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Does preheating affect the microleakage of bulk-fill composite restorations?

Sultan Aktuğ Karademir[1], Serdar Akarsu[1]

[1] Ordu University, Faculty of Dentistry, Department of Restorative Dentistry, Ordu, Türkiye

Abstract

Aim: Microleakage is one of the most important factors in restoration failure in today's dentistry. The aim of this in vitro study is to evaluate the microleakage of preheated bulk-fill composites.

Methodology: In the study, 160 slot cavities prepared in the mesial and distal of 80 molar teeth were used. In the gingival of the cavities, Estelite Bulk Fill Flow (Tokuyama, Tokyo, Japan) in the mesial and SDR® Posterior Bulk Fill Flowable Base (Dentsply, DeTrey, Konstanz, Germany) in the distal were used, and the remaining occlusal parts were restored using Filtek™ One Bulk Fill Restorative (3M, St Paul, MN, US). Composites were applied at four different temperatures (4ºC, room temperature, 39ºC and 55ºC). After finishing and polishing, specimens were thermocycled and then placed in 0.5% methylene blue for 24 hours. Specimens were sectioned into two parts in a mesiodistal direction, and the microleakage levels were examined by a stereomicroscope (x40).

Results: In the statistical evaluation of the microleakage levels of Filtek™ One Bulk Fill Restorative applied at four different temperatures on the occlusal surface with One-way ANOVA, it was observed that there was a significant difference between the groups (p=0.02). In the comparison of the microleakage values of Estelite Bulk Fill Flow applied in the gingival region with the one-way ANOVA test at four different temperatures, there was no statistically significant difference between the groups (p>0.05), while there was a significant difference between the groups using SDR® Posterior Bulk Fill Flowable Base (p=0.002). The highest microleakage values were observed at 4ºC in all groups.

Conclusion: The results obtained in the study show that heating bulk fill composites before polymerization is effective in reducing microleakages, but longer in vivo studies are required.

Keywords: Bulk-fill composite, preheating, microleakage, composite restorations

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Correspondence:

Dr. Sultan AKTUĞ KARADEMİR
Ordu University, Faculty of Dentistry, Department Restorative Dentistry, Ordu, Türkiye.
E-mail: sultan_721@hotmail.com

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Introduction

According to Rafael Bowen (1957), composite resins changed the practice of dentistry and became one of the most important restorative materials (1). In today's dentistry, composite resins have become one of the most frequently used materials in both anterior and posterior restorations because of the increasing concerns and esthetic demands of patients about mercury levels in amalgam restorations (2). However, one of the major shortcomings of conventional composite resins is that polymerization is difficult, and there can be polymerization shrinkage in deep cavities due to limited light penetration in posterior restorations. The resultant polymerization shrinkage stress manifests clinically with several potential complications, such as cusp deflection, microcracking and fracture of enamel margins, microleakage, debonding, postoperative sensitivity, and pulpal irritation (3-5).

To overcome these problems, an incremental technique for placing composite resins has been introduced. This technique provides proper light polymerization of the composite resin and reduces the polymerization shrinkage stress (6, 7). The incremental technique has been used in the polymerization of the posterior teeth for a long time, but it has some disadvantages, such as long process times, gaps between layers, and a risk of contamination.

In recent years, the use of composite resins for the restoration of posterior teeth has improved (8, 9). However, there are still concerns about their use in high-stress areas. The high viscosity and adhesion of these materials make them difficult to fit and conform to the cavity walls. Poor adhesion between the dentin and the restorative material leads to gap formation (10). Ultimately, marginal cavity formation leads to microleakage, which may be responsible for increased postoperative sensitivity, pulpal inflammation, discoloration and recurrent caries (11, 12). Material adaptation to the cavity walls is essential for perfectly sealed, long-lasting restorations (13, 14).

