μCT-3D visualization analysis of resin composite polymerization and dye penetration test of composite adaptation

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This study evaluated the effects of the light curing methods and resin composite composition on composite polymerization contraction behavior and resin composite adaptation to the cavity wall using μCT-3D visualization analysis and dye penetration test. Cylindrical cavities were restored using Clearfil tri-S Bond ND Quick adhesive and filled with Clearfil AP-X or Clearfil Photo Bright composite. The composites were cured using the conventional or the slow-start curing method. The light-cured resin composite, which had increased contrast ratio during polymerization, improved adaptation to the cavity wall using the slow-start curing method. In the μCT-3D visualization method, the slow-start curing method reduced polymerization shrinkage volume of resin composite restoration to half of that produced by the conventional curing method in the cavity with adhesive for both composites. μCT-3D visualization method can be used to detect and analyze resin composite polymerization contraction behavior and shrinkage volume as 3D image in the cavity.

Keywords: μCT, 3D visualization, Polymerization, Dye penetration, Adaptation

INTRODUCTION

Resin composite polymerization results in volumetric shrinkage, and the resultant stress created leads to formation of gaps between the resin and cavity surfaces^{1–4}. Such marginal gaps and the subsequent micro-leakage may cause marginal staining, postoperative sensitivity^{5,6}, and secondary caries. Light-cured resin composites are widely used in clinical practice. However, the polymerization reaction of light-cured resin composites is faster than that of self-cured composites, which leads to the development of higher setting stresses than self-cured materials⁷. Therefore, the maximum interfacial stress generated at the cavity wall in light-cured resin composite restorations is twice as large as that seen for self-cured resin composite restorations⁸. This stress has been shown to lead to greater gap formation between the resin and cavity surfaces than self-cured resin composite¹.

On the other hand, when the bond strength exceeds the polymerization shrinkage stresses, a crack is initiated in the tooth structure, usually in the enamel^{1,9–15}, leading to a direct communication with the oral cavity. We previously reported that white margins represented cracks in the enamel surrounding resin composite restorations, and these cracks were located 25–100 μm from the composite–enamel interface, as observed using environmental scanning electron microscopy (SEM) under the 100% saturated water vapor condition^{14,15}.

There are several ways to overcome the curing stresses generated by light-cured, bulk-filled resin composites. One technique involves utilization of a flowable resin composite as a lining material¹⁶. The low shrinkage stress¹⁷ contributes to improved adaptation

to the cavity wall^{16,18}. However, the poor mechanical properties of flowable composite¹⁹ decrease the bond strength to the dentin wall^{20,21}. The incremental filling technique is used by many clinicians²². This technique was thought to decrease the curing stress at the tooth–resin interface, which occurs when a cavity is bulk-filled with light-cured resin composites. However, in a theoretical study using finite element analysis, it was reported that the incremental filling technique could produce greater polymerization shrinkage at the restoration–enamel interface compared with the bulk filling technique²³. In addition, it was demonstrated that incremental filling could not improve the bond strength to the floor of a box-like cavity²⁴.

Alternatively, increasing the curing velocity of light-cured resin composite decreased the adaptation to the cavity wall when a resin with a different composition was used²⁵. Therefore, the polymerization rate has a significant effect on the development of strain. The deep internal hardness of cured resin composites increases with argon ion laser output along with increasing intensity, but the maximum internal hardness was reduced with intense light intensity²⁶.

The use of an intense light source may lead to more frequent marginal and wall gap formation^{3,4,27,28}. A slow-start light curing method with an initial low-intensity light followed by a high-intensity light has been used to cure composite and decrease the stresses produced. This method was found to produce excellent marginal sealing and cavity adaptation^{3,4,13–16,29–32}. Previous studies have reported that when a composite is light cured with an initial light intensity of 270 mW/cm² for 10 s, followed by a 5 s interval, and then a light intensity of 600 mW/cm² for 50 s, the resin composite

hardened at the resin composite base faster than at the surface adjacent to the light source^{3,4,33}. This procedure, known as the slow-start curing method^{31,32}, allowed most polymerization contraction to be completed during the initial flowable stage of resin composite polymerization.

