

Influence of artificial saliva contamination on adhesion in class V restorations

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This study aimed to evaluate the effects of artificial saliva contamination on a glass ionomer cement (GIC), a resin-modified GIC (RMGIC), and a composite resin (CR) that was used with two different etching adhesive systems. Three surface conditions were created on bovine teeth using artificial saliva: control (group I), mild saliva contamination (group II), and severe saliva contamination (group III). The microtensile bond strength (μ TBS) of CR with dentin was significantly lower in group III than in group I. However, the μ TBS of GIC and RMGIC with both enamel and dentin showed no significant intergroup differences. Moreover, CR exhibited significantly greater microleakage on cementum in group III than in group I, whereas both GIC and RMGIC showed no significant differences for both enamel and cementum. Thus, GIC and RMGIC may be suitable for preventing secondary caries after class V restorative treatments when contamination by saliva cannot be avoided.

Keywords: Glass ionomer cements, Composite resins, Microtensile bond strength, Failure mode, Microleakage

INTRODUCTION

Contamination of the dental operation area by saliva and other fluids is detrimental to positive restoration outcomes and should be controlled. The use of a rubber dam enables the control of such contamination, allowing the dentist to focus on the actual clinical procedure¹⁻⁴. The etiology of class V cervical lesions varies, including occlusal factors, brushing habits, dietary regimens, and psychological manifestations. Diagnosis of these lesions includes abrasion caused from incorrect brushing techniques, erosion from improper dietary or chemical occupational exposure, abfraction through malocclusion and caries caused by dissolution of tooth structure from bacterial by-products^{5,6}. However, class V restorations (cervical caries) are difficult to perform under a rubber dam when the cavity approaches the attachment part of the rubber dam clamp^{7,8}.

Composite resins (CRs) are commonly used in restoration procedures, even though they show reduced bond strength when contaminated by saliva either before or after primer application⁹⁻¹². In contrast, glass ionomer cements (GICs) chemically adhere to mineralized dental tissues, but incomplete chemical reactions and their sensitivity to water during the first stage of the GIC-setting reaction can lead to softening and cracking of the cement surface, reducing wear resistance and fracture toughness¹³. According to Yamazaki *et al.*, resin-modified GICs (RMGICs) have greater shear bond strength than conventional GICs in the case of luting by water immersion¹⁴. Our previous study suggested that CR exhibited significantly reduced shear bond

strength and greater microleakage after artificial saliva contamination, whereas no significant differences were found for GIC and RMGIC¹⁵.

According to Siegwad *et al.*, microtensile bond strength (μ TBS) was evaluated to determine retention loss, marginal discoloration, and marginal integrity on cervical (class V) restorations¹⁶. However, few studies have compared μ TBS and the degrees of microleakage exhibited by GICs, RMGICs, and CRs in a moist environment on class V restorations.

The purpose of this *in vitro* study was to evaluate the influence of artificial saliva contamination on the μ TBS of three restorative materials (GIC, RMGIC, and CR) used for class V restorations and the effect on the degree of microleakage exhibited by the restorative materials after thermocycling.

MATERIALS AND METHODS

The materials used in this study, namely GIC (Fuji IX extra capsule [F9E], GC, Tokyo, Japan), RMGIC (Fuji II LC capsule [2LC], GC), and CR (CLEARFIL AP-X, Kuraray Noritake Dental, Tokyo, Japan), are listed in Table 1. For the CR, we used two different etching adhesive systems: a total-etching adhesive (OptiBond Solo Plus [CR-OBS], Kerr, Orange, CA, USA) and a self-etching primer (Scotchbond Universal Adhesive [CR-SUA], 3M ESPE, St Paul, MN, USA).