To compensate for these problems, the heating of composite resins before polymerization has recently been proposed (12, 15, 16). Although the preheating process varies based on the brand of the material, preheated composite resins show more fluidity. In addition, the polymerization of composites at high temperatures increases the curing rate and degree of conversion. This can result in improved mechanical properties. Seeking methods that are less sensitive to removing these disadvantages and placing techniques has resulted in the introduction of bulk-fill composite resins that can be placed and photopolymerized in a single layer with a thickness of 4-8 mm (17).

Some changes have been made to the chemistry, particle size, and shape of these resins' monomers to allow these materials to be used in bulk. While some bulk-fill composites are produced as posterior restorative materials, others are produced as base materials intended to be covered with composite resin (18).

The clinical success of composite restorations is highly dependent on polymerization and the degree of monomer conversion. High-viscosity composites are very difficult to adapt to cavities correctly, and gap formation might occur in the course of application. Therefore, more fluid composites with less filler have been produced (19).

One of the major limitations of direct composite resin restorations is their high polymerization shrinkage (5). The C-factor, also known as the configuration factor, is the ratio of the bonded surface of the restoration to the nonbonded surface and is a factor that affects polymerization shrinkage. Only the nonbonded surfaces of a restoration can act as a reservoir for plastic deformation during polymerization. Thus, if the C-factor can be reduced clinically, polymerization shrinkage can also be reduced (19).

Recent research has focused on heating composite resins to improve their properties. Different preheating devices and methods, including the Micerium Ena Heat conditioner, the Calset Composite heater, and the Therma-flo heating system, and the microwave heating system, have been used in dentistry (20).

Friedman defined a polymerization method in which a higher monomer conversion degree was obtained by heating a composite resin at 54-60 degrees prior to light activation (21). It was found that heating composite resins before photopolymerization increases the degree of conversion, decreases viscosity, and increases adaptation ability. As the temperature increases, both the radical and monomer mobility of the composite resin increase, resulting in a higher rate of cross-linked polymer chains (22).

With this increase in transformation, the mechanical and physical properties of the composite resin, such as surface hardness and polymerization depth, improve. In addition, the composite, whose fluidity increases as a result of preheating, becomes more compatible with the cavity walls, which causes a decrease in microleakage and a decrease in secondary caries in the restoration (23). There have been many studies on the microleakage of preheated composite resins in the dental literature. However, there have been a limited number of microleakage studies on Class II bulk-fill composite restorations close to the cemento-enamel junction.

The aim of this in vitro study is to evaluate the microleakage of preheated bulk-fill composites. The null hypothesis of this study is that the temperature of bulk-fill composite resins before polymerization has no effect on microleakage.

Materials and Methods

This study was approved by the Ordu University Ethics Committee (2019-76). The sample size calculated per group was 10 sound molars with an alpha of 0.05 and a beta of less than 0.2 (power > 80%).

This study had five stages: cavity preparation, restoration placement, thermal cycle, dye penetration, and microleakage evaluation.

Cavity preparation

A total of 80 freshly extracted sound human molars were used in the study. The remaining connective tissue and calculus on the teeth were removed by scaling, and the teeth were stored in 0.1% thymol solution at room temperature until the study was carried out. Samples were prepared from 80 non-carious molar teeth stored in saline at room temperature. A total of 160 standardized Class II cavities on the mesial and distal parts of the teeth were prepared under water cooling. Class II cavities were prepared at 6 mm depth, 3 mm mesiodistal width, and 4 mm buccolingual width. The gingival walls of the cavities were located 1 mm above the cement enamel junction. Cavity depths were measured using a periodontal probe.

Restoration placement

In the study, one high-viscosity bulk-fill composite Filtek™ One Bulk-fill Restorative and two flowable bulk-fill composites Estelite Bulk-fill Flow and SDR® Posterior Bulk-fill Flowable Base were used.

A matrix band (SuperMat, KerrHawe, Uxbridge,UK) was placed around the teeth for restorative procedures and was held by finger pressure against the gingival margin of the cavity. Clearfil SE-Bond (Kuraray, Okayama, Japan) was used for all restorations.

