Structural integrity of MIH-affected teeth after treatment with fluoride varnish or resin infiltration: An 18-Month randomized clinical trial

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ARTICLE INFO
Keywords:
Molar incisor hypomineralization
Enamel defects
Resin infiltration
Dental enamel
Fluoride therapy
Icon

ABSTRACT
Objective: To evaluate the influence of fluoride varnish (FV) therapies or resin infiltration (RI) to maintain the structural integrity of Molar Incisor Hypomineralization (MIH) affected teeth.

Methods: Fifty-one children aged 6–12 years with at least one incisor and one first permanent molar with yellow/brown MIH opacities were included. Patients were randomly allocated into three groups: FV–Fluoride Varnish (Duraphat); FV+etch–Fluoride Varnish (Duraphat) after enamel etching with 37% phosphoric acid; or RI–Resin Infiltration system (Icon). Opacities were monitored for 18 months. The primary outcome was the loss of integrity due to post-eruptive enamel breakdown (PEB). Covariables included sex, age, DMFT index, opacity colour, plaque index, number of MIH-affected teeth, and number of MIH-affected surfaces. Fisher’s Exact was used to test the association of treatments with PEB, the Kaplan-Meyer method analysed the survival rates and Cox-regression determined which covariables would predict failure (\(\alpha=0.05\)).

Results: From a total of 235 teeth, the PEB rate for RI (6.1%) was significantly lower (\(p<0.05\)) than FV (17.9%; OR 3.0, 95%CI 1.07, 8.48) and FV+etch (17.3%; OR 3.1, 95%CI 1.13, 8.73). DMFT index >3, brown opacities, cusp involvement, and age between 6–8 years predicted PEB (\(p<0.05\)).

Conclusions: Resin infiltration positively influenced the structural integrity maintenance of MIH-affected teeth by decreasing the risk of enamel breakdown over 18 months follow-up. Registry of Clinical Trials (RBR-8wwk3n).

Clinical Relevance: Resin infiltration proved to be a more efficacious intervention to maintain the structural integrity of MIH-affected teeth than fluoride varnish therapies.

1. Introduction

Molar Incisor Hypomineralization (MIH) was first defined in 2001 as demarcated enamel qualitative developmental defects affecting a minimum of one first permanent molar with or without the involvement of the incisors [1]. The hypomineralized lesions vary in colour from creamy/white through yellow to brown [2].

MIH-affected enamel shows decreased mechanical properties due to a disorganized prismatic structure with lower mineral density and higher protein content, leading to increased porosity, especially in yellow and brown lesions [3]. Therefore, the affected enamel is more likely to develop post-eruptive enamel breakdown (PEB), potentially occurring soon after tooth eruption, facilitating plaque accumulation and the development of carious lesions in those at caries risk [4]. According to a recent review, the global MIH mean prevalence is 13% with 878 million people affected, with 4.8 million cases per year requiring invasive treatments [5]. Also, MIH-affected children undergo dental treatment on their first permanent molars close to ten times as often as children

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https://doi.org/10.1016/j.jdent.2020.103570
Received 16 September 2020; Received in revised form 28 November 2020; Accepted 19 December 2020
Available online 29 December 2020
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without MIH [6]. MIH-affected teeth frequently present structural loss and hypersensitivity, the subsequent treatments become even more challenging to both patient and clinician, with unfavourable restorative outcomes [2–4].

Fluoride (F⁻) based materials are commonly used in clinical practice to support remineralization of the lesion surface and hamper further demineralization. However, about 20% of yellow and brown MIH defects develop PEB even after fluoride varnish (FV) therapy, most likely due to poor physicochemical lesion characteristics [7][8]. Faced with this consequence, the pre-treatment of the enamel surface has been proposed in an attempt to improve adhesion and mineralizing therapies for MIH-affected teeth. As options, the use of 10% carbamide peroxide CH₃N₂O₅, hydrogen peroxide (H₂O₂), and 37% phosphoric acid (H₃PO₄) has been reported with varying results [9–11]. The techniques use the premise of ‘opening the surface’ to increase penetration/diffusion of materials.

