Bacterial Leakage Assessment for Different Types of Resin-Based Dental Restorations Applied Using Various Placement Methods

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Abstract

Introduction: Although composite resins have improved greatly since their introduction, microleakage is one of the most frequently encountered problems. This study compared the effects of different monomer systems and layering techniques on the bacterial leakage of Cl I composite resin restorations. **Methods:** Eighty-two sound human third molars were used. The teeth were randomly divided into six groups of 12 teeth each and two positive and negative control groups of five teeth. Class I cavities, measuring 4×4×2 mm, were prepared. The first three groups were filled with a silorane-based composite (Filtek P90) using three different methods of filling (bulk, incremental and snowplow) and the remaining three groups were filled with a methacrylate-based composite (Clearfil AP-X) using the same techniques. The specimens were stored for 24 hours at 37°C and then thermocycled up to 1000 cycles. The bacterial leakage of the specimens was assessed in a microbiological laboratory and statistical analyses of data were performed by Fisher's exact and chi-squared tests (P<0.05). **Results:** There were no significant differences between Filtek P90 and Clearfil AP-X (P=1) in terms of microleakage. The difference between the outputs related to three filling techniques was not significant, either (P>0.05). **Conclusion:** Leakage occurred similarly in both silorane- and methacrylate-based composite resins and three filling techniques.

Introduction

Composite resins are the materials of choice for most restorations in today's clinical dentistry (1) because of their biocompatibility and absence of mercury, ability to match tooth color, thermal non-conductivity and ability to bond to tooth structures (2,3). Although composite resins have improved greatly since their introduction, polymerization shrinkage of 1.5-5% and microleakage as a result, are the most frequently encountered problems (4). Microleakage is defined as the passage of fluids, bacteria or molecules between the cavity walls and restorative materials (5). Microleakage may lead to postoperative sensitivity, enamel fracture, marginal staining, recurrent caries, eventual failure of restorations, and the development of pulpal pathology (6). Although the protective functions of dentin and the capacity of the pulp to sustain bacterial challenges have been demonstrated (7), the defense mechanisms of the dentin/pulp complex must not be put to the test.

Therefore, bacterial leakage at restoration margins is a major concern. To minimize volumetric shrinkage, efforts have been directed toward slowing down the composite resin polymerization rate (8), using an incremental placement technique (9), placing thicker adhesive layers under the composite (10) or use of low-modulus intermediate layers (11). Various studies have also reported efforts to develop a non-shrinking high-performance polymer for use as a matrix material for dental composite resins (12). Siloranes, a new category of ring-opening monomers, were introduced to overcome the problems associated with volumetric shrinkage. The volumetric shrinkage of silorane-based composite resins is less than 1% (13), which is due to opening and extending the oxirane rings during polymerization, compensating for volume reduction (13,14). The use of flowable composite resins as liners has been suggested to improve adaptation to cavity walls, reducing microleakage. These effects may be due to its low viscosity, increased elasticity and wettability (15). Various techniques have been used in microleakage studies. Bacterial penetration test is a non-destructive technique that was originally described to test bacterial leakage around filling materials (16), and later it found applications in endodontics (17). Since there is a clear relationship between dentin infection, pulp inflammation and loss of pulp vitality, the question regarding possible differences in capacity of different restorative materials to prevent bacterial microleakage is very important. The aim of this study was to compare the effects of different resin-based dental restorations and layering techniques on bacterial microleakage of Cl I composite resin restorations.

Materials and Methods

This in vitro study was carried out on 82 newly erupted non-carious, non-restored, human third molars, gathered following informed consent, approved by the Commission for Medical Ethics of Mashhad University of Medical Sciences (N0. 900672). The teeth were disinfected by storage in 0.02% thymol solution for 24 hours and stored in normal saline solution until use (18). The occlusal enamel was trimmed at the level of the main grooves using a slow-speed disc (KG Sorensen, Barueri, SP-Brazil) under copious running water, exposing an occlusal flat enamel surface. Uniform box-shaped Class I cavities were prepared measuring approximately 4 mm (mesial-distal) $\times$ 4 mm (buccal-lingual) $\times$ 2 mm (in depth) at the occlusal crown center, using a high-speed handpiece with fissure burs # 109/008 (Brasseler, Savannah, GA, USA) under constant water irrigation for all the cavities. Cavity dimensions were measured by a periodontal probe. The burs were changed every five preparations. The cavosurface margins were prepared at 90°. All the prepared Cl I cavities consisted of enamel and dentinal walls and the pulpal floor was located on dentin. These dimensions yielded a box-shaped cavity with a C-factor of 4 (bonded surface/unbonded surface area = 64 mm^2/16 mm^2 = 4).

