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Effect of saliva contamination on microleakage of alkasite restorative material

Huseyin Hatirli[a,*], Sura Boyraz[b]

[a] *Gaziosmanpasa University, Faculty of Dentistry, Department of Restorative Dentistry, Tokat, Turkey*
[b] *Yozgat Bozok University, Faculty of Dentistry, Department of Restorative Dentistry, Yozgat, Turkey*

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Abstract

Aim: The aim of this study was to evaluate the effect of saliva contamination on the microleakage of resin composite, high-viscosity glass ionomer cement, and an alkasite restorative material.
Materials and Methods: Eighty class II cavities were prepared on the mesial and distal surfaces of 40 extracted human molar teeth. Teeth were divided into four restorative material groups [resin composite (Solare X, GC), glass ionomer cement (Fuji IX GP, GC), an alkasite restorative material with and without adhesive (Cention N, Ivoclar Vivadent)] and mesial cavities were contaminated with human saliva (n=10). Restorative materials were applied and after the setting time of materials, restorations were finished. Specimens were thermo-aged, and were subsequently immersed in 0.5% methylene blue dye solution. Teeth were sectioned through the center of the restorations in mesio-distal direction. The extent of dye penetration was assessed using a stereomicroscope. The data was analyzed using the Kruskal-Wallis and Wilcoxon nonparametric tests ($p < 0.05$)
Results: Among the non-contaminated and saliva contaminated groups Fuji IXGP (FGP) showed the highest microleakage scores ($p < 0.05$). Cention N with adhesive showed the least microleakage, the difference between the alkasite groups and Solare X was not significant ($p > 0.05$). Both of the Cention N groups showed a lower microleakage when contaminated by saliva than FGP ($p < 0.05$). Saliva contamination significantly increased the microleakage scores of all groups except FGP ($p < 0.05$).
Conclusions: An alkasite restorative material applied with or without adhesive revealed lower microleakage than high-viscosity glass ionomer cement in both non-contaminated and saliva contaminated groups.

Introduction

Contamination of cavity surfaces by saliva, blood, and other contaminants is the most insidious factor that negatively affects the adhesion, bond durability, and longevity of the restoration. Rubber dam use is considered the most effective method to control the contamination of adherent surfaces [1]. However the proper placement of rubber dam can be particularly difficult on a tooth with equigingival or subgingival cavity margins and the improper placement of rubber dam can be ineffective to avoid contamination. In addition, most dentists disregard the use of rubber dam for many restorative procedures [2].

Recently, resin composites are the first choice of restorative materials used for the posterior restorations. Despite developments in recent years, adhesive bonding continues to be a technique sensitive process. The literature knowledge about the consequences of saliva contamination during restorative procedures on the quality of adhesion is controversial. This could be because of the reduction of adhesion is related to the type of adhesive system and to the stage of the adhesive application when contamination occur. Some studies demonstrated that bond strength and microleakage of the restorations are not affected by saliva contamination [3-5]. In contrast, there are several studies in the literature confirming the detrimental consequences of saliva contamination on adhesive quality [6-8] and decontamination by rinsing is recommended [9]. To overcome the polymerization shrinkage, adequate polymerization, and technique sensitivity problems of resin composites on sub-gingival class II restorations, use of the sandwich technique together with glass ionomer cement could be beneficial [10]. On the other hand, during the setting reaction of conventional glass ionomer cements saliva contamination may give rise to an incomplete chemical reaction that leads to the softening and cracking of the cement

*Corresponding author:
Email address: huseyinhatirli@gmail.com (Huseyin Hatirli)

[11].

One of the main components of composite resins are filler particles which composite resins has been classified based on the particle size and quantity. Nano-fill composite resins are produced to provide enhanced mechanical properties, as well as aesthetic features by using recent composite technology [12]. However, incremental placement of composite resins may cause contamination between increments and extends clinical time. With the purpose of simplifying the restorative procedure and shorten the chair time, a recent self-adhesive and tooth colored alkasite restorative material [Cention N, Ivoclar Vivadent, Schaan, Liechtenstein), was introduced and indicated for permanent Class I and Class II restorations [13]. Cention N (CNR) capable of releasing calcium, fluoride and hydroxide ions and consists of powder and liquid that are hand mixed before the application. According to the manufacturer, the hydrophilic liquid monomer, PEG-400 DMA, enhances flowing ability and promotes the ability to wet tooth substrate [14]. In addition this restorative material has self-cure or light-cure polymerization options and can be used with or without an adhesive system.

