MICRO COMPUTED TOMOGRAPHIC EVALUATION OF COMPOSITE RESIN MICROLEAKAGE AND SHRINKAGE BY USING DIFFERENT BONDING AGENTS

Microleakage and shrinkage evaluation of different bonding agents

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Introduction: Microleakage is a major problem in adhesive dentistry. Introduction of contemporary bonding agents with different techniques and contents is essential and comparative assessment of them is the key to find the best for eliminating microleakage.

Purpose: Microleakage is the major challenge in resin-based restorations. The aim of the present study was to determine the amount of microleakage occurs for different adhesive systems by using micro computed tomography (Micro CT).

Materials and Methods: Thirty extracted human premolars were used by preparing standardized class V cavities. Thirty premolars were divided into 3 groups of each comprising 10 specimens as follows: A; One up bond F plus, Tokuyama dentals (6th generation), group B; Palfique bond, Tokuyama dentals (7th generation), and group C; Palfique universal bond, Tokuyama dentals (8th generation) adhesive systems were utilized. Palfique LX5, Tokuyama dentals composite resins were used for the restoration of cavities. Micro computed tomography scanning was conducted for each specimen before and after thermocycling (1000 cycles). Kruskal-Wallis H and Mann Whitney U tests were used to compare the amount of the silver nitrate penetration along with total volumetric changes occurred among the groups. The level of significance was set at ($P<0.05$).

Results: Group C showed the least amount of microleakage followed by group B while group A adhesive system showed maximum amount of microleakage. Group A presented a statistically significant higher microleakage value than Group B and group C. In terms of volumetric chances, no significant difference was found between the groups (A, B, C) ($p<0.05$).

Conclusion: Newer adhesive systems were found to have better success achievements and were recommended for routine use in clinical practice.

Keywords: Micro computed tomography, bonding agents, adhesion, class V, microleakage, shrinkage.

INTRODUCTION

Currently composite resins are the main and most frequently used materials in dental restorations. As a result of several innovations and improvements, such as; the use of different novel particles with low-shrinkage monomers, impressive rise occurred in the successful use of bonded composite restorations.[1] Formerly, retention, resistance, and stability of the restorations required excessive removal of sound tooth structure in order to create undercuts in an attempt to aid in retention. Nowadays, this issue is mainly solved by the introduction of newer generation dentin-bonding agents.[2] Dental adhesive generations underwent several changes through years in their chemistry, mechanism, strength, number of steps, application techniques and clinical effectiveness. When self-etching systems were compared with etch-and-rinse systems, they offered several advantages, such as being less technique sensitive, causing less post-operative pain, and leaving hydroxyapatite crystals available for chemical bonding of functional monomers to calcium that contributes to interface stability.[3-6] With the introducing of self-etch systems, acid etching step was eliminated by mixing acid and prime. Marketing of 6th generation bonding agents had the strategy of superficially demineralize dentin and simultaneously penetrate it with monomers while remaining strong enough (23MPa) under stresses. [(2,5)] Introduction of one-step self-etching adhesive system as 7th generation bonding agent which combined acid etcher, primer, and adhesive in one bottle made the practical work less technique sensitive and reduced the procedural time needed.[4,5] Currently the newest system is light- and self-cured bonding agent that came into market as the 8th generation dentin bonding agent. Light cure step is eliminated, and this brings time saving and prevents evaporation of solvents. [5,6]

Several techniques were used in order to evaluate microleakage; which occurs as a result of external invasion through the restoration margins by penetration or movement of bacteria, fluids, molecules and ions into the tooth-resin interface. Dye penetration technique was the most frequently preferred technique in the assessment of microleakage, however; main drawback of this technique was that it only provided qualitative and subjective assessment. Therefore, researchers focused on the development new methods in order to accurately quantify interfacial leakage.[7-11] Micro-computed tomography (Micro-CT) is an innovative technique that was developed for the *in vitro* imaging and assessment of relatively small samples with ultra-high accuracy and ultra-high radiation doses incompatible with human. Recently, Micro-CT was utilized in the evaluation of microleakage and gap formation in resin-based restorations. Micro CT reconstructs 3D images of entire dental restorations and dental tissues. In this method the specimens need to be immersed in a solution like silver nitrate which has radiopacity higher than dental tissues so that micro-CT can differentiate between the dental tissue, resin composite material and produced gap and leakage between the resin-dentine interface. [12-14]

In consideration to increasing number of newly introduced dentin bonding agents and possible differences between microleakage levels of different materials, the aim of the present study was to compare microleakage levels of 6th, 7th, and 8th generation dentin bonding agents and volumetric changes in resin bulk by using micro-computed tomography. A null hypothesis chosen that there are differences in microleakge and polymerization shrinkage amount between the tested materials.