In an attempt to improve the physical characteristics of the MIH lesion, infiltration with low viscosity resin material has been investigated [9–11]. Initially approached for masking non-cavitated carious lesions [12,13], the resin infiltration (RI) therapy is based on the penetration of a low-viscosity TEGDMA-based resin into the lesion body by capillary forces, promoting the obliteration of porosities and preventing lesion progression. Considering this mechanism of action, RI has been proposed for the treatment of developmental defects of enamel, including MIH, presenting unpredictable variability in the depth of resin penetration in vitro [9–11].

Since most investigations are conducted in vitro, the actual clinical effects of MIH-affected teeth treatments to improve the physical characteristics of the MIH lesion remain unknown to the authors’ knowledge. Therefore, this randomized controlled trial aims to evaluate the influence of fluoride varnish therapies or resin infiltration to maintain the structural integrity of Molar Incisor Hypomineralization (MIH) affected teeth. The primary null hypothesis tested was that FV therapies or RI would not differ in maintain the structural integrity of teeth with yellow and/or brown MIH opacities. The primary outcome was the loss of integrity due to PEB.

2. Materials and methods

The experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statements [14].

2.1. Ethics approval and protocol registration

This study was conducted in full accordance with The Code of Ethics of the World Medical Association Declaration of Helsinki. The local Ethics Committee on Involving Human Subjects reviewed and approved this study (protocol n°. CAAE 494.734.15.0.0000.5416) registered in the Brazilian Registry of Clinical Trials (REBEC) by BBR-8wvk3n. The interventions were only performed after written informed consent from the parents or legal guardians of all children who agreed to participate.

2.2. Study design and participant recruitment

This was a single-blind randomized controlled clinical trial. A convenience sample of participants was recruited while having routine treatment in the local Faculty’s Paediatric Dental Clinic during the period Sep/2016 to Jan/2017.

2.3. Sample calculation

The sample size was calculated based on the relative risk of PEB development in a total of 185 MIH-affected permanent incisors and first molars of 45 children that received FV applications weekly over 1 month [7]. Results reported being 2% for yellow and 17% for brown opacities at 1-year follow-up [7]. Given two experimental proportions design, a minimal of 62 teeth would be required for FV intervention to achieve a power of 0.85 at a 5% significance level. Considering a dropout rate of 20% and three experimental protocols, it was determined that at least 202 teeth would be required for all interventions.

2.4. Eligibility criteria

A total of 223 children aged from 6 to 12-years were examined to determine whether the participants met the inclusion and exclusion criteria. Examinations were performed by a calibrated researcher (A) in the dental clinical environment using a triple syringe and a reflector light.

Inclusion criteria included children born and living in Araraquara (São Paulo, Brazil), registered for regular treatment at the Paediatric Clinic of the Araraquara School of Dentistry, and having at least one permanent incisor and one permanent molar affected with yellow and/or brown MIH lesions. The exclusion criteria were developmental enamel defects related to syndromes, dental fluorosis, amelogenesis imperfecta, dentinogenesis imperfecta, and the presence of orthodontic appliances. MIH teeth with restorations, PEB, or carious lesions classified as score >0 according to the International Caries Detection and Assessment System (ICDAS) [15] were also not included. Children would no longer be part of the study if they received any dental treatment outside the Faculty’s Paediatric Dental Clinic. At the end of assessment, 54 participants were considered eligible (235 teeth).

