Enamel margins resealing by low-viscosity resin infiltration

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This study evaluated low-viscosity resin potential as enamel marginal gap sealant for existing composite restoration. Standard restorations with marginal gaps were created in bovine teeth; gaps were resealed using G-Bond Plus (GB, GC, Tokyo, Japan) or Icon (ICN, DMG, Hamburg, Germany) with or without HCl pretreatment (n=8). Swept-source optical coherence tomography (SS-OCT) images were taken before and after resealing of the margin and thermal cycling to calculate enamel marginal gap extent. Cross-sectional microscopy was performed to confirm SS-OCT findings. SS-OCT showed remarkable reduction of backscatter signal at enamel margins after application of the low-viscosity resin. Enamel margin resealing significantly decreased gap and there was a significant difference between ICN (regardless of HCl pretreatment) and GB, while thermal cycling increased gaps (p<0.05). The low-viscosity resin could effectively infiltrate micro-gaps at enamel margins and improve sealing of an existing composite restoration. Resin infiltration is a viable option for resealing intact restorations with open margins.

Keywords: Resin infiltration, SS-OCT, Resealing, Hydrochloric acid

INTRODUCTION

Resin composite has predominantly become the material of choice to directly restore the function and morphology of the missing tooth structure. However, despite improvements in their mechanical properties, shrinkage due to polymerization remains an intrinsic, often challenging property. Furthermore, long-term bond degradation and residual stresses may affect integrity of the restoration which may cause marginal gap formation and a loss of seal¹. Several studies have evaluated the association between marginal gaps and marginal caries²,³. Such gaps are known to cause microleakage which remains one of the reasons of failure and requires re-restoration.

In case of small morphologic discrepancies at the margins and in the absence of a diagnosis of secondary caries, polishing could address the issue. Nevertheless, with overzealous use of polishing procedure, there is wearing of the superficial tooth structure leading to more accumulation of local deposits. This also removes the fluoride-rich outer layer of enamel, which takes a period of 3 months to rebuild⁴,⁵. Also, the decision of when to repair and when to replace is often taken based on subjective criteria. Dentists may opt for replacement of a restoration with defective margins; however, the procedure is usually accompanied by the loss of additional tooth structure, and involves replacement of an otherwise intact restorative material.

Therefore, a limited treatment such as re-sealing is desirable to save time, cost and most importantly to preserve tooth structure. However, re-sealing agents for marginal gaps in restorations have not been extensively investigated due to limited choice of materials, techniques and evaluation methods.

Previous studies have evaluated re-bonding techniques reporting improvements in the wear resistance⁶, the marginal integrity and minimizing microleakage⁷. However, the efficacy was reported to be material-dependent and some discrepancies in the results were found⁸. This may be partly caused by the use of assessment methods based on visual inspection. Furthermore, conventional assessment methods for microleakage require destruction of the specimen and do not allow evaluation in vivo. Hence, an objective assessment tool is desirable for clinical dentistry.

Swept-source optical coherence tomography (SS-OCT) is a non-invasive and non-destructive diagnostic method that provides high-resolution cross-sectional images⁹ for the evaluation of materials and dental structures without the use of ionizing radiation. In SS-OCT, a coherent light is projected over the sample, and the image is reconstructed in real time from depth-resolved backscattering. SS-OCT has the ability to characterize physiological changes in dentin, evaluate restins composites adaptation, assess natural enamel lesions and observe gaps and defects in dental restorations¹⁰,¹¹. Low-viscosity light curing resins have been developed recently for use as micro-invasive treatment for caries infiltration extended radiographically up to the outer third of dentin¹²,¹³. Such triethyleneglycol...
dimethacrylate (TEGDMA) based infiltrant resin driven by capillary forces aims at occluding the porosities of carious lesions consequently obstructing the diffusion of acids and dissolved minerals. Thus, the lesion progression is reduced or arrested. However, acid-dissolution of the intact surface layer using hydrochloric acid (HCl) is required. Such surface layer hampers the penetration of the infiltrant into the lesion body. Previous investigations also demonstrated that the resin infiltration can mask enamel white spot lesions\(^{16,19}\).

