Original Article

Effect of Lactic Acid on Microleakage of Class V Low-Shrinkage Composite Restorations

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Abstract

Objectives: To assess the effect of lactic acid (LA) on microleakage of silorane-based composite restorations and methacrylate-based composites with self-etch and etch-and-rinse bonding systems.

Materials and Methods: Class V cavities were prepared in 120 extracted human teeth, divided into four groups and restored as follows: 1. Silorane-based composite+P90 adhesive system (P90); 2. Filtek Z250+SE Bond (Z250SE); 3. Filtek Z350+SE Bond (Z350SE) and 4. Filtek Z250+Single Bond (Z250SB). Half of the samples in each group were immersed in LA and the other half in distilled water (DW) for seven days. Degree of microleakage was determined by dye penetration. Data were analyzed using Kruskal Wallis and Mann Whitney-U tests (type 1 error was considered 0.05 for primary and 0.017 for post-hoc tests).

Results: No significant difference was found in microleakage between LA and DW groups. The difference among groups in gingival margin microleakage was significant ($P<0.05$). The highest degree of microleakage was seen in Z250SB; which was significantly higher than Z250SE (DW: P=0.012 and LA: P=0.002) and Z350SE (DW: P=0.002 and LA: P=0.014). Microleakage was not significantly different between Z250SE and Z350SE (DW: P=0.683 and LA: P=0.533). The degree of microleakage of P90 in both media was lower than Z250SB and higher than that of Z250SE and Z350SE; but these differences were not significant.

Conclusions: Immersion in LA has no effect on microleakage of class V composite restorations regardless of the type of composite and adhesive system. At gingival margins, the highest microleakage occurred in Z250SB followed by P90 and self-etch groups.

Keywords: Silorane Composite Resin; Dental Leakage; Lactic Acid

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INTRODUCTION

Presence of dental biofilm is fundamental for development of caries. Dental biofilm contains acidogenic bacteria [1] and high concentrations of lactic acid (LA), acetic acid and propionic acid [2,3]. It has been documented that presence of dental biofilm is not related to oral hygiene or technique of plaque removal by the patient [4]. Oral biofilm has the potential of producing organic acids in every individual [5].

Tooth-colored restorative materials, and particularly composite resins are highly popular in contemporary dentistry [6,7]. However, drawbacks such as stress due to polymerization shrinkage compromise their clinical success. Stress accumulation in the cavity walls restored with composite resin causes gap and subsequent microleakage [7]. The gap at the tooth-restoration interface allows passage of bacteria and ions leading to consequent tooth hypersensitivity, pulp irritation and marginal discoloration [8,9]. There are ways to decrease polymerization shrinkage stress including deceleration of composite polymerization [10], replacing dual-cure cements with self-cure cements [11], placing a thick layer of adhesive beneath the composite resin [12] and incremental application of composite resin [13]. Altering the

resin matrix and producing low-shrinkage composites like silorane-based composites is one recent solution to decrease polymerization shrinkage [7]. The manufacturers claim that silorane composite has two main advantages: first, its low polymerization shrinkage due to the ring-opening reaction of oxirane molecule and second its increased hydrophobicity due to the presence of siloxane molecule [9,14,15].

Many clinical and laboratory studies have demonstrated that the adhesive interface is the weakest area in adhesive restorations [16-18]. The mechanism of action of the currently used bonding systems (both self-etch and etch and rinse) is based on the formation of a hybrid layer [19,20]. Previous studies showed that hybrid layer is very susceptible to hydrolysis and thus, weakens the dentin-adhesive interface [21,22].

Silva et al, [5] showed that acids present in oral biofilm may affect the bond strength of adhesive systems to human dentin. Lactic acid is a carboxylic acid with –OH and –COOH functional groups in its formulation. It is highly likely that these functional groups form hydrogen bonds with the polar end of methacrylate monomers present in the bonding agent matrix such as –OH in Bis-GMA, –O- in TEGDMA and Bis-EMA and N-H in UDMA, causing greater softening of the matrix [23].

There is controversy regarding the marginal adaptation of silorane composites versus methacrylate-based composites before and after seven days of water storage [24-26]. Low-shrinkage composites may have less destructive effect during polymerization on the adhesive and the cavity walls [15,24]. Thus, the adhesive layer might remain intact. According to this theory and the hydrophobicity of silorane composites, the degradation pattern of silorane composites due to organic acids in biofilm might be different from that of methacrylate-based composites. Therefore, this study aimed to assess the effect of LA (as a main organic acid in dental biofilm) on microleakage of Class V silorane-based composite restorations compared to restorations with two methacrylate-based (nanofilled and microhybrid) composites following the application of self-etch and etch and rinse bonding systems.

