Surface Properties and Color Stability of Resin-Infiltrated Enamel Lesions

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Clinical Relevance
Resin infiltration increases surface hardness of white spot lesions and remains stable under thermocycling challenges, but its surface polish and color stability may be of concern when used in the esthetic zone.

SUMMARY
Objectives: To examine the surface topographies, microhardness, and color stability of resin-infiltrated enamel lesions before and after aging challenges in vitro using three-dimensional laser scanning profilometry, surface microhardness testing, spectrophotometry, and scanning electron microscopy.

Methods: Forty human third molars were embedded in epoxy resin, and each tooth was prepared to have two white spot lesions and one sound enamel area. One white spot lesion received resin infiltration and the other was untreated. Ten specimens were subjected to thermocycling for 10,000 cycles, 10 specimens were immersed in coffee solutions, and 10 specimens were placed in water storage. Surface area roughness (Sa), Vickers microhardness (VHN), and CIE L*a*b* color values were measured on sound enamel, resin-infiltrated lesions, and untreated lesions before and after aging. The surface morphology of resin-infiltrated lesions was observed after aging under scanning electron microscopy and compared with 10 specimens that were not subjected to aging challenge.

Results: Resin infiltration increased the surface microhardness of the enamel lesions from 89.3 to 212.0 VHN. The surface microhardness of resin-infiltrated enamel lesions was not significantly affected by aging. The surface roughness of resin-infiltrated lesions (0.32–0.37 \( \mu \)m) was greater than that of sound enamel (0.05–0.06 \( \mu \)m) and untreated lesions (0.12–0.13 \( \mu \)m). Thermocycling and water storage further increased surface roughness of resin-infiltrated surfaces. Resin-infiltrated enamel lesions showed greater discoloration than sound enamel surfaces. Surface microfissures and microcracks were observed on resin-infiltrated enamel lesions after thermocycling.

Conclusions: Surface hardness of enamel lesions increased significantly after resin infiltration and remained stable following thermocycling. Surface roughness and color
stability of resin-infiltrated enamel lesions were less than ideal and might further deteriorate after aging in the oral environment.

INTRODUCTION

Although frequent application of fluoride is often recommended as the treatment of choice for initial enamel caries on smooth or proximal surfaces, the effectiveness of this approach depends strongly on the patient’s oral hygiene practice and is therefore not suitable for noncompliant patients. An alternative therapy to arrest initial caries lesions is infiltration of the pores and microspaces within enamel lesions using a low-viscosity liquid resin. It has been shown that artificial and natural caries lesions on smooth, interproximal, and occlusal surfaces can be successfully infiltrated using this microinvasive technique.

In comparison to fluoride therapy, resin infiltration was found to result in greater surface hardness of enamel carious lesions. Resin infiltration was effective, at least in the short-term, in arresting both the smooth surface and interproximal surface enamel lesions in randomized and controlled clinical trials, and the procedure was well received by both the practitioners and the patients. As this technique is relatively new, there is a lack of data on its long-term outcomes. In a recent study that followed 45 patients for a period of 12 months, the infiltrated surface showed excellent marginal adaptation but significant discoloration. This finding was further substantiated by an experiment in vitro, which found that infiltration resin had the highest staining susceptibility as compared with several resin-based dental bonding and adhesive materials.

As smooth surface white spot lesions are often present in the esthetic zone, color stability of resin-infiltrated surfaces is an important determinant for long-term success. It is generally accepted that all resin-based dental materials degrade to some extent in the oral environment. Water sorption and surface degradation are considered factors associated with discoloration of resin-based dental materials. In addition, polishability and surface roughness contribute significantly to color stability and discoloration of this type of material.

