td>
<td>Experimental resin infiltrant: 75% TEGDMA; 25% Bis-EMA; 1% EDAB; 0.5% CQ</td>
</tr>
<tr>
<td>Experimental containing 10% nano-hydroxyapatite (EH)</td>
<td>Experimental resin infiltrant: 75% TEGDMA; 25% Bis-EMA; 1% EDAB; 0.5% CQ; 10% nano-hydroxyapatite</td>
</tr>
</tbody>
</table>

*According to manufacturer DMG, Hamburg, Germany.

Experimental resin infiltrants: ethoxy bisphenol A glycidyl dimethacrylate (Bis-Ema - Sigma Aldrich, St. Louis, EUA); triethylene glycol dimethacrylate (TEGDMA - Sigma Aldrich, St. Louis, EUA); camphorquinone (CQ - Sigma Aldrich, St. Louis, EUA); tertiary amine dimethylaminoethyl benzoate (EDAB - Sigma Aldrich, St. Louis, EUA); nano-hydroxyapatite (Sigma Aldrich, Steinheim, German)

Flowchart 1. Experimental design.

1. **Induction of initial superficial white spot lesion**

After obtaining the specimens, three surface microhardness readings were performed using a 50 g load for 10 seconds on the exposed surface of each specimen. The specimens were randomized into the healthy enamel group, and for the other groups, randomization occurred after a new surface microhardness reading following the demineralization-remineralization (DES-RE) protocol. The solutions was composed as described: DES 0.1 M acetate buffer containing 1.28 mM Ca, 0.74 mM Pi and 0.03 µg F/mL (3 mL/mm2) with pH = 5; RE composed of 1.5 mM Ca, 0.9 mM Pi, 150 mM KCl, 0.05 µg F/mL, 0.1M
Tris buffer, pH 7.0 (25). To simulate the demineralization cycle, the specimens were individually immersed in the DES solution for 4 hours at 37°C in an incubator, in an amount of solution in milliliters based on their surface area, then rinsed with deionized water and immersed in the RE solution for 20 hours. Seven cycles of DES-RE were performed, with each cycle corresponding to 24 hours, and on the eighth day the specimens remained in RE solution for 24 hours. The solutions were changed on the fourth day [25].

Specimens designated for color evaluation were not subjected to surface microhardness testing and were randomized before the DES-RE protocol based on the Lightness (L*) value obtained from three readings on each specimen using a light hanger with a spectrophotometer (Konica Minolta Sensing Americas, Inc, USA).

2. Experimental infiltrants formulation

The experimental infiltrant are composed of Bis-EMA (25%) and TEGDMA (75%) as the monomeric base, using Edab (1%), and camphorquinone as photoinitiator at 0.5% concentration [14, 26, 27]. All components were calculated for the manipulation of 5ml of the material and weighed on an analytical scale under a yellow light environment. They were then stored in sealed vials and taken to the high-frequency mixer SpeedMixer (FlackTeck, Inc, Landrum-SC, USA) for 5 minutes at 3000 rotations to ensure homogeneity.

For the manipulation of the material containing nano-hydroxyapatite, the same monomeric base described for the experimental group was used, the only difference being the addition of commercially acquired hydroxyapatite nanoparticles (Sigma-Aldrich, Steinheim, Germany) at a concentration of 10% [15, 17] so it could later be taken to the SpeedMixer. Prior to use, both materials were stirred in a magnetic stirrer (Model M089, Piracicaba-SP, Brazil) for 5 minutes.

3. Resin infiltrants application

To apply the material onto the specimen surfaces the protocol suggested by the manufacturer DMG (Hamburg, Germany) was followed. Etching was performed with 15% hydrochloric acid for 2 minutes, followed by thorough rinsing, Icon dry application for 30 seconds, infiltrant application for 3 minutes, photopolymerization (Valo corded, 395-480nm, irradiance 1000mW/cm², Ultradent, South Jordan-UT, USA), followed by a new 1-minute application and polymerization. Then finally polishing the surface using low-speed abrasive rubbers (TDV Dental, Pomerode-SC, Brazil) for 20 seconds.