MATERIALS AND METHODS

Preparation of specimens: A total of 120 human molar and premolar teeth extracted within the past three months prior to the experiment were collected. The teeth were free from caries, cracks or restorations and had been extracted for orthodontic or periodontal reasons. To remain hydrated, the teeth were stored in distilled water (DW). The collected teeth were rinsed with water and the tissue residues, and debris were removed by a curette and then, an ultrasonic scaler. Teeth surfaces were then polished by low-speed handpiece, a prophy brush and pumice paste. The specimens were disinfected by immersion in 0.5% chloramine T solution for one week followed by storage in DW at 4°C in a refrigerator. The teeth were randomly divided into four groups of 30 (A-D).

Cavity preparation: Standard class V cavities were prepared on the buccal surfaces of all teeth measuring 1.5 in depth, 2 mm occlusogingivally and 4 mm mesiodistally using a high speed handpiece and 008 diamond fissure bur (Stoddard, Garden City, England) under water spray in such a way that the cavities extended to 1mm below the cementoenamel junction. The bur was replaced for every five teeth. The teeth were stored in DW during the experiment.

Material application: The teeth received the following restorations (Table 1 summarizes the characteristics of the materials used in this study):

Group A: Teeth in this group were restored with a silorane based composite (P90, 3M ESPE. St. Paul, MN, USA) and adhesive (P90 3M ESPE. St. Paul, MN, USA) according to the

Table 1. Materials and their Composition

Type	Material	Content	Manufacturer
Filtek Z250	Microhybrid methacrylate-based composite	Bis-GMA, Bis-EMA, UDMA, TEGDMA **Filler:** zirconia, silica, 78wt%, 60v% **Particle size:** 0.01-3.5 µm	3M ESPE, St. Paul, MN, USA
Filtek Z350XT Enamel	Nanofilled methacrylate-based composite	Bis-GMA, UDMA, Bis-EMA, silica, zirconia, nanoparticles (20µm) and nanoagglomerates (0.4-0.6 µm) **Enamel shade:** 78.5wt%, 63.3v%	3M-ESPE. St. Paul MN, USA
Filtek P90	Silorane-based composite (microhybrid)	**Matrix:** 3,4 Epoxycyclohexyl ethyl cyclo poly-methyl siloxane, bis-3,4 epoxycyclohexyl-ethyl-phenyl-methyl silane **Filler**: Silanized, quartz, yttrium fluoride 76wt%– 55v%, **Particle size:** 0.04-1.7µm	3M ESPE, St. Paul MN, USA
P90 System Adhesive	Two step self-etch	**Primer**: phosphorylated-methacrylate, Vitrebond copolymer Bis-GMA, HEMA, water, ethanol, Silane-treated silica filler, camphorquinone stabilizer **Bond**: hydrophobic dimethacrylate, phosphorylated-methacrylate, TEGDMA, Silane-treated silica filler, initiator, stabilizer	3M ESPE, St. Paul, MN, USA
SE Bond	Self- etch adhesive	**Primer:** 10-Methacryloyloxydecyldihydrogen phosphate (MDP), 2-Hydroxyethyl methacrylate, hydrophilic dimethacrylate, dl-camphorquinone, accelerators, water **Bond:** 10- Methacryloyloxydecyl dihydrogen phosphate (MDP), bisphenol a diglycidyl methacrylate (Bis-GMA), 2-Hydroxyethyl methacrylate (HEMA), hydrophobic dimethacrylate, dl-Camphorquinone, accelerators, silanized colloidal silica, surface treated sodium fluoride	Kuraray Noritake Dental Inc., Osaka, Japan
Single Bond	Total- etch adhesive	Bis-GMA, HEMA, dimethacrylate, ethanol, water, novel photoinitiator system, methacrylate functional copolymer of polyacrylic, polyitaconic acids	3M-ESPE. St. Paul, MN, USA

manufacturer's instructions: The primer was applied to the cavity walls (both enamel and dentin) using a microbrush and agitated for 15 seconds. The primer layer was gently air dried and cured with a LED light curing unit (Valo, Ultradent Products Inc., South Jordan, UT, USA) with a light intensity of 1000 mW/cm^2 for 10 seconds.

Using a new microbrush, bonding agent was applied to the cavity walls and after gentle air drying, it was cured for 10 seconds. P90 composite resin was applied incrementally to the cavity in three increments. The first increment was applied on the occlusal margin and the axial wall, the second increment was applied over the previous layer to the middle third of the axial wall and the third layer was applied over the previous ones to the outer third of the gingival margin obliquely. Each layer was cured for 10 seconds.

Finishing and polishing procedures were carried out using a fine flame diamond bur and flexible coarse, medium, fine and super fine polishing discs (Soflex, 3M ESPE. St. Paul, MN, USA), respectively. The samples were post cured for 20 seconds.