Measurement of resin composite polymerization shrinkage is important for resin composite adaptation to the cavity surface. Polymerization shrinkage behavior of resin composite is analyzed using the finite element method^{34,35} and photoelastic analysis^{8,36}. However, the finite element method and photoelastic analysis are unsuitable methods for the measurement of resin composite polymerization in the tooth cavity. The finite element method assumes that the dentin bonding is uniform and is not influenced by dentin depth. Photoelastic analysis requires mold cavity. However, the geometry and region of a clinically prepared tooth surfaces are complex, and therefore it may be difficult to obtain reliable information on the influence of clinical cavity on the actual polymerization behavior by the finite element method and photoelastic analysis.

On the other hand, the micro-focus X-ray computed tomography machine (μ CT) can observe resin composite restoration in the clinical cavity and can be used non-invasively. However, a previous study was only able to detect regional shrinkage within experimental light-cured resin composite with large sized filler^{37,38} or bubble³⁹ markers. Recently, three-dimensional (3D) visualization software provides analysis and modeling system using μ CT scan data. There is no report on polymerization shrinkage volume of resin composite restoration in the cavity with adhesive system.

It is hypothesized that the slow-start curing method will improve the resin composite adaptation to the cavity wall using dye penetration test and decrease composite

polymerization contraction amount in the cavity with adhesive system using μ CT-3D data visualization method.

MATERIALS AND METHODS

Specimen preparation

The materials, components, manufacturers, batch numbers, and bonding procedures used in this study are listed in Table 1. An experimental quartz-tungsten halogen light-curing unit (GC, Tokyo, Japan) (Fig. 1) that was connected to a slide regulator was used. This light curing unit had a control system for lamp voltage and adjustable light intensity, which was

Fig. 1 Experimental quartz-tungsten halogen light curing unit: control systems for two types of lamp voltage (right two knobs); control systems for irradiation time and interval time (left two knobs).

Table 1 Study materials

Material/Manufacturer	Components ^a	Batch No.	Bonding Instruction ^b
Clearfil tri-S Bond ND Quick (TS) (Kuraray Noritake Dental, Tokyo, Japan)	Bond: Bis-GMA, MDP, HEMA, dimethacrylates, sodium fluoride, photoinitiator, water, micro filler	8N0022	a, b (5 s), c (10 s)
Clearfil Photo Bright (Kuraray Noritake Dental) Shade: US	Silanated colloidal silica, prepolymerized organic filler containing colloidal silica, Bis-GMA, dimethacrylates, photoinitiator, others Filler load: 82 weight %	470002	—
Clearfil AP-X (Kuraray Noritake Dental) Shade: A3	Silanated barium glass filler, Silanated silica filler, Silanated colloidal silica, Bis-GMA, TEGDMA, barium glass, micro filler, photoinitiator, others Filler load: 85 weight %	6J0038	—

^a Abbreviations: Bis-GMA, bisphenol A-glycidyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethyleneglycol dimethacrylate

^b Procedures: (a) apply adhesive; (b) dry with gentle air-blowing; (c) light-cure

Fig. 2 Preparation of a bonding substrate: cylindrical cavity with 1/2 enamel and 1/2 dentin margins, 2 mm depth, 3 mm diameter (C-factor=3.7).

measured using a curing radiometer (model 100, Sybron Kerr, Milwaukee, WI, USA). A spectroradiometer (Spectroradiometer 40 V, Ushio, Tokyo, Japan) was used to measure the wavelength distribution and spectral radiant illuminance from 2 to 40 s after the start of light irradiation. Cylindrical cavities with 1/2 enamel and 1/2 dentin margins, 2 mm depth, 3 mm diameter, and a C-factor of 3.7 were prepared on the labial cervical region of extracted intact erupted bovine incisors (Fig. 2) using a diamond point (# B12, GC) under copious water spray. Each of the 32 cavities was treated with the adhesive Clearfil tri-S Bond ND Quick (Kuraray Noritake Dental, Tokyo, Japan). After this adhesive was cured, the cavities were bulk-filled with Clearfil AP-X (Kuraray Noritake Dental) resin composite or Clearfil Photo Bright (Kuraray Noritake Dental) resin composite. The shade of resin composites used in this study corresponds to Vita classical shade A3 (Vitapan Classical, Vita Zahnfabrik, Bad Säckingen, Germany).