4. Color stability

The specimens were fixed in acrylic plates with sticky wax on the back wall and sides, exposing only the buccal surface, and were immersed in a container with 1.5 liters of coffee solution (Mellita do Brasil Indústria e Comércio, Avaré-SP, Brazil) for a period of 28 days in an incubator at 37°C, with daily solution changes [23, 28]. For each color evaluation, the samples were removed from the acrylic plate, rinsed thoroughly with distilled water, color assessed, and then reattached to the plates.
The color evaluation was performed using a spectrophotometer (Konica Minolta Sensing Americas, Inc, USA) at three distinct time periods: (T0) before immersion, (T1) 14 days after immersion, and (T2) 28 days after immersion.

The data obtained from the CIEL*a*b* system, which correspond to L* for lightness, a* for chromaticity (red-green), and b* (blue-yellow), were used in the CIEDE2000 color variation formula (ΔE00) between the T0-T1, T0-T2, and T1-T2 intervals.

5. Cross-sectional microhardness

The specimens for this analysis were sectioned in the buccolingual direction right in the center of the sample using a cutting disk on a metallographic cutter (Buehler LTD., Lake Bluff, IL, USA). Half of each sample was randomly selected and all were placed in an embedding device (Arotec S/A Indústria e Comércio, Cotia-SP, Brasil) with the surface to be analyzed facing the metal platform to be embedded in clear acrylic resin (VIPICril Plus, Pirassununga-SP, Brazil). Once the stubs with the samples were obtained, they were taken to the polisher (Arotec S/A Indústria e Comércio, Cotia-SP, Brasil) where they were polished for 10 minutes at low speed with 600-grit sandpaper, for 13 minutes at high speed on 1200-grit sandpaper, and finally for 15 minutes on felt with a polishing solution. At each interval they were placed in an ultrasonic bath for 5 minutes.

Next, the samples were taken to the microdurometer (Future Tech FM Hardness Tester, Future Tech Corporation, Kawasaki, Japan) for analysis. After a pilot test, the distances were established as follows: 3 columns spaced 200 µm apart, each containing 10 rows spaced 30 µm apart, with the first reading also taken 30 µm from the surface. A load of 25 g was used for 5 seconds.

The data were allocated into a spreadsheet where the calculation of ΔS was performed. This value is attained from the areas calculated by numerical integration of the hardness values versus depth (kg/mm² × µm), using the trapezoidal rule [29].

6. Polarized Light Optical Microscopy

From the halves not used for microhardness testing, 5 samples from each group were selected to be taken to the Polarized Light Microscope (Leica DMLP, Optika Microscopes, Ponteranica-BG, Italy). To prepare these samples, they were polished in a polisher with 1200-grit sandpaper until they reached a thickness of 0.6mm. Then, they were manually polished with 600-grit sandpaper until they reached a thickness of 0.3mm, followed by 1200-grit sandpaper until they reached a thickness of 0.2mm, and finally, 2000-grit sandpaper until they had a thickness between 110 to 170 µm. At last, they were polished for 1 minute on felt with diamond paste. They were stored in a humid environment under refrigeration and taken to the microscope, where they were analyzed between a glass slide and coverslip, containing distilled water at the interface. The images were obtained with a 40x objective.

7. Statistical analysis
For color stability, R program version 4.2.2 was used. The normality assumption of the data was accepted through the Shapiro-Wilk test, and a one-way ANOVA test was applied for each $\Delta E_{00}$ interval, followed by Bonferroni post hoc test. For internal hardness ($\Delta S$), SigmaStat 4.0 program was used. The data were square root transformed, and the normality assumption was accepted using the Shapiro-Wilk test, followed by one-way ANOVA and Tukey's post hoc. In both statistics, the significance level was set at 5%.

The images obtained through polarized light microscopy were analyzed qualitatively.

RESULTS

1. Color stability

One-way ANOVA revealed a significant difference in $\Delta E_{00}$ T0-T1 between the treatment groups. Multiple comparisons (post hoc) exposed significant differences in averages between the H and MB groups compared to the infiltrated groups. H and MB did not differ from each Other (Table 2).

