Published in final edited form as:

Dent Mater. 2017 November ; 33(11): 1315–1323. doi:10.1016/j.dental.2017.08.186.

Calibration of a lactic-acid model for simulating biofilm-induced degradation of the dentin-composite interface

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Abstract

Objective—To verify and calibrate a chemical model for simulating the degradation of the dentin-composite interface induced by multi-species oral biofilms *in vitro*.

Methods—Dentin-composite disks (5-mm dia. × 2-mm thick) were made from bovine incisor roots and filled with either Z100™ (Z100) or Filtek™ LS (LS) composite. The disks, which were covered with nail varnish, but with one of the dentin-composite margins exposed, were immersed in lactic acid solution at pH 4.5 for up to 48 hr. Diametral compression was performed to measure the reduction in bond strength of the dentin-composite disks following acid challenge. Scanning electron microscopy (SEM) was used to examine decalcification of dentin and fracture modes of the disks. To better understand the degradation process, micro-computed tomography, in combination with a radiopaque dye (AgNO₃), was used to assess interfacial leakage in 3D longitudinally, while SEM was used to determine the path of leakage. One-way analysis of variance (ANOVA) was used to analyze the results, with the level of statistical significance set at $p < 0.05$. The results were compared with those obtained previously using multi-species biofilms for verification and calibration purposes.

Results—After 48 hr of acid challenge, the debonding load of both the LS- and Z100-filled disks reduced significantly ($p < 0.05$). In the Z100-filled disks, debonding mostly occurred at the adhesive-dentin interface, while in the LS-filled disks, this happened at the adhesive-composite interface, instead. The degree of dentin demineralization, the reduction in debonding load and the

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modes of failure observed were very similar to those induced by multi-species oral biofilms found in the previous work. Leakage of AgNO₃ occurred mainly along the hybrid layer. The specimens filled with Z100 had a thicker hybrid layer (~6.5 µm), which exhibited more interfacial leakage than those filled with LS.

Significance—The chemical model with lactic acid used in this study can induce degradation to the dentin-composite interface similar to those produced by multi-species biofilms. With appropriate calibration, this could provide an effective *in vitro* method for ageing composite restorations in assessing their potential clinical performance.

Keywords

Secondary caries; Composite resins; Bond strength; Interfacial degradation; Microleakage

1. Introduction

In recent years, resin-based composites have become the most widely used restorative material to restore decayed and damaged teeth because of their esthetics and improved clinical performance [1-3]. About 121 million composite restorations were placed in the US by 2006 [4]. However, moderate to large composite restorations have higher failure rates and, as a result, more frequent replacement when compared to amalgam restorations [5]. Composite restorations fail for various reasons, among which secondary caries is the main one [6, 7], accounting for up to 55% of the failures reported by dentists [8].

Secondary caries is defined as caries recurring at the interface between tooth and restoration [9], and is the most common reason for the replacement of different kinds of dental restorations [10, 11]. Similar to primary caries, secondary caries are caused by substances harmful to the tooth tissues and resins, e.g. acids and enzymes produced by biofilms accumulating at the restoration margins [12]. It has been reported that biofilms accumulate more easily on composite than amalgam restorations due to the former's lack of antibacterial properties [13, 14]. Moreover, biofilms have been shown to increase the surface roughness of resin composites [15], which could enhance the adherence of biofilms/plaque on their surfaces. Interfacial defects, which can be inherent of the restorative materials or caused by operator errors, biofilm challenge, occlusal forces or polymerization shrinkage of the resin composite, facilitate the leakage of harmful substances into the interfacial space. The exposed collagens in the hybrid layer are subject to hydrolytic and enzymatic degradation [5], leading to interfacial breakdown, the development of secondary caries and finally failure of the composite restoration. Although an amalgam restoration is not bonded to the surrounding tooth tissues, its corrosive products provide an effective seal against the infiltration of the harmful substances. These are the major reasons cited for the higher failure rate seen in composite restorations when compared to amalgam restorations.

