Comparing Impact of Two Resin Infiltration Systems on Microhardness of Demineralized Human Enamel after Exposure to Acidic Challenge

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Abstract

AIM: This study compared the impact of two resin infiltration systems on microhardness of demineralized enamel before and after an acidic challenge.

MATERIALS AND METHODS: A total of forty human maxillary molar teeth were used in this study. Each tooth has 4 groups (four standardized windows onto each tooth). Group A1: Untreated sound enamel surface (positive control), Group A2: Artificially demineralized enamel surface (negative control), Group A3: Icon resin infiltrating to demineralized enamel, while Group A4: Single bond universal adhesive applied to the demineralized enamel surface. All teeth were immersed in a demineralizing solution. The groups (A3 and A4) were further subdivided into two subgroups according to acidic ethanol challenge Subgroup B1: Specimens tested before an acidic challenge and B2: Specimens tested after an acidic challenge. Vickers microhardness test was done for all groups. One-way analysis of variance (ANOVA) was used to study the difference between tested groups on mean microhardness within each group. Tukey’s post-hoc test was used for pair-wise comparison between the means when ANOVA test was performed, and the significance level was set at p ≤ 0.05.

RESULTS: Icon resin infiltration and single bond universal adhesive showed significantly higher mean microhardness than negative control, but significantly lower mean microhardness than positive control. However, insignificant difference was found between Icon and single bond universal adhesive. After the acidic challenge, icon resin infiltration was more successful than single bond universal total-etch adhesive system in microhardness.

CONCLUSION: After an acidic challenge, Icon resin infiltration was more successful than single bond universal total-etch adhesive system in microhardness.

RECOMMENDATION: Icon resin infiltration technique is a promising, non-invasive approach that prevents the progress of the carious lesion.

Introduction

Dental caries is one of the most common diseases around the world. It has a dynamic nature and controlling caries is unexpected on the remineralization of initial carious lesions [1]. Remineralization is considered as a non-invasive management of early carious lesions and it represents a major progress in clinical management of the disease. As the subsurface carious lesions advances, the enamel minerals start to dissolve, resulting in increased porosities that can present clinically as “white-spot” lesions (WSLs) [2]. However, natural remineralization occurs by the saliva which results in mineral reuptake into the surface layer of WSLs which improves the esthetics and structural properties of the subsequent lesions [3]. Hence, it is important to apply remineralizing agents to repair the deeper layers of WSLs for improved esthetics. Various studies have examined the differences between different types of remineralizing agents or made attempts to identify the best possible option [1]. Resin infiltration technique seemed a promising micro-invasive approach that inhibited demineralization and mechanically stabilizes the demineralized lesions. In addition, it has been proved that it improves the microhardness of demineralized dental hard tissues significantly and reduces the mineral loss [4]. Icon resin infiltration formulated in 2009, by DMG Hamburg, Germany was solve numerous dental problems that it improves the microhardness of demineralized lesions. In addition, it has been proved that it inhibits demineralization and mechanically stabilizes the demineralized lesions. In addition, it has been proved that it improves the microhardness of demineralized dental hard tissues significantly and reduces the mineral loss [4]. Icon resin infiltration formulated in 2009, by DMG Hamburg, Germany was solve numerous dental problems such as incipient caries, primary caries, and secondary caries [5]. Many studies were done on the Icon effect, and they provided promising results [6], [7], [8]. However, it is still unconfirmed if the resin infiltration system will act differently than regular resin systems especially after further demineralization challenge. Subsequently, it would be advantageous to compare the impact of different resin infiltration systems on enamel microhardness before and after acidic demineralization challenge. This study compared the effect of two resin infiltration systems on microhardness of demineralized enamel before and after an acidic challenge, to observe the durability of the materials against acid attacks.
Materials and Methods

A total of forty caries free, human maxillary 1st molar teeth were collected from the oral surgery department clinics. Teeth were extracted from diabetic patients (45–65 years old) after obtaining written informed consent. Ethics approval was obtained from the Research Ethical Committee, Al-Farabi Private Colleges in Jeddah-KSA (Approval No 20-06/1). The teeth were washed under water and scaled to remove any plaque, calculus, or soft tissue remnants, and were cleaned using polishing brush and non-fluoride polishing paste (Nupro cups, Dentsply, USA) with low speed handpiece under water coolant then stored in saline solution containing sodium azide (10%) at 4°C which had no effect on structure or composition of enamel and dentin as described in dental literature [9].

