**In vitro** evaluation of microleakage in Class II composite restorations: High-viscosity bulk-fill vs conventional composites

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This study compared marginal microleakage of Class II cavities restored with bulk-fill resin (Filtek™ Bulk Fill) and conventional composite resin (Filtek™ Supreme XTE). Two standardized Class II cavities were prepared in forty extracted human molars. The gingival margin was located above the cemento-enamel junction for twenty molars (groups 1 and 2) and apically for the other twenty (groups 3 and 4) (n=20). The occlusomesial cavity was filled with bulk-fill resin by insertion in bulk (groups 1 and 3) and the occlusodistal cavity was restored with conventional composite using incremental technique (groups 2 and 4). The teeth were thermocycled (500 cycles 5–55°C), stained and observed under light microscope. The microleakage was significantly lower in gingival margins located in enamel compared with dentin margins (p<0.01). There was no statistically significant difference between groups 1 and 2 (p=0.86) and groups 3 and 4 (p=0.26). Bulk-Fill resins presents gingival microleakage similar to conventional composites.

**Keywords:** Bulk-fill composite, Bulk-fill technique, Microleakage, Marginal adaptation, Class II restoration

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**INTRODUCTION**

Dental composite resins are the most frequently used direct restorative materials for restoring posterior teeth. Despite the developments experienced in recent years, resin-based composites are technique sensitive and direct restoration technique continues to be complex1-5.

Effective marginal adaptation is of critical concern with posterior restorations. Microleakage is one of the important contributing factors in the failure of resin restorations so, to achieve successful posterior direct composite restorations, it is vital to maintain the marginal integrity of the composite-tooth6. The marginal sealing of direct composites involves several factors: the cavity configuration, the physical-mechanical and viscoelastic properties of the composite resin, the adhesive bond, the restoration technique and the curing method, etc.5,6. The ability to generate adequate marginal integrity of the restorations is closely related to the shrinkage and polymerization stress of the materials used, which is considered one of the major drawbacks of direct composite restorations5. Polymerization shrinkage produces stress between the tooth-restoration interface, weakening its integrity. Polymerization shrinkage can result in cuspal deflection, tooth cracking or failure of interfacial bonding, permitting the passage of bacteria that will produce recurrent caries or postoperative sensitivity, and therefore, will reduce the longevity of the restoration5,7,11. Hence, several methods have been developed to reduce the effects of shrinkage stress.

Conventional composite resins need to be placed in increments no thicker than 2 mm to ensure proper monomer to polymer conversion at the bottom of the increment. This will reduce polymerization shrinkage, thus obtaining an effective marginal sealing of the restoration1,10,11. This is even more critical at the bottom of a proximal box of a large Class II restoration, where light access is often compromised.

In an attempt to simplify this incremental layering technique and in order to shorten the chair time, a new generation of composite resins has been introduced, known as “bulk-fill resins”. These materials are a miscellaneous group of composite resins suitable for polymerization in thicknesses of 4–5 mm, thus avoiding the stratification technique and filling the cavity in bulk. The manufacturers ensure this depth of cure by improving the translucency, the inclusion of new photo-initiators in its composition and the reduction of volumetric shrinkage. With these monobloc resins, the restoration can be placed in one go or with two increments, depending on the classification of the bulk-fill material (base or full-body bulk-fill composites), because with flowable bulk-fill composites capping with a standard composite is required to improve the mechanical properties of the filling5,10,12-18.

Bulk-fill resins are indicated in the posterior sector. When the cavity presents all the cavosurface margin carved in solid and consistent enamel, the marginal seal is strong. However, the most critical area of the restorations is the gingival floor, where detachments and microleakage are more frequent. Below the cement-enamel junction the bond with dentin is weaker5,10.

The aim of the current study was to compare cervical microleakage of bulk-fill resins and conventional composite resins in Class II restorations with enamel and cement margins. The null hypothesis of the study was that there is no significant difference in microleakage of the different evaluated composite resins.
MATERIALS AND METHODS

This research work was approved by the Human Research Ethics Committee of the University of Valencia, under approval number H1449244809884.

A total of 40 human molars were used in this research. The molars were extracted for periodontal or orthodontic reasons and were included in the study according to the following criteria: intact crown, caries-free, hypoplastic defect-free, restoration-free, and crack-free. All calculus deposits and remaining connective tissue were removed by scaling (hand instrumentation and ultrasonic scaling) and the teeth were stored in 0.1% thymol solution for up to 4 months after extraction, at room temperature, until the study was carried out.

The teeth were divided into two main groups according to the location of the gingival margin. Twenty molars with approximately 4 mm long from the marginal ridge to 1 mm above the enamel-cementum junction were randomly assigned to Groups 1 and 2. Another 20 molars with a length of approximately 4 mm from the marginal ridge to 1 mm below the cemento-enamel junction were randomly assigned to Groups 3 and 4.

Cavity preparation

A single operator made 80 Class II cavities using a cylindrical round end diamond bur (838-314-012 Komet Dental, Lemgo, Germany) at high-speed handpiece with plenty of water spray. A new bur was used for every ten preparations in order to maintain cutting efficiency. In each molar, two independent cavities (occlusomesial and occlusodistal) were carved. The overall dimensions of the cavities were standardized as follows: 4 mm occlusal-gingival height, 4 mm buccolingual width and axial wall at 2 mm from the mesial or distal tooth surface. In Groups 1 and 2 the proximal gingival margin was placed in enamel, 1 mm above the cementum-enamel junction, and was bevelled with gingival margin trimmers. As for Groups 3 and 4 the gingival wall was located in cementum/dentin, 1 mm below the cemento-enamel junction. The teeth were stored in physiological solution until the restorative procedure.