Group B: Teeth in this group were restored with Z250 composite resin (3M ESPE. St. Paul, MN, USA) and SE bond (Kuraray Noritake Dental Inc., Osaka, Japan). Primer was applied to enamel and dentin cavity surfaces by a microbrush and agitated for 20 seconds followed by gentle air-drying. The bonding agent was then applied to cavity surfaces using a microbrush, gently air dried and light cured for 10 seconds using a LED light-curing unit. Cavities were then restored with Z250 composite resin according to the protocol in group A.

Group C: Procedures were performed as in group B except that the cavities were filled with Z350

composite resin (3M ESPE. St. Paul, MN, USA) and SE bond.

Group D: Teeth in this group were restored with Z250 composite resin (3M ESPE. St. Paul, MN, USA) and Single Bond (3M ESPE. St. Paul, MN, USA). The cavity was first etched with 37% phosphoric acid (3M ESPE. St. Paul, MN, USA). Enamel was etched for 20 seconds and dentin for 15 seconds. The cavity was then rinsed for 20 seconds and gently air-dried. The adhesive was then applied by an applicator according to the manufacturer's instructions, agitated for 15 seconds and gently air dried for five seconds. The second layer of adhesive was applied to the cavity walls and light cured for 10 seconds using a LED light curing unit with a light intensity of 1000 mW/cm^2. Then, half of the teeth in each group were immersed in LA (pH = 4, 0.01M) and the other half in DW (pH=7) as the control group and stored in an incubator at 37°C for seven days.

Dye penetration technique: Before the immersion of teeth in dye solution, the apices, root surfaces and the furcation area were sealed with sticky wax. All teeth surfaces except for the restoration area and 1 mm around the restoration margins were sealed with two coats of nail varnish. The teeth were immersed in 2% basic fuchsin (Sigma Aldrich, Taufkirchen Germany) in DW and stored in an incubator at 37°C for 24 hours. The samples were rinsed, dried and mounted in clear polyester acrylic resin and longitudinally sectioned in buccolingual direction by a saw with a blade thickness of 0.82mm in a cutting machine (Mecatome, T201A, Persi, France). Gingival and occlusal margin microleakage was assessed under a stereomicroscope (SMZ 800, Nikon, Tokyo, Japan) at x40 magnification and scored as follows:

0: No dye penetration

1: Dye penetration extending up to half the gingival/occlusal wall

2: Dye penetration extending to more than half the gingival/occlusal wall

3: Dye penetration extending into the axial wall and pulp

Statistical analysis: Data were analyzed using SPSS 18. Non-parametric Kruskal Wallis test was used for the analysis of microleakage (ordinal variable). The Mann Whitney U test was used to compare the degree of microleakage at each margin and for each composite in different media. Also, the Kruskal Wallis test was applied to compare the four groups of composites based on the margin and the media. The type of post hoc test was Mann-Whitney U with Bonferroni adjustment. (type 1 error was considered 0.05 for primary and 0.017 for post-hoc tests). Dunn procedure with P-value adjustment was used for pairwise comparisons. Confidence level was set at 95% (α= 0.05).

RESULTS

Table 2 shows the frequency of degrees of microleakage in the studied groups.

Table 2: Microleakage values at both occlusal and gingival margins in the studied groups

Microleakage	Distilled water				Lactic acid			
Level	0	1	2	3	0	1	2	3
Occlusal								
P90	3	9	1	2	6	8	0	0
Z350	5	9	1	0	8	5	1	0
Z250 SB	2	7	2	1	8	6	1	0
Z250 SE	6	9	0	0	12	3	0	0
Gingival								
P90	4	6	0	5	5	7	2	0
Z350	9	5	0	1	10	3	0	1
Z250 SB	1	4	3	4	3	8	1	3
Z250 SE	8	5	0	2	13	1	0	1

The Mann Whitney test found no significant difference in microleakage between distilled water and LA at each margin and for each type of composite (P>0.05). The Kruskal Wallis test revealed that the microleakage at the occlusal margin was not significantly different between groups in either media (P>0.05) but significant differences were found in this respect at the

gingival margin (P<0.05). Pairwise comparisons were made using Dunn procedure with P value adjustment. The highest degree of microleakage was seen in Z250+SB group, being significantly higher than that of Z250+SE (P=0.012 in DW and P=0.002 in LA) and Z350+SE (P=0.002 in DW and P=0.014 in LA). The degree of microleakage was not significantly different in Z250+SE and Z350+SE groups (P=0.683 in DW and P=0.533 in LA). The microleakage of P90 in both media was less than that of Z250+SB and more than that of Z250+SE and Z350+SE; but none of the differences were statistically significant (P>0.017). Microleakage was not different in occlusal and gingival margins (P>0.05) in all groups except for Z250SB (P=0.039 in DW and P=0.008 in LA).

