The use of a ‘bleach-etch-seal’ deproteinization technique on MIH affected enamel

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Aims. To ascertain whether deproteinization pre-treatment of molar-incisor hypomineralization (MIH) enamel affects resin sealant infiltration.

Design. Thirty one extracted MIH teeth were divided into three sections and randomly allocated into the Control (etch and FS), Treatment 1 (5% NaOCl, etched and fissure sealed), and Treatment 2 (5% NaOCl and fissure sealed with no etch) groups. Two hundred seventy nine sealant tag/enamel grade observations were recorded by scanning electron microscopy.

Results. Control and Treatment 1 were similar in their outcomes, and Treatment 2 was markedly different. There was no statistical evidence to suggest that there was any difference between Treatment 1 and the Control Treatment (95% CI, 0.52, 1.51; \( P = 0.6 \)). There was a marked difference between Treatment 2 and the Control Treatment (95% CI, 0.07, 0.25; \( P < 0.001 \)). All treatments also demonstrated a high-predicted probability of obtaining ‘poor’ sealant tags (Control = 47%, Treatment 1 = 49%, and Treatment 2 = 40%).

Conclusions. The findings suggest that there was no significant difference in the tag quality between the conventional technique (Control) and the ‘bleach-etch-seal’ technique (Treatment 1). There was no benefit in pre-treating with NaOCl alone (without etch) before sealing. This research also showed that there was a high-predicted probability of obtaining ‘poor’ sealant tags in MIH enamel, regardless of which of the three treatments was used.

Introduction

The micromechanical bonding of dental materials with tooth enamel was established in the 1950s, when it was identified that chemical pretreatment of enamel with acid etch could create a surface into which resin could infiltrate, thus resulting in microtag formation and a sound resin–enamel bond¹⁻⁴. It was shown at the time that the depth of resinous penetration was related to the concentration of phosphoric acid used⁵⁻⁷; 37% phosphoric acid produced the optimal etch relief for sound enamel, and this has since been used as the ‘gold’ standard⁷,⁸.

These findings were deemed pivotal at the time as the more destructive techniques involved for caries prevention and ‘prophylactic odontotomoy’ could be superseded with these newer, minimally invasive techniques¹². Unfortunately, these same techniques have not proved so successful for the restorative management of molars affected with molar-incisor hypomineralization (MIH) (the ‘hypomineralization of systemic origin affecting one or more first permanent molar teeth (FPM) and frequently associated with affected incisors’)⁹. It is suggested that sealants and restorations on children with MIH-affected molars may require more treatment and intervention than children without MIH¹⁰,¹¹. Kotsanos et al. found that MIH teeth were eleven times more likely to undergo restorative treatments on at least one-first permanent molar and were three times more at risk of their ‘fillings and sealants’ failing, whereas Lygidakis et al. have reported that only a quarter of fissure sealants remained on MIH teeth four years after application¹¹,¹².

Despite this, there appears to be a paucity of restorative techniques specific to the
treatment and management of MIH-affected enamel; the techniques still used and advocated originally stem from the management of otherwise healthy enamel.

It has been shown that the enamel–resin interface for MIH enamel is weak in comparison with sound enamel, possibly due to the limited inter-rod dissolution, intercrystal porosity, and microtag formation seen in MIH enamel. The proposed aetiology of defective MIH enamel is disruption of ameloblast function during the maturation phase of tooth development. This is thought to cause a less dense prism structure with loosely packed appetite crystals and a wider sheath region, possibly as a result of retained organic material in the form of proteins during enamel maturation.

The high organic content seen in MIH, as well as the poor bond between its enamel–resin interface, has led to similarities being drawn between the composition, treatment, and management of amelogenesis imperfecta (AI)-affected enamel. It has been shown in numerous studies that, as a result of its protein denaturing ability, ‘bleach’ pretreatment in the form of 5% sodium hypochlorite can have a beneficial affect on the enamel–resin interface of AI and dysmineralized teeth. Drawing from the similarities between the two types of enamel, Wright and Williams postulated that the aforementioned ‘bleach-etch-seal’ technique could be used for enhancing the resin–enamel interface on MIH-affected enamel.