A main topic in dental materials research has been the search for a representative laboratory test to simulate the ageing, for example by biofilm challenge, of dental restorations observed clinically. In a previous study, degradation of the dentin-composite interface was effected by using multi-species oral biofilms grown in a CDC reactor [16]. The multi-species biofilms were produced directly from plaque samples collected from donors with a history of early

childhood caries [17]. Therefore, they were considered to be more realistic than single-species biofilms. The CDC reactor was set up in such a way that, when pulsed with sucrose solution to simulate food intake, the pH of the medium followed the Stephan Curve as seen in the oral environment. That is, it fell rapidly and remained below the threshold of 5.5 for dentin demineralization during the course of sucrose pulsing, and it returned quickly to a value above 6.0 when sucrose-pulsing was stopped. Significant dentin demineralization was found in dentin-composite disks challenged with the sucrose-pulsed biofilms for 2 days [16]. The bond strength of these disks was also significantly reduced ($p < 0.001$). In contrast, dentin-composite disks subjected to the same biofilms but without sucrose pulsing did not exhibit significant demineralization or loss of bond strength. Since the drop in pH with sucrose-pulsing was the main difference in the challenges experienced by the two groups of specimens, we hypothesize that a low-pH chemical model can simulate the above degrading effects of the multi-species biofilms on the dentin-composite interface.

The inorganic acids produced by biofilms, e.g. lactic, acetic, propionic, succinic and formic acids, are the main cause of caries formation [18]. Among these, lactic acid has long been used to produce artificial carious lesions [19-21]. Fatigue failure of dentin is also accelerated when exposed to lactic acid [22]. Moreover, lactic acid may degrade resin composites, changing their surface hardness [23]. In this study, therefore, a simple chemical model using lactic acid is used to simulate biofilm-induced degradation of the dentin-composite interface. The results are compared to those obtained previously *in vitro* using multi-species biofilms [16] for verification and calibration purposes.

2. Materials and methods

2.1. Preparation of dentin-composite disks

Dentin-composite disks were prepared using bovine incisors and the same restorative materials as reported previously [16]. As shown in Fig. 1, after removing the soft tissues, the crown portion above the cementum-enamel junction (CEJ) and the apical portion of ~3-mm long were cut off from the bovine incisors with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The root canals were enlarged to 2 mm in diameter, and the roots trimmed into cylinders of 5 mm concentric and parallel with the enlarged root canals. After that, the root dentin cylinders were filled incrementally with the composites Z100™ or Filtek™ LS (3M ESPE, St. Paul, MN, USA) using the corresponding adhesives (Single Bond Plus and LS Adhesive System, respectively) and following the manufacturer's protocols. Finally, the filled cylinders were sliced into 2-mm thick disks and stored in de-ionized water at 4°C before testing.

2.2. Lactic acid challenges

A static chemical model using a lactic acid solution of pH 4.5 (0.1 M lactic acid, 2.2 mM CaCl_2 and 2.2 mM K_2HPO_4) was employed in this study. For each material, one group of dentin-composite disks ($n = 14$) were first incubated in the lactic acid solution for 24 hr at 37°C and then in a phosphate-buffered saline (PBS) buffer for 48 hr to simulate the sucrose-pulsed biofilm challenge [16]. This group of specimens was subjected to chemical challenge with the same time-averaged pH as the biofilm model. Another group was incubated in the

acid solution for 48 hr and then in the PBS buffer for 24 hr to provide longer acid challenge. In addition, control groups of dentin-composite disks were run at pH 7.4 for 72 hr in the PBS buffer. The relatively basic value was chosen based on pH measurements of dental plaques taken before breakfast [24]. All the disks were painted with an acid-resistant nail varnish, but leaving one side of the composite filling and a perimeter of ~0.3-mm wide around it exposed (Fig. 1). After the 72-hr incubation, the disks were collected and the nail varnish was removed for mechanical tests.

2.3. Mechanical testing

The interfacial bond strength of the dentin-composite disks was determined by diametral compression using a universal test machine (858 Mini Bionix II, MTS, MN, USA) with two parallel horizontal steel plates [25]. The load was applied in a stroke-control mode at a speed of 0.5 mm/min. The fracture loads of the disks were determined from the first load drop in the load-displacement curves.

2.4. Assessment of interfacial leakage

To better understand the difference in degradation behavior between the two material groups, micro-computed tomography (micro-CT), in combination with a radiopaque dye (silver nitrate, AgNO_3), was used to assess leakage through the restoration margins [26]. Three freshly prepared disks for each material group were stored in a 50% (w/w) solution of AgNO_3 at room temperature. After 6, 12 and 24 h of storage, they were taken out and scanned using a micro-CT machine (XT H 225, Nikon Metrology Inc., Brighton, MI, USA). The scanning parameters were 90 kV, 90 μA , 708 ms of exposure, 720 projections and 4 frames per projection. After scanning, 3D reconstructions were performed using the software CT Pro 3D (Nikon metrology, Inc., Brighton, MI, USA). A cylindrical volume of interest (VOI), ~2 mm in diameter, encircling the composite filling was generated to calculate the volume of silver nitrate that had penetrated into the dentin-composite disks.