Although discoloration of infiltration resin has been reported in the aforementioned studies in vivo and in vitro, the mechanism underlying the color change remains unclear. In contrast to conventional resin-based restorative materials, currently available infiltration resin is composed mainly of hydrophilic triethylene glycol-dimethacrylate (TEGDMA). Infiltration resin has two major differences from the other resin-based materials: it is an unfilled liquid resin composed of mostly TEGDMA and it does not have a polishing step after its application per the manufacturer's instructions. TEGDMA is important for maintaining the extremely low viscosity that allows penetration of the resin to the demineralized lesion. But it is well known that TEGDMA has a high water sorption rate and is prone to discoloration, and a nonpolished surface may mean a rougher surface. Aging challenges under thermal stress might further affect the physical property and color stability of resin-infiltrated enamel surfaces and compromise the long-term outcomes. The aim of the present study was to evaluate the influence of aging challenge on the physical properties and color stability of resin-infiltrated enamel surfaces in vitro.

METHODS AND MATERIALS

A total of 40 freshly extracted permanent third molars were collected from oral surgery and general dentistry clinics following ethic guidelines from the authors’ institution. The teeth were cleaned from soft tissues and stored in a refrigerator in 0.1% thymol solution for no more than two months before use.

Sample Preparation

The teeth were sectioned at the cemento-enamel junction using a high-speed handpiece (TF12, Mani, Inc., Tochigi, Japan) with water coolant. The crowns were placed in cylindrical plastic molds (20 mm in diameter and 20 mm in height) with cusps facing down on a flat surface and embedded in epoxy resin. The occlusal surfaces of the embedded crowns were ground flat with 400-grit waterproof SiC paper (Softflex, Matador GmbH, Remscheid, Germany) under water cooling until at least three flat areas of the enamel, each measuring at least 2 mm × 2 mm, were exposed. The exposed enamel areas were typically the two buccal cusps and one lingual cusp of the molar crown (Figure 1a, b). Exposed enamel surfaces were then polished in sequence with 800-, 1200-, 2400-, and 4000-grit waterproof SiC paper using running tap water as a coolant. The prepared specimens were examined under a stereomicroscope to verify that the enamel surfaces were exposed, with absence of cracks or other surface defects. After preparation, the specimens were stored in 0.1% thymol solution to avoid dehydration.
Artificial Enamel Caries Lesions

Of the three exposed enamel surface areas, one area was covered with acid-resistant nail polish to serve as a sound enamel control. The other two areas were left exposed. Artificial lesions were created within the two exposed enamel areas by immersing each tooth into a 50-mL aliquot of a Ca/PO₄/acetate solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate maintained at pH 4.5 and a temperature of 37°C for 48 hours. The artificial white spot lesions created presented depths between 100 and 150 μm and exhibited optical properties of early-stage caries lesions.²¹

The artificial caries model prepared this way has two separate artificial white spot lesions on the same specimen and one sound enamel area as internal control (Figure 1c). The white spot lesion was identified as an opaque and chalky white area on the enamel surface in contrast to the semi-translucent sound enamel.²² Each specimen was inspected visually to ensure that the white spot lesions were successfully created on the enamel surfaces.

Resin Infiltration of Enamel Surface Lesions

Of the two lesions on the enamel surface of the specimen, one was randomly chosen to receive the ICON (ICON DMG, Hamburg, Germany) resin infiltration treatment, while the other served as the control. A computerized simple randomization scheme was used to select the lesion for treatment (see Supplementary Materials). The resin infiltration treatment followed a protocol described in detail in a previous report (Figure 1d).²³ Briefly, the selected lesion was etched with 37% phosphoric acid gel (Gluma Etch 35 Gel, Heraeus Kulzer GmbH, Hanau, Germany) for five seconds, rinsed with air-water spray for 30 seconds, and air dried for 10 seconds. Pure ethanol was then applied to the lesion surface for 10 seconds, followed by air drying for another 10 seconds to render surface desiccation. The ICON infiltration resin was applied to the enamel caries lesions for three minutes, and resin excess was removed with a cotton roll. The resin-infiltrated surface was then light-cured for 40 seconds. The infiltration resin was applied for a second time as above for one minute and light cured for another 40 seconds. After light curing, the resin surface was polished with 4000-grit aluminum oxide abrasive paper for 20 seconds.