Four standardized 3 × 3 mm² square shape windows marked onto each tooth: Two on the mesial surface and two on the distal surface (Figure 1). The windows were covered with adhesive tape, then the rest of the entire tooth was coated with nail varnish. The adhesive tapes were then removed from all windows except positive control ones. The teeth were soaked in a demineralizing solution in a proportion of 2 ml solution/mm² of exposed enamel for 72 h at 35°C to induce caries like lesion [10]. The demineralizing solution had the following composition: CaCl₂ = 2.2 mM, NaH₂PO₄ = 2.2 mM, lactic acid = 0.05 M, fluoride = 0.2 ppm, adjusted with 50% NaOH to a pH 4.5 [11]. Then, the teeth were removed from the demineralizing solution, washed with water, and dried on absorbent paper. The adhesive tape was removed from the positive control sound enamel windows. Afterward, the teeth were sectioned into two halves in a buccolingual direction using a perforated diamond cutting disc (Rong Mai, China) with low-speed handpiece and water coolant. Each sectioned tooth half was then embedded in a built-in scaled microscope and Vickers values were measured using Digital Display Vickers Microhardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20× objective lens. A load of 200 g was applied onto the surface of the specimens for 10 s. Three indentations were made on the surface of each window (Figure 2). The indentations were placed equidistant by 0.5 mm over a circle. The diagonal lengths of the indentations were measured by a built-in scaled microscope and Vickers values were converted into microhardness values. Microhardness was obtained using the following equation: $HV = 1.854 P/d^2$ where $HV$ is Vickers hardness in Kgf/mm², $P$ is the load in Kgf and $d$ is the average length of the two diagonals in mm. All treated specimens (distal tooth halves) were further subjected to acidic challenge, by soaking in a demineralizing solution for 72 h at 35°C. The same solution used for inducing artificial caries-like lesions were used for the acidic challenge and re-tested for enamel microhardness.

Figure 1: Two windows on the tooth surface
Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics Version 22 for Windows (SPSS Inc., IBM Corporation, NY, USA). The significance level was set at $p \leq 0.05$. Collected data were explored for normality using D’Agostino-Pearson test for normal distribution. Microhardness data showed a normal distribution, so one way-analysis of variance (ANOVA) was used to study the difference between tested groups on mean microhardness within each group. Tukey’s post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. Independent $t$-test was used to compare between materials after acidic challenge. Meanwhile dependent $t$-test was performed to compare between treatments and after acidic challenge. The significance level was set at $p \leq 0.05$.

Results

The results showed that icon resin infiltration and single bond universal adhesive had significantly higher mean microhardness values than negative control, but significantly lower mean microhardness than positive control. However, an insignificant difference in microhardness values was found between icon and single bond universal adhesive. After the acidic challenge, icon resin infiltration showed significantly higher mean microhardness than negative control. In addition, single bond universal adhesive showed insignificant difference than negative control (Table 1).