DISCUSSION

In the current study, seven days of immersion in LA did not cause a significant change in microleakage compared to immersion in distilled water. Silva et al, [5] showed that seven days of immersion in LA decreased the bond strength of composite restorations with Clearfil SE Bond and Adper Single Bond compared to artificial saliva. However, chemical agents such as the acids present in dentinal fluid, saliva, dental biofilm, foods and drinks can impact on the tooth-restoration interface as well as the hybrid layer and cause variable patterns of degradation of collagen fibrils and resin [21,27]. In addition, LA is a carboxylic acid with –OH and –COOH functional groups in its formulation. It is highly likely that these functional groups form hydrogen bonds with the polar end of methacrylate monomers present in the bonding agent matrix such as –OH in Bis-GMA, –O- in TEGDMA and Bis-EMA and N-H in UDMA, causing greater softening of the matrix [23].

The current study aimed to assess the early effect of immersion in LA on degree of microleakage and thus, immersion was only performed for seven days and no significant diffidence was noted in microleakage following immersion of teeth in LA and DW. It might be related to short-term storage. However, long-term immersion may have destructive effects on the hybrid layer and resin and cause degradation of tooth-restoration interface. Further studies with longer storage time and thermocycling are required to better elucidate this phenomenon.

The results revealed no significant difference in microleakage of groups restored with silorane-based composite and Z250 bonded with SE bond. In a study by Kermanshah et al, [28] no difference was reported in microleakage of cavities restored with a silorane-based composite and SE bond. In contrast, Al-Boni and Raja [14] demonstrated that microleakage of silorane after thermocycling was less than that of Z250 bonded with self-etch adhesive.

In the current study, the highest degree of microleakage in gingival margin was seen in Z250+SB group. In a study by Hooshmand et al, [29] the microleakage of silorane-based composites was reported to be less than that of specimens bonded with Exite (an etch and rinse adhesive).

Single Bond is a two-step, etch and rinse adhesive system. This adhesive system is susceptible to nano-leakage due to inadequate impregnation of adhesive at the resin-dentin interface after polymerization [30]. On the other hand, higher content of hydrophilic monomers in two-step etch and rinse adhesives (compared to three-step systems) [31] allows greater penetration following polymerization, facilitates water sorption and increases leakage [32]. However, all adhesive systems have shown some degrees of incomplete polymerization and subsequent microleakage [33-35]; this is especially true about simplified systems like one-step self-etch and two-step etch and rinse systems due to higher hydrophilic monomer content [33-35]. SE-Bond is a two-step, self-etch adhesive. In self-etch adhesives, acidic co-monomers demineralize dentin and penetrate it at the same

time. Complete infiltration of adhesive into the substrate is necessary to achieve a stable bond [36]. On the other hand, in mild (pH=2) two-step self-etch adhesives, phosphate or carboxylate groups present in monomers form a chemical bond with the residual hydroxyapatite crystals in dentin collagen network, reinforcing long-term stability [37,38]. In the current study, the degree of microleakage of silorane group was somewhere between that of Z250+SB and the remaining two groups; but none of these differences were statistically significant. Silorane composite resins are polymerized via a cationic ring-opening mechanism [7-9]. These new monomers are formed by the reaction of oxirane and siloxane molecules and thus the name silorane [7,8,39, 40]. Silorane primer has a pH of 2.7 and according to the manufacturer's claim, it causes mild etching of the tooth surface and provides a strong, stable bond [41,42]. Moreover, Mine et al, [43] demonstrated that silorane primer forms a chemical bond with hydroxyapatite crystals. On the other hand, P90 primer and bonding agent are supplied in separate bottles and each one is photo-cured separately after application. Santini and Miletic [44] reported the presence of an intermediate layer with 1µm thickness between the silorane primer and bonding agent using micro-Raman spectroscopy. This area might be the weakest area involved in the mechanism of failure of silorane restorations; further investigations are warranted in this respect.

In the current study, the degree of microleakage of Z350+SE was similar to that of Z250+SE. But, Sharma et al, [45] indicated higher microleakage of Z350 compared to that of Z250 after thermocycling. In our study, similar results were obtained for samples restored with composites in conjunction with SE adhesive.

Z350XT is a nanofilled composite with high filler content. It undergoes less linear shrinkage than microhybrid composites due to smaller monomers and higher filler volume [46]. Low shrinkage stress may improve marginal fit [24]. On the other hand, Filtek Z250 composite has shown acceptable results with regard to marginal fit [45,47].

CONCLUSION

Immersion in LA had no effect on microleakage of class V composite restorations regardless of the type of composite and adhesive system. At gingival margins, the highest microleakage was seen in Z250SB followed by P90 and self-etch groups.

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