The gingival halves of the mesial Cl II cavities were restored with Estelite Bulk-fill Flow (Tokuyama, Tokyo, Japan) (3 mm dept); the gingival halves of distal Cl II cavities were restored with SDR® Posterior Bulk-fill Flowable Base (Dentsply, DeTrey, Konstanz, Germany) composite (3 mm depth); and the remaining occlusal halves of the cavity were restored with Filtek™ One Bulk-fill Restorative (3M, St Paul, MN, US) (3 mm depth). All composite restorations were applied by an operator at a room temperature of 21°C on the same day. A schematic of the restoration procedure is shown in Figure 1. The composites were applied at four different temperatures: 4ºC, room temperature (21ºC), 39°C, and 55°C.

For the groups at 4°C, composites were used as soon as they came out of the refrigerator. For the samples at 39°C and 55°C, composites were applied at the desired temperature in the Micerium Ena Heat (Micerium S.p.A, Avegno, Italy) composite heating device according to the manufacturer's recommendations. The composite resins were polymerized from the occlusal part of the cavities with a high-performance LED light device (Elipar™ S10, 3M, Seefeld, Germany) for 20 s.

The batch numbers and the respective manufacturers of the bulk-fill composite resins used in the study are shown in Table 1.

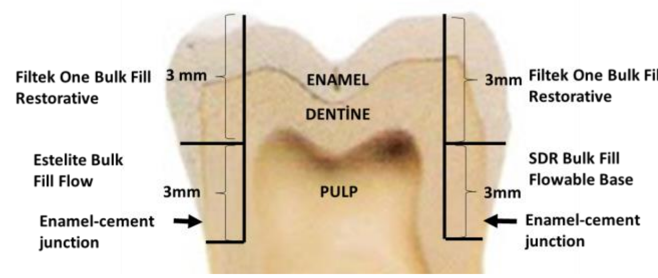

Figure 1. Schematic view of the restoration procedure

Table 1. Composite resins used in the study and their compositions (composition obtained directly from manufacturer information)

Materials/ Brand name	Viscosity type	Filler type	Fillers	Matrix	Filler load (vol%)	Manufacturer/ LOT number
Filtek™ One Bulk-Fill Restorative	High viscosity	Nanofilled	YbF3, Silica, Zirconium, Zirconium/silica	AUDMA, UDMA, DDDMA, AFM	58.5 %	3M, St Paul, MN, US LOT:NA29615
Estelite Bulk-Fill Flow	Low viscosity	Suprananofilled	SiO_2, ZrO_2	Bis-GMA, Bis-MPEPP, TEGDMA	56%	Tokuyama, Tokyo, Japan LOT:025E38
SDR® Posterior Bulk-Fill Flowable Base	Low viscosity	Undeclared	Ba-Al-F borosilicate glass, Sr-Al-F-silicate glass	SDR® patented UDMA, Bis-EMA, TEGDMA	45%	Dentsply, DeTrey, Konstanz, Germany LOT:1812000757

Abbreviations: **AUDMA:** Aromatic urethane dimethacrylate; **UDMA:** Urethane dimethacrylate; **DDDMA:** 1,10-decanediol dimethacrylate; **AFM:** Additional fragmentation monomer; **YbF3:** Ytterbium fluoride; **SiO_2:** Silicon dioxide; **ZrO_2:** Zirconium dioxide; **Ba-Al-F**: Barium-alumino-fluoro; **Sr-Al-F:** Strontium alumino-fluoro; **Bis-GMA**: Bisfenol-A-glycidyl dimethacrylate; **TEGDMA:** Triethylene glycol dimethacrylate; **Bis-MPEPP:** 2,2-bis [4-methacryloxy poly-ethoxyphenyl] propane; **Bis-EMA:** ethoxylated bisphenol-A dimethacrylate.

