Microleakage of resin infiltration in artificial white-spot lesions

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Abstract: This study examined the effectiveness of resin infiltration in inhibiting microleakage from artificial white-spot lesions (AWL) in enamel. Fifty sound extracted premolars were selected and randomly divided into five groups (n = 10 each). Group 1 included sound teeth. In contrast, an AWL was created in all specimens in groups 2 to 5, as follows—Group 2: AWL with no treatment; Group 3: AWL treated with resin infiltration; Group 4: AWL treated with resin infiltration and 10,000 cycles of thermocycling; Group 5: AWL treated with resin infiltration and 10,000 cycles of thermocycling. All specimens were then coated with nail varnish, except for a 4 × 4 mm² area on the buccal surface (the measurement area), immersed in 2% methylene blue solution, and sectioned buccolingually. Microleakage was evaluated with a stereomicroscope. Data were analyzed by using the Kruskal-Wallis test and Bonferroni post-hoc correction. Application of resin infiltrant reduced microleakage in AWL. No microleakage was seen in Group 3, and differences between Groups 3, 4, and 5 were not significant (P > 0.05). The resin infiltration technique appears to aid in sealing enamel AWL and may help provide long-term protection against microleakage in enamel AWL.

Keywords: enamel, microleakage, resin infiltration, white-spot lesion

Introduction

Minimally invasive interventions are an important approach for management of white-spot lesions on teeth, as they preserve the structure of sound teeth, improve esthetics, and enhance clinical outcomes of preventive and restorative dentistry. A white-spot lesion is defined as a subsurface enamel porosity caused by carious demineralization and is associated with biofilm retention. They appear as milky white opaque lesions and are rough and microporoused when located on a smooth surface [1]. Without management, white-spot lesions can be remineralized by minerals in saliva. Enan et al. reported that 57.1% of white-spot lesions had improved without treatment after a 1-year follow-up [2]. However, Al-Khateeb et al. observed that white-spot lesions could not be completely eliminated [3]. Thus, there is a critical need to inhibit progression of these lesions, which is the first-line approach before cavitation.

Resin infiltration is a novel microinvasive treatment for white-spot lesions that involves filling, reinforcing, and protecting demineralized enamel. Resin infiltration inhibited progression of enamel demineralization and improved esthetics of white-spot lesions [4]. This technique uses a light-cured low-viscosity resin monomer, triethylene glycol dimethacrylate (TEGDMA), as the active ingredient to fill demineralized porosities on the enamel surface, thereby slowing progression of carious lesions and masking the opacity of white-spot lesions [5]. Enan et al. reported that resin infiltrant reduced surface resistance of demineralized enamel against acidic challenge [6]. Similarly, other studies showed that esthetic outcomes were better for demineralized enamel treated with resin than for demineralized enamel treated with fluoride or casein phosphopeptide-amorphous calcium phosphate [7,8].

Thermocycling is used to simulate artificial aging of restorations. This technique yields information on adhesive failure between the bonding resin and tooth structure, which is caused by dissimilar coefficients of thermal expansion in the restorative material and tooth structure [9]. Various thermocycling regimens have been used to simulate aging; however, Gale et al. reported that a cyclic regimen of 10,000 cycles per year was sufficient for investigating adhesive failure of tooth-restorations [10].

The success of preventive and restorative dentistry depends on achieving a complete seal in restorations. However, a persistent concern is microleakage, defined as clinically undetectable passage of bacterial, fluids, molecules, or ions between the tooth and restoration material applied to it [11]. Several approaches have been proposed for management of microleakage; however, none has resulted in successful clinical outcomes [7,8]. Alternatively, resin infiltrant/monomer can be applied to minimize microleakage at the tooth and restoration interface. Unfortunately, few studies have investigated the effect of resin infiltrant on microleakage of white-spot lesions. Therefore, this study examined the ability of a resin infiltration technique to limit microleakage from white-spot lesions in artificial enamel.

Materials and Methods

Specimen preparation

A total of fifty sound premolars were collected and stored in 0.1% thymol solution until the study was conducted. Ethical approval for the study was granted by the ethics subcommittee for human research in the sciences of Thammasat University: No. 3 (ECScTU No. 3) COE number 072/2560.

The collected teeth were randomly divided into five groups. Group 1 included sound teeth. In contrast, an artificial white-spot lesion (AWL) was created in all specimens in Groups 2 to 5, as follows—Group 2: AWL with no treatment; Group 3: AWL treated with resin infiltration; Group 4: AWL treated with resin infiltration and 10,000 cycles of thermocycling; Group 5: AWL treated with resin infiltration and 10,000 cycles of thermocycling.