Therefore, the aim of this study was to ascertain whether or not differences were seen at a microscopic level in the quality of fissure sealant infiltration on MIH-affected enamel that had been (i) conventionally treated with etch and fissure sealant (Control), (ii) pre-treated with 5% sodium hypochlorite and then conventionally treated with etch and fissure sealant (Treatment 1), or (iii) only pre-treated with 5% sodium hypochlorite and then fissure sealed with no etch (Treatment 2). Thus, the aim was to investigate the quality of infiltration of fissure sealant resin into variously treated MIH-affected enamel.

The objective of this study was to test the null hypotheses that the deproteinization pre-treatment of MIH teeth using 5% sodium hypochlorite (Treatment 1 and Treatment 2) will not increase the quality of fissure sealant infiltration compared with conventional etch and sealant preparatory techniques (Control). Control = Treatment 1 = Treatment 2.

Materials and methods

Ethics

This study was an in vitro randomized controlled study. The regional NHS Ethics Board approved the study, and it was registered according to and complied with the Human Tissue Act (2006).

Recruitment

Children attending the Child Dental Health department (Bristol Dental Hospital) for routine examinations, GA pre-assessment, and joint Orthodontic–Paediatric Dental Clinics had their first permanent molar teeth assessed by the author (SG) according to Weerheijm’s MIH judgment criteria. The patients were considered to be suitable for the study if they required extraction of their FPM. Positive consent was obtained for the donation of teeth for this research.

Each extracted tooth was stored in labelled pot containing 70% ethanol. Clinical observations were reconfirmed by the author with reference to in vitro photographed images of each tooth: (i) tooth notation, (ii) opacity type, (iii) signs of post eruption breakdown, (iv) signs of atypical restoration, and (v) additionally, it was assessed whether the tooth had three hypomineralized areas suitable for use in this research. From the aforementioned information, it was determined whether or not the tooth was MIH in nature and whether or not it could be used for the study. These observations were confirmed by two further examiners (PJMC and PS).

Sample size

Thirty one of 49 teeth were identified as being suitable for the study.
Randomization

The 31 teeth were organized randomly, using a random number generator (using http://www.random.org).

The three suitable areas per tooth which were deemed suitable for experimentation were labelled A, B, and C (Fig. 1). Each area was then sectioned accordingly and received the randomized treatment.

Treatment protocol

The occlusal surface of each specimen was washed with deionized water using a three in one syringe (60 s). This area was pumiced using a small cup bristle brush on a slow-speed handpiece (60 s). The area was washed again until all visible debris was removed (30 s). The area was dried using the three in one syringe (30 s). Following this, each specimen received one of the three randomized treatments listed below.

Control group protocol (etch and sealant alone)

The etchant (35% Delton® Phosphoric Acid, Denstply) was applied onto the occlusal surface of the tooth using a Microbrush® (Microbrush Co., Waterford, Ireland) (30 s). The area was washed with the three in one syringe (30 s) followed with air drying (30 s). Delton® Light Cure Fissure Sealant was applied onto the occlusal surface with a Microbrush, and this was light cured (Dentsply Smartlite™ PS for 20 s).

Treatment group 1 protocol (5% sodium hypochlorite, followed by etch and sealant)

Five percentage sodium hypochlorite (10% sodium hypochlorite mixed 1 : 1 with deionized water) was applied onto the occlusal surface of the tooth using a Microbrush® (60 s). The area was washed with the three in one syringe (30 s) followed with air drying (30 s). These teeth were etched and sealed as per the Control Group protocol.

Treatment Group 2 Protocol (5% sodium hypochlorite/sealant)

Five percentage sodium hypochlorite was applied onto the occlusal surface of the tooth using a Microbrush® (60 s). The area was washed with the three in one syringe (30 s) followed with air drying (30 s). No etch was used in this group. Delton® Light Cure Fissure Sealant was applied onto the occlusal surface with a Microbrush, and this was light cured (Dentsply Smartlite™ PS for 20 s).

Each specimen was placed into an individual container.