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine decalcification of the dentin, fracture modes of the disks and the path of AgNO_3 leakage in more detail. For the latter, dentin-composite disks ($n = 3$ per group) with or without prior acid challenge (24-hr duration) were used. The SEM was conducted in a tabletop scanning electron microscope (TM-3000, Hitachi, Japan) using an acceleration voltage of 15 kV and combo observation mode. The disks treated by silver nitrate were sectioned axially along a diameter and polished with 1200 grit sandpapers before SEM scanning. All specimens were fixed on the top of an aluminum stub with conductive carbon tapes.

2.6. Statistical analysis

Assumptions of normal distribution were evaluated by Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) was carried out with OriginPro8.0 (OriginLab, USA) to compare the impact of lactic acid on the degradation of the two groups of disk specimens. The extent of silver nitrate penetration in the disks was also analyzed using one-way ANOVA with Tukey's post-hoc test. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Bond strength of the dentin-composite disks

Most of the disks deformed approximately linearly with increasing load until debonding took place (Fig. 2A). The debonding loads of the dentin-composite disks after different durations of acid challenge are shown in Fig. 2B. After 24 hr of lactic acid challenge at pH 4.5, the fracture loads of both Z100 (352.31 ± 58.09 N) and LS (378.76 ± 51.38 N) specimens were lower than those of the control groups (376.77 ± 41.14 N for Z100 and 402.11 ± 40.02 N for LS), but the difference was not statistically significant. When the lactic-acid challenge was extended to 48 hr, the debonding loads of the disks (342.52 ± 43.31 N for Z100, 363.45 ± 43.64 N for LS) became significantly lower than those of the control groups ($p < 0.05$). Also, the debonding loads of the Z100-filled specimens were all lower than those of the LS-filled ones, although the difference was not statistically significant. The average debonding loads, with or without acid challenge, were similar to those found with the biofilm model [16], Fig. 2C, except for the control group for Z100, which showed a lower debonding load.

3.2. Failure mode analysis

The failure modes of the different groups of dentin-composite disks after diametral compression are presented in Table 1. The LS- and Z100-filled disks exhibited different failure modes. Within the LS disks, adhesive failure at the adhesive-composite (AC) interface, and that mixed with some cohesive failure of the composite (C), were the most frequent failure modes (>83%). With acid challenge, a small number of LS disks failed at the adhesive-dentin (AD) interface (<17%). In contrast, Z100 disks failed predominantly with cohesive failure of the adhesive (A) and delamination at the adhesive-dentin (AD) interface (>85%), with most of the failed disks showing resin tags pulled out from the dentin. A small number of Z100 disks also showed cohesive failure of the composite (C) mixed with adhesive failure at the adhesive-composite (AC) interface (<15%). Acid challenge did not change the major failure modes of the Z100-filled disks. These results were consistent with our previous finding using multi-species biofilms [16].

3.3. Scanning electron microscopy

As shown in Fig. 3, the exposed surface dentin in the disk specimens subjected to acid challenge was decalcified extensively. A decalcification depth of ~ 30 μm could be seen as a dark band at the exposed end of the fracture surfaces of both the LS and the Z100 disks after 24 hr of acid challenge. When exposed to a longer time, 48 hr, the decalcification depth increased to around 80 μm . A similar extent of dentin decalcification (~ 50 μm) had been seen in the disks challenged by multi-species biofilms [16].

The respective interfaces formed between the LS and Z100 composites and dentin were also examined by SEM for comparison to better understand the difference in leakage behavior (see later). As illustrated in Fig. 4, a hybrid layer of ~ 6.5 μm thick was observed in the Z100 disks. In contrast, the LS disks had a much thinner hybrid layer, which was hard to discern in the SEM images.

3.4. Interfacial leakage

Marginal leakage along the dentin-composite interface was traced using micro-CT in combination with AgNO₃ as a radiopaque dye; see Fig. 5. Z100-filled disks exhibited more extensive interfacial leakage than LS-filled ones. After 6, 12, and 24 hr of immersion in the dye solution, the volume of silver nitrate penetrated into the Z100-filled disks was $0.272 \pm 0.049 \text{ mm}^3$, $0.473 \pm 0.161 \text{ mm}^3$, and $0.730 \pm 0.102 \text{ mm}^3$, respectively. In the LS-filled disks, it was $0.127 \pm 0.006 \text{ mm}^3$, $0.171 \pm 0.028 \text{ mm}^3$, and $0.306 \pm 0.022 \text{ mm}^3$, respectively. The difference between the two material groups was statistically significant at all the time points ($p < 0.01$). Fig. 6 shows that the rate of leakage was rather constant for both material groups ($R^2 > 0.99$) within the 24 hr of assessment.