Thermocycling, Staining Challenge, and Water Storage

Following resin infiltration treatments, the specimens were randomly divided into the following four groups using a random list generator: 10 specimens for thermocycling challenges, 10 specimens for staining challenges in coffee at 37°C, 10 specimens for water storage at 37°C as a control, and 10 specimens for scanning electron microscopy (SEM) to obtain baseline surface morphology data on resin-infiltrated enamel lesions.

For the thermocycling group, the specimens were placed in a thermocycling machine programmed to perform 10,000 cycles in 180 hours (7 days 12 hours) between two water baths at temperatures of 5°C and 55°C, respectively, with a dwell time of 30 seconds at each bath temperature.

For the staining challenge group, the specimens were immersed in coffee solution prepared with 75 g instant coffee (Nescafé, Nestlé, Vevey, Switzerland) in 750 mL boiling water and stored at 37°C for 180 hours. The coffee solution was refreshed every day for seven days.

For the water storage group, the specimens were stored in distilled water at 37°C for 180 hours, the
same duration as the thermocycling and the staining challenge group.

3D Laser Scanning Microscopy
Specimens in the thermocycling and water storage group were subjected to surface roughness testing using a three-dimensional (3D) laser scanning microscope (VK-X100/X200, Keyence, Osaka, Japan) at 3000× magnification before and after the aging challenges. For each area on each specimen, three different locations (95.7993 × 71.8495 μm² in size) were randomly chosen and scanned for 3D surface area profiling. Surface roughness was measured in average arithmetic roughness (Sa) values. The mean values of the three measurements for each area were used as the Sa value.

Surface Microhardness Testing
Surface microhardness of sound enamel, resin-infiltrated lesions, and untreated lesions on each specimen in the thermocycling and water storage group was determined using a Shimadzu microhardness tester (HMV-2T, Shimadzu Corporation, Kyoto, Japan) with a Vickers diamond indenter. Three microhardness indentations were performed with 25-g load and 10 seconds dwell time on each area. The Vickers microhardness (VHN) value for each specimen was measured before and after thermocycling or water storage. The mean values of the three indentations for each area were used as the VHN value.

Spectrophotometry
Specimens in the staining challenge group were subjected to color measurement using an Olympus CrystalEye Spectrophotometer (Olympus, Tokyo, Japan) before and after coffee storage. For each area on the specimen, three different sites were measured according to the CIE L*a*b* system, and the mean values were used as the color of the area.

Scanning Electron Microscopy
To qualitatively assess the surface morphology of resin-infiltrated lesions before aging challenges, 10 specimens were examined with an SEM (BCPCAS4800, JEOL, Tokyo, Japan) immediately following infiltration resin treatment. All specimens were coated with a gold layer approximately 10-nm thick before examination, and the scanning was operated at 4000× and 20,000× magnifications with an accelerating voltage of 1.5 kV. All 20 specimens in the thermocycling and the water storage groups were also scanned in the same manner to qualitatively assess the surface morphology of the resin-infiltrated lesions after the 180-hour aging challenges.

Statistical Analysis
The primary outcome measures of the present study were the changes in the surface roughness of the resin-infiltrated enamel surfaces following thermocycling challenges. Our pilot testing showed that the surface roughness of enamel lesions treated with the ICON infiltrant resin was approximately 0.4 with a standard deviation of approximately 0.05. We considered that a 20% difference in surface roughness was clinically significant as it correlated to an increase of ΔE from 2.9 to 3.5.24 It has been shown that a ΔE smaller than 3.3 is not clinically significant.25,26 Based on the effect size of 20% difference in surface roughness, we needed nine samples in each group to achieve 90% power at an alpha level of 0.05. We decided to use 10 specimens in each group. Surface roughness, microhardness, and coffee staining data were compared among the three areas (untreated lesions, resin-infiltrated lesions, and sound enamel) using analysis of variance and the post hoc Fisher PLSD test. The surface roughness and microhardness data were compared before and after thermocycling or water storage using paired t tests with Bonferroni corrections. Data were analyzed using StatView (SAS Institute Inc., Cary, NC, USA) for two-tailed tests, and a P value smaller than 0.05 was considered statistically significant.