The specimens were covered with black plastic sacks, immediately placed in the μ CT imaging chamber, and scanned before light curing, as explained later in this section. Thereafter, the composites were light cured using the conventional curing method (600 mW/cm² for 40 s) or the slow-start curing method (270 mW/cm² for 10 s+5 s interval+600 mW/cm² for 30 s). The tip of the light guide contacted the cavity surfaces. The composites were cured while the sample was fixed to the stage in the chamber, and all samples were re-scanned after light curing using the μ CT.

Micro-focus X-ray computed tomography, 3D data visualization analysis, and modeling

The samples were fixed upright with utility wax and double-sided tape at the marked position on the μ CT sample holder. The operating conditions for the micro-focus X-ray computed tomography machine (InspeXio SMX-100CT, Shimadzu, Kyoto, Japan) were 100 kV and 100 μ A. Metal filters (0.5-mm-thick Al and 0.2-mm-thick Cu) were placed in front of the detector for hardware beam-hardening correction. These resin composite restorations were subjected to segmentation of images observed using μ CT before and after polymerization. This acquired scan data was then analyzed and modeled using 3D data visualization software (Avizo 6.2, FEI, Visualization Sciences Group, Hillsboro, OR, USA) that presents polymerization shrinkage volume as 3D images

by generating grids from voxel data that represents image volume. Voxel data of 3D polymerization shrinkage volume was converted to mm³ using the following formula: 1 voxel data=6×6×6 μ m³ and 1 μ m³=0.000,000,001 mm³.

Marginal seal and cavity wall adaptation

The specimens were stored in water maintained at 37°C in the dark for 24 h. Thereafter, the restorations were finished and polished using wet 600-grit SiC paper, and thermocycled between 5 and 55°C for 500 cycles⁴⁰⁾, with a 30 s dwell time. The dye penetration test was used to determine the degree of adaptation to the cavity margins and walls. This test was performed by placing a 1.0% acid red propylene glycol solution (Caries Detector, Kuraray Noritake Dental) at the margin of the restoration for 5 s, followed by rinsing with water and gentle blow-drying. The extent of dye penetration was observed with a mesoscope (20× magnification). A photographic record of each specimen was acquired at this stage.

The specimens were then longitudinally cut in half using a diamond saw microtome (MC-110, Maruto, Tokyo, Japan) under running water, the dye was reapplied to the sections, and images were acquired to observe the gaps. In these images, the extent (length) of dye penetration along the cavity margins and walls was measured using a digitizer (KD4300 model, Graphtec, Tokyo, Japan). Areas of marginal dye penetration in the enamel were considered to be enamel cracks. The degree of marginal defect (enamel crack formation and dentin marginal leakage) was defined as the length of dye penetration, measured as a percentage of the total length of the cavity margin. Dye penetration along the cavity walls was calculated as a percentage of the total cavity wall length. This area was referred to as the cavity wall–resin gap. The dye penetration test scores were compared using the Mann-Whitney U test.

RESULTS

The peak wavelength of the experimental quartz-tungsten halogen light curing unit was 480–490 nm, and it exhibited a sudden increase in spectral radiant illuminance up to 4 s after the start of light irradiation, followed by a gradual decrease until 40 s after the start of light irradiation.

The polymerization shrinkage volume of resin composite restoration for the conventional curing method with Clearfil AP-X was 0.2996 mm³ (Fig. 3A), while that of the slow-start curing method was 0.1426 mm³ (Fig. 3B). The polymerization shrinkage volume of resin composite restoration for the conventional curing method with Clearfil Photo Bright was 0.0133 mm³ (Fig. 4A), while that of the slow-start curing method was 0.0069 mm³ (Fig. 4B).