2.5. Baseline assessments and calibration procedures

Participants’ sex and age were recorded. MIH diagnosis was made according to the European Academy of Paediatric Dentistry (EAPD) criteria [16]. The number of MIH-affected teeth and the affected surface(s) were categorized regarding the presence of incisel or cusp involvement. Only surfaces that allowed direct visualization were included. Opacities were classified as ‘yellow’ or ‘brown’ considering the most predominant colour. Dental plaque was evaluated using the Visible Plaque Index (VPI), recorded as presence or absence considering only the teeth of interest. Participants’ caries experience was recorded using the Decayed, Missing, and Filled Primary Teeth index (DMFT) [17], and the presence of carious lesions were scored according to ICDAS criteria [15]. Assessment included digital photographs (Canon – EOS 7D SLR / Lens Canon 100 mm f2.8 L Macro IS USM EF D67/Flash Macro Ring Lite MR-14EX, Canon Inc., Tokyo, Japan) to allow reassessment.

Assessments were performed by a single examiner (A) calibrated according to the DMFT, EAPD, and ICDAS indices. Criteria were initially studied and thoroughly discussed by the examiner (A) with a group of experienced researchers. For inter-examiner reproducibility, digital photographs from patients that were not part of the study sample were used. These images were examined and classified independently by examiner (A) and the experienced researchers, resulting in inter-examiner Cohen’s Kappa agreements ≥0.86. For intra-examiner reproducibility, clinical evaluation of patients who were not part of the study sample was used. The examiner (A) classified the patients according to the above-mentioned criteria twice with a one-week-interval, resulting in intra-examiner Cohen’s Kappa agreements ≥0.89.

2.6. Randomisation and interventions

Considering that different interventions performed in the same oral cavity would confound the results, the unit of randomization was the participant. Thus, block randomization (number of included teeth <4 and ≥4; block size: 6) was designed to allocate the participants with a proportional teeth allocation ratio. A statistician independent of the study organized the participant allocation sequence according to an online tool (https://www.randomizer.org) in opaque and sealed envelopes that were opened right before the treatment performance.

All treatments were performed by a single researcher (B), who was
not involved with the assessments/reassessments, according to the manufacturers’ specifications. Another researcher (C) facilitated the handling of materials and recorded the data. Treatments protocols are described below.

**FV. Fluoride Varnish**: pumice prophylaxis; cotton-roll isolation; air jet drying for 5 s; fluoride varnish (5% NaF; Duraphat; Colgate-Palmolive, Germany) application using a micro-applicator; guidance for no food/water intake for 30 min. Frequency: four applications with a one-week interval between applications.

**FV+etch. Fluoride Varnish + Phosphoric Acid Pre-treatment**: pumice prophylaxis; cotton-roll isolation; enamel etching for 30 s (37% Phosphoric Acid; Condac; FGM; Brazil); 30 s water rinsing; 5 s air jet drying. 5 s delicate air dryings; fluoride varnish (Duraphat) application using a micro-applicator; guidance for no food/water intake for 30 min. Frequency: four applications with a one-week interval between applications.

**RI. Resin infiltration**: local anaesthesia; rubber dam isolation; prophylaxis; application of Icon Etch (DMG, Germany) for 2 min; 30 s plentiful water rinsing; 5 s air jet drying; 30 s application of Icon Dry (DMG, Germany); 5 s delicate drying with air-jet; application of Icon Infiltrant (DMG, Germany) for 2 min; 40 s light curing (1000 mW/cm²; Radii-cal, SDI, Australia); a second application of Icon Infiltrant for 1 min; 40 s light curing; rubber dam removal. Frequency: one application (Fig. 1). Considering that the assigned interventions (fluoride varnish therapies or resin infiltration) differed severely concerning the technique (isolation of the operative field, anaesthesia, and frequency), the procedure of blinding the participants was not practicable. During the study, all children underwent an oral hygiene instruction session (toothbrushing and flossing) after receiving treatment, and fluoride dentifrice (1450 ppm; Colgate-Palmolive) was recommended for oral hygiene twice-a-day.