Cross-sectional SEM images of dentin-composite disks after immersion in silver nitrate for 24 hr demonstrated that marginal leakage occurred along the hybrid layer, followed by sideways penetration along the dentinal tubules (Fig. 7). The depth of dentin on the exposed surface stained by silver nitrate without prior acid challenge was approximately 100-200 μm . The extent of staining and penetration increased with prior acid challenge for both material groups, with the depth of stained dentin increasing to approximately 200-300 μm . Compared with the LS disks, the hybrid layer of the Z100 disks was more highly stained with silver nitrate.

4. Discussion

Resin-based composite restorations are degraded by mechanical, biological and environmental loads [2]. Our previous study [16] demonstrated that multi-species oral biofilms caused significant surface demineralization and bond strength reduction in dentin-composite disk specimens only when there was a significant drop in pH induced by sucrose pulsing. In this study, we hypothesized that a static chemical model could simulate the same degrading effects of the biofilms on the dentin-composite disks. Lactic acid of pH 4.5 was used as the static chemical model as it is a major product of the metabolism of fermentable carbohydrates by oral bacteria such as *Streptococcus mutans* (*S. mutans*) [27]. As far as the authors are aware, the work presented here represents the first study to verify and calibrate a chemical model against a multispecies biofilm model in its degrading effects on the dentin-composite interface. With an equivalent duration of challenge (~24 hr) that provided the same time-averaged pH, specimens subjected to lactic acid were found to have lower bond strength reduction (Fig. 2B and 2C) and less dentin demineralization (Fig. 3) than those subjected to multi-species biofilms. The difference could be due to the fact that biofilms also produced other organic acids (e.g., acetic, propionic, succinic and formic) and biomolecules that could degrade the tooth-composite interface. A study by Bourbia et al. showed that *S. mutans* could degrade dental resin composites and adhesives through esterase activities [28]. Such destruction of the resin-based materials could accelerate the marginal degradation that leads to secondary caries [7, 28]. The constant flow of the medium in the CDC reactor could also increase the degradation. As the duration of the lactic acid challenge was increased to 48 hr, a more significant reduction of bond strength and a greater demineralization depth, similar to those produced by multi-species biofilms in our previous study [16], were found. These results indicated that a static chemical model, with appropriate durations established

through calibration, could be used to simulate the biodegradation of dentin-composite specimens induced by biofilms *in vitro*, at least in terms of dentin demineralization and bond strength reduction.

Hybrid layers and resin tags are formed by infiltration of the adhesive or primer into the etched dentin and its tubules, which are important in the formation of a seal and reliable micro-mechanical retention between resin and dentin [29]. Leakage as a result of material permeability or interfacial defects could lead to the diffusion of harmful substances which attack the exposed dentin collagens and underlying tooth structure [30], resulting in discoloration, sensitivity, interfacial degradation and recurrent caries. In this study, we used the same two restorative systems as those used previously [16]: Z100 with a total-etch adhesive, and LS with a self-etch adhesive. The materials were chosen to provide, mainly, different dentin preconditioning and shrinkage stress levels at the interface. Fig. 4 shows that the self-etch LS adhesive system produced a much thinner hybrid layer than the total-etch system of Z100. With the latter, etch-and-rinse was performed using a 37% phosphoric acid gel, which removed the smear layer and exposed the partially demineralized dentin. A thicker hybrid layer of around 6.5 μm and longer resin tags were observed in Z100-filled disks, which should provide stronger bonding than LS-filled disks. However, both the current study and the previous one using biofilms [16] demonstrated a greater reduction following biochemical challenges in the dentin-composite bond strength for the Z100-filled disks than for the LS-filled disks. As shown by the current study, this was probably because the hybrid layer was the weakest zone within the composite-dentin interface in terms of leakage, and that of the Z100 disks was more prone to degradation by acid challenge. It has been observed that the Single Bond adhesive system does not fully cover the etched dentin, even with the application of two consecutive layers. This may explain why the volume of silver nitrate penetration in the Z100 disks was much higher than that in the LS disks at all the time points. Another reason for this difference between the two material groups could be that Z100 is a high-shrinkage composite. Although incremental filling was used to restore the dentin disks in order to minimize shrinkage stress, the latter might still have been sufficiently high to cause partial debonding at the dentin-composite interface. The non-uniform AgNO_3 penetration around the interface seemed to support this notion. Walshaw and McComb [31] also found gaps at dentin-composite interface formed with Single Bond Plus, which they concluded might lead to microleakage and affect the longevity of the bonds, as found in the current study. As a self-etching adhesive system, LS adhesive could demineralize and infiltrate into the dentin simultaneously [32]. Although the hybrid layer in the LS-filled disks was thin and might not have provided micromechanical interlocking as strong as that in the Z100-filled disks, the etched dentin was well covered by a thicker adhesive layer without any observable gaps or voids [16]. This again may explain why silver nitrate could not penetrate along the interface of LS specimens as easily as it did in those restored with Single Bond Plus and Z100. As presented in Table 1, a mixture of adhesive-dentin interfacial failure and cohesive failure of the adhesive was the predominant failure mode in Z100-filled disks, confirming that the dentin-adhesive interface was the weakest link in this group of specimens. In contrast, only a few LS-filled specimens failed at the adhesive-dentin interface.