Various surface conditions were created on the enamel or dentin/cementum of bovine incisors using artificial saliva. The artificial saliva contained 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 30 mM KCl, 4 mM KH_2PO_4 , and 0.7 mM CaCl_2 ¹⁷. The rationale for using artificial saliva was that the presence

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Table 1 Materials used in the study

Material	Composition (batch no.)	Application conditions
Fuji IX extra capsule (F9E)	Powder: fluoroaluminosilicate glass, polycarboxylic acid. Liquid: polycarboxylic acid, water, polybasic carboxylic acid. Shade: A2 (1504161).	—
Fuji II LC capsule (2LC)	Powder: fluoroaluminosilicate glass. Liquid: methacrylic acid ester, polycarboxylic acid, water. Shade: A2 (1501151).	—
Cavity conditioner	Water, polycarboxylic acid, aluminum chloride (140129).	Apply the conditioner for 10 s, rinse for 10 s, and gently air dry.
CLEARFIL AP-X	Monomer: Bis-GMA, TEGDMA. Fillers: surface-treatment glass powder, surface-treatment silica-based micro filler. Additional contents: photo-initiator, colorant. Shade: A3 (CG0056).	—
OptiBond Solo Plus (CR-OBS)	Bond: Bis-GMA, hydroxyethyl methacrylate, ethyl alcohol, fillers (5450989). Gel etchant: 37.5% phosphoric acid (5225747).	Apply the etchant for 15 s, rinse for 15 s, and gently air dry. Bond for 15 s, gently air dry for 10 s, and cure under light for 20 s.
Scotchbond Universal Adhesive (CR-SUA)	Monomer: Bis-GMA, MDP, HEMA, Decamethylene dimethacrylate. Filler: Silane treated silica. Additional contents: ethyl alcohol, photo-initiator, water (587216).	Apply the adhesive to the prepared tooth and rub it in 20 s. Gently air dry the adhesive for approximately 5 s. Light cure for 10 s.

Bis-GMA: bisphenol A-diglycidyl methacrylate; TEGDMA: triethyleneglycol dimethacrylate; MDP: methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; DMA: dimethacrylate.

of calcium and phosphate ions would prevent additional demineralization, which can alter the etching depth for enamel and dentin/cementum surfaces treated with acid or adhesives. The artificial saliva did not contain sodium azide because there was no need to store the specimens in sodium azide. The test samples were divided into the following groups: group I (control), in which the bonding surface remained dry; group II (mild saliva contamination), in which 0.1 mL of the artificial saliva was placed on the bonding surface and dried slightly; and group III (severe saliva contamination), in which 0.1 mL of the artificial saliva was used as is.

μTBS test

After confirming the absence of abnormalities such as discoloration or hypoplasia on the labial side, the enamel and dentin on the cervical area of bovine incisors were cut into blocks using a low-speed diamond blade (IsoMet, Buehler, Lake Bluff, IL, USA). These blocks were embedded in an acrylic resin (Unifast II, GC). Next, the enamel or dentin surfaces of the blocks were polished using 600-grit sandpaper. The blocks were then divided into the three groups. Blocks in each of the three groups were then further divided into four material categories: F9E, 2LC, CR-OBS, and CR-SUA. The surfaces of the F9E, 2LC, and CR-OBS blocks were treated in accordance with the instructions in their respective

manuals; a cavity conditioner was used for F9E and 2LC, and a gel etchant was used for CR-OBS. Artificial saliva was applied to the group II and III blocks, after which the appropriate adhesives were applied to the CR-OBS and CR-SUA blocks, in accordance with their respective manufacturers' instructions. The specimens were prepared using a silicone mold. The 2LC, CR-OBS, and CR-SUA blocks were light-cured for 20 s using a visible-light curing unit (G-Light Prima-II; GC), whereas the F9E blocks were stored for 5 min at 37°C and 100% relative humidity. After the silicone molds were removed, all specimens were stored in water at 37°C for 24 h. Then, eight specimens were prepared for each of the four materials in each of the three surface condition groups (approximate dimensions, 1×1×3 mm) (*i.e.*, a total of 96 specimens each for enamel and dentin). The specimens were loaded to failure under tension at a crosshead speed of 1.0 mm/min using a universal testing machine (Autograph EZ-S; Shimadzu, Kyoto, Japan).

The enamel/dentin side of all fractured specimens from each group was air-dried and examined with a scanning electron microscope (Miniscope TM3000; Hitachi High-Technologies, Tokyo, Japan) operating at 15 kV. The fracture modes were classified as follows: (1) cohesive failure in the restorative material; (2) mixed fractures; (3) adhesive failure at the enamel/dentin-restorative materials interface; (4) cohesive failure in

enamel/dentin.