This is achieved by infiltration of the lesions using a resin with a refractive index similar to the enamel’s (1.52 vs. 1.62–1.65). Hence, visually the lesions appear similar to the adjacent enamel sound and white spots are aesthetically masked. Despite having proven their efficacy both in situ\(^{20}\) and in vivo\(^{17,21}\), the infiltrant potential as sealant for marginal gaps in restorations have not been extensively investigated.

Thus, the aim of this study was to evaluate the efficacy of the low-viscosity resin to reseal marginal gaps of composites using SS-OCT as evaluation method. The null hypotheses were that there was no significant difference regarding enamel marginal gap extent before and after the use of low viscosity resins, and that there was no difference between the infiltrant resin with or without the HCl pretreatment and a bonding agent to reseal the margins.

**MATERIALS AND METHODS**

The materials used in this study are listed in Table 1. The enamel marginal gaps resealing ability of the infiltrant resin Icon (ICN; DMG, Hamburg, Germany) with and without HCl pretreatment was compared to that of an all-in-one self-etching adhesive G-Bond Plus (GB; GC, Tokyo, Japan) in 4 experimental groups:

1. Gap resealed with ICN resin and HCl pretreatment (RE-ICH)
2. Gap resealed with ICN resin without HCl pretreatment (RE-ICN)
3. Gap resealed with GB (RE-GBN)
4. Positive control group bonded (no intentional gap) and not resealed (CNTRL)

**Preparation of the specimens**

A schematic illustration of the experiment is shown in Fig. 1. Freshly extracted sound bovine teeth were selected for this study. The buccal enamel was lightly polished with 1000-grit silicon carbide (SiC) paper in order to expose enamel and remove the superficial layer. Tapered round cavities 4 mm in diameter and 2 mm in depth were prepared using a diamond bur (149FG, 100-μm grit, Shofu, Kyoto, Japan) attached to a high-speed air turbine under water coolant, and then a superfine finishing bur (SF109R, 25-μm grit with flat end-tapered cylinder, Shofu)\(^{3}\). SS-OCT was used for monitoring the standardization of the prepared cavities. The specimens were divided into 4 groups (n=8) as mentioned above.

**Creating the gap**

In order to create standard marginal gaps in the resealing specimens (24 of 32 cavities), the cavity floor and almost half of the axial walls and margin were treated with a bonding agent (Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan), while the remaining part of the axial walls and margin were left without bonding agent. The treated cavities in all groups were restored with a flowable resin composite (Estelite Flow Quick, Tokuyama

| Material       | Composition                                                                 | Lot No. | Manufacturer  |
|----------------|------------------------------------------------------------------------------|---------|---------------|
| Clearfil SE Bond Primer: | MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-CQ, N,N-Diethanol-p-toluidine, water | 7M0160  | Kuraray Noritake Dental |
|                | Bond:                          |         |               |
|                | MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, dl-CQ, N,N-Diethanol-p-toluidine, colloidal silica | 7L0257  |               |
| G-Bond Plus    | Phosphoric acid ester monomer, 4-MET, UDMA, dimethacrylate monomer, water, acetone, PI, stabilizer, nano-silica filler | 1604201 | GC            |
| Estelite Flow Quick | Bis-MPEPP, TEGDMA, UDMA, silica-zirconia filler, silica-titania fillers (53% filler by volume, 0.04 to 0.6 μm particle size), CQ | J061    | Tokuyama Dental |
| Icon-Etch      | 15% HCl, pyrogenic silicic acid, surface-active agents                        | 724869  | DMG           |
| Icon-Dry       | 99% ethanol                                                                  | 724862  | DMG           |
| Icon-Infiltrant | methacrylate-based resin matrix (TEGDMA), initiators, additives              | 724856  | DMG           |

MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; CQ: camphorquinone; Bis-GMA: bisphenol-A-diglycidyl methacrylate; 4-MET: 4-methacryloxyethyl trimellitic acid; UDMA: urethane dimethacrylate; Bis-MPEPP: bisphenol A polyethoxy methacrylate; TEGDMA: triethyleneglycol dimethacrylate
Dental, Tokyo, Japan) in a single increment, and cured for 20 s using a halogen light unit (Optilux 501, Kerr, Orange, CA, USA) with an output power density of 600 mW/cm². In this manner the polymerization shrinkage of the composite reproducibly created marginal gaps that extended along the unbounded axial walls. In the bonded positive control group CNTRL, the whole cavity was treated with the all-in-one adhesive (GB) according to the manufacturer’s instructions and filled in the same manner as the other groups. After storage in normal water for 24 h at 37°C, excess of resin was polished away using 2000-grit (SiC) paper.