RESULTS
Surface Roughness
Representative 3D laser scanning images of sound enamel, resin-infiltrated lesions, and untreated lesions are shown in Figure 2. Surface roughness data before and after thermocycling or water storage are listed in Tables 1 and 2. Before aging challenges, the surface roughness of the untreated enamel lesions was greater than that of the sound enamel but less than that of resin-infiltrated lesions (p<0.01; Figure 2; Tables 1 and 2), indicating resin infiltration increased the surface roughness of the enamel lesions. Thermocycling and water storage caused a further increase in surface roughness of the resin-infiltrated surfaces (p<0.01) but did not have a significant impact on the sound enamel and untreated lesion surfaces (p >0.05; Figure 2; Tables 1 and 2). The average surface roughness (Sa) of the resin-infiltrated surface increased from 0.373 μm to 0.621
µm after thermocycling for 180 hours. Water storage had a similar effect on the resin-infiltrated surfaces but was smaller in magnitude (from 0.317 µm to 0.472 µm on average).

Surface Microhardness
Surface microhardness measured in VHN before and after thermocycling or water storage is listed in Tables 3 and 4. Compared with that of sound enamel, the mean surface microhardness of the artificial carious lesion was reduced by more than threefold ($p<0.05$; Tables 3 and 4). Surface microhardness of resin-infiltrated enamel lesions decreased slightly after thermocycling and water storage, but this change did not reach the a priori level of statistical significance ($p>0.05$).

Surface Staining
The overall color change of the specimens after coffee storage for all experimental sites is shown in Table 5. Color changes happened to all experimental sites (sound enamel, resin-infiltrated lesions, and untreated lesions). Resin-infiltrated surfaces showed significantly higher color alteration ($\Delta E=12.7\pm4.7$) than sound enamel ($\Delta E=4.3\pm0.8$) but much less than untreated lesions ($\Delta E=31.1\pm4.4$; $p<0.05$). Analysis of $L^*a^*b^*$ values showed that color change of resin-infiltrated lesions was largely due to decreased lightness ($\Delta L^*$; $p<0.05$), while changes in $\Delta a$ and $\Delta b$ were not statistically significant as compared with the sound enamel ($p>0.05$). Untreated lesions showed significant increases in $\Delta a$ and $\Delta b$ values ($p<0.05$) in addition to decreased lightness.

Surface Morphology Under SEM
As shown in Figure 3, resin-infiltrated surfaces were largely intact and uniform before aging challenges (Figure 3a). Surface microcracks and microfissures appeared on resin-infiltrated lesions following thermocycling challenges (Figure 3b). Minor changes could also be observed following water storage but were much less distinct than those after thermocycling (Figure 3c). At high magnification (20,000x), hydroxyapatite crystals were shown to be embedded in the resin matrix to form a relatively uniform and intact surface (Figure 3d). After aging challenges, microfissures and microcracks could be observed on

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<th>Table 1: Effect of Thermocycling on Surface Roughness (Sa) of Resin-Infiltrated Enamel Lesions (Mean ± SD)</th>
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<td>Before Thermocycling*</td>
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<tr>
<td>Sound enamel</td>
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<td>Analysis of variance</td>
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* Different letters in the same column denote statistically significant differences between each other with analysis of variance post hoc tests. NS, not significant.
the surfaces, especially after thermocycling (Figure 3e,f).

**DISCUSSION**

The findings of the present study indicate that resin infiltration significantly increases the surface microhardness of enamel lesions and remains stable after thermocycling challenges. However, surface properties of resin-infiltrated enamel lesions may deteriorate with time in the oral environment and result in an increase in surface roughness and discoloration. Microcracks may appear on the resin-infiltrated surfaces after thermocycling challenges, which may further render the surface vulnerable to staining and discoloration.