Microleakage analysis

Cavities (1.5 mm in depth and 3.0 mm in diameter) were created at the occlusal (enamel) and gingival (cementum) margins of the labial aspect of the bovine incisor enamel. The specimens were randomly divided into one of the three surface condition groups, namely, group I, group II, and group III. After application of artificial saliva, the cavities were filled with the respective materials (F9E, 2LC, CR-OBS, and CR-SUA) as described above ($n=5$). The filled cavities were covered with a polyester film. The 2LC, CR-OBS, and CR-SUA blocks were irradiated for 20 s, whereas the F9E blocks were stored for 5 min at 37°C and 100% relative humidity. Next, all specimens were stored for 24 h at 37°C in distilled water. After removing the polyester film, the specimens were gently polished using #600 silicon carbide paper under water irrigation. All specimens were then thermocycled for 10,000 cycles at 5 and 55°C; the dwell time was 30 s^{18,19}. They were then soaked in a 0.1% methylene blue solution at 37°C for 20 h. The specimens were subsequently rinsed in distilled water, embedded in acrylic resin (Unifast II; GC), and sectioned longitudinally on either side of the cavity midline using a low-speed diamond blade (IsoMet, Buehler). The microleakage distances were assessed using a digital microscope (One-shot 3D Measurement Microscope VR-3000; Keyence, Osaka, Japan, Fig. 1).

Statistical analysis

Before performing any analyses for multiple group comparisons, the homogeneity of variance was assessed with Levene's test. As Levene's test revealed no significant differences among the groups, one-way analysis of variance (ANOVA) was used to analyze group differences in enamel/dentin μ TBS and enamel/cementum microleakage distances in each material. A p -value <0.05 was considered statistically significant. When one-way ANOVA showed a significant difference

among groups, Tukey's honestly significant difference (HSD) *post-hoc* test was used to identify group differences accounting for the significant p -value. All analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Japan, Tokyo, Japan).

RESULTS

Figure 2 shows the enamel and dentin μ TBS values of the tested materials at the different surface conditions. F9E and 2LC exhibited no significant intergroup differences in both enamel and dentin μ TBS (Figs. 2a and b). Conversely, for CR-OBS and CR-SUA, the dentin μ TBS was significantly lower in group III than in group I (control) (CR-OBS: $p=0.009$, CR-SUA: $p<0.001$), but there were no significant differences in the enamel μ TBS (Fig. 2a). Figure 3 illustrates the percentage of each fracture type in each group, and Fig. 4 shows the scanning electron microscopy micrographs of the enamel/dentin side of a representative fractured beam in each material. Most (46–83%) of the F9E and 2LC post-test specimens showed cohesive failure in restorative material, while most (67–100%) of the CR post-test specimens showed mixed fractures (Figs. 3a and b). For CR with dentin, the percentage of specimens showing interfacial failure

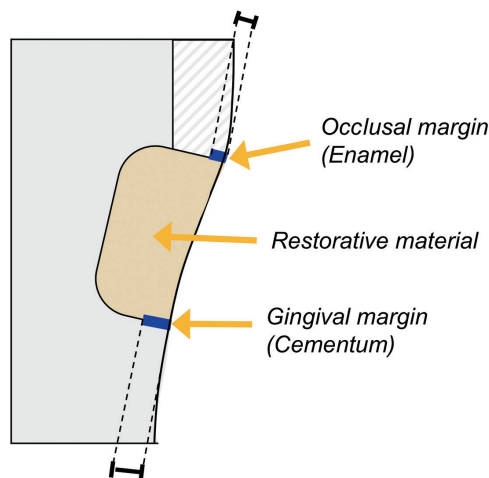


Fig. 1 Measurement of the dye penetration distances.