Thermocycling

The restored teeth were then stored in distilled water at 37°C for 24 h to ensure polymerization. After 24 h, the restored teeth were subjected to a thermocycle (MTE101 Thermocycling Machine, Esetron, Ankara, Türkiye) and alternated between 5ºC and 55ºC water baths with a dwell time of 20 seconds and a rest time of 20 seconds, 1000 times.

Dye penetration

After the thermal cycle application, the root ends of the samples were covered with a flowable composite to prevent the passage of methylene blue from the foramen apical and lateral canals into the pulp cavity. Next, two layers of nail polish were applied to all tooth surfaces 1 mm closer to the restoration edges. The prepared samples were kept in 0.5% methylene blue dye for 24 h. The teeth were then rinsed under running water to remove residual paint and allowed to dry at room temperature. Afterward, the samples were cut in two along the mesiodistal direction with water cooling.

Microleakage evaluation

The dye penetration depth in the sections was examined by a different researcher at a magnification of × 40 (1280 × 960 resolution) on a stereomicroscope (Olympus, Tokyo, Japan) and was quantitatively evaluated using the NIS Element D image analysis software. Occlusal and gingival microleakage amounts were measured in µm under the same magnification on stereomicroscope images. Examples of microleakage belonging to the groups are shown in Figures 2-5.

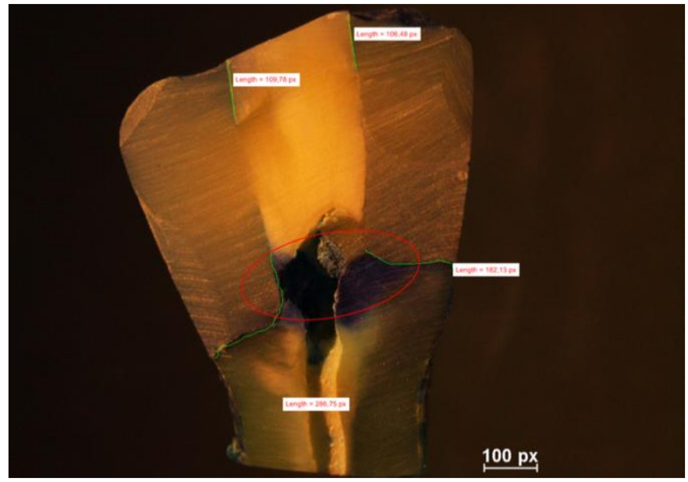

Figure 3. Amount of microleakage at room temperature in the lingual section of the sample.

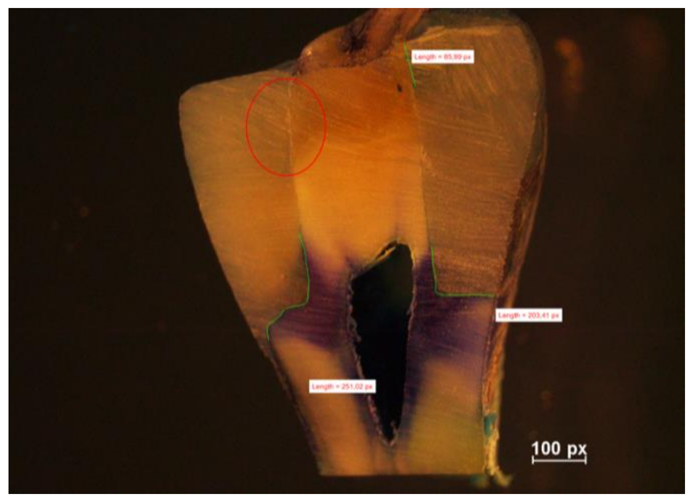

Figure 4. Amount of microleakage at 39ºC in the sample with the lingual cross section (microleakage is not observed in the mesial-occlusal margin restored with Filtek™ One Bulk Fill Restorative)