MATERIALS AND METHODS

Sample Selection

Thirty (n=30) intact human premolar teeth extracted for orthodontic purposes were collected from our data after obtaining local ethical approval. The teeth were debrided of blood, saliva, then scaled, polished, and examined under a magnification of 3× using the surgical loupes and stereomicroscope to eliminate any cracks. Thereafter, specimens were kept in distilled water at 4°C up to 3 months in order to keep them hydrated. Besides, each week samples were washed and distilled water was renewed in order to avoid bacterial growth and minimize deterioration according to International Standardization Organization (ISO), TS 11405 for testing of adhesion to tooth structure.[15]

Cavity Preparation

Class V cavities were prepared 1 mm above the cement-enamel junction (CEJ). Standardized box shaped class V cavities with a uniform mesio-distal extension of 3 mm, occluso-cervical length of 2 mm and depth of 2 mm were prepared with a round tungsten diamond bur. The walls were aligned by fissure diamond bur and finishing was done by fine round and fissure diamond bur with a high speed handpiece with air/water spray. All the dimensions were evaluated using a digital sliding caliper and a periodontal probe. 30 teeth were divided into 3 groups of each one comprising 10 specimens as follows: A; One up bond F plus, 6th generation self-etching bonding agent (Tokuyama Dental Corporation, Tokyo, Japan), B; Palfique bond, self-etching single step 7th generation bonding agent (Tokuyama Dental Corporation, Tokyo, Japan), and C; Palfique universal bond, self-cured 8th generation single step universal adhesive of Tokuyama Dentals (Tokuyama Dental Corporation, Tokyo, Japan), figure 1 shows the sample grouping.

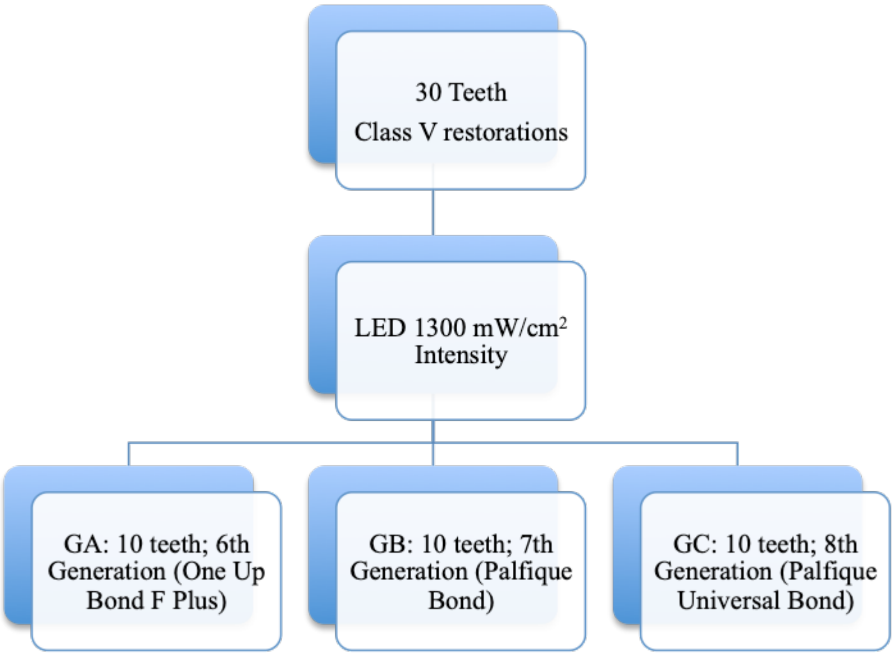

Figure 1: A schematic diagram showing sample grouping.

Sample Preparation

After the application of bonding agents to all three groups according to the manufacturer's instructions, the cavities were restored by Palfique LX5 resin-based dental restorative material of "Tokuyama Dentals" with A2 shade according to the manufacturer's instructions (Tokuyama Dental Corporation, Tokyo, Japan). In all three groups Zenolite LED curing light unit (President Dental, Allershausen, Germany) was used with 1300mW/cm^2 (Table 1).

Table 1: Schematic representation of the materials used in the study.