A few previous studies have evaluated the microleakage of alkasite restorative material; however results of these studies are somewhat conflictive [15-17]. Shailendra et al. [18] compared the apical sealing capability of CNR with mineral trioxide aggregate (MTA) and they concluded that CNR can be preferred as an alternative restorative material to MTA as a retrograde filling material. For this reason, performance of CNR on contaminated cavities can be issue of concern. However, no study has evaluated the effect of salivary contamination on restorations performed with alkasite restorative material.

Therefore, this study aimed to evaluate the effect of saliva contamination on the microleakage of an alkasite restorative material, a high viscosity glass ionomer and a nano filled composite resin. The null hypothesis tested was that restorative material type and saliva contamination have no effect on the extent of microleakage.

Materials and Methods

Specimen Preparation

The study protocol was approved by the Ethics Committee of the Tokat Gaziosmanpasa University (21-KAEK-098). Forty sound mandibular human third molar teeth, extracted for periodontal reasons, were collected, and disinfected in 0.5 % chloramine T solution for 48 hours, and then stored in distilled water for up to 6 months after extraction.

The sample size was calculated considering 80% power and a significance level of 0.05 using data obtained from a previous study (effect size=0.45) conducted by Moteveasselian et al. [19].

The teeth were placed in plastic molds and embedded in auto polymerizing acrylic 3 mm below the cement-enamel junction. A single operator prepared class II slot cavities on mesial and distal parts of the teeth (Figure 1).

The diamond burs (No: 856, Meisinger Dental Burs, Hager & Meisinger GmbH, Neuss, Germany) that were used for cavity preparation at high-speed with water coolant, replaced after every ten preparations. The buccolingual cavity width was 1/3 of the intercuspal distance, the axiopulpal dimension of the cavities was 2 mm, and the gingival margins of the cavities were 1 mm below the cementoenamel junction. All the internal line angles were rounded and cavosurface angles were about 90°. All the cavity dimensions were confirmed with a periodontal probe.

According to the restorative materials used in the study, prepared teeth were randomly divided into four groups (Table 1).

Mesial and distal cavities were further subdivided into saliva contaminated and non-contaminated subgroups respectively (n=10). Mesial cavities were contaminated with one drop (0.025 ml) unstimulated human saliva for 20 s and dried for 5 s whereas distal cavities were not contaminated with saliva. The saliva used was collected from a healthy volunteer at least one hour after the any food or drink consumption. A contoured circumferential matrix system (Adapt SuperCap, Kerr Co, Orange, CA, USA) was placed and gingival part of the matrix was tightened with a dental floss ligature that was placed below the gingival margins. Restorative materials were placed according to the manufacturers' instructions as stated below;

Group A: Nano filled composite resin (Solare-X; SXC)

According to self-etch procedure, Single Bond Universal (3M ESPE, St Paul, MN, USA) adhesive was applied to the whole cavity surface with a micro brush and rubbed for 20 seconds. Then a gentle air stream was applied for 5 s and polymerized for 10 s with a Valo LED unit at the standard power of $1000mW/cm^2$ (Ultradent Products Inc., South Jordan, UT, USA). The nano-filled composite resin (Solare-X, GC, Tokyo, Japan), shade A2, was applied with incremental technique and light cured for 20 seconds.

Group B: High-viscosity glass ionomer cement (Fuji IX GP; FGP)

A cavity conditioner (GC, Tokyo, Japan) was applied to the entire cavity for 10 seconds, the cavity was rinsed and gently air dried. FGP capsules were mixed in a capsule mixing device (Ultramat 2, SDI Limited, Bayswater, VIC, Australia) for 10 seconds and slowly injected into the cavity as a single increment. Glass ionomer cement contoured with hand instruments and left untouched for 120 s for the completion of the initial setting.

Group C: Alkasite Restorative Material (Cention N) with adhesive (CNR with UA)

A universal adhesive (Single Bond Universal) was applied as stated previously. Two measuring spoons of powder and two drops of liquid were mixed manually on a mixing pad for 60 s. CNR inserted into the cavity with a spatula. The material was left untouched for 5 min and light curing was not executed.

Group D: Alkasite Restorative Material (Cention N; CNR) without adhesive

The powder and liquid of CNR was mixed as stated previously and inserted into the cavity with spatula. CNR was left untouched for 5 min and light curing was not executed.