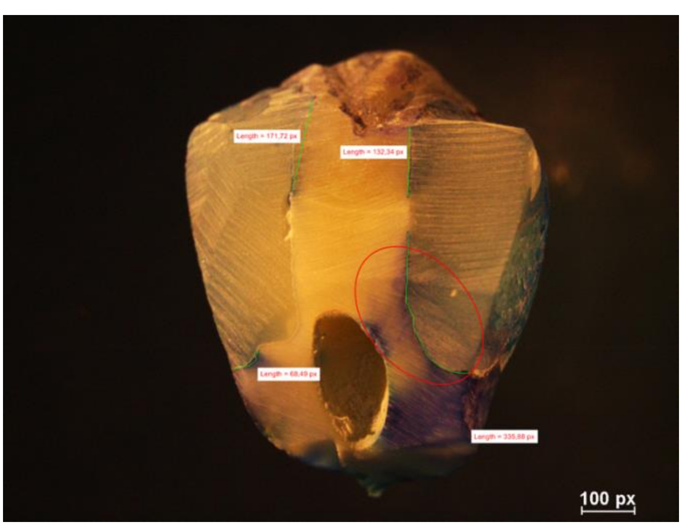

Figure 2. Amount of microleakage at 4ºC in the lingual section of the sample (microleakage involving the axial wall is observed at the distal-gingival edge restored with SDR® Posterior Bulk Fill Flowable Base)

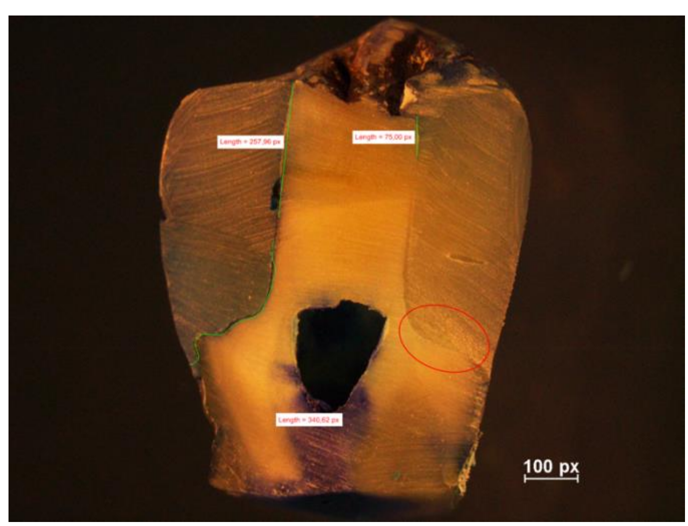

Figure 5. Amount of microleakage at 55 ºC in the lingual section of the sample (microleakage is not observed in the distal-gingival edge restored with SDR® Posterior Bulk Fill Flowable Base)

Statistical analysis

Analyses were performed by using SPSS software (IBM SPSS Statistics version 22.0, IBM Inc., Armonk, NY, USA).

The data normality distribution was tested using the Kolmogrov-Smirnov test, which found a normal distribution. A one-way ANOVA was used to compare the microleakage values of each composite resin at four different temperatures. The post-hoc Tukey test was used to compare the data of the groups in pairs.

An independent-sample t-test was used to compare the microleakage values of composites applied at four different temperatures in the gingiva. The threshold for statistical significance was set at $p<0.05$.

Results

As a result of the comparison of the microleakage values of the Filtek™ One Bulk-fill Restorative at four different temperatures on the occlusal surface, there was a statistically significant difference ($p<0.05$) observed among the four temperatures. To determine which groups caused this difference, the difference between the 4ºC -39ºC (p=0.013) and 4ºC-55ºC (p=0.005) groups was statistically significant in the group comparisons. However, the differences between the 4ºC -20ºC (p=0.208), 20˚C-39˚C (p=0.216), 20ºC-55ºC (p=0.113), and 39ºC -55ºC (p=0.726) groups were not statistically significant. The highest microleakage value was observed at 4ºC (1701.35 µm), while the lowest microleakage value was observed at 55ºC (1385.88 µm). The mean microleakage depths, standard deviation values, and p-values for the groups are shown in Table 2.