The results for marginal defect and cavity–wall gap formation are shown in Table 2. Enamel crack formation (Figs. 3B (arrow) and 5) was observed in all specimens. No significant differences in marginal defect were observed between the two light-curing methods with

Fig. 3 3D image of polymerization shrinkage of Clearfil AP-X restoration using μ CT-3D visualization method. (A) is 600 mW/cm², (B) is 270/600 mW/cm² light irradiation. The polymerization shrinkage volume of (A) is 0.2996 mm³, (B) is 0.1426 mm³. Arrow in (B) shows 3D image of enamel crack formation.

Fig. 4 3D image of polymerization shrinkage of Clearfil Photo Bright restoration using μ CT-3D visualization method. (A) is 600 mW/cm², (B) is 270/600 mW/cm² light irradiation. The polymerization shrinkage volume of (A) is 0.0133 mm³, (B) is 0.0069 mm³.

Table 2 Marginal defect and cavity-wall gap formation [%: mean (SD)]

Light-curing method	Clearfil AP-X		Clearfil Photo Bright	
	Marginal defect	Cavity-wall gap formation	Marginal defect	Cavity-wall gap formation
600 mW/cm ² 40 s	36.8 (22.9)	63.4 (10.4) ^A	41.7 (22.6)	32.9 (9.5) ^{a,A}
270 mW/cm ² 10 s +5-s interval +600 mW/cm ² 30 s	35.2 (17.2)	45.2 (11.1) ^B	37.2 (15.6)	1.0 (2.6) ^{a,B}

Intergroup data with the same superscripted lowercase letters for each light curing method are significantly different ($p < 0.05$).

Intergroup data with the same superscripted uppercase letters for each resin composite are significantly different ($p < 0.05$).

both resin composites ($p > 0.05$). There were no significant differences in cavity-wall gap formation between the conventional curing method and the slow-start curing method for Clearfil AP-X ($p > 0.05$). However, Clearfil Photo Bright using the slow-start curing method

showed significantly less cavity-wall gap formation than the conventional curing method did ($p < 0.05$).

Fig. 5 Photographic image of enamel cracks on the margin around Clearfil AP-X resin composite restoration after dye penetration (2×).

DISCUSSION

Clearfil AP-X exhibited significantly greater cavity-wall gap formation than that of Clearfil Photo Bright with both the conventional curing method and slow-start curing method. In this μ CT-3D visualization analysis, polymerization shrinkage amount of Clearfil AP-X resin composite restoration that showed twenty times the volume of Clearfil Photo Bright resin composite restoration in the cavity with adhesive. Therefore, large polymerization shrinkage amount of Clearfil AP-X caused greater cavity-wall gap formation than Clearfil Photo Bright.

The conventional curing method resulted in the highest cavity-wall gap formation of Clearfil AP-X to the cavity wall. Several factors associated with the curing pattern of light-cured composite resins, such as the direction and speed of polymerization shrinkage, depth of curing, and polymerization stress, may compromise the ability to achieve an excellent seal along the cavity wall. A previous report indicated that, at a certain light intensity, the amount of activated starter radicals was optimal for the formation of cross-linked, long-chain molecules³⁰. Moreover, higher concentrations of radicals inhibit a chain reaction of molecules early and make short-chain molecules³⁰, suggesting that curing with a high-intensity light was more likely to result in marginal gaps and poor adaptation of resin composite to the cavity wall^{3,4,27,28}.