Groups	**Generations of adhesives**	**Materials used**
A. 10 specimen	6th Generation	One-Up Bond F Plus, Tokuyama Dentals
B. 10 specimen	7th Generation	Palfique Bond, Tokuyama Dentals
C. 10 specimen	8th Generation	Palfique Universal Bond, Tokuyama Dentals

Micro-CT Analysis → First scanning

A PET/ Micro-CT (Super Aargus: Sedecal USA Inc, Madrid, Spain) device was used for the Micro-CT imaging. The images were obtained at 68 × 68 mm FOV, 40 kVp and 140 mA with 0.06 voxel size. The duration of the irradiation was 10 min 30 s. The scanning was conducted in high resolution with 2 shots of 360 projections having 1040 radial pixel projection size (x) and 1144 axial pixel projection size (z), with 3 bed positions. **Figure 2** shows samples placed in the Micro-CT machine for 1st scanning. Initial volume of composite restorations and the pattern of infiltration was digitally assessed after 3-D reconstruction in Amide software (version 1.0.4) in coronal, sagittal, and axial planes. Amide is a Medical Imaging Data Examiner (AMIDE) and it is a completely free tool for viewing, analyzing, and registering volumetric medical imaging data sets. Data viewer and its functions, such as brightness change, color mode, and magnification, were used for image analysis which allowed observing of all micro scans. Three-dimensional 3D images were obtained by juxtaposition of 2D images of adjacent slices. Therefore, after standardizing the system for each scan, first scanning was done in which each sample was molded with their corresponding number in each group and each tooth number was written from 1 to 10, since the ordered scanning was mandatory to make the second scanning in the same order.

Figure 2: Sedecal/ Super Argus micro-tomography machine.

Thermo-cycling Procedure

All specimens were thermo-cycled (SD Mechatronik, Feldkirchen-Westerham, Germany) in distilled water for 1000 cycles (5–55°C) with dwell time of 30 s and draining time of 10 s between cycles.[1] After thermocycling procedure, all the specimens were varnished with nail polish, leaving 1mm around the restoration area in order Silver Nitrate ($AgNo^3$) solution could penetrate the resin-tooth interface. The specimens were ordered in number by coloring each sample with a different nail varnish color from 1 to 10. Then samples of each group were immersed into 50% Silver Nitrate ($AgNO^3$) solution for 12 hours (Figure 3, 4). Finally, specimens were washed and rinsed before second scanning.[11]

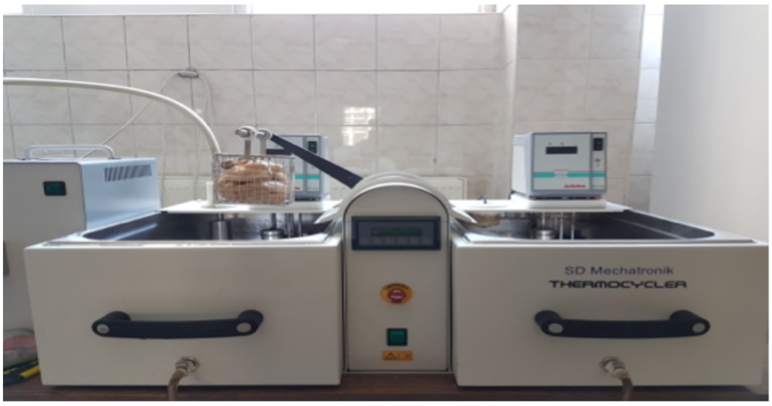

Figure 3: SD Mechatronik Thermocycler machine used in the study.

Figure 4: Samples of each group varnished with nail polish leaving one mm around the restoration.

Micro-CT Analysis → Second scanning

Second scanning was done by using the same standard shown in **Figure 5** and in the same order of teeth as in the first scanning to compare first and second scanning images with a dedicated computer software program (Amide) which allowed calculation of the volume change within each specimen and the amount of microleakage by $AgNO^3$ penetration. The calculation of volumetric changes was conducted by free hand technique that allowed observer to focus on region of interest (ROI). For each specimen, ROI was drawn in a way that occupied all the area of interest and size of each sample was measured, and then by using volume rendering setting volumes were measured. Thereafter, each volume calculation from the first scanning of each sample was compared with that of the same sample of the second scanning for the evaluation of polymerization shrinkage within each group in mm^3. Microleakage was analyzed by Point Analysis, the pattern of infiltration digitally assessed with the same software program Amide. Data obtained from the second scans were assessed after DICOM images were transferred to Amide software. Thereafter, 0.06mm slice thickness was formed, after managing the threshold percentages the infiltration areas were observed and measured twice and then average was taken from the areas that microleakage observed in each sample. From the beginning to the end of the procedure, microleakage could be observed in each scan and the infiltration was measured with the accuracy up to 0.001mm. 1 researcher conducted microleakage assessment throughout the study.