The prepared teeth were then randomly divided into two main groups of 36 teeth each and two positive and negative control groups of five teeth each as follows.

Group 1: a silorane-based composite resin (Filtek P90, 3M ESPE)

Group 2: a methacrylate-based composite resin (Clearfil AP-X, Kurary, Japan)

Either FiltekSilorane System Adhesive with Filtek P90 composite resin or Clearfil SE Bond with Clearfil AP-X composite resin was applied to the cavity as follows:

The Filtek P90 primer was applied to the entire cavity wall and left over the entire area for 15 sec. A gentle stream of air was used and the primer was cured for 10 seconds. The bonding agent was applied to the entire cavity walls and cured for 10 sec.

The Clearfil SE primer was applied to the entire cavity wall and left in place for 20 seconds.

Then, the volatile ingredients evaporated with a mild air stream. The bonding agent was applied to the entire cavity walls and cured for 10 seconds.

The adhesives were light-polymerized using an Optilux 501 quartz tungsten halogen unit (Demetron–Kerr, Orange, CA, USA) set at 600 mW/cm^2. All the bonding procedures were carried out by a single operator at a room temperature of 24°C. Each of the two main groups was subdivided into three subgroups A, B and C (n = 12) based on three different placement techniques:

Subgroup A (bulk filling): Shade A_2 composite resin was placed in one bulk and cured for 40 seconds.

Subgroup B (incremental filling): Shade A_2 composite resin build-ups were constructed in two 1-mm-thick horizontal increments, which were individually light-polymerized for 20 seconds.

Subgroup C (snowplow): The first layer consisted of 0.5-mm-thick Filtek Z 350 flowable composite resin. Then shade A_2 composite resin was condensed over the uncured flowable composite resin; excess flowable composite resin was removed with an explorer and then light-cured for 20 seconds. The rest of the cavity was filled similar to that in subgroup B. After finishing the restorations, additional curing of occlusal aspects of each tooth was carried out for 40 seconds. Then the teeth were finished and polished with rubber cups and points (Identoflex, Kerr Hawe SA, Bioggio, Switzerland).

Positive control group: The five teeth used in this group were left empty after cavity preparation to provide a passage for bacterial leakage.

Negative control group: In this group (n=5), sound teeth were used and all the surfaces were coated with two layers of nail varnish to prevent bacterial leakage.

All the restored specimens were stored for 24 hours in distilled water at 37°C and subjected to 1000 thermal cycles at 5°C/55°C with a 30-second dwell time.

Bacterial Leakage Assessment

Following the removal of the roots, the dentin between the furcation and the pulp chamber floor was also removed. The external surfaces of all the specimens, except for 2 mm around the restoration, were covered with two layers of nail varnish. The microbial test consisted of a 2-chamber method with some modifications (16). Each specimen was embedded in one end of a plastic tube with epoxy resin (Meliodent, Heraeus-Kulzer, Germany). The junctions between the crown, epoxy resin and the tube were sealed with cyanoacrylate adhesive. The mounts were sterilized for 8 hours in an ethylene oxide sterilizer (Anprolene, AN 74C, Andersen Products Inc., Haw River, NC, USA) at room temperature. After sterilization, the apparatus was placed in a glass flask (the lower chamber) containing sterile brain-heart infusion broth (BHI, ScharlauChemie S.A., Barcelona, Spain). 2-3 mm of the specimens was immersed in the broth. The junctions between the plastic tubes and the glass flasks were tightly sealed with Parafilm and cyanoacrylate adhesive. An initial bacterial suspension containing 1.5×10^8 CFU/mL of *S. mutans* (ATCC 25175) was used. The upper chambers were filled with 8 mL of the initial suspension keeping the bacterial suspension in contact with the occlusal surface of the specimens (Fig 1). The mounts were always handled in sterile conditions under a laminar flow hood (Nuaire, Plymouth, MN, USA) to avoid bacterial contamination. They were placed in an incubator at 37°C for 3 days. The lower chambers of all the mounts were observed daily and the turbidity time was recorded for each specimen. Once turbidity was present, a sample of the turbid broth was streaked onto blood agar plates and the bacteria were identified to ensure that there was no contamination other than *S. mutans*. The results were analyzed using Fisher's exact and chi-squared tests. Statistical significance was set at P<0.05.
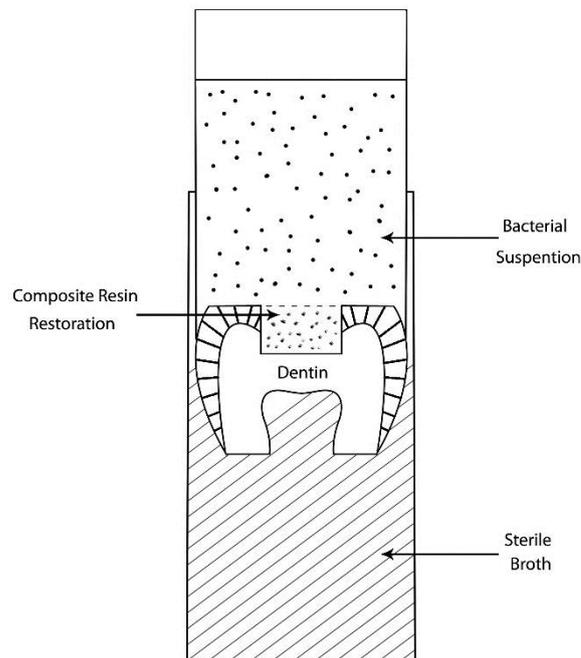
Figure 1. The assembly used for testing bacterial leakage.