For $\Delta E_{00}$ at T0-T2, one-way ANOVA also showed a statistically significant difference between the groups. Multiple comparisons showed differences between the H, E, and EH groups, but H did not differ significantly from I and MB groups. MB, on the other hand, differed from all three infiltrated groups (Table 2).

Regarding $\Delta E_{00}$ between T1-T2 time intervals, the one-way ANOVA revealed no significant difference between the groups.

Table 2
Color stability through CIEDE2000 ($\Delta E_{00}$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>$H$, N = 15$^1$</th>
<th>$MB$, N = 15$^1$</th>
<th>$I$, N = 15$^1$</th>
<th>$E$, N = 15$^1$</th>
<th>$EH$, N = 15$^1$</th>
<th>$p$-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0-T1</td>
<td>9.6 ± 2.1 A</td>
<td>9.7 ± 3.3 A</td>
<td>15.0 ± 3.9 B</td>
<td>17.6 ± 4.0 B</td>
<td>18.4 ± 3.2 B</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>T0 – T2</td>
<td>16.9 ± 2.1 AB</td>
<td>15.0 ± 3.0 A</td>
<td>20.7 ± 5.0 BC</td>
<td>23.7 ± 4.1 C</td>
<td>24.8 ± 3.9 C</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>T1 – T2</td>
<td>9.30 ± 1.85 A</td>
<td>7.19 ± 1.29 A</td>
<td>8.93 ± 4.89 A</td>
<td>8.43 ± 1.49 A</td>
<td>9.19 ± 1.65 A</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$^1$Mean ± SD

$^2$One-way ANOVA

Different letters on the lines means statistical difference between groups.

2. Cross-sectional microhardness
The distribution of $\Delta S$ data is shown in Graphic 1. As described, microhardness was performed in all groups; however, due to the healthy group not presenting demineralization, its $\Delta S$ formula calculation resulted in 0 for all samples since it presented a mineral plateau, making the statistical analysis of this group impossible. Nonetheless, data were obtained to verify the absence of demineralization and it was possible to draw a graph to visually compare with the other groups (Graphic 1).

The MB and E groups did not show a statistically significant difference between them, as did the I and EH groups. However, MB and E differed statistically from the I and EH groups (Table 3).

**Graphic 1.** Microhardness in function of distance.

H: Healthy; MB: White spot; I: resin infiltrant Icon; E: Experimental infiltrant; EH: Experimental infiltrant containing 10% nano-hydroxyapatite.

<table>
<thead>
<tr>
<th>$\Delta S$</th>
<th>Mean (SD)</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MB</td>
</tr>
<tr>
<td></td>
<td>107,75 (26,63)</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>55.58 (15.5)</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>88.36 (20.37)</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EH</td>
</tr>
<tr>
<td></td>
<td>65.48 (18.55)</td>
<td>B</td>
</tr>
</tbody>
</table>

Distinct letters represent significant statistical difference ($p < 0.05$).

H: Healthy; MB: White spot; I: resin infiltrant Icon; E: Experimental infiltrant; EH: Experimental infiltrant containing 10% nano-hydroxyapatite.

**3. Polarized Light Optical Microscopy**

The images were obtained using a 40 objective, as can be seen in the following figures. In the image captured from the H (Fig. 1) group the uniformity of the enamel structure can be observed, while in the MB (Fig. 2) group, an initial subsurface lesion is visible. In the infiltrated groups (Figs. 3, 4 and 5) there is a superficial deposition of resin material, and near the edges of the specimen a decline in the mineral surface can be noticed (Fig. 6). No initial white spot lesion was detected in the infiltrated groups.

**DISCUSSION**

The first hypothesis that there would be no difference between the infiltrated groups and the untreated surface groups was rejected. Due to the material's high sorption properties [13, 30], the infiltrant tends to incorporate pigments, represented by the $\Delta E00$ evaluation. It was noticed that the three infiltrated groups
(I, E, EH) showed higher staining compared to the healthy surface and initial white spot lesion groups during the 14-day period. This conclusion is in line with the study by Leland et al. from 2016, where they found a higher $\Delta E$ in surfaces treated with Icon compared to the healthy surface.