Figure 5: Samples placed for scanning into the machine.

Statistical Analysis

Descriptive statistics tests were used to compare and amount of the silver nitrate penetration along with total volumetric changes occurred among the groups in Statistical Package for Social Sciences (SPSS) software version 23. The level of significance was set to ($P<0.05$).

RESULTS

Amount of silver-nitrate penetration among groups

Microleakage infiltration was evaluated based on the findings analyzed from Silver Nitrate ($AgNO^3$) infiltration within the samples expressed in mm on micro-CT images. Maximum mean amount of microleakage occurred in group A with 0.721mm, followed by Group B with 0.223mm. Minimum mean amount of microleakage was found for Group C with 0.022mm. Statistically significant differences were found between the bonding agents (Table 2) group A showed significantly higher microleakage values ($P<0.05$) than Group B and C whereas there was no significant difference between Group B and Group C. Figure 6 show patterns of silver nitrate penetration through the samples for each group.

Table 2: Descriptive statistics for the depth of the microleakage in mm.

Main Group	Subgroup	N	Mean Size	Std. Deviation
LED 1300mW/cm^2 Intensity	A (One up bond F plus)	10	0.721	0.648
	B (Palfique bond)	10	0.223	0.520
	C (Palfique universal bond)	10	0.022	0.035

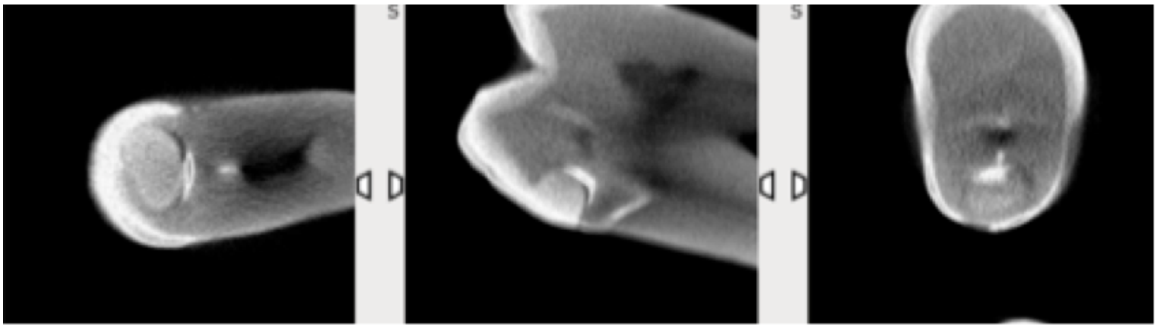

Figure 6: Shows the patterns of silver nitrate penetration through the samples in Group (mm).

Amount of total volumetric changes among groups

The total volumetric changes due to the initial polymerization shrinkage and expansion of composite resin during water storage were assessed based on the findings obtained by micro-CT before and after the thermocycling. Statistically significant differences were not found between Group A (One up bond F plus) and Group B (Palfique bond) and Group C (Palfique universal bond) (Table 3). Figure 7 and 8 shows the volumetric changes in resin restorations.

Table 3: Descriptive statistics of First-scanning, Second scanning and their difference in mm^3 for shrinkage.

	Pre-test Mean (volume mm^3)	SD	Post-test Mean (volume mm^3)	SD	Difference Mean (volume mm^3)	SD
LED 1300mW/cm^2 Intensity						
A (One up bond F plus)	1.094	0.149	1.024	0.162	0.091	0.058
B (Palfique bond)	1.224	0.135	1.085	0.343	0.140	0.277
C (Palfique universal bond)	1.221	0.199	1.171	0.202	0.049	0.048