Water sorption and surface degradation caused by thermocycling may affect the mechanical properties of resin-based dental materials. Surface microhardness of many resin composites decreased following thermocycling challenges.27,28 Plasticization of the resin matrix by water sorption and hydrolytic breakdown of the resin–filler interface were considered to be the causes of the reduced surface hardness of resin composite materials.28 However, the surface microhardness of resin-infiltrated enamel lesions was not significantly altered following thermocycling in the present study. Although the TEGDMA-based infiltration resin is unfilled and prone to water sorption and matrix degradation, the microhardness of the resin-infiltrated surfaces found in the present study and other studies ranged from 150 to 240 VHN (median 185 VHN)5,20,29 which is significantly higher than most of the highly filled resin composites with a range from 40 to 150 VHN and a median of 72 VHN.28,30 Such high surface hardness is obviously not a function of the resin matrix as polymerized TEGDMA is the softest (26 VHN) among the resin polymers used in dental restorative materials.31 The infiltration resin was designed to penetrate the porous lesions left after acid etching and to fill the voids and spaces of the demineralized zone in a white spot lesion, thus preventing further demineralization and lesion progression.32,33 It appears that the infiltration resin was able to encapsulate the hydroxyapatite crystals in the white spot lesion and form a relatively uniform resin-hydroxyapatite complex (Figure 3a,d) that exhibits high surface hardness. Although microcracks and microfissures may occur on the surface of the resin–hydroxyapatite complex, its surface hardness remained stable following thermocycling challenges. A stable resin–hydroxyapatite complex may be the foundation for clinical success of the resin infiltration technology.

Surface roughness of resin-infiltrated carious lesions was reported to be as high as 6.9 μm on average using the ICON infiltration resin,20 which is considerably higher than the 0.2 μm threshold generally regarded as acceptable for a restorative material to resist plaque accumulation.34-36 We found that the average surface roughness of resin-infiltrated areas was approximately 0.32 μm to 0.37 μm immediately after treatments, which was generally in agreement with that of Mueller and others.36 After thermocycling challenge at temperatures between 5°C and 55°C for 10,000 cycles, simulating one-year of clinical service,37 the surface roughness of resin-infiltrated lesions further deteriorated to 0.62 μm on average (Table 2; Figure 2), signifying a 70% increase compared with baseline. Repeated temperature fluctuations in the oral cavity may

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<th>Table 2: Effect of Water Storage on Surface Roughness (Sa) of Resin-Infiltrated Enamel Lesions (Mean ± SD)*</th>
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<td>Before Water Storage</td>
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<th>Table 3: Effect of Thermocycling on Surface Microhardness (VHN) of Resin-Infiltrated Enamel Lesions (Mean ± SD)*</th>
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induce degradation of resin-hydroxyapatite bonds due to differences in thermal expansion coefficients between enamel hydroxyapatite and the infiltration resin.\textsuperscript{38,39} Thermal stress may also affect the surface integrity of resin-infiltrated enamel lesions as indicated by the presence of surface microcracks and microfissures after thermocycling challenges (Figure 3). These changes in surface properties may contribute to staining and discoloration of resin-infiltrated surfaces. The findings of the present study are in agreement with recent reports that ICON resin-infiltrated carious lesions were prone to discoloration under staining challenges.\textsuperscript{40,41}