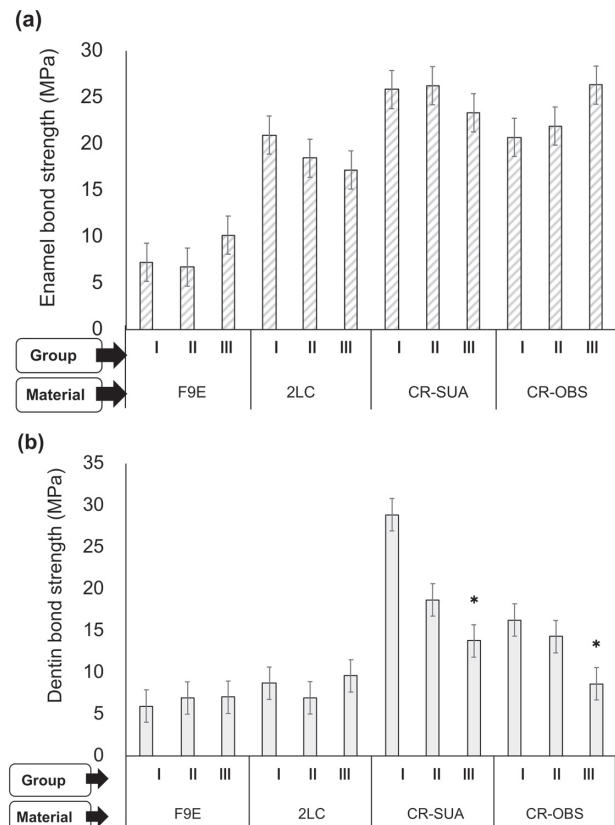


Fig. 2 (a) Enamel μ TBSs and (b) dentin μ TBSs. *ANOVA with Tukey HSD (compare with group I of each material), $p<0.05$.

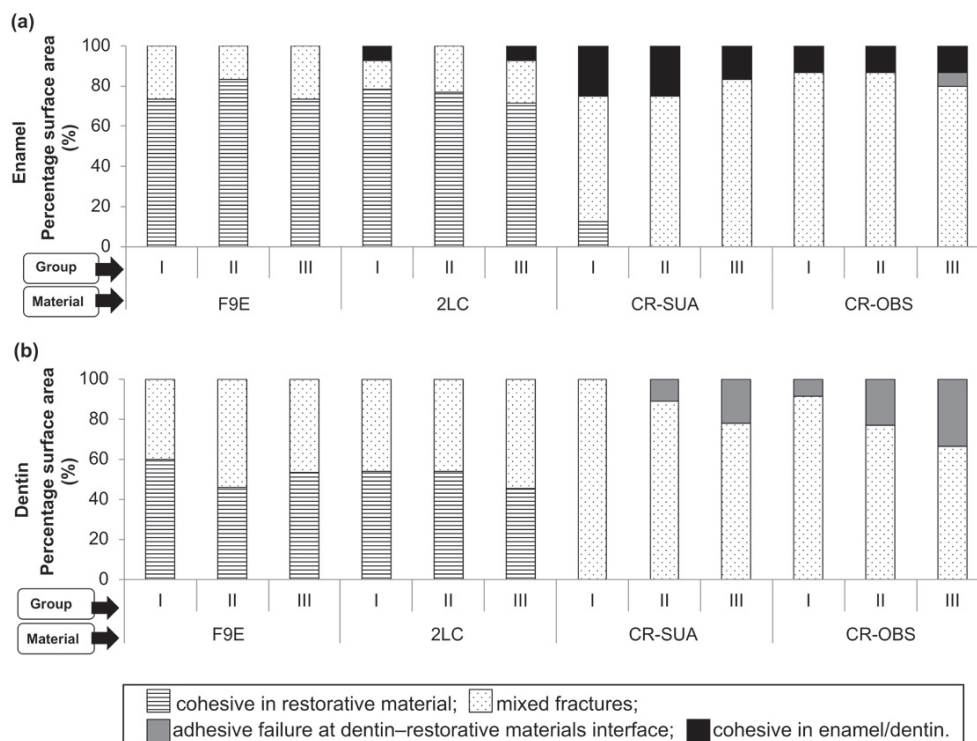


Fig. 3 The distribution of failure modes in each material for the three groups. The percentage surface area of a particular failure mode in each group represented the ratio of the fracture surface area exhibited by that failure mode to the total surface area in all the fracture specimens. (a) Enamel surface area and (b) dentin surface area.