**Infiltration treatment**

In the RE-ICH group, a 15% HCl etching gel (DMG) was applied by screw syringe on the area for 120 s in accordance to the manufacturer’s instructions. The gel was washed off with air-water-spray for 30 s and then dried by oil-free and water-free air for 10 s. A second screw syringe containing ethanol was applied, let set for 30 s, and dried again by air-blowing. Finally, the third screw syringe containing the infiltrant was applied. After 3 min of penetration and setting time, excess material was removed, and the infiltrant was light-cured for 60 s. A new tip was screwed onto the infiltration syringe and the infiltration step was repeated once with a setting time of 1 min and light-cure again for 60 s. The infiltration step was repeated once with a setting time of 1 min and light-cure again for 60 s. In the RE-ICN group, ICN resin was applied without HCl using the methodology above described. Finally, in the RE-GBN group, GB was applied as resealant according to manufacturer’s instructions. All the specimens were again subjected to SS-OCT imaging (IVS-2000, Santec, Aichi, Japan) after the infiltration treatment following the procedure above described at exactly the same cross-section.

**Thermal cycling procedure**

All the samples were subjected to thermal cycling procedure immersing them in deionized water baths at 5 and 55°C with a dwell time of 30 s in each temperature and a transfer time of 2 s between baths (Cool Line CL200 and Cool Mate TE200, Yamato Scientific, Tokyo, Japan) for 10,000 cycles, which has been reported to represent approximately one year of clinical function²²).
The samples were subjected to SS-OCT imaging again after the thermal cycling treatment at the exact same cross-section using the same methodology as described below.

**SS-OCT system and imaging**

Each sample was mounted and fixed on a micrometer head stage and subjected to SS-OCT imaging that incorporates a high-speed frequency-swept laser that sweeps the wavelength from 1,260 to 1,360 nm (centered at 1,310 nm) at a 20 kHz rate. The axial and lateral resolutions of the system in air are 12 μm (7–8 μm in tissues with a refractive index of about 1.5) and 20 μm, respectively. The sample is scanned at the desired X and Z dimensions by the laser beam emitted from the probe. The collected backscattered light from each scan point is returned to the system, digitized in a time scaled, and then analyzed in the Fourier domain forming a depth-resolving scan (A-scan). Several A-scans along the sample can make a cross-sectional B-scan. By converting B-scan raw data into a grayscale image, high-resolution 2-dimensional images can be obtained. The SS-OCT hand-held scanning probe was set at 5-cm distance from the samples, with the scanning beam oriented about 90 degrees to the surface. The imaging range in this study was 5 mm (width) by 4.98 mm (depth), forming a 1,002×1,000 pixel image.

**SS-OCT image analysis**

Both pre-infiltration treatment and post-infiltration raw SS-OCT data were imported to image analysis software (ImageJ v1.47, Wayne Rasband, NIH, Bethesda, MD, USA) where the region of interest (ROI) was selected along the thickness of the enamel as a polygon selection being the ROI width ~130 pixels. The enamel marginal gap extent was calculated by means of a custom plugin as shown in Fig. 1. Such plugin distinguished and considered pixels with increased signal intensity as interfacial gap: target pixels (white) are designated as a median values when the higher intensity values pixels were equal or greater than the background noise sum. All other pixels were designated as null (black). The plugin calculated automatically the enamel marginal gap extent of such white target pixels (gap) over the ROI length.

**Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) observation**

The actual gaps sealing was confirmed under CLSM and SEM. For this purpose, after SS-OCT imaging representative specimens were embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) and then cut across the restoration by means of a low-speed diamond saw (Isomet, Buehler). Specimens were then polished up to the same SS-OCT cross-section of interest using 2000-grit SiC paper and diamond pastes down to 0.25 μm in particles sizes under running water in circular motions. Samples were ultrasonicated at the end of each polishing step and finally examined under CLSM (VK-X150, Keyence, Osaka, Japan) at low magnification (×250). The specimens were then air-dried for 24 h in the room temperature (23°C), sputter-coated with gold and viewed under the SEM (JSM-5310LV, JEOL, Tokyo, Japan) at high magnification (×2,000) with an accelerating voltage of 15 kV.