The choice of coffee as a pigmentation agent was based on studies that also evaluated the color stability of resin infiltrants, such as the studies by Leland et al. from 2016, Ayad et al. from 2022, and Ceci et al. from 2017 [23, 28, 31]. Coffee was chosen because it is commonly consumed by patients in their daily lives and has a high pigmentation capacity. Most studies use the CIEL*a*b* calculation to obtain $\Delta E$ for color variation between periods. However, this study used the CIEDE2000 calculation, which is more sensitive in identifying color changes and better mimics human vision [32].

In the 28-day period (T0-T2), the I group did not differ statistically from the H group, suggesting that its long-term pigment incorporation was similar to that of the dental structure. Nevertheless, considering the perceptibility and acceptability thresholds for $\Delta E00$, which are 0.8 (perception) and 1.8 (acceptance), it can be assumed that a value higher than 0.8 makes the color difference perceptible, and when higher than 1.8, it can be considered as clinically acceptable or not by the observer [33]. Thus, although there is no significant difference between these two groups, it can be inferred that under clinical conditions this difference may be perceptible and potentially aesthetically unfavorable, which could result in the need for clinical intervention to ensure aesthetic outcomes for the patient.

In terms of color stability, the experimental groups behaved similarly to the commercial group, with no statistical difference between them. This can be explained by their composition, which includes a higher constitution in the monomeric base such as TEGDMA that incorporates pigments, similar to Icon, as described by Alqahtani and Ceci [24, 28]. The formulation of the experimental infiltrants without the addition of nanoparticles is also supported by other studies, such as Cerqueira and Pedreira [34, 14], where the depth of penetration, sorption and solubility results, cohesive strength, and degree of conversion were found to be similar or superior to Icon.

There was no statistical difference between the groups in the T1-T2 period, suggesting that the resin infiltrants reach color saturation during this time, i.e. having sufficient pigment impregnation. But as described previously, even if there is no statistically significant difference, these values may indicate a clinical perception, leading to aesthetic dissatisfaction.

Although not evaluated in this study, other research has reported that polishing can provide some reversibility of staining [23, 35]. Therefore, it is important to consider that patients should be instructed to undergo regular check-ups for control and guidance regarding the material [36, 37].

The calculation of mineral loss ($\Delta S$) is widely used in the fields of biochemistry and cariology to assess the amount of superficial enamel demineralization [29, 38, 39]. The calculation is based on data obtained from cross-sectional microhardness, which allows the evaluation of the internal enamel surface. As the premise of the resin infiltrants is to penetrate and reinforce the dental structure, this methodology was
applied to determine if there was indeed a difference in mineral loss compared to the untreated initial lesions.

As observed in the Table 2, the MB and E groups did not show a statistical difference, and we can infer that the E group was not able to reinforce the mineral structure. This may be due to its composition, consisting only of TEGDMA, BisEMA, EDAB, and camphorquinone, which did not provide it with mechanical strength [14, 27, 40].

On the other hand, the I and EH groups exhibited lower mineral loss, suggesting that they were able to reinforce the demineralized enamel. Therefore, the second hypothesis was also rejected. EH showed this behavior most likely because of its hydroxyapatite nanoparticles, which, as reported by Elambaby et al. [20], were able to improve the penetration of the Icon material and the mineral density. Another important characteristic for new materials is the degree of conversion, and Souza et al. reported that the experimental infiltrant containing 10% nano-hydroxyapatite achieved a high degree of conversion when compared to other infiltrants, including Icon [15].

As described by Meyer-Lueckel and Paris [1], the penetration depth of the infiltrant composed of TEGDMA is approximately 200 µm in natural lesions in human enamel. Therefore, it is important that the methodology employed for the internal surface evaluation be established accordingly. In this study, the distance was designated at 30 µm, extending up to 300 µm to ensure we were evaluating this infiltrated area.

The study by Ayad et al. [22] demonstrated that the initial white spot lesion group treated with Icon was able to reinforce the dental structure. However, despite corroborating the results presented in the table, some data differ, such as the distance used in the test. They employed an initial distance of 100 µm and did not calculate ∆S but rather the average at each distance to determine hardness.