Specimen preparation for scanning electron microscope

The techniques implemented were in accordance with the successful methods used and described in previous studies. Each specimen was sectioned vertically using a 0.3-mm-thickness diamond blade (Microslice II, Metals Research, UK). Each cut surface was polished – P600-1200-graded silicon carbide paper (10 s), 1200 grit silicon carbide power (60 s), and aluminium polishing powder (60 s). Each specimen was placed into a beaker of water, so that debris could be removed via the ultrasonic bath (FB 11011 Bath, Germany).

To reveal the interface between restoration and tooth, each specimen was immersed in two Molar hydrochloric acid (50 s), followed by placement into a beaker of deionized water (60 s). Specimens were freeze-dried to avoid the precipitation of mineral at the
enamel surface and to avoid collapse of surface structure through surface-tension effects that can result during a drying process. Liquid nitrogen was used to freeze each specimen and phosphorus pentapeptide used to absorb water. Freeze drying was completed under vacuum at \(-60^\circ\text{C}\) for 24 h.

Each specimen was mounted on a SEM Stub using Glue (Loctite, Cyanoacrylate Adhesive), and after air drying (1 h), the mounted specimens were sputter coated with platinum on two separate occasions \((2 \times 60 \text{ s})\) (SC7620; Quorum Technologies, UK). The specimens were viewed under scanning electron microscope \((20 \text{ kV secondary emission mode, ISI 60; SEM Tech Ltd, Derbyshire, UK})\).

**Data collection**

**Blinding.** The observer (SG) was blinded and unaware as to which treatment the specimens had received whilst grading the sealant tag and the enamel.

**Interpretation of sealant tag and enamel profile.** Each MIH tooth had provided three subspecimens suitable for receipt of one of the three randomized treatments. The left-hand side, centre, and the right-hand side of each subspecimen were photographed using the SEM software \((750\times \text{ and } 1500\times \text{ magnification: SEM Tech Ltd})\). These photographs were used for assessing the sealant tag and enamel quality.

A classification system was used to categorize the qualitative blinded observations (Refer to Fig. 2 and text).

The form of the sealant tags varied in terms of consistency. It could be seen that some of the specimens demonstrated harmonious sealant-tag-like extensions, appropriate in quantity and effectively infiltrating the depth of the etched enamel prisms. By contrast, some specimens displayed sealant tags that were sparse in quantity, irregular in profile and depth, or even completely void of resin.

Variations were also observed in the enamel quality of the specimens. In some of the specimens, it could be seen that the enamel rod structures were very well defined, whereas this was less so in other specimens. Likewise, variation in the organization of the enamel could be seen, with some specimens being densely packed and well organized, whereas others were disorganized with the enamel structure being atypical and haphazard in appearance.

**Data classification of sealant tag and enamel profile.** A \(5 \times 4\) grid model was used (one of up to 20 different combinations could arise): the sealant tag grade being defined as A, B, C, D, or X, and the enamel grade being defined as 1, 2, 3, or 4.

In total, 31 teeth were divided into three sections each \((93),\) and each specimen was observed in three separate areas \((279)\).

**Statistical analysis**

In this study, ordered logistic regression was used to demonstrated which one of the three treatments (Control, Treatment 1, and Treatment 2) offered the greatest chance of obtaining Excellent (A), Good (B), Fair (C), Poor (D), and No Tags (X), respectively, when enamel quality \((1, 2, 3, \text{ or } 4)\) had also been considered.
Results

Results were considered for the three groups:

1. Control Intervention (etch and sealant alone)
2. Treatment 1 Intervention (5% sodium hypochlorite, followed by etch and sealant)
3. Treatment 2 Intervention (5% sodium hypochlorite and sealant)

A range of appearances of both tag quality and enamel quality were seen and are represented in Table 1.

Data analyses

The quality of sealant tag and enamel types appeared to be similar between the Control

Table 1. Observations seen in each Treatment Group.