2.7. Reassessments

Participants returned at 1, 3, 6, 12, and 18 months after interventions. At these periods, VPI and DMFT were reassessed by the researcher (A), who was fully blinded regarding the assigned interventions. The teeth were photographed to compare enamel structural integrity throughout the subsequent evaluations (Fig. 2). The cut-off for the presence of carious lesions (CL) was determined using an ICDAS score \( \geq 3 \), due to the difficulty of differentiating white spots lesions masked by MIH lesions. The same oral hygiene instructions given on the day of treatment were reinforced at all reassessments.

2.8. Statistical analyses

Data were analysed using the Statistical Package for Social Sciences, version 16.0 for Windows (SPSS Inc., IL, USA). The development of PEB was the primary outcome and considered a failure. The association of the three treatments with failure was assessed with Fisher’s Exact test. The Kaplan-Meyer method was used to analyse the survival rates within treatments. Covariables (sex, age, DMFT index, opacity colour, plaque, number of MIH-affected teeth and surfaces) were dichotomized and Cox-regression was used to determine failure predictors. Odds Ratios (OR) were calculated to test the cumulative association of covariables with failure using Fisher’s Exact test. All the analyses were conducted at a pre-established significance level of 5%.

Initially, all analyses were performed separately for molars and incisors, since the former are more likely to develop PEB [7], and then the same analyses were performed for the complete sample. Since the addition of the incisors did not significantly change the overall results, the authors have chosen to describe the results of tests relating to the sample containing molars and incisors.

3. Results

At baseline, 54 children were included in the study, totalling 249 teeth, randomly allocated at FV: 86 teeth; FV+etch: 81 teeth; RI: 82 teeth (Fig. 3). Throughout the study, three children withdrew their participation. Thus, at the end of 18 months, 51 participants were evaluated: 50.9% were female, the mean age was 8.1 years (median = 8.2). The teeth distribution was FV: 45 molars and 33 incisors; FV+etch: 43 molars and 32 incisors; RI: 47 molars and 35 incisors, with a mean value of 4±2 (±SD) affected teeth per participant.

After the 18-months assessments, the frequency of failure was 17.9% for FV, 17.3% for FV+etch, and 6.10% for RI, the latter being significantly lower (Fisher’s Exact test; \( p<0.05 \)) (Table 1). Failures prevailed in molars. Carious lesions development (CL) occurred only associated with PEB and for molars treated with FV (2 teeth; 4.4%) or FV+etch (1 tooth,
2.3%) (Table 1). The survival of FV and FV+etch was significantly lower compared to RI at 6 months (Mantel-Cox: p = 0.04), and presented an increased risk of failure of 3.1 (OR) and 3.0 (OR), respectively (p < 0.05; Fig. 4).

DMFT, opacity colour, tooth surface, and age were significantly associated with failure (Fisher’s Exact Test; p < 0.05; Table 2). Participants aged 6–9 years presenting DMFT >3, brown opacities, and cusp involvement together presented the lowest survival rates in all follow-up sessions, especially at 18 months (FV: 10.7%; FV+etch: 10.4%; and RI: 20.2%) (Table 3).

4. Discussion

In an attempt to investigate efficacious treatments to prevent MIH-affected teeth structural losses, this randomized clinical trial evaluated the performance of FV therapy preceded or not by enamel etching using 37% H3PO4 and resin infiltration on yellow and/or brown MIH lesions over 18 months. Since the primary outcome of PEB rate in RI was
follow-up. Significance level at five percent.

Note.

with failure development in MIH-affected teeth after 18 months.