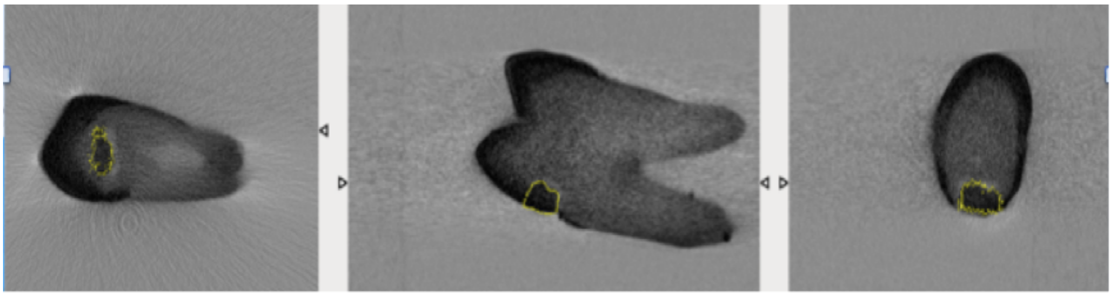

Figure 7: Shows the volumetric changes in resin restorations.

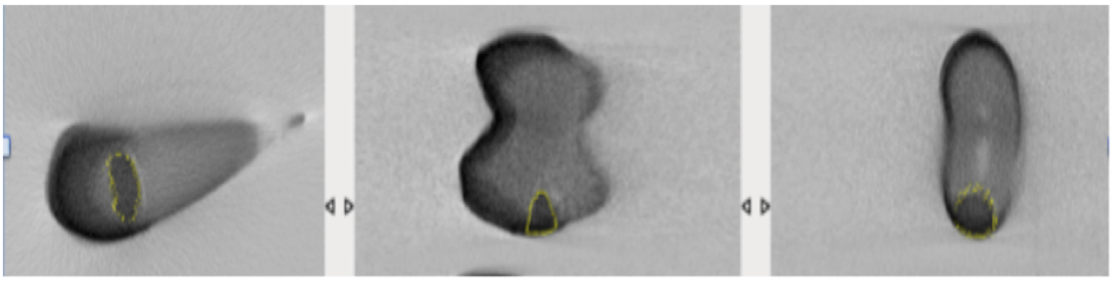

Figure 8: Shows the volumetric changes in resin restorations.

DISCUSSION

In the present study, by using micro-CT analyses, the amount of microleakage along resin-tooth interface of three different generation bonding agents was compared, and the total volumetric change in resin bulk was evaluated. The results of the present study showed that 6th generation dentin bonding agent (Group A) presented the highest microleakage values when compared to the 7th generation (Group B) and the 8th generation (Group C) dentin bonding agent. Therefore, the first null hypothesis was partially accepted. Regarding the total volumetric chances in resin bulk, a volumetric loss occurred at some degree in all groups. Therefore, the second null hypothesis was accepted.

Microleakage have always been the major challenge in resin-based restorations. Newly introduced materials were all aimed at overcoming and solving this issue. In class V restorations microleakage is the major problem since it has the highest amount of C-factor 'is the ratio of bonded surface of the restoration to the unbonded surfaces' leading to consequent higher polymerization shrinkage. In addition, its complex morphology makes it more difficult to manage class V cavities as they are located approximately 1 mm from the CEJ, and dentinal tubules are oriented parallel to the cervical wall. A classic hybrid layer formation is absent in the dentinal margins, and this absence is an important cause of leakage, therefore we focused on assessing class V cavities in the present study.[6,16] Thermocycling procedure was used since it is a commonly used method in dental studies to simulate temperature changes that take place in the actual oral environment.[17]

In the present study, the maximum microleakage shown in 6th generation adhesive system (One up bond F plus), which is HEMA-free, when compared to group B and group C. Both Palfique Bond (7th generation) and Palfique Universal Bond (8th generation) contain same ingredients of Hydroxyl Methacrylate (HEMA), Triethylene Glycol Dimethacrylate (TEGDMA) and Bisphenol A-glycidyl Methacrylate (Bis-GMA). The results of present study could be attributable to the absence of HEMA in 6th generation adhesive system (One up bond F plus). Since the hydrophilicity of HEMA makes it an excellent adhesion promoting monomer and by enhancing wetting of dentin it significantly improves bond strength, thereby reducing microleakage. HEMA also generates hydrogen bonds inside the micro-porosities of demineralized dentin, mechanically interlocking into the substrate by undergoing hygroscopic expansion after polymerization, thereby resulting in stronger bonds to the dentin surface. Therefore, presence of HEMA makes the 7th generation (Palfique bond) and 8th generation (Palfique universal bond) adhesive system results in less microleakage when compared to the 6th generation (One up bond F plus) adhesive system.[6]