Despite the results showing the lowest mineral loss, a finding of the present study was the presence of a decline in the majority of samples analyzed under polarized light microscopy. This decline can be attributed to the acid etching carried out with 15% hydrochloric acid for 2 minutes, as established by the manufacturer DMG, which is capable of removing the superficial micrometric layer (40 µm) and allowing the resin material to settle, as the surface was subtly removed, exposing the fully demineralized area [41].

Esteves-Oliveira [21] employed the same ∆S calculation methodology to evaluate the internal surface of specimens treated with resin infiltrant and different polishing protocols, differing from this study only in the distance employed. However, the values assumed for the first indentation were similar to the I and EH groups.

Considering the color and ∆S analyses, the EH group presented great promise by presenting results similar to the commercial product and, combined with its well-established biocompatibility potential in the literature, should be investigated with other methodologies capable of accurately mimicking or predicting the performance of resin infiltrants. As expected, the MB group had the highest mineral loss.
and also the greatest variability, yet it did not differ from the E group, demonstrating that this group was not able to provide reinforcement for the demineralized enamel region.

The premise of using resin infiltrants is highly beneficial for non-invasive dentistry and for halting the progression of early lesions. However, attempts to improve the properties of the material and, most importantly, maintain the integrity of demineralized enamel are valid. Hence, it is of utmost importance that this line of research be maintained and new methodologies be developed that more precisely access the mechanism of action of these materials and find further ways to enhance their viability.

CONCLUSION

- The infiltrated groups I, E, and EH exhibited similar behavior, with greater staining compared to the untreated H and MB groups at the 14-day evaluation, while at 28 days, group I showed results similar to H;
- The infiltrated groups I and EH showed lower mineral loss than the E and MB groups, suggesting a reinforcement of the demineralized dental structure;
- The EH experimental group exhibited a behavior similar to the commercial Icon in terms of structural reinforcement and staining, proving to be promising.

Declarations

Acknowledgments

The authors acknowledge the great contribution of Flávia Sammartino Mariano Rodrigues from the Microscopy and Image Center at Piracicaba Dental School (University of Campinas, Piracicaba, São Paulo, Brazil) for the assistance in polarized light microscopy analyses.

A. Author Contribution

- J.L.G.Z. Conceptualization, performed all methodology, analysis, obtained datas and performed statistical analysis, wrote original draft and final manuscript.
- G.A.C. Performed microscopy methodology and prepared samples to polaryzed light microscope.
- R.F.S. Performed cross-sectional microhardness.
- F.H.B.A. Conceptualization, reviewed methodology.
- C.P.M.T. Resources, performed cross-sectional microhardness.
- G.M.M. Conceptualization, reviewed methodology, review and editing the manuscript.
- All authors reviewed the manuscript.

B. Ethics Approval and Consent to Participate

Not Applicable
C. Funding

This study was financed in part by the Fundação de Amparo à Pesquisa de São Paulo (FAPESP) – Process Code 2021/14881-5. This research is part of the dissertation of JLG Zago, to whom a scholarship funded by FAPESP was awarded.

D. Conflict of Interests

This study has no conflicts of interest.

References


Figures
Figure 1

Healthy group, 40x magnification.
Figure 2

White spot (MB), 40x magnification.
Figure 3

Resin infiltrant Icon (I), 40x magnification.
Figure 4

Experimental resin infiltrant (E), 40x magnification.
Figure 5

Experimental resin infiltrant containing 10% nano-hydroxyapatite (EH), 40x magnification.

Figure 6
Declines on surface, Icon (I) in 5x magnification (left), Experimental (E) in 10x magnification (center), Experimental containing 10% nano-hydroxyapatite (EH) in 10x magnification.

**Figure 7**

**Flowchart 1.** Experimental design.
Figure 8

Graphic 1. Microhardness in function of distance.

H: Healthy; MB: White spot; I: resin infiltrant Icon; E: Experimental infiltrant; EH: Experimental infiltrant containing 10% nano-hydroxyapatite.