The comparison of microleakage values of Estelite Bulk-fill Flow applied in the gingival region at four different temperatures with the one-way ANOVA test revealed no statistically significant ($p>0.05$) differences between the four groups. When comparing the groups in pairs using the post-hoc Tukey test, there was a statistically significant difference between the 4ºC -39ºC (p=0.030) and 4ºC -55ºC (p=0.044) groups.

Among all the groups of Estelite Bulk-fill, the highest microleakage value was observed at 4˚C with an average of 2773.02 µm, with the lowest value at 39˚C and an average of 2227.59 µm. Average microleakage depths, standard deviation values, and P-values for all groups are shown in Table 3.

Table 2. Comparison of microleakage depths (in µm) of Filtek™ Bulk Fill Posterior applied to the occlusal surface at four different temperatures

	ONE-WAY ANOVA					POST-HOC TUKEY					
	GROUPS					4ºC-20ºC	4ºC-39ºC	4ºC-55ºC	20ºC-39ºC	20ºC-55ºC	39ºC-55ºC
	4ºC	20ºC	39ºC	55ºC	P	P	P	P	P	P	P
MEAN	1701.35	1561.58	1424.59	1385.88	0.020	0.208	0.013	0.005	0.216	0.113	0.726
SD	625.14	439.70	475.48	406.50							

SD: Standard deviation, Statistically significant difference p<0.05.

Table 3. Comparison of microleakage depths (in µm) of Estelite Bulk Fill applied in the gingival at four different temperatures

	ONE-WAY ANOVA					POST-HOC TUKEY					
	GROUPS					4ºC -20ºC	4ºC-39ºC	4ºC-55ºC	20ºC-39ºC	20ºC-55ºC	39ºC-55ºC
	4ºC	20ºC	39ºC	55ºC	P	P	P	P	P	P	P
MEAN	2773.02	2462.27	2227.59	2268.89	0.116	0.211	0.030	0.044	0.344	0.435	0.867
SD	908.16	754.50	692.92	744.90							

SD: Standard deviation, Statistically significant difference $p<0.05$.

Upon examination of the results of the comparison between the microleakage values of SDR® Posterior Bulk-fill Flowable Base applied in the gingival at four different temperatures and the one-way ANOVA test, the difference between the four temperatures was found to be statistically 0.005. When the average microleakage depths were examined, the highest microleakage value was observed at 4ºC, with an average of 2792.49 µm, and the lowest value was observed at 2079.23 µm, at 55ºC. The difference was statistically significant at 4ºC -20ºC (p=0.007), 4ºC-39ºC (p=0.008), and 4ºC -55ºC (p=0.000) in the post-hoc Tukey's test, while it was observed that the difference between the 20ºC-39ºC (p=0.980), 20ºC-55ºC (p=0.282), and 39ºC-55ºC (p=0.271) groups were not statistically significant. Average microleakage depths, standard deviation values, and p-values for the groups are shown in Table 4.

In the comparison between the microleakage depths of the Estelite Bulk-fill Flow and the SDR® Posterior Bulk-fill Flowable Base applied in the gingival with the independent t-test, no statistically significant difference was found between the two composites at four different temperatures (p>0.05). The average microleakage depths, standard deviation values, and p-values of the groups are shown in Table 5.