Traditional methods to detect leakage evaluate the penetration of a specific tracer, such as rhodamine B, methylene blue and silver nitrate, along cut surfaces. At best, they can only provide semi-quantitative assessment and, as they are destructive to the specimens, they cannot readily provide longitudinal information. In contrast, X-ray micro-CT is a nondestructive technique. Micro-CT has been used for the assessment of dental materials, including the evaluation of polymerization shrinkage of dental resin-based composites [33], quantification of interfacial gaps in composite restorations [26, 34] and assessment of microleakage patterns along the tooth-restoration interface [35]. When used in conjunction with the electron dense and radiopaque silver nitrate, it can provide 3D, longitudinal information on microleakage. Here, we used micro-CT to quantify the volume of silver nitrate penetrant in dentin-composite disks as a function of incubation time in order to better understand the interfacial degradation process. The results showed that the infiltration of silver nitrate along the dentin-composite interface was a diffusion-like process with its volume increasing linearly with time during the period of observation (Fig. 6). However, it should be pointed out that a considerable amount of silver nitrate penetrated through the exposed dentin of the disc specimens (Fig. 5), which conceivably occurred early in the incubation. This could explain the similar nonzero penetrant volumes at time zero as indicated by the linear regression lines for the two composite systems (Fig. 6). Also, note that the depth of exposed dentin stained by the silver nitrate solution, which was acidic, was similar to that of the exposed dentin demineralized by the chemical or biofilm challenge. Thus, while it was clear that the two systems had significantly different permeability, the actual rates of leakage through the interface were likely to be lower than those presented in Fig. 6. Due to the comparatively low resolution of micro-CT ($\sim 10\ \mu\text{m}$), SEM was used to reveal more clearly the path of silver nitrate penetration along the dentin-composite interface. As shown in Fig. 7, when the penetration through the exposed dentin was ignored, it can be seen that, axially, the silver nitrate penetrated mainly along the hybrid layer, which clearly became more permeable after acid challenge and from which the penetration branched into the dentinal tubules. It is conceivable that similar degradation of the hybrid layer can be caused by acidic products and other biomolecules produced by oral biofilms. The gaps thus formed may allow the invasion of bacteria and subsequent biofilm formation along the interface and exposed dentinal tubules.

Bovine dentin was used in this study because it is more readily available than human dentin. Numerous studies have concluded that bovine dentin could be a suitable substitute for the human form in dental materials research, although there are position-dependent differences in permeability and bond strength values they provide [36-38]. Orientations of the dentinal tubules relative to the restoration in our dentin-composite specimens are rather different from those in a proximal restoration which is most prone to secondary caries. However, the aim of this study is to verify and calibrate the lactic acid model against the multi-species biofilm model used previously [16]. As long as the methods are consistent between the two studies, the substrate used should be of secondary concern.