**Statistical analysis**

The gap data were analyzed using repeated-measure ANOVA comparing the four treatment groups and three imaging stages before infiltration, after infiltration (except CNTRL group), and after thermal cycling procedure, and the interaction between the factors. A significant interaction was followed by pair comparisons with Bonferroni correction. The statistical procedures were performed at a significance level of α=0.05 with the statistical package for social science (SPSS for windows, Version 16.0, IBM, Chicago, IL, USA).

**RESULTS**

Figure 2 represents SS-OCT cross-sectional B-scans images before and after the infiltration with GB and ICN resin and confirmatory CLSM and SEM images of the enamel margins and gaps (Figs. 3 and 4). SS-OCT showed remarkable reduction of backscatter signal at
Representative CLSM images (×250 magnification) confirming the good sealing results found in SS-OCT (b, c). Images correspond before the application of sealant (a), after Icon and HCl pretreatment (RE-ICH) (b), Icon without HCl pretreatment (RE-ICN) (c), and G-Bond Plus (RE-GBN) (d). The white arrows (b, c) show Icon (ICN) reaching the dentin enamel junction (DEJ). E, enamel; C, composite.

Representative SEM images (×2,000 magnification) before the application of sealant (a), and after the application of Icon with HCl pretreatment (RE-ICH) (b), without HCl pretreatment (RE-ICN) (c), and G-Bond Plus (RE-GBN) (d). Icon (ICN) showed good penetration (b, c) whereas the bonding agent sealing capability was inferior to that of Icon (ICN) (d). These images confirmed the results obtained with SS-OCT. E, enamel; C, composite.

Enamel margins after application of the low-viscosity resin ICN. Such decrease in signal intensity revealed an improved sealing in confirmatory microscopy images.

Repeated-measure ANOVA analysis of the SS-OCT data before and after the infiltration revealed a significant difference in enamel marginal gap extent ($p<0.05$) in all resealed groups (RE-ICH, RE-ICN, RE-GBN). The average enamel marginal gap extent values for all groups with their standard deviations are plotted in Fig. 5. When enamel gap was present, the application of a resealant reduced the enamel marginal gap extent significantly. A significant difference ($p<0.05$) was found between the use of ICN, regardless of the usage of HCl, and GB as resealant. This was also confirmed through CLSM and SEM images where ICN (Figs. 3b, 3c, 4b and 4c) shows better sealing capabilities than GB (Figs. 3d and 4d). No significant difference ($p>0.05$) was found with or without the use of HCl in ICN. Thermal cycling regimens caused a significant increase in the enamel marginal gap extent in all groups as shown in Fig. 5 ($p<0.05$).

**DISCUSSION**

In the present study our aim was to evaluate the resealing performance of composite restoration margins by low-viscosity resin infiltration with SS-OCT. Gaps at the enamel margins appeared as a peak in the signal intensity. Lack of seal between composite resin and enamel causes such increased signal intensity at the interface. This is due to the increased scattering of light at an interface between two media with different refractive indices. This discrepancy in the refractive index ($\eta$) between air filling the gaps or defects ($\eta=1$) and
that of composite resin and enamel (η=1.55 and 1.63, respectively) produces such backscattering of light at the interface, as shown in Fig. 2. Refractive index of the low viscosity resin used in this study, ICN, is similar to that of enamel (η=1.52) making easy to visualize such discrepancies on the gray-scale image. The power of SS-OCT for reliable detection of lateral wall gap depends on the incidence angle of the laser beam and the interface; a wall inclination over 35° to the incident laser beam has been reported as adequate. Results of the present study showed a reduction of backscattering light on the enamel margins after the application of the low-viscosity resin that was confirmed through CLSM and SEM images.