After the recommended setting time for the materials was reached, the matrix bands were removed and the restorations were finished and polished with fine diamond burs

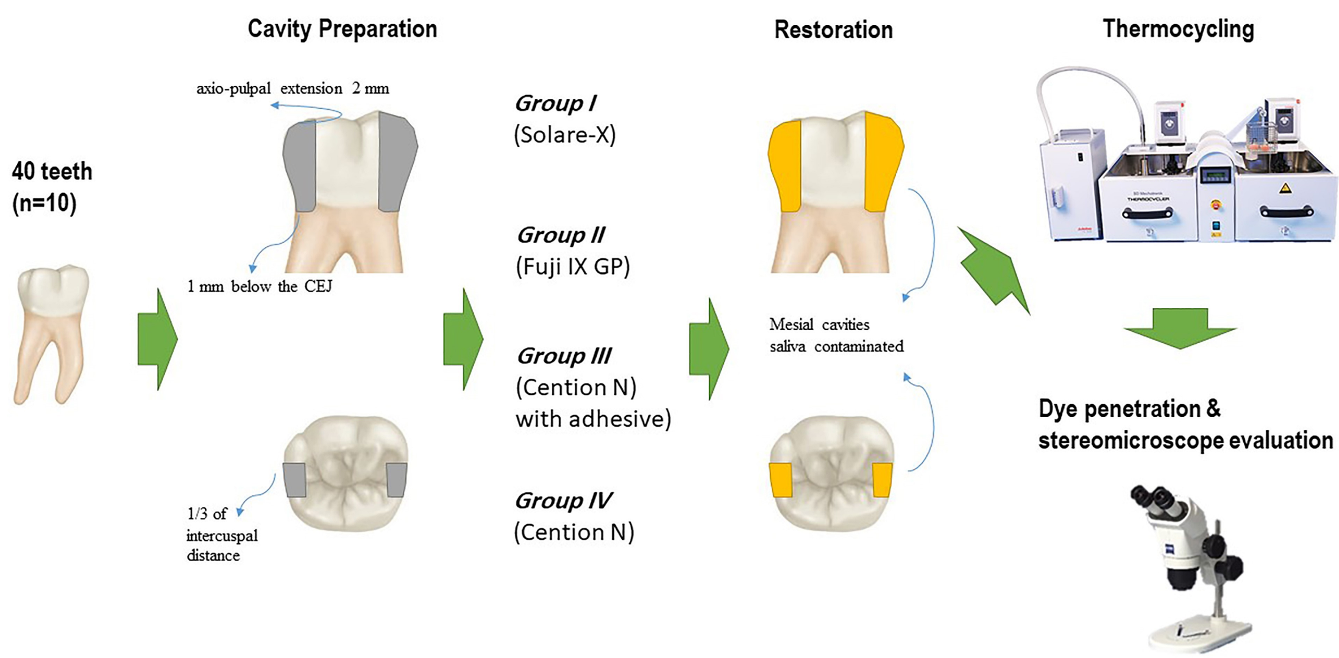

Figure 1. Summary diagram of experimental design

Table 1. Compositions and manufacturer details of the tested restorative materials

Material (Abbreviation)	Material Category	Composition	Manufacturer
Solare X (SXC)	Resin Composite	UDMA, silica nanoparticles, prepolymerized fillers containing silica nanoparticles, fluoroaluminosilicate glass fillers, filler content (wt/vl): 77% / 65%.	C Corporation Tokyo, Japan
Fuji IX GP (FGP)	High-Viscous Glass Ionomer Cement	Powder: %95 aluminum-fluoro-silicate glass, 5% polyacrylic acid powder Liquid: 50% distilled water, 40% polyacrylic acid, and 10% polybasic carboxylic acid	GC Corporation, Tokyo, Japan
Cention N (CNR)	Alkasite, Restorative Material	Powder: Barium aluminium silicate glass, ytterbium trifluoride, isofiller, calcium barium aluminium fluorosilicate glass, Calcium fluoro silicate glass. Liquid: UDMA, DCP, Tetramethylxylylen diurethane, dimethacrylate, PEG-400 DMA	Ivoclar Vivadent AG, Liechtenstein

Abbreviations: UDMA: Urethane dimethacrylate; DCP: Tricyclodecan-dimethanol dimethacrtylate; PEG-400 DMA: polyethylene glycol 400 dimethacrylate;