Odds Ratios (OR) and the cumulative association of dichotomized covariables

Table 2

<table>
<thead>
<tr>
<th>Covariable</th>
<th>Dichotomization</th>
<th>OR (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
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<tr>
<td>DMFT index</td>
<td>0 – 3</td>
<td>0.12</td>
<td>(0.05-0.24)</td>
</tr>
<tr>
<td></td>
<td>4 – 9</td>
<td>8.01</td>
<td>(7.70-11.15)</td>
</tr>
<tr>
<td>Surface involvement</td>
<td>Free surface</td>
<td>0.07</td>
<td>(0.03-0.19)</td>
</tr>
<tr>
<td></td>
<td>Incisal/Occlusal</td>
<td>15.25</td>
<td>(6.50-35.73)</td>
</tr>
<tr>
<td></td>
<td>Yellow</td>
<td>0.35</td>
<td>(0.029-0.74)</td>
</tr>
<tr>
<td>Opacity colour†</td>
<td>Brown</td>
<td>2.62</td>
<td>(1.27-6.25)</td>
</tr>
<tr>
<td></td>
<td>6 – 8</td>
<td>8.12</td>
<td>(6.96-10.85)</td>
</tr>
<tr>
<td>Age</td>
<td>9 – 12</td>
<td>0.12</td>
<td>(0.07-0.28)</td>
</tr>
<tr>
<td>Number of affected teeth</td>
<td>2 – 5</td>
<td>0.93</td>
<td>(0.80-1.05)</td>
</tr>
<tr>
<td></td>
<td>6 – 9</td>
<td>1.07</td>
<td>(0.98-1.12)</td>
</tr>
<tr>
<td>Visible Plaque Index</td>
<td>Absent</td>
<td>0.74</td>
<td>(0.74 – 0.97)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>1.22</td>
<td>(0.87-3.01)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.96</td>
<td>(0.79-1.18)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>1.04</td>
<td>(0.91-2. 50)</td>
</tr>
</tbody>
</table>

Note. *p value established by Fisher’s Exact test (α=0.05). DMFT, Decayed Missed, and Filled Teeth. †no white MIH-lesions were included.

significantly lower than FV and FV+etch, the primary null hypothesis tested was that FV therapies or RI would not differ in maintain the structural integrity of teeth with yellow and/or brown MIH opacities was rejected.

Currently, two clinical trials [7,18] evaluated treatments that attempt to maintain the surface integrity of MIH-affected teeth to the authors’ knowledge. The use of a crème containing 10 % casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in MIH-affected molars improved the mineral content and decreased the porosity of the lesion surface [18]. However, the effects of mineralizing therapies (including FV) for MIH-affected teeth may fail to reach the severely hypomineralized body of the lesion in the short term and rely on application compliance [8]. Thus, the susceptibility to PEB and possibly CL development indicates that the application of a single treatment that requires little compliance would have significant advantages.

In the present study, 17.9% of the teeth that received FV applications (FV group) presented failure after 18 months, which corroborates to a previous study (20%) that used a similar methodology [7]. In an attempt to improve the action of FV via increasing the porosity of the surface layer, we used 37% phosphoric acid before the FV application in FV+etch group. The idea was based on the hypothesis that the demineralization of the MIH enamel surface layer would increase surface porosity, allowing deeper remineralization and consequent improved structural support. Pre-treatment of the enamel surface to improve the physical characteristics of the MIH lesion, mostly with phosphoric acid and sodium hypochlorite, has been investigated previously, but the results are quite discordant [9,19-21]. Our results showed no difference regarding failure development between FV+etch that received 37% phosphoric acid pre-treatment compared to FV without pre-treatment. The failure ratio in FV (3.1 OR) and FV+etch (3.0 OR) were also similar, and one outstanding and clinically important fact was that the failures began after 6 months of FV application in both groups. Also, it is
noteworthy that the presence of PEB led to the development of caries lesions in some teeth submitted to treatment with FV or FV+etch, indicating a transient action of this type of therapy. We cannot assume that this is the maximum action period of FV, nor if an additional application in this period would change the final results, but this leaves a question for further investigation and indicates that individuals with yellow and brown MIH defects should be closely monitored in clinical practice.