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<tr>
<td>Total</td>
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Summary of results

The three major findings from the study include the following:

1. When the Control intervention was compared with the Treatment 1 intervention: the findings suggest that when sealant tags were compared, there was little difference in the tag quality whether the Control (etch and fissure sealant) or Treatment 1 method (5% NaOCl, etch and fissure sealant) was employed (Table 3). There is no statistical evidence to suggest that there was any difference between the results found for Treatment 1 (5% NaOCl, etch and fissure sealant) and the Control Treatment (etch and fissure sealant) (95% CI, 0.52, 1.51; \( P = 0.6 \)). The null hypothesis is thus supported; the use of NaOCl adds nothing to the success of the technique.

2. When the Control intervention was compared with the Treatment 2 intervention: the findings suggest that when the sealant tags were compared, there was a difference in the tag quality whether the Control (etch and fissure sealant) or Treatment 2 Group and Treatment Group 1, and different, when compared with those seen in Treatment Group 2 (Table 1).

Predicted probabilities (ordered logistic regression) analysis suggested that Control and Treatment 1 were very similar in their outcomes, but that Treatment 2 was markedly different (Table 2). The probability of obtaining a ‘Poor’ tag type was between 40% and 50% for all groups; the Control, Treatment 1, and Treatment 2 Group probabilities were 47%, 49%, and 40% respectively.

Proportional odds ratio (ordered logistic regression) analysis suggests that:

1. Treatment Group 1 would have an 88% chance of obtaining a sealant tag type or multiple groups of sealant tag types (in ordinal order), similar to those seen in the Control Group (Table 3) and

2. Treatment Group 2 would have a 14% chance of obtaining a sealant tag type or multiple groups of sealant tag types (in ordinal order), similar to the Control Group (Table 3).
method (5% NaOCl and fissure sealant only) was employed (Table 3). There is statistical evidence ($P < 0.001$) to suggest that there was a difference between the results found for Treatment 2 (5% sodium hypochlorite/sealant) and the Control Treatment (etch and fissure sealant) (95% CI, 0.07, 0.25; $P < 0.001$). The null hypothesis is thus not supported; the omission of etching seriously compromises the effectiveness of the treatment. Considering the quality of sealant tags and by means of predicted probabilities (ordered logistic regression) (Table 2): the probability of obtaining a ‘Poor’ quality of sealant tag for all interventions was close to 50%. The Control, Treatment 1, and Treatment 2 group probabilities were 47%, 49%, and 40%, respectively.

### Discussion

During this study, it was shown that the conventional etch and fissure sealant techniques were not different from a bleach-etch-seal technique (5% NaOCl, etch and fissure sealant) – no statistical significant differences could be seen between the two groups.

Previous studies have described clinical success when a ‘bleach-etch-seal’ technique has been used on AI-affected enamel\(^\text{18}\). Also, subtle variations of ‘deproteinization’ techniques have been reported; Saroglu \textit{et al.} reported that a ‘etch-bleach-seal’ technique significantly enhanced enamel shear bond strengths on AI-affected primary teeth, whereas Sonmez \textit{et al.} showed that the ‘etch-bleach-seal’ technique had no significant effect on the success of restorations on AI-affected enamel\(^\text{22,23}\). In our own study, attempts were made to ‘modify’ (through deproteinization) the enamel surface; however, no advantages were identified with the use of the ‘bleach-etch-seal’ technique on MIH enamel.

Possible explanations for the different conclusions reached in our own study could be due to the fact that only mild MIH enamel, with subtle differences in organic composition was used. For example, the areas investigated were not so severe to be affected with post-eruptive breakdown. It could be suggested that as a result, any enamel modification

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**Table 2. Predicted probabilities (ordered logistic regression).**

<table>
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<th>Sealant tag type</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
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</thead>
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<tr>
<td></td>
<td>Control</td>
<td>Treatment 1</td>
</tr>
<tr>
<td>A (Excellent)</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>B (Good)</td>
<td>0.11</td>
<td>0.1</td>
</tr>
<tr>
<td>C (Fair)</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>D (Poor)</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>X (No Tags)</td>
<td>0.11</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*Adjusted for enamel grade.