Table 1: Mean, standard deviation (SD), minimum, and maximum of microhardness for different groups after acidic challenge

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>p-value</th>
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<tr>
<td></td>
<td>Positive control</td>
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<tr>
<td>Mean</td>
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<td>SD</td>
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<td>Minimum</td>
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<tr>
<td>Maximum</td>
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<td></td>
<td>Negative control</td>
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<tr>
<td>SD</td>
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<td>Minimum</td>
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<tr>
<td>Maximum</td>
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<td></td>
<td>Icon resin infiltration</td>
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<td>Minimum</td>
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<tr>
<td>Maximum</td>
<td>363.89</td>
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<tr>
<td></td>
<td>Single bond universal adhesive</td>
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<tr>
<td>Mean</td>
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<tr>
<td>SD</td>
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<td>Maximum</td>
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</table>

*Significant. Same letter within each row is not significantly different at $p \leq 0.05$.

Discussion

Oral cavity is a battlefield of demineralization and remineralization processes, the balance between them is a critical factor that affects the caries process [12]. Remineralization of the early carious lesions bridges the traditional gap between prevention and surgical procedures, accurately measure the degree of mineral loss, guaranteeing that the correct intervention will be applied [13]. Infiltration techniques were used to create a diffusion barrier inside the lesion, by replacing the lost minerals with resin [14]. Icon has reported successful depth within the WSLs, and it has been proved to be effective in hypomineralized lesions. This good penetration also tends to increase the microhardness of the structure [15]. It is applied in three sequential steps: Hydrochloric acid etching, ethanol drying, and resin infiltration. Comparison between single bond universal adhesive and icon resin infiltration was done in this study to conclude if the clinicians could use an ordinary adhesive system rather than Icon in treatment of white enamel lesions. One of the commonly used dental adhesive is the single bond universal adhesive and it could be used in total-etch, self-etch or selective-etch mode. In this study, total-etch mode was used to compare the use of phosphoric versus hydrochloric acid. An ethanol drying step was performed with both treatment modalities to standardize the intermediate drying procedure. Maxillary molar teeth were selected as they have wide, flat surface area in its proximal surface to fulfill the testing procedure requirements and to enable performing 4 windows on both proximal surfaces, such that every tooth serves as its own control. Acid resistant nail varnish coated the entire tooth except the windows to ensure that the demineralizing solution act on the exposed area of enamel only in a proportion of 2 ml solution/mm$^2$ to avoid over or under demineralization of the exposed enamel area [10]. Specimens were immersed in the demineralizing solution (CaCl$_2$, NaH$_2$PO$_4$, lactic acid, and fluoride) to make subsurface demineralization of approximately 150 microns width, with intact surface like the early enamel lesion [11]. Calcium and phosphate concentrations were 50% of the saturation level in the demineralization solution which allows dissolution of enamel subsurface. Fluoride was added to reduce the demineralization by forming fluorapatite at the surface, which simulated the happening early enamel lesions.
having nearly intact surface layer [10]. Vickers microhardness tester was used to assess the microhardness of all specimens. Microhardness measurement is suitable for a material having fine microstructure, nonhomogeneous or prone to cracking like enamel. Surface microhardness indentation provides a relatively simple, non-destructive, and rapid method in demineralization and remineralization studies. It was measured at points per specimen and the mean was calculated to try to abolish the slight variations substructure [16]. Concerning the acidic challenge, the same demineralizing solution that was initially utilized to induce the artificial caries lesion was utilized to test the durability of the tested materials against the acid attacks and further progression of the carious lesion. During those procedures, specimens were kept in a water to avoid dehydration which might affect the obtained microhardness results [17]. The results of that study showed that bothIcon and single bond had significantly higher microhardness values than the negative demineralized control, on the other hand, those values were still significantly lower than positive sound enamel control. Moreover, no significant difference was found between icon and single bond groups. Infiltration of enamel WSLs is a