The use of resin infiltrant when compared to FV reduced the risk of failure in yellow and brown MIH opacities. Notably, there was no development of caries lesions for teeth treated with RI independently of PEB. Also, 6.1% of teeth in the RI group had failed at 18-months, significantly lower than FV and FV+etch. The major limitation of the infiltration technique is reported to be the unpredictable quality of pore obliteration and penetration depth in the opacities [9–11,22,23]. The great variation of mineral density, organic content, and the surface layer thickness may directly contribute; however, this assumption should be investigated. Our favourable results for resin infiltration may be related to the inclusion of only yellow and brown lesions in the sample, which presents greater porosity and structural disorganization, allowing greater penetration of the resin infiltrant and also hampering the demineralization process [10]. Although this is not fully supported by previous in vitro studies, none of these evaluated the in vivo performance of this material [9,10,22,23].

Since molar teeth are exposed to greater mechanical stress than incisors, it was expected in all groups that molars would show the highest failure rates. Importantly, teeth with cup or incisal involvement presented a significantly higher risk (15.25 OR) of failure than teeth with involvement of only free surfaces. Additionally, brown opacities had almost three times more risk of failure than yellow opacities. These characteristics are extremely important and may assist risk classification and treatment planning of MIH patients, especially during tooth eruption when PEB may occur [4].

Another important variable was age. The chances of PEB occurring are higher at age 6–8 y compared to 9–12 y. Potentially, the first age group could be going through this event whereas the other group has already completed it and would have been excluded from the study [2]. Besides age, MIH patients with DMFT >3 were more likely to develop PEB. Usually, patients with MIH have higher DMFT values due to the MIH condition itself [4], therefore, clinicians should carefully evaluate caries risk.

The survival rates decreased over time and the lowest was observed 18 months post-intervention for all groups of patients with DMFT >3, brown opacities, with cup involvement, and age between 6–8 y together. Nevertheless, RI had a survival rate of 20.2% for these characteristics at 18-months, whereas FV and FV + etch had lower survival rates, 10.7%, and 10.4%, respectively. These results reinforce that resin infiltrant treatment may be a strategy that decreases the risk of failure in yellow and/or brown MIH defects, although a survival rate of 20.2% translates to a PEB rate of 79.8%. This also reflects how weak the brown lesions are, however, it may be better elucidated when different characteristics are compared. For example, patients with DMFT <3, yellow opacities, without cup involvement, and age between 6–8 y presented survival rates of 90.2% (FV), 89.6% (FV+etch), and 92.5% (RI) at 18 months.

We understand that the inclusion of only yellow and brown opacities in our study limits our results and extrapolation to MIH overall. Also, the intrinsic structural variability of each lesion, the unpredictability, and the sensibility of the resin infiltration technique are limiting factors. Moreover, the covariables present in the study and the randomization on patient level could act as confounders, but this influence was controlled by regression analyses and also by the block randomization process. Regarding sampling at patient level, this served to control the dilution effect of FV on others tooth that could be treated with RI and present results influenced by the fluoride action.

To the authors’ knowledge, this is the first clinical trial to evaluate the clinical performance of resin infiltration to maintain the enamel structural integrity of yellow and/or brown MIH defects. Despite the positive findings, MIH is still challenging and stimulates the scientific search for answers and solutions. It was observed that patient and opacity related characteristics might influence the risk of developing PEB, so clinicians must be closely attentive. Even considering the limitations of this study, resin infiltration proved to be an efficacious intervention to reduce PEB in MIH-affected teeth.

5. Conclusions

Among the strategies evaluated, resin infiltration positively influenced the structural integrity of MIH-affected teeth by decreasing the risk of enamel breakdown after 18 months follow-up.

CRediT authorship contribution statement

Vinicius Krieger Costa Nogueira: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing - original draft. Igor Paulino Mendes Soares: Methodology, Data
Declaration of Competing Interest

None of the authors of this manuscript have any conflict of interest to declare.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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