**Table 3. Proportional odds ratio (ordered logistic regression).**

<table>
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<th>Tag type</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
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<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Control group</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Treatment group 1</td>
<td>0.89</td>
<td>0.52, 1.51</td>
</tr>
<tr>
<td>Treatment group 2</td>
<td>0.12</td>
<td>0.06, 0.21</td>
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OR, odds ratio; CI, Confidence interval.

*Adjusted for enamel grade The proportional odds ratio in this case is used to determine the odds of a sealant tag type or multiple groups of sealant tag types occurring, in ordinal order, in one treatment group ‘compared’ with another treatment group, that is ‘What are the odds of the Control and a Test Group ending up with similar results’?
procedures would require an exaggerated type of ‘intervention’ for any discernible differences to be seen between the groups. It could also be suggested that the NaOCl ‘bleach-etch-seal’ pretreatment may be enhanced if the deproteinization agents were intensified, prior to acid etching the enamel surface; for example, if a strengthened solution of sodium hypochlorite was to be used or if the chemical pretreatment exposure times were increased further.

During the study, the severities of the MIH defects were not recorded even though it is known that as the severity of MIH enamel hypomineralization increases, then so too does the organic content near the enamel surface24. If such studies were to be repeated, then it could be suggested that the profile of the subspecimen could be analysed by X-ray microtomography analysis, to help establish the organic composition and the quality of the enamel25.

In this study, depth of in vitro enamel infiltration (or the lack of it) was used to measure the success of each treatment. This was chosen as it has been identified as being one of the factors influencing mechanical bond strength of restorations in enamel3–5,7,8,26. If future studies were to be completed on MIH enamel, then other outcome measures such as microhardness, microtensile strength, microleakage, and in vivo analysis could be used to evaluate bond strength and the clinical efficacy of new treatments13.

In the past, when the ‘bleach-etch-seal’ technique was advocated, intermediate hydrophilic bonding agents were not established in practice. More recently, a ‘bleach-etch-bond-seal’ technique has been described 23. It has been shown that the use of an (i) ‘etch-bleach-bond-seal’ technique and an ‘etch-bond-seal’ technique on AI-affected enamel has been deemed clinically successful over a 3-year period23 and (ii) the use of fifth-generation bonds during fissure sealing MIH enamel has been shown to be significantly more retentive compared with conventional ‘etch-seal’ techniques12. It may be the case that the merits in using a deproteinization technique have been superseded with the advantages in using fifth-generation bonding materials. This, however, has not been proven on MIH-affected enamel, and this could be an area for future investigation.

Conclusions

Tag formation was generally poor throughout all three groups; there was a high probability of obtaining a ‘Poor’ sealant tag, regardless of which intervention was used, in 47%, 49%, and 40% of Control Group, Treatment Group 1, and Treatment Group 2, respectively (Table 2).

The high probability of obtaining a ‘Poor’ sealant tag in this study may help explain why resinous treatment of MIH enamel in the past has fared so poorly10. These findings may help provide insight as to why MIH teeth are eleven times more likely to undergo restorative treatments on at least one-first permanent molar and are three times more at risk of their ‘fillings and sealants’ needing replacement compared with children from the Control Group11. It also helps confirm the findings of William et al13, who concluded from their own study that ‘the limited bonding (of the resin) to hypomineralized enamel was attributed to inadequate microtag formation’.

This is one of the first studies that have attempted to investigate this novel technique for the treatment of MIH-affected enamel. So far, it has been shown that there is no advantage in using the ‘bleach-etch-seal’ technique on MIH enamel.

Why this paper is important to paediatric dentists

- The ‘bleach-etch-seal’ technique does not improve resinous infiltration of fissure sealants in MIH enamel.
- There is a high possibility that a ‘Poor’ sealant tag would result in MIH enamel, irrespective of whether enamel is pre-treated with 5% sodium hypochlorite or not.
- In view of the high probability of obtaining ‘Poor’ sealant tags regardless of treatment type, further research may be advantageous to seek alternative techniques for infiltrating MIH enamel, whether pre-treated with NaOCl bleach or not.

Conflict of Interest

The authors declare no conflict of interest.
References