Table 4. Comparison of microleakage depths (in µm) of SDR® Bulk Fill applied in gingival at four different temperatures

ONE-WAY ANOVA						POST-HOC TUKEY					
	GROUPS					4ºC-20ºC	4ºC-39ºC	4ºC-55ºC	20ºC-39ºC	20ºC-55ºC	39ºC-55ºC
	4ºC	20ºC	39ºC	55ºC	P	P	P	P	P	P	P
MEAN	2792.49	2280.25	2284.88	2079.23	0.002	0.007	0.008	0.000	0.980	0.282	0.271
SD	679.77	556.87	620.53	466.67							

SD: Standard deviation, Statistically significant difference p<0.05

Table 5. Independent t-test comparison of Estelite and SDR® microleakage depths (µm) at four different temperatures

	4ºC		20ºC		39ºC		55ºC	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Estelite	2773.02	908.16	2462.27	754.50	2227.59	692.92	2268.89	744.90
SDR®	2792.49	679.77	2280.25	556.87	2284.88	620.53	2079.23	466.67
p	0.939		0.391		0.784		0.342	

Discussion

Microleakage tests are a method of evaluating the sealing efficiency of restorative materials (24). This study compares the microleakage of different types and temperatures of bulk-fill composites. The results of our study led us to reject our null hypothesis that the prepolymerization temperature of bulk-fill composite resins has no effect on microleakage.

Preheating is performed by placing composite resin tubes or syringes in a heating device (20). The use of this method resulted in simpler placement and greater adaptation to the cavity walls compared to resins used at room temperature. The preheated composite resin provides higher monomer conversion even when less energy is used and reduces irradiation time by up to 75% (25). By heating the material before polymerization, it is possible to increase its fluidity without sacrificing the amount of inorganic filler and to increase its marginal adaptation and physical and mechanical properties, resulting in a reduction in microleakage (21).

There are many studies in the literature on microleakage in bulk-fill composite restorations (26–32). However, studies investigating the effect of preheating on the microleakage of bulk-fill composite resins are limited in number. Moustafa et al. reported that, when preheated to 60ºC, the bulk-fill composite Filtek™ Bulk-fill showed less microleakage than the same non-preheated composite resin and conventional composite (Filtek™ Z350) when heated to 60ºC (25).

The study by Moustafa et al. yielded results similar to ours. The lowest microleakage values of Filtek™ Bulk Fill Restorative applied to the occlusal surface and of SDR® Bulk Fill Flowable Base applied to the gingival surface were observed at 55ºC, whereas the lowest microleakage value of Estelite Bulk Fill Flow applied to the gingival surface was observed at 39ºC. The highest microleakage value of all three composites was observed in samples used immediately after they were removed from the refrigerator (4ºC).

In their studies, Daronch et al. (33) observed that increasing the polymerization temperature increases the conversion of dimethacrylate monomers up to a certain temperature limit, but the conversion of monomers decreases with a further temperature increase after reaching this limit. The authors suggest that the reduction in monomer conversion is due to reactant evaporation and photoinitiator breakdown (33). This may explain why the lowest microleakage value of the Estelite Bulk Fill Flow composite applied to the gingival surface in our study was observed at 39ºC. The manufacturer provides no data regarding a temperature limit of the Estelite Bulk Fill Flow composite we used. Therefore, more studies are needed to determine the accuracy of this result.

Yang et al. investigated the effect of preheating on microleakage in microhybrid composites (Heraeus Charisma Smile) and report that the composite resin preheated at 50ºC showed the least microleakage (19). Choudhary et al. who implemented restoration procedures similar to those in our study in class II cavities, examined the microleakage values of the nanofile universal hybrid composite Filtek™ Z350 (3M) and the condensable Filtek™ P60 (3M) at room temperature, at 37ºC, and at 54ºC. Up to 54ºC, significantly lower microleakage scores were obtained in both composite groups (13). These results are similar to the lowest microleakage value of SDR® Posterior Bulk Fill Flowable base applied at the gingival surface in our study, which was observed at 55ºC.