Discoloration of resin-based restorative materials may arrive from intrinsic and/or extrinsic stains. Intrinsic stain is associated with the properties of the polymeric networks such as water sorption and the presence of unreacted methacrylate in the resin matrix, while extrinsic stain is caused by external colorants such as those in beverages and foods.\textsuperscript{42-44} The ICON infiltration resin is primarily a TEGDMA-based polymer with high penetration efficiencies.\textsuperscript{45,46} Compared with other resin polymers commonly used in dental materials, such as UDMA and Bis-GMA, TEGDMA has the highest degree of water sorption owing to the presence of hydrophilic ether linkages.\textsuperscript{19,42,47} A high degree of water sorption has long been linked to color stability issues and discoloration of resin-based dental materials.\textsuperscript{13,14,18} On the other hand, surface roughness was recognized as the most important extrinsic factor for discoloration of resin-based dental materials.\textsuperscript{15-17} Most modern resin composite materials for esthetic restorations could achieve a high glossy finish with surface roughness below the acceptable threshold of 0.2 μm after finishing and polishing.\textsuperscript{35} Such a high degree of polishability appears to be difficult to achieve with the infiltration resin, as its surface roughness was not improved even after polishing with the Sof-Lex finishing and polishing system.\textsuperscript{36} Therefore, the mechanisms underlying the discoloration of infiltration resin are likely twofold: one is intrinsically associated with its primary constituent TEGDMA, which has a high degree of water sorption, and the other is extrinsically related to a less than ideal surface polish that deteriorates with time in the oral cavity. To ensure long-term success, further research is warranted to improve surface polish and esthetic outcomes of resin infiltration, especially when smooth surface white spot lesions are involved in the esthetic zone.

As TEGDMA is prone to water sorption and may absorb twice as much water as compared with Bis-GMA, TEGDMA-based materials may be more susceptible to degradation than Bis-GMA- or UDMA-based materials.\textsuperscript{42,48,49} The presence of water in the resin matrix may increase internal stress, leading to microcracking.\textsuperscript{50} It was also shown that water sorption by TEGDMA increases with the elevation of temperatures,\textsuperscript{49} which in combination with the thermal expansion and contraction effects of temperature fluctuation may further affect the integrity of the TEGDMA-based infiltration resin. The surface microcracks and microfissures observed on the resin-infiltrated lesions in the present study may be a result of such internal and thermal stresses due to water sorption and thermal expansion and contraction.

| Table 4: Effect of Water Storage on Surface Microhardness (VHN) of Resin-Infiltrated Enamel Lesions (mean ± SD)\textsuperscript{a} |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Before Water Storage | After Water Storage | Paired t Test |
| Sound enamel                   | 313.2 ± 25.5a    | 312.3 ± 17.5a    | NS             |
| Resin-infiltrated lesion        | 219.1 ± 25.2b    | 209.6 ± 35.6b    | NS             |
| Untreated lesion                | 93.8 ± 25.6c     | 90.2 ± 23.0c     | NS             |
| Analysis of variance            | < 0.01           | < 0.01           |                 |

\textsuperscript{a} Different letters in the same column denote statistically significant differences between each other with analysis of variance post hoc tests. NS, not significant.

| Table 5: Effect of Staining Challenge on Surface Color Change of Resin-Infiltrated Enamel Lesions (Mean ± SD) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | ΔL              | Δa              | Δb              | ΔE              |
| Sound enamel                   | -3.0 ± 0.8a     | 0.7 ± 0.4a      | 2.9 ± 0.7a      | 4.3 ± 0.8a      |
| Resin-infiltrated lesion        | -9.7 ± 3.5b     | 1.7 ± 1.7a      | 6.4 ± 5.8a      | 12.7 ± 4.7b     |
| Untreated lesion                | -25.3 ± 4.9c    | 5.5 ± 2.6c      | 16.2 ± 5.1c     | 31.1 ± 4.4c     |
| Analysis of variance            | < 0.01          | < 0.01          | < 0.01          | < 0.01          |

\textsuperscript{a} Different letters in the same column denote statistically significant differences with analysis of variance post hoc tests.
CONCLUSION

Within the limitations of this study, we conclude that the surface hardness of resin-infiltrated enamel lesions was high and remained stable following the thermocycling challenges. Surface roughness and color stability of resin-infiltrated enamel lesions was less than ideal and might further deteriorate after aging in the oral environment. Surface microcracks and microfissures could occur on the surface of the resin-hydroxyapatite complex after aging challenges. These changes may render the resin-infiltrated areas susceptible to discoloration.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Rochester.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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