The penetration of a liquid (light curing resin) has been described by the Washburn equation, where penetration of a liquid into a solid substrate depends on the liquid’s surface tension to air and its contact angle to the substrate surface and is inversely related to the dynamic viscosity of the liquid. This means that high surface tensions, low contact angles and low viscosity will obtain better penetration. The ICN resin used for enamel caries infiltration, penetrates into the subsurface lesion body by capillary forces. The system consists of 3 components: Etch (HCl 15%), dry (ethanol) and the infiltrant (TEGDMA, initiators). It has been reported that higher amounts of TEGDMA and ethanol decrease viscosity and contact angle to enamel, resulting in an increased penetration. This, in addition to the fact of being an unfilled infiltrant, is in accordance to the good sealing found on the CLSM and SEM images as shown in Figs. 3 and 4.

In the present study, GB was selected for the comparison. This all-in-one adhesive does not comprise the hydrophilic monomer HEMA and is fluoride-free. The results are in line with previous works; although adhesives have shown infiltration of shallow lesions, deeper structures could not be achieved. Commercially available adhesives showed low penetration, being in some cases ten times lower than experimental infiltrant resins. Higher molecular weight monomers in GB lack of adequate water displacement by acetone and nano-filler potentially affects its penetration ability negatively in comparison to ICN. In addition, the use of ethanol solution prior to ICN is thought to displace water, further clean debris and improve infiltration of the infiltrant into the space.

In case of a white spot lesion, after removal of the surface layer with HCl, ICN infiltrates, occludes the porosities and prevents acid penetration into the lesion. Nevertheless, in the present study, no such surface layer was present to be removed for gaps infiltration at the enamel margins. Consequently, using another approach, ICN without HCl was used following the same methodology that with etchant and with GB. The results are consistent with a previous study that reported gap impregnation by a low-viscosity resin at unetched margins. Despite these findings, the present authors consider that it is recommended to use the etchant specially in old restorations where the enamel margins are stained; HCl has been known to act by decalcifying the enamel as well as the stain contained intrinsically. These impurities could obstruct the resin penetration by reduction of the enamel free energy. Moreover, TEGDMA is not a functional monomer and believed to not have adhering capacity to the apatite surface. Thus, micromechanical interlocking with enamel by penetration of the low-viscosity resin into the porosities created by the etchant are necessaries for the adaptation and long-term durability of ICN.

The results of the current study showed increase in the gap percentage at enamel margins after the specimens were subjected to thermal cycling, which was significant in both ICN infiltrated groups and bonded specimens. This deterioration is related to the higher thermal contraction/expansion coefficient of the restorative material (as compared with that of tooth tissue) and the accelerated hydrolysis of interface components due to the hot water. Thus, repetitive contraction/expansion stress is generated at the interface that may lead to gap formation (Fig. 5). All-in-one adhesives are by design hydrophilic and previous studies have shown that the hydrophilic resin formulations absorb water. While it was reported that polymerized TEGDMA had a relatively high water sorption, a recent study reported that thermal cycling stress did not affect the sealing ability of ICN on enamel even though the integrity of the resin layer was affected. It was assumed that ICN was not chemically affected as it had no hydrolysable chemical bond.

It should be noted that although in this study the resealing was aimed to seal the enamel margins, the infiltrant ICN could reach as deep as dentin enamel junction (DEJ) in some cases (Fig. 3b, c). Moreover, an interesting finding of this study was that enamel cracks

Fig. 6 The white arrows of this CLSM image show the penetration of the low-viscosity resin Icon (ICN) into enamel cracks, sealing them.
were sealed by ICN, as shown in Fig. 6. Such cracks develop along the cavosurface cavity margin, result in “white-line” appearance and have been attributed to polymerization shrinkage. The low-viscosity resin could penetrate these enamel cracks and seal them as shown in Fig. 6.

It has been shown that the extent of the gap along the enamel margin correlated with the demineralization progress at the margin. Therefore, resealing such margins should improve the resistance of margins against caries progress at the margins of an existing restoration.

Based on the results of the study, the null hypotheses of this study were rejected, as there was significant difference regarding enamel marginal gap extent before and after the use of low viscosity resins, and there was difference between the infiltrant resin ICN (regardless of HCl pretreatment) and a bonding agent to reseal the margins.

CONCLUSION

The low-viscosity resin ICN could effectively infiltrate micro gaps formed at enamel margins and improve sealing of an existing composite restoration. Resin infiltration is a viable option for resealing intact restorations with open margins.

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