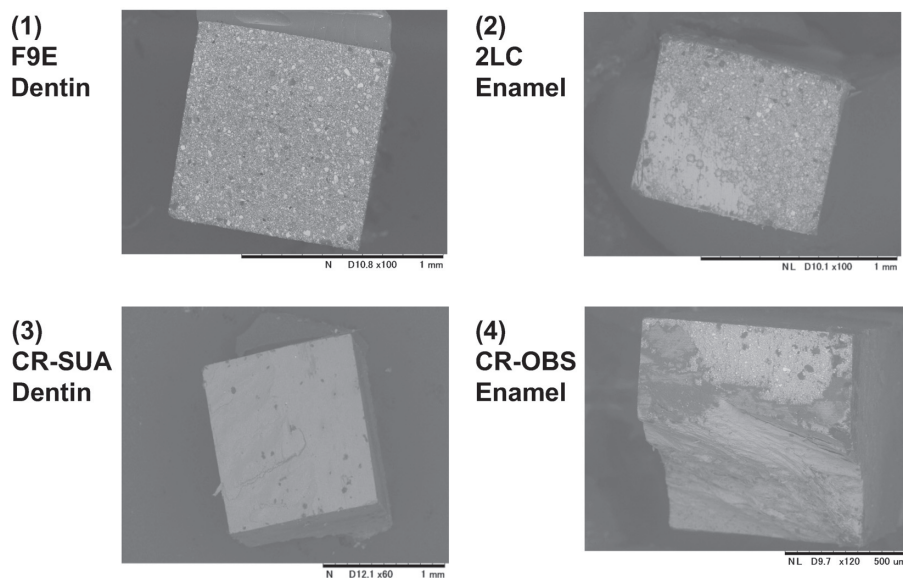


Fig. 4 Scanning electron microscopy micrographs of the enamel/dentin side of a representative fractured beam in each material.

(1) Cohesive failure in restorative material; (2) mixed fractures; (3) adhesive failure at the dentin–restorative material interface; and (4) cohesive failure in enamel.

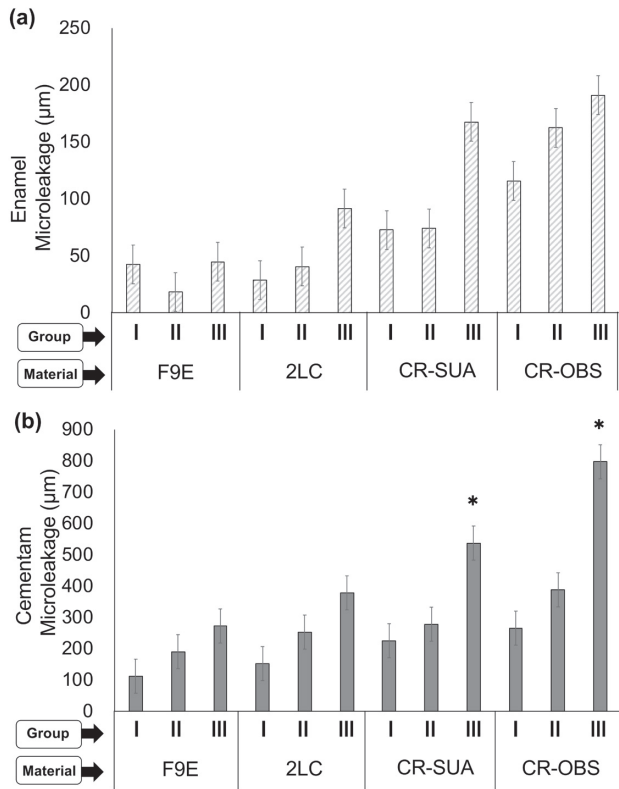


Fig. 5 (a) Enamel microleakage distances and (b) cementum microleakage distances.
*ANOVA with Tukey HSD (compare with group I of each material), $p < 0.05$.

increased with the level of artificial saliva contamination (Fig. 3b).