Formation of artificial white-spot lesions

To create AWL, each specimen in Groups 2 to 5 was immersed in demineralizing and remineralizing solutions. The demineralizing solution was prepared by mixing 50 mM of acetic acid, 1.5 mM of CaCl₂, and 0.9 mM of KH₂PO₄. The pH of the solution was adjusted to 5.0 with 1 M of KOH. The pH value was periodically monitored with a pH meter (Orion 3-Star: Expotech USA, Houston, TX, USA). The specimens were immersed in the prepared solution in separate containers at 37°C for 14 days, and the solution was renewed daily. The specimens were then removed and thoroughly rinsed with distilled water for 1 min. To produce a natural surface layer of white-spot lesions, demineralized teeth were placed individually in a remineralizing solution containing 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 130 mM KCl, and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The pH of the remineralizing solution was adjusted to 7.0 with 1 M of KOH, and the pH value was periodically monitored with a pH meter. The demineralized specimens were immersed in the remineralizing solution in separate containers at 37°C for 7 days and the solution was renewed daily. The specimens were then rinsed again with distilled water for 1 min after being removed from the remineralizing solution [12].
**Table 1** Composition and application of resin infiltration material

| Material                                      | Composition                              | Treatment procedure                                                                 |
|-----------------------------------------------|------------------------------------------|--------------------------------------------------------------------------------------|
| Resin infiltration material; ICON             | ICON-etch: 15% hydrochloric acid         | Apply ICON-etch and leave undisturbed for 2 min, remove using high-power suction, and rinse thoroughly with air-water spray for 30 s. |
| (DMG, Hamburg, Germany) Lot number: 733275    | ICON-dry: 99% ethanol                    | Apply ICON-dry for 30 s and dry for 5 s (repeat etching process if white spot lesions are still visible after application of ICON-dry). |
|                                              | ICON-infiltrant: TEGDMA-based resin, initiators, stabilizers | Apply ICON-infiltrant and let set for 3 min, remove excess material with microbrush, and light cure for 40 s, reapply and leave for 1 min, and light cure for 40 s. |

**Thermocycling process**

The specimens were stored in 37°C and 100% humidity for 24 h in an incubator (Contherm 160M, Contherm Scientific Ltd., Lower Hutt, New Zealand). Specimens from Groups 4 and 5 were then thermocycled for 5,000 and 10,000 cycles, respectively. Thermocycling was performed at a bath temperature of 5°C and 55°C, a dwell time of 30 s in each bath, and a transfer time of 5 s.

**Microleakage of AWL**

All specimens were coated with nail varnish, except for an area measuring $4 \times 4 \text{ mm}^2$ on the buccal surface, which was used for measuring microleakage. Then, the specimens were immersed in 2% methylene blue solution at 37°C for 24 h (immersion time specified by ISO/TS11405). All specimens were rinsed under running tap water and then sectioned buccolingually with a slow cutting machine (Isomet, Buehler, IL, USA). The sectioned specimens were examined under a stereomicroscope (ML 9300, Meiji Techno Co. Ltd., Saitama, Japan) at ×40 magnification. Microleakage was scored by assessing penetration of methylene blue, as follows:

- 0 = no penetration of methylene blue
- 1 = methylene blue penetrates to outer half of enamel
- 2 = methylene blue penetrates to inner half of enamel
- 3 = methylene blue penetrates to outer half of dentin
- 4 = methylene blue penetrates to inner half of dentin

**Statistical analysis**

Microleakage scores were compared between groups with statistical software (SPSS 17.0; SPSS, Chicago, IL, USA) by the Kruskal-Wallis test and post-hoc Bonferroni correction for pairwise comparison at 95% confidence. The significance level was set at $P < 0.05$.

**Results**

In this study, resin infiltration inhibited microleakage. Table 2 shows methylene blue penetration scores. Methylene blue penetrated to the outer half of the enamel (100%) in Group 1 but only to the inner half of the enamel (50%) and outer half of the dentin (50%) in Group 2. Group 3 exhibited almost no penetration of methylene blue (90%); only 10% of specimens exhibited penetration to the outer half of the enamel. Methylene blue was noted in the outer half of enamel in only 30% of Group 4 specimens but penetrated the outer half of enamel in up to 70% of Group 5 specimens. The values for Groups 3, 4, and 5 did not significantly differ ($P > 0.05$; Fig. 1 and Table 3). The extent of methylene blue penetration was assessed at ×40 original magnification with a stereomicroscope, as shown in Fig. 2.
Resin infiltration is a novel treatment for white-spot lesions of enamel. However, microleakage, attributable to various factors in the oral cavity, remains a concern. Thus, this study examined the effectiveness of a resin infiltrant (ICON) in limiting microleakage from enamel AWL.