Additionally, the 8th generation (Palfique universal bond) adhesive system has up to 50 MPa micro-tensile bond strength to dentin, and has over 30MPa shear bond strength while 6th generation (One up bond F plus) adhesive system has only 20 MPa shear bond strength to dentin, which makes 8th generation (Palfique universal bond) adhesive system more strong, durable and effective when compared to the 6th generation (One up bond F plus) adhesive system, which explains why One-up bond F plus turned up to be the least successful material among three materials used. [6, 24] In line with the results of the present study, Vinay and Shivanna[16] reported that 6th generation adhesive system showed the maximum amount of microleakage. They claimed that these results can be explained by the absence of the fillers in the 6th generation dentin bonding agents and insufficient amount of strength. The absence of a separate primer may reduce the infiltration depth or the wettability of the dentin adhesives, thereby reducing the sealing adhesion and the sealing capacity. However, these findings are inconsistent with the result of Hoseinfer and Kermanshah[25] who found no significant difference between 6th and 7th generation bonding agents in terms of microleakage.

In the current study, Micro-computed tomography (Micro-CT) was used as an assessment tool since it is introduced to dentistry as a microleakage and gap formation examination tool in resin based restorations, which has several advantages over conventional methods such as; application times are standardized; a comparison among different studies is possible, micro-CT analysis offers three-dimensional visualization so it can evaluate all aspects of the pattern of microleakage, it does not require cutting of the specimens, it is a non-destructive method and repeated measurements of the same specimen is possible. [(8, 9, 11, 20)] We used specimens immersed in a solution like silver nitrate which has radiopacity higher than dental tissues. Thereby, observers may easily differentiate between the dental tissue, resin composite material and produced gap and leakage between the resin-dentine interface on micro-CT images.[13, 14] As reported by Neves et al.,[21] this technique showed good sensitivity to evaluate the pattern of silver nitrate infiltration at the resin-tooth interface.

Total volumetric changes among groups

The shrinkage of the materials contains both effects, the chemical shrinkage due to the formation of bonds forming denser networks and the thermal expansion because of the exothermal curing reaction of resin and hardener followed by a cooling phase. Thermal shrinkage takes place when the reaction rate decreases and the temperature drops.[26] As the reaction starts and the material begins to shrink. In the following range the shrinkage is proportional to the not-yet-reacted polymer and therefore it follows an exponential decay. In general, many chemical reactions can be described by exponential equations depending on time and a reaction constant so it can be assumed that proper results can be obtained also for the chemical shrinkage of a polymer. [26]

LED units can result in a C=C conversion of the resin composite within a range. Generally, increased curing intensity leads to a better conversion rate, assuming that the spectrum of the curing unit, irradiation time, and light-guide tip diameter are very similar. Regarding spectra emission, LED units differ greatly from conventional QTHs. It would be expected that the narrow light spectrum of LEDs, with peak intensity at 465 to 475 nm, better fit the absorption peak of Camphorquinone than the broader spectrum of QTH light-curing units. Theoretically, LED units should be more efficient than QTH units in curing activation. [27-30]

According to results obtained from the statistical analysis there are volumetric changes in all the groups, which means that in all the groups there are gap formation due to the polymerization shrinkage, and when we compared group A1, A2 and A3 according to the results there are no significant differences in polymerization shrinkage. The results obtained, indicate that none of the used materials could prevent polymerization shrinkage and none of them showed a better success over one another in terms of polymerization shrinkage.

In this study the percentage of linear polymerization shrinkage was examined by comparing pre- and post- scanned results of the samples, which were analyzed by Amide computer software program, in three-dimensional views, the changes measured occurred among the samples in composite resin. The percentage of shrinkage of the curing units was similar. The type and concentration of the monomers and fillers in a resin composite, as well as the degree of monomer conversion, account for the polymerization shrinkage value, because only one resin composite brand was used in the study (Palfique LX5, Tokuyama dentals), the principal contributor factor accounting for differences in percentage of linear shrinkage among both techniques was degree of conversion. As there were no significant differences in degree of conversion among the groups, percentage of linear shrinkage was similar as well. [30]

CONCLUSION

The 8th generation (Palfique universal bond, Tokuyama dentals) adhesive system showed better results in preventing microleakage in comparison to the 6th generation (One up bond F plus) and 7th generation (Palfique bond) adhesive systems. None of the adhesive bonding generations could totally prevent microleakage at the resin-tooth interface. Group A, B and C showed the same amount of polymerization shrinkage with no significant difference among them.

Conflict of Interest:

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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