The slow-start curing method resulted in the highest adaptation of Clearfil Photo Bright to the cavity wall. Polymerization shrinkage that occurs after gelation or curing results in the build-up of large stresses in a resin composite⁴¹. However, a decreased rate of surface hardness development due to a prolonged gel state and the accompanying absence of dye penetration suggest that this particular protocol results in increased material flow, which provides stress relief in spite of its high elastic modulus and photosensitivity of the resin

composite¹⁰. In this μ CT-3D visualization analysis, the slow-start curing method reduced a polymerization shrinkage volume to half of that produced by the conventional curing method for both Clearfil AP-X and Clearfil Photo Bright resin composite restorations in the cavities with adhesive. This confirms and supports the findings of previous studies that reported the time required for complete polymerization shrinkage after the slow-start curing method was 2 min, which was less than half that required after the conventional curing method^{3,4}. The slow-start curing method resulted in a lower microhardness at the top surface of Clearfil Photo Bright resin composite compared with the conventional curing method up to 60 s from the start of curing^{3,4}. The viscosity of resin composite until 40 s from the start of curing was lower when initial low-intensity curing followed by high-intensity curing was used than when only high-intensity curing was used⁴¹. The use of a light intensity lower than the maximum resulted in a significant decrease in post-gel contraction without significantly affecting the degree of conversion⁴². This irradiation allowed for most of the resin composite polymerization shrinkage to occur during the initial flowable stage^{3,4}.

It was reported there was a high correlation between dye penetration test using caries detector and environmental SEM observation of gap¹⁶. The slow-start curing method resulted in significantly decreased dye penetration, namely better adaptation (almost 100%) of Clearfil Photo Bright to the cavity wall than the conventional curing method did. Previous studies have shown that, when Clearfil Photo Bright resin composite is light-cured using the slow-start curing method, the resin hardens faster at the bottom surface than at the top surface^{3,4,33}. Light transmission through light-cured resin composites is strongly affected by its opacity and shade, opacity changes during polymerization. The opacity of the resin composite is indicated by the refractive index mis-match between the matrix and filler⁴⁴ or the contrast ratio^{45,46}, and optimizing the filler/resin refractive index mis-match increases the curing depth⁴⁴. The contrast ratio decreases as the transparency of the resin composite increases, and is equal to 1 for a completely opaque material and ranges between 0 and 1 for a translucent material⁴⁵. Moreover, most resin composite materials have a tendency to exhibit a decrease in the contrast ratio during polymerization⁴⁵. However, the contrast ratio of Clearfil Photo Bright increases during polymerization (increased opacity), while that of Clearfil AP-X decreases during polymerization (increased transparency)⁴⁶. Therefore, the delay in hardening of Clearfil Photo Bright, particularly at the top surface^{3,4,33}, may decrease curing stresses and allow more time for relief.

Moreover, the curing rate of resin directly adjacent to the cavity wall may be enhanced by free radicals that are already present in the bonding resin. An initial low intensity light was thought to enhance the polymerization rate at this location, rather than at the resin surface. The process of polymerization is then

completed by high-intensity radiation, which allows for more uniform curing throughout the bulk of the resin composite. Therefore, most of the polymerization shrinkage occurs during the initial flowable stage of the resin composite polymerization, thus enabling the resin to flow within itself and preventing it from pulling away from the cavity walls^{3,4,13-15,29-32}.

The most marginal defects were observed in the enamel. Enamel crack formations were observed in all specimens. There were no significant differences in marginal defects between the slow-start curing method and the conventional curing method. Enamel is a highly mineralized tissue with a modulus of elasticity higher than that of dentin. The enamel prisms are extremely fragile, and the tensile strength of enamel is under 10 MPa when pulled vertical to these enamel prisms^{47,48}. It has been reported that the enamel cavosurface exhibited disruption even with the use of a diminished power density of 100 mW/cm^{2,10}. Therefore, enamel crack formation could not be avoided, even with the slow-start curing method.

CONCLUSION

The light-cured resin composite, which had increased contrast ratio during polymerization, improved adaptation to the cavity wall using the slow-start curing method. In the μ CT-3D visualization method, the slow-start curing method reduced the polymerization shrinkage volume of resin composite restoration to half of that produced by the conventional curing method in the cavity with adhesive for both Clearfil AP-X and Clearfil Photo Bright. μ CT-3D visualization method can be used to detect and analyze resin composite polymerization contraction behavior and shrinkage volume as 3D image in the cavity.

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