The microleakage distances of the four materials and the significance of the three different surface conditions for each material are presented in Fig. 5. F9E and 2LC showed no significant differences with both enamel and cementum (Figs. 5a and b), whereas CR exhibited significantly greater microleakage distances in group III than in group I with the cementum (CR-OBS: $p = 0.027$, CR-SUB: $p = 0.025$, Fig. 5b).

DISCUSSION

Saliva and gingival crevice fluid can often cause contamination of a filling even when the caries is present under the edge of the gingiva. Therefore, for successful prevention of secondary caries in restored teeth, it is critical to maintain the restorative material intact over time. Achievement of good moisture control is a common challenge in restorative dentistry, especially when isolation using a rubber dam is not feasible^{18,20}.

In this study, we evaluated the enamel and dentin μ TBS values and degrees of microleakage for three restorative materials (GIC, RMGIC, and CR) exposed to artificial saliva contamination for a class V restoration.

The results of the statistical analyses showed that artificial saliva contamination decreased the dentin μ TBSs of CR-SUA and CR-OBS; however, their enamel μ TBSs were not significantly affected. Similarly, the degree of microleakage for cementum with CR-OBS and CR-TSB after thermocycling increased significantly with artificial saliva contamination.

The micromechanical bonds formed on enamel surfaces demineralized by acid etching may explain the differences between the dentin and enamel bond strengths. However, artificial saliva contamination did not affect the μ TBSs for F9E and 2LC; this was true for both enamel and dentin. This result may be explained by the chemical self-adhesion of enamel and dentin even in the absence of applied adhesives, which are affected by saliva.

The degree of microleakage after thermocycling in F9E and 2LC was not affected by artificial saliva contamination for both enamel and dentin. However, for CR-SUA and CR-OBS, the dentin bond strengths were significantly lower in severe saliva contamination than in the control. The resulting microleakage may engender staining, marginal breakdown, hypersensitivity, secondary caries, and the development of pulpal pathology²¹. Thus, these results indicate that CR with a high bond strength was more effective for preventing secondary caries in environments where a rubber dam can be utilized, while GIC and RMGIC are suitable in environments in which the use of a rubber dam is not possible.

Assessments of the fracture surface area indicated cohesive failure in restorative material in most (46–83%) of the F9E and 2LC post-test specimens. This may be attributable to the fact that the strength of F9E and 2LC is lower than that of enamel/dentin, and adhesion is tight. Under clinical conditions, it is possible to restrict the progression of caries, since some material remains on the dental surface even after removal of the restorative materials. The CR specimens showed mixed fractures or adhesive failure at the dentin-restorative material interface, while cohesive failure in enamel was noted in 13–25% of the specimens, which may be because the strength of CR is higher than that of the enamel surface, and adhesion is tight. Therefore, when the adhesive force decreases as a result of the saliva mixture, severe secondary caries occurs due to exposure of the enamel/dentin.

Clinically, class V cavities in the vicinity of the cervical region of the tooth are located at the site where the clamp is placed; therefore, isolation using a rubber dam is difficult. Gingival crevice fluid contamination is a common occurrence during restoration procedures. This suggests that GIC or RMGICs are suitable for the repair of class V cavities when isolation is not feasible.

There are some limitations in the present study. Since we utilized artificial saliva, it is necessary to conduct experiments using solutions that replicate blood and gingival crevice fluid. Moreover, we used bovine incisors for the experiment. Human and bovine teeth are regarded as essentially homogeneous structures^{22,23},

and the teeth used in this study were less affected by environmental conditions, such as the frequent use of fluorine.

CONCLUSIONS

Based on the results of this study, the following conclusions could be drawn:

1. Contamination with artificial saliva does not affect the enamel/dentin μ TBSs of GICs and RMGICs.
2. The μ TBS of CRs with dentin is reduced significantly when a total-etching adhesive or a self-etching primer is used under severe saliva contamination.
3. The microleakages in cavities with enamel and cementum margins filled with a GIC or an RMGIC do not increase under artificial saliva contamination after thermocycling.
4. For CR with dentin, the percentage of interfacial failure increased with the level of artificial saliva contamination.

The presence of microleakage and the subsequent decrease in bond strength may result in the formation of secondary caries around the restorative material. Therefore, GIC or RMGIC restorative materials are suitable for positive class V restorations.

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