It should be emphasized that the lactic acid used in this study represents a rather simple static chemical model, and only dentin demineralization and bond strength reduction have been considered in assessing degradation. The actual oral environment is very complex in its composition and the pH fluctuates significantly. Other possible degradation mechanisms,

such as enzymatic cleaving of the ester bonds within the composite and adhesive resins, were not considered. Neither was the possibility of dentin remineralization. Care must therefore be taken in extrapolating the current results to the clinical situation. Nevertheless, with appropriate calibration and for the parameters considered (dentin demineralization and bond strength reduction), this simple chemical model can provide an effective *in vitro* means for ageing composite restorations in assessing their potential clinical performance.

5. Conclusion

With suitable calibration, a static chemical model using lactic acid can be used to simulate the degrading effects of multi-species oral biofilms on the dentin-composite interface *in vitro*. These include dentin demineralization, reduced bond strength and increased permeability of the hybrid layer.

Acknowledgments

This work was partially supported by the National Institute of Health (Grant No. R01 DE021366); the National Natural Science Foundation of China (Grant No. 81200774); and Shanghai Colleges and Universities Young Teachers Training Funding (Grant No. ZZjdyx13120).

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Fig.1.

A flow diagram showing preparation of the dentin-composite disks. The crown and a short apical portion were removed from the bovine incisor. The root canal was enlarged to 2 mm in diameter and the root trimmed into a concentric cylinder of 5 mm in diameter. The cylinder was then filled with a composite (Z100 or LS) using the corresponding adhesive, and light cured. Finally, the filled cylinder was transversely cut to produce 2-mm thick round disks. The photo on the right shows the side of the dentin-composite disks which had the composite and a perimeter of ~0.3-mm wide around it exposed to acid challenge.

Fig. 2.

(A) A typical load-displacement curve from the diametral compression tests of dentin-composite disks after acid challenge. (B) Debonding loads of the dentin-composite disks with or without lactic acid challenge. Control Group: Specimens were incubated in PBS at pH 7.4 for 3 days. 24-hr Group: Specimens were challenged with lactic acid at pH 4.5 for 24 hr, followed with 48 hr of incubation in PBS at pH 7.4. 48-hr Group: Specimens were challenged with lactic acid for 48 hr, followed with 24 hr of incubation in PBS at pH 7.4. The data shown are the means $\pm$ standard deviations of the debonding loads. (C) The debonding loads of dentin-composite disks subjected to biofilm challenge were obtained from [16] and given here for comparison. * indicates significant difference between groups ($p < 0.05$).

Fig. 3.

SEM images of fracture surfaces showing demineralization of exposed surface dentin after lactic acid challenge: LS-filled disks after 24 hr (A) and 48 hr (B) of acid challenge; Z100-filled disks after 24 hr (C) and 48 hr (D) of acid challenge.

Fig. 4. Cross-sectional SEM images showing the hybrid layers of the dentin-composite disks. In the LS-filled disks (A and B), the hybrid layer could not be clearly seen. In the Z100-filled disks (C and D), the hybrid layer was about 6.5-µm thick.

Fig. 5. 3D micro-CT images of AgNO₃ penetrant along the dentin-composite interface. (A), (B) and (C): leakage in a representative LS specimen after 6, 12 and 24 hr of immersion in AgNO₃ solution. (D), (E) and (F): leakage in a representative Z100 specimen after 6, 12 and 24 hr of immersion in AgNO₃ solution.

Fig. 6.
The volume of AgNO_3 penetrant along the dentin-composite interface after 6, 12 and 24 h of immersion in the solution.

Fig. 7.

Cross-sectional SEM images of control and acid-challenged dentin-composite disks after silver nitrate penetration for 24 hr. (A) and (B): a LS disk with 72 hr of incubation in PBS (control). (C) and (D): a LS disk with 24 hr of prior lactic acid challenge. (E) and (F): a Z100 disk with 72 hr of incubation in PBS (control). (G) and (H): a Z100 disk with 24 hr of prior lactic acid challenge.

Table 1

Number (percentage) of specimens failed with a particular fracture mode for all experimental groups.

LS	Ctrl*	24 hr*	48 hr	Z100	Ctrl	24 hr	48 hr
AD	0(0)	2(16.7)	1(7.1)	AD+A	13(92.9)	13(92.9)	12(85.7)
AC	9(69.2)	7(58.3)	9(64.3)	AC	0(0)	0(0)	0(0)
AC+C	4(30.8)	3(25.0)	4(28.6)	AC+C	1(7.1)	1(7.1)	2(14.3)

AD: adhesive-dentin interfacial failure. AC: adhesive-composite interfacial failure. A: cohesive failure in the adhesive layer. C: cohesive failure in the resin composite.

* A few specimens were lost during the mechanical test.