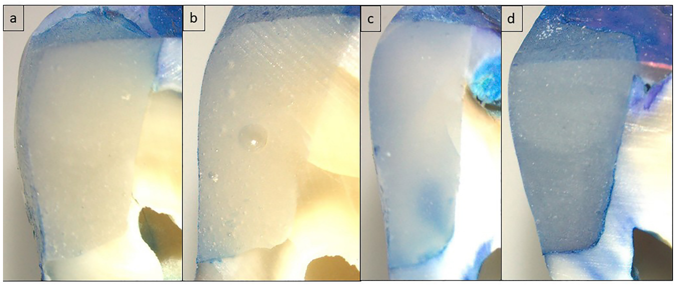

Figure 2. Representative images of microleakage. a Score 0; b Score 1; c score 2, and d Score 3

(852FG; Hager&Meisinger GmbH, Neuss Germany) and rubber polishing cups (HilusterPLUS polishing systems, KerrHawe, Bioggio, Switzerland). The specimens were waited in distilled water for 24 h at 37 ^oC and subsequently thermo-cycled between 5 ^oC and 55 ^oC with a dwell time of 30 s for 5000 cycles in distilled water (SD Mechatronic thermocycler THE-1100, SD Mechatronics, Westerham, Germany).

Dye Penetration Test

The entire surfaces of the teeth were covered with nail varnish up to the 0.5 mm of the restoration margins. Specimens were immersed in 0.5% methylene blue solution at 37 ^oC for 24 h. The teeth were rinsed thoroughly with distilled water. All the specimens were sectioned in half through the center of the restorations in mesio-distal direction with a diamond saw (Microcut 201, Metkon, Bursa, Turkey) under a water coolant. The extent of dye penetration at the gingival wall was assessed by two evaluators, who were blinded to the methods, at 20X magnification by using a stereomicroscope (Stemi2000, Axiovison4.8; Carl Zeiss, Jena, Germany). The section with the greatest microleakage was scored using the scale as follows (Figure 2) [20];

Score 0 no dye penetration
Score 1 dye penetration to half of the gingival wall
Score 2 dye penetration along the gingival wall
Score 3 dye penetration along the gingival and axial walls.

In case of disagreement between the evaluators, the final score resulted as a common decision of both evaluators.

Statistical Analysis

The statistical analyses of gingival microleakage data were performed using SPSS version 19 (SPSS Inc, Chicago, IL, USA). The dye penetration data of contamination conditions were compared using the Mann Whitney U test ($p < 0.05$). The dye-penetration scores for the each of the four restorative material (SXC, FGP, CNR with UA, CNR) were compared using the Kruskal-Wallis test with Bonferroni correction ($p < 0.008$).

Results

The distribution of the microleakage scores of the restorative material groups was shown in Figure 3.

Among the non-contaminated groups, the FGP showed the significantly highest microleakage scores ($p < 0.05$)

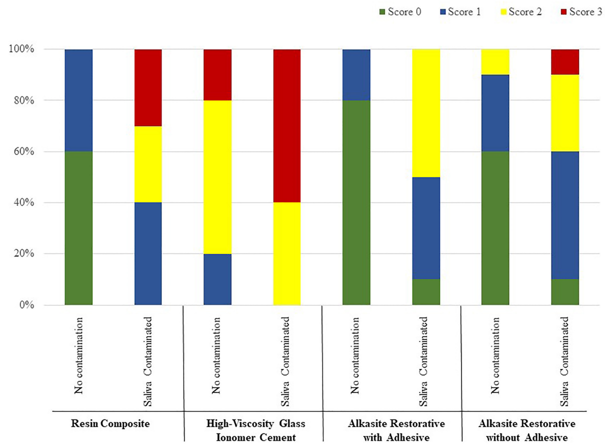

Figure 3. The distribution of microleakage scores for each group

(Table 2). The CNR with UA group showed the least microleakage however, no significant differences were observed between the alkasite groups and SXC ($p > 0.05$). Among saliva contaminated groups FGP showed the highest microleakage. No significant difference was observed between the SXC and FGP groups ($p < 0.05$). Both CNR groups showed significantly lower microleakage than FGP ($p < 0.05$). In addition, both CNR groups showed similar microleakage values to SXC ($p < 0.05$).

Saliva contamination increased the microleakage scores of all groups. The difference between groups that were non-contaminated and saliva contaminated groups was significant for the SXC and both of the CNR groups ($p < 0.05$). On the other hand, similar microleakage values were observed for non-contaminated and saliva contaminated FGP groups ($p < 0.05$).