Results

Complete leakage was recorded in positive controls, while negative samples showed no leakage during the experiment. The results of bacterial leakage are shown in Tables 2 and 3.

Statistical analysis revealed no significant differences between Filtek P90 and Clearfil AP-X (P=1) in terms of microleakage. The differences between the results, which were related to different filling techniques, were not significant, either (P>0.05).

Table 1. Materials, chemical compositions and application procedures

Material (Manufacturer)		Chemical composition
FiltekSiloraneSystem Adhesive (3M ESPE)	Filtek P90 Primer	phosphorylated methacrylates, Vitrebond copolymer, Bis-GMA, HEMA, water, ethanol,silane-treated silica filler, initiators, stabilizers
	Filtek P90 Bond	hydrophobic dimethacrylate, phosphorylated methacrylates, TEGDMA, silane-treated silica filler, initiators, stabilizers
Clearfil SE Bond (Kuraray,Tokyo, Japan)	Clearfil SE Primer	HEMA , 10-MDP ,hydrophilic dimethacrylate, water, accelerators, dyes, camphorquinone
	Clearfil SE Bond	Bis-GMA , HEMA,10-MDP , hydrophilic dimethacrylate, colloidal silica , initiators , accelerators, dyes , camphorquinone
Resin composite	Filtek P90 (3M ESPE)	Silane treated quartz ,3,4-epoxycyclohexylcyclopolymethylsiloxane, Bis-3,4-epoxycyclohexylethyl-phenylmethylsilane, yttrium trifluoride
	Clearfil AP-X(Kuraray,Tokyo, Japan)	Bis-GMA, TEGDMA, silanated barium glass filler, silanated silica filler, silanated colloidal silica, dl-camphorquinone, catalysts, accelerators, pigments
	Filtek Z350 flowable restorative (3M ESPE)	Silane treated ceramic, BIS-GMA,TEGDMA,BIS-EMA,silanetreted silica and zirconium oxide, functionalized dimethacrylate polymer

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol A diglycidylmethacrylate; TEGDMA, triethylene glycol dimethacrylate; 10-MDP, 10-Methacryloyloxydecyl dihydrogen phosphate; BIS-EMA, ethoxylatedbisphenol A glycol dimethacrylate.

Table 2. Comparison of bacterial microleakage in different subgroups of thetested materials

Resin composite	Filling method	Microleakage	No microbial growth	N	P-value
Filtek P90	Bulk	9	3	12	
	Incremental	4	8	12	.062
	Snow-plow	4	8	12	
Clearfil AP-X	Bulk	10	2	12	
	Incremental	5	7	12	.060
	Snow-plow	5	7	12	

Table 3. Comparison of bacterial microleakage in two resin composites

Filling method	Resin composite	Microleakage	No microbial growth	N	P-value
Bulk	Filtek P90	9	3	12	P=1.00
	Clearfil AP-X	10	2	12	
Incremental	Filtek P90	4	8	12	P=1.00
	Clearfil AP-X	5	7	12	
Snow-plow	Filtek P90	4	8	12	P=1.00
	Clearfil AP-X	5	7	12	