Methods of inducing artificial white caries that promote superficial deminerization or AWL include use of acidified gels, buffered solutions, and incubation with natural biofilm. Buffered solutions use demineralizing and remineralizing solutions to provide alternate periods of demineralization and remineralization, and constant changes in the solutions inhibit saturation. Because it produces a thicker layer of demineralization, the buffered solution method is adequate for studies of enamel caries and more effective than acidified gels [13]. Sound enamel has a refractive index or index of refraction (IR) of 1.62. However, enamel white-spot lesions have numerous porosities filled with water (IR = 1.33) or air (IR = 1.00). The difference in IR between enamel and water/air in these porosities affects light scattering and gives these lesions an opaque/white appearance. When the pores of white-spot lesions are filled with resin infiltrant (IR = 1.46), which has an IR similar to that of sound enamel, white-spot lesions resemble normal enamel. For this reason, resin infiltration stops progression of white-spot lesions and enhances tooth esthetics. Ciftci et al. reported that the resin infiltration technique significantly decreased AWL opacity [14]. Moreover, Pintanon et al. concluded that resin infiltration immediately improved the esthetics and surface hardness of AWL [12].

The ability of a resin infiltrant/monomer to minimize microleakage at the tooth and restoration interface is an important factor in predicting clinical success. Organic dyes are commonly used to detect leakage in vitro. Such dyes include basic fuchsin, methylene blue, eosin, crystal violet, erythrosine, and aniline blue [15]. Methylene blue dye was used to detect leakage of resin infiltrant and the tooth interface in this study because its absorption at a wavelength of 660 nm is close to the red region of the visible spectrum. Moreover, Pintanon et al. reported that the duration of specimen immersion in dye varied from 4 h to 72 h or longer [17]. Moreover, Tulunoglu et al. reported that resin infiltration decreased microleakage in class II resin composite restorations [18].

The goal of resin infiltration is for a low-viscosity resin monomer to penetrate enamel white-spot lesions [19]. Micromechanical bonding with enamel by TEGDMA penetration of microporosities created by the HCl etchant are necessary for the adaptation and durability of resin infiltration [20]. After the enamel caries surface has been etched with hydrochloric acid (ICON-etch) and is completely dried with ethanol (ICON-dry), the low-viscosity resin monomer (TEGDMA; ICON-infiltrant) penetrates the lesion to a depth of a few hundred micrometers by means of capillary action [19,21]. After polymerization, the resin monomer seals and rehardens lesions by increasing the surface hardness of the enamel, which improves esthetics [12]. Previous studies found that resin infiltration exhibited better penetration depth in enamel lesions as compared with colloidal silica and remineralizing agents [21,22].

The present results indicate that the depth of methylene blue penetration significantly differed between specimens that were and were not treated with resin infiltration. Groups 3, 4, and 5 had the least microleakage. Thermal cycling is often used to evaluate the effects of thermal changes on an enamel AWL-bonded filled resin monomer. Any deficiency in sealing is an interface failure and leads to gaps or leakage. However, Groups 3, 4, and 5 did not significantly differ. The low-viscosity resin monomer (TEGDMA) penetrates AWL [19]. After polymerization, the resin monomer seals the lesions and protects against microleakage in enamel lesions immediately and in the long term. In sound teeth (Group 1), microleakage was greater than in specimens treated with resin infiltration. Similarly, Lee et al. found that the depth of methylene blue penetration was significantly less for resin-infiltrated enamel lesions than for untreated enamel surfaces [23]. Sound enamel has microporosities that are penetrated by methylene blue, thus resulting in microleakage in the outer half of the enamel. The greatest microleakage was seen in Group 2. Deminerization of AWL is the process by which mineral ions are removed from hydroxyapatite crystals of enamel. Deficiencies in the integrity of the hydroxyapatite latticework result in porosities. When immersed in methylene blue, the dye penetrates the inner half of enamel (50%) and outer half of dentin (50%). In conclusion, the resin infiltration technique appears to aid in sealing enamel AWL, and may help ensure long-term protection against microleakage in enamel AWL.

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Conflict of interest
The authors declare no conflict of interest.

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