promising alternative treatment of remineralization and restorative treatment. The resin used in this technique had low viscosity, low contact angles to enamel, and high surface tension. Therefore, the pretreatment of the impermeable surface layer by acid etching is effective and allows deeper infiltration of the resin inside the body of the lesion [15], [18]. Infiltration of enamel lesions is related to the capillary forces. The pore volume and the capillary radius of the material to be penetrated directly affect the resin infiltration. Consequently, the low pore volume on the enamel superficial layer will obstruct the resin penetration and the use of acid etching will perforate and open these pores and enhance the resin infiltration. As a result, the removed parts of the surface layer by acid etching are used to improve the surface porosity and thus make the body of lesion accessible for mineral ions [19]. In this study, 37% phosphoric acid etch was applied for 20 s then the single bond universal adhesive was applied, compared to 15% hydrochloric acid etching for 120 s with icon. Meyer-Lueckel et al. [18] used 15% hydrochloric acid gel etchant for 90–120 s and he stated that it was more successful in eroding the enamel surface for all application times and result in complete removal of the surface layer. Thus, more profound penetration of resin infiltrant might be achieved [14]. Absolute alcohol (99% ethanol) was applied for 30 s before application of the Icon infiltrant, as well as with single bond adhesive. Addition of ethanol increases the penetration coefficient by decreasing the viscosity and contact angle [14]. It acts as a water-chaser which utilized to chemically dehydrate the demineralized enamel lesion. In addition, ethanol-saturated substrate is much more hydrophobic than water-saturated one, and its solubility parameter is closer to the resin monomer [20], which will permit superior diffusion of resin and improve the durability of the resin infiltration. Icon infiltration is a low molecular weight, methacrylate-based resin matrix containing bisphenol A-glycidyl methacrylate and tri-ethylene glycol dimethacrylate (TEGDMA), and single bond also contain dimethacrylate resins with hydroxyethyl methacrylate (HEMA) and an ethanol-water solvent. Resinous mixtures containing high amounts of HEMA, TEGDMA, and ethanol are associated with high penetration coefficients and satisfactory hardening results subsequently, they can be effective in caries penetration [21]. As a result, HEMA and ethanol are important factors in the infiltrating agents. On the other hand, Meyer-Lueckel and Paris [22] contended that a solvent-free resin that contained TEGDMA is much better in use. This could explain that both Icon and single bond had significantly higher microhardness values than the negative demineralized control, with insignificant difference between both treatments. The results of this study agreed with Gray and Shells [23] who found excellent results when they used short time etch with 36% phosphoric acid, dehydration with ethanol for 2 min, and multiple layers of bonding resin. The results of this study agreed with Subramaniam et al. [24] who concluded that the resin infiltration penetrated the artificially created WSL effectively. Although significantly higher microhardness values than negative demineralized enamel, both resin infiltration treatments showed significantly lower results than sound enamel (positive control). On the other hand, Taher et al. [2] stated that enamel treated with the resin infiltration had nearly the same microhardness as sound enamel, indicating that this material may be effective in the treatment of enamel subsurface lesions. This might be due to the application of icon on sound enamel and in this study; we used the Icon with demineralized enamel. After the acidic challenge, the microhardness results of both resin treatments decreased than after treatment and Icon appeared significantly higher microhardness than negative demineralized control, whereas single bond showed non-significant difference as compared to the negative demineralized control. The success of resin infiltration was depending on the type of solvent used within the adhesives systems [25] and the penetration of the resin into the pores of the body of the lesion and the depth of the areas, in that manner, it will protect the lesion against further demineralization. Mueller et al. [6] stated that penetration of adhesives into initial caries lesions inhibited further demineralization and the application of a second layer of the adhesive was important to reduce the progress of the lesion. Furthermore, Paris et al. [26] stated that increasing the application time which improves the penetrative effect of the adhesive by up to 30 s and decreases the lesion progression by 15 s application time and in this this study, double coat of Icon infiltration was applied for 3 and 1 min, respectively, according to manufacturer instructions.
Conclusion

Exposed to acidic challenge, icon resin infiltration system was more resistant than single bond universal total-etch adhesive system in microhardness.

Recommendations

Icon resin infiltration technique seems promising, noninvasive approach that hinders the progress of the carious lesion and it is expected to increase the span of microinvasive dentistry.

References