A study conducted by Walter found that the heated resin lost approximately 50% of the increased temperature within 2 min after being removed from the device, and the loss was approximately 90% within 5 min. The rate of heat loss can be affected by placing the resin in the cavity preparation, which can act as a heat sink (34). In our study, the composites were placed in the cavity as quickly as possible after their removal from the heating device, but heat loss was inevitable. In Estelite Bulk Fill Flow, as in other composites, the lowest microleakage value is expected to be observed at 55ºC; one of the reasons for monitoring at 39ºC may be the heat loss. In the results of our study, the difference between the 39ºC and 55ºC groups was not found to be statistically significant for Estelite Bulk Fill Flow ($p>0.05$).

Given that the composite cools at a very rapid rate after removal from the warming unit, it seems advantageous to place the preheated composite quickly into the cavity to improve its performance and to place the capsule directly into the dispensing syringe during preheating rather than preheating only the individual capsules themselves (23).

It is recommended that the preheated composites be placed as soon as the preheating process begins, without delay. In this way, the positive effects of preheating in reducing microleakage could be monitored without the negative effects of delayed elastic deformation (15). Wagner et al. In their research, they cured preheated composite resin with a 15-second delay; as expected, preheating resulted in the lowest microleakage. Delayed curing has been proposed to reduce the amount of thermal shrinkage that can occur when the heated composite is initially cured at a high temperature.

The highest microleakage value was not expected in the group cured with a delay of 15 seconds, but this group had it. This can be explained by the fact that the 15-second delay allows the composite to move away from the cavity walls due to its viscoelastic behavior (15).

Studies have reported that preheating does not reduce microleakage in composite restorations (35, 36). This may be due to the sudden drop in the placement of the composite resin in the cavity (35). In our study, the composite resin heated in the Micerium Ena Heat device was placed in the cavity without waiting.

The literature contains various results regarding composite restorations and microleakage. The variation in these results might be due to the many factors affecting microleakage in laboratory studies.

Restorative material amount, cavity geometry, C factor, light source position and intensity, thermal aging application, and the mineral structure of the teeth are factors affecting microleakage. To eliminate the deviations caused by these factors in our study, a light source was applied to cavities prepared using the same depth and width in such a way that it touched the matrix at the same distance in all samples. The same adhesive system was also used in all samples, and the amount of restorative material was consistent. Thermal cycles were applied at 5-55ºC at 1,000 cycles in all groups.

The mineral structure of the teeth is another factor that affects the results of microleakage studies in composite restorations. The extracted teeth this study used contained no caries, discoloration, or fractures. However, micro defects that form in the cervical region during shooting, which are not visually noticeable, may cause increased microleakage values at the gingival edge.

Scoring methods are generally used to detect edge leakage in microleakage studies using dye penetration (37). However, thanks to recent developments in image analysis software, quantitative microleakage measurement methods have become popular. In this study, images obtained at 40× magnification under the stereomicroscope were evaluated quantitatively using NIS Element D image analysis software instead of the traditional scoring method.

Although the current difference is not statistically significant in terms of microleakage between the fluid bulk-fill composites applied in the gingival, it might be attributable to the differences in monomers, elasticity modulus, and filler particle ratios.

Conclusion

Based on our study's results, not using bulk-fill composites immediately after removing them from the refrigerator, but preheating the bulk-fill composites while placing them in the cavity may be a useful method to reduce microleakage. However, more studies are needed to verify these results' accuracy. Our study was conducted under in vitro conditions, and in vivo studies are needed to determine the advantages and disadvantages of preheating in clinical conditions.

Disclosures

Ethical Approval: Ethics committee approval was received for a part of this study from the Ordu University Ethics Committee, in accordance the World Medical Association Declaration of Helsinki, with the approval number: 2019-76.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception – S.A.K.; Design – S.A.K., S.A.; Supervision – S.A.; Materials – S.A.K., S.A.; Data Collection and/or Processing – S.A.K.; Analysis and/or Interpretation – S.A.K., S.A.; Literature Review – S.A.K.; Writer -S.A.K.; Critical Review – S.A.K., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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