Discussion

Stresses produced along the tooth-restoration interface from polymerization shrinkage and mechanical fatigue through repetitive masticatory loading and temperature changes in the oral environment result in microleakage of composite resin restorations (19). Various methods have been used to detect microleakage; however, there is no gold standard method for microleakage evaluation. Dye penetration studies are commonly used in vitro to detect bond failure at the enamel-resin interface; however, this technique has no clinical relevance (20). In the present study the bacterial penetration test was used. An important advantage of this method is its clinical relevancy (21). We had made some modifications in the method introduced by Mortensen et al. (16). The roots and pulp chamber floors of the teeth were removed to avoid the effects of root canal systems on bacterial penetration.

Uniform box-shaped Class I cavities that had high C-factor were prepared. The uniformity of cavity preparation was a critical factor for the study, because having cavities with similar dimensions is essential to inserting and photo-activating a standardized volume of composite resin in each sample.

The samples were subjected to thermocycling according to the ISOTR 11405 standard in order to simulate the degradation of bond in the oral cavity due to the difference in the coefficient of thermal expansion of the restoration and the tooth interface (22). In the current study, none of the restorative materials showed complete prevention of bacterial leakage. P90 composite resin showed microleakage results similar to APX. In general, it was reported that microleakage scores of silorane-based composite resins were lower or similar to methacrylate-based ones (23-28). Similar to this study, Schmidt et al (29) did not find significant differences in marginal adaptation of the low-shrinkage silorane-based composite resins compared to methacrylate-based composite resins in vivo, either. The polymerization process of siloranes occurs via a cationic ring-opening reaction which helps in gaining space and counteracts the loss of volume due to bond formation. Furthermore, silorane-based composite resins showed longer time to gel point, allowing for flow of material and stress relaxation (27). These phenomena can explain their low polymerization shrinkage and stress. However, the lower polymerization stress of P90 was no guarantee of the best marginal integrity. There are some other factors influencing the marginal integrity of restorations, such as adhesive system and stiffness of uncured composite resin. Clearfil SE Bond Adhesive contains microfillers in the bonding resin and its adhesive resin layer has a thickness of about 40-200 µm (30). This thick adhesive layer could absorb some of the shrinkage stress. On the other hand, the uncured Filtek P90 is rather stiff compared to the relatively soft Clearfil AP-X (31), and its good adaptation to the cavity walls in the narrow cavities may have been problematic. Another issue evaluated by researchers is antibacterial properties and inhibition of bacterial growth by restorative materials (32-34). Buergers et al. (14) reported that Filtek silorane composite resins had significantly lower susceptibility to *Streptococci* adherence than conventional methacrylate-based composite resins. This factor may be attributed to increased hydrophobicity of silorane-based composite resins and its influence on predicting long-term performance of restorations in clinical situations. In the present study, the filling technique had no significant effect on microleakage of restorations.

According to the results, 9 specimens in the bulk group of Filtek P90, and 10 specimens in the bulk group of Clearfil AP-X showed leakage that were higher compared to incremental and snowplow groups. However, this difference was not statistically significant, but it can be extremely important clinically, since it is essential to reduce degree of leakage, pain and sensitivity after restoration. Lower number of leaked samples in the incremental groups may be attributed to the effect of incremental insertion of composite resins on decreasing polymerization shrinkage. Incremental technique can lower the configuration factor (C-factor). High C-factor values can break down the bond between the restorative system and the cavity walls (35). The use of a liner has been suggested for relieving the stress induced by polymerization shrinkage (15,36). It has been reported that the lower Young's modulus of elasticity of flowable composites could help dissipate the shrinkage stress that occurs during polymerization of restorative composite resins (37). However, in vitro studies have shown conflicting results regarding the ability of an elastic liner to decrease microleakage of restorations (38–41). This study showed that use of flowable composite resin liner does not improve marginal integrity of restorations compared to the incremental technique. This finding is consistent with that of Kasraei et al. (42). They found no significant differences between restoration with flowable composite resin liners and those without the liner. In spite of comparable sealing ability with different filling techniques, further in vivo investigations might be necessary.

It is suggested that future studies focus on developing bioactive materials that inhibit plaque collection, suppress bacterial activity and inhibit caries.

Conclusion

Under the limitations of the present laboratory study, it was concluded that leakage occurred in both silorane- and methacrylate-based composite resins but the difference was not statistically significant. Based on the results of this study, filling technique had no significant effects on microleakage of restored teeth.

Acknowledgment

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