Discussion

This study evaluated the microleakage of subgingival restorations performed with a nano-filled composite resin, a high-viscosity glass ionomer, and alkasite restorative material (with and without adhesive) under saliva contamination. The main finding of this study was that both alkasite restorative material groups applied with or without adhesive showed similar microleakage scores with nano-filled composite resin and a lower microleakage scores than high-viscosity glass ionomer in saliva contaminated and non-contaminated groups. The result of this study demonstrates that saliva contamination increased the microleakage of composite resin and alkasite restorative material groups, however high-viscosity glass ionomer was not significantly affected. Therefore the null hypothesis was partially rejected.

Obtaining a good marginal seal, adequate bond strength, and producing a durable interface between the tooth surface and restoration are critical factors for the success of restorations [21]. The microleakage test is a method used to evaluate the marginal and internal adaptation of restorative materials. The dye penetration method, which is one of the microleakage test methods, is still one of the

Table 2. Median, Minimum (Min), and Maximum (Max) Microleakage scores for tested materials under contamination conditions

Material	No contamination Number/percentage			Saliva contaminated Number/percentage			p-value^a
	Median	Min-Max		Median	Min-Max		
A Solare-X	0	0-1	a	22	1-3	ab	0.004*
B Fuji IX GP	2	1-3	b	33	2-3	b	0.063
C Cention N with adhesive	0	0-1	a	2	0-2	a	0.006*
D Cention N without adhesive	0	0-2	a	11	0-3	a	0.007*
p-value^b			p < 0.001			p = 0.005	

* p-value^a p value indicates differences between contamination conditions (Mann Whitney U test)

** p-value ^{b} Different letters indicate significant difference in column (Kruskal Wallis test with Bonferroni correction) (p<0.0085).

most preferred methods because it is easy and inexpensive[11, 19, 20, 22].

When the gingival margins of the cavities are below the CEJ, restoration of class II cavities is especially challenging in terms of isolation, marginal sealing, and the polymerization of composite resin because of the distance to the light polymerization unit [20, 23]. However, the results of this study revealed that resin composite and alkasite restorative material showed lower microleakage than high-viscosity glass ionomer in both non-contaminated and saliva contaminated groups. Similarly, previous studies revealed that the highest microleakage was observed in high-viscosity glass ionomer groups [10, 19, 24]. The high viscosity characteristic of FGP, due to the reduced particle size and increased powder-liquid ratio, might be responsible not wetting the cavity surface properly and then fails to form an adequate seal.

In this study, higher microleakage was observed in the saliva-contaminated and universal adhesive-applied groups (SXC and CNR with UA) compared to the groups without saliva contamination (SXC and CNR with UA), and the difference was statistically significant. Contamination of the cavity surfaces with moisture can block required contact of the adhesive and adherent and salivary proteins fill the micro-gaps on tooth surfaces [25]. Absorption of salivary constituents results in the decrease of the surface energy and make the surface unfavorable for adhesion. In addition, water content may give rise to the incomplete polymerization of the adhesive monomers [9, 26]. Universal adhesives contain both hydrophilic and hydrophobic monomers in their composition and previous studies indicated reduced bond qualities when contaminated with saliva [3, 6].

CNR with UA demonstrated the lowest microleakage in this study and both CNR groups presented lower microleakage than FGP. Presence of a special filler (Isofiller) in the material which acts as a stress reliever and minimizes shrinkage force might provide marginal sealing [17]. Similarly, previous studies have indicated a lower microleakage and superior marginal adaptation for alkasite restorative material when compared to glass ionomer cements and resin composites [16, 17, 27-29]. In addition, Meshram et al. [15] stated that marginal adaptation of CNR is enhanced by adhesive application and the findings of the present study is in accordance with their results.

Saliva contamination significantly increased the microleakage in CNR groups. However, the microleakage degrees of the CNR groups were lower than both SXC and FGP. This result could be associated with the presence of a PEG-400 DMA liquid monomer which has a hydrophilic character in the content of CNR. To the best of our knowledge, previous studies only focused on the microleakage of alkasite restorative material and this is the first study that evaluates the effect of saliva contamination.

A limitation of this study is that the environmental conditions of the mouth could not be precisely simulated by only using thermal aging. Consequently, the effects of the mechanical loading on the marginal adaptation and microleakage of restorative materials should be investigated in further clinical studies.

Conclusion

The following conclusions can be drawn from the present study;

- Alkasite restorative materials applied with or without adhesive revealed a lower microleakage than high-viscosity glass ionomer cement in both saliva contaminated and non-contaminated groups.
- High-viscosity glass ionomer showed the highest microleakage in both saliva contaminated and non-contaminated groups and salivary contamination increases the microleakage of alkasite restorative material with or without universal adhesive and composite resin restorations.

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