Restorative procedure

All restorations were made by one operator. In Groups 1 and 3, occlusomesial cavities were filled with Filtek™ Bulk Fill A2. In Groups 2 and 4, occlusodistal cavities were restored with Filtek™ Supreme XTE A2B.

Materials used in the restorations and their compositions are shown in Table 1.

First, a circumferential metal matrix (Automatrix® MT, Dentsply, Milford, DE, USA) was adjusted around the cavity and secured. To remove loose preparation debris, all cavities were sprayed with water and were dried using lightly air blast. Subsequently, a total-etch technique was used in both cavities, occlusomesial and occlusodistal. The cavities were etched with 37% phosphoric acid etching gel (Octacid®, Clarben Laboratories, Madrid, Spain). The fosforic acid etchant was applied to the enamel first, and then to the dentin for 15 s, rinsed with water for 15 s and dried without overdrying.

In the occlusodistal cavity, an independent monodose L-Pop adhesive (Scotchbond™ UniversalL-Pop, 3M ESPE, St. Paul, MN, USA) was used following the manufacturer’s instructions. The bond was applied to the entire preparation’s walls using a disposable saturated applicator and rubbed in for 20 s, followed by a gentle stream of air over the liquid for about 5 s until it no longer moved and the solvent evaporated completely. The bonding agent was cured with the LED curing light (Led Elipar® 3M ESPE) for 20 s. Next, in this cavity, a first 2 mm increment of Filtek™ Supreme XTE A2 body was placed horizontally and measured by graduated periodontal probe. A ball and pear shape instrument were used to adapt the material into the cavity corners, undercuts and against cavity walls. Immediately after that, an independent L-Pop adhesive monodose (Scotchbond™ UniversalL-Pop) was applied to the occlusomesial cavity, also following the manufacturer’s instructions.

Table 1 Materials used in this study

| Product                  | Manufacturer    | Lot       | Expiration date | Composition                                                                 |
|--------------------------|-----------------|-----------|-----------------|-----------------------------------------------------------------------------|
| Octacid®                 | Laboratorios Clarben SA | 150528    | 2018-06         | 37% phosphoric acid gel                                                    |
| Scotchbond™ Universal Adhesive L-Pop | 3M ESPE       | 612785    | 2017-11         | MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond™ copolymer filler, ethanol, water, initiators, silan |
| Filtek™ Supreme XTE A2B | 3M ESPE        | N735773   | 2018-10         | Silica 20 nm particle and zirconia 4–11 nm particle                        |
| Filtek™ Bulk Fill A2     | 3M ESPE        | N721167   | 2018-08         | Zirconia and silica 4–11 nm particle, and ytterbium trifluoride 100 nm particle |
|                          |                 |           |                 | Bis-GMA, UDMA, TEGDMA, PEGDMA and Bis-EMA                                  |
|                          |                 |           |                 | ERGP-DMA, diuretano-DMA, and 1, 12-dodecane-DMA                            |
directions. Both cavities were polymerized at the same time for 20 s with the LED curing lamp (Led Elipar®, 3M ESPE). Next, in the occlusodistal cavity a second horizontal 2 mm increment of Filtek Supreme XTE A2 body was placed. Immediately after that, a single increment of Filtek™ Bulk Fill A2 was inserted into the occlusomesial cavity, covering the entire cavities and then modelling them with a ball and pear shape instrument. Both cavities were polymerized maintaining the lamp as close as possible to the restorative material 20 s per occlusal. Then the metal matrix was removed and an additional 20 s curing of the buccal and palatal aspects was performed. The finishing and polishing was carried out with a fine grain diamond rugby ball mill (8368.314 Komet® Dental) and the Sof-Lex™ Spiral finishing and polishing discs (3M ESPE). The restorative sequence is shown in Fig. 1.

The teeth were stored in physiological solution until processed.

Thermocycling
The restored teeth were subjected to artificial aging by thermocycling (SD Mechatronik Chewing Simulator CS-4®). All teeth were immersed alternately in cold water baths at a temperature of 5°C (±5°C) and hot water at a temperature of 55°C (±5°C) with a dwell time of 30 s in each bath for 500 cycles.

Staining of the samples
All the root apices were sealed with cyanoacrylate (LOCTITE® Superglue, Düsseldorf, Germany) and all tooth surfaces were coated with two layers of nail varnish (Rimmel® 60 seconds, London, UK) up to 1 mm from the restoration margins to prevent dye penetration into the tooth except at the resin-tooth interface. The teeth were immersed in a 0.5% basic fuchsine dye solution at room temperature for 24 h. Then the specimens were rinsed under running water and sectioned mid-sagitally in the mesiodistal plane using a cutting diamond disc (918B/104/220 Komet® Dental) mounted in handpiece.

Microleakage analysis
One same observer scored all the sectioned restorations blindly in an optical microscope (Leica DMS 1000®, Leica Microsystems, Wetzlar, Germany). Totally 160 sections belonging to the 80 restorations were evaluated. Dye penetration tooth-restoration was measured on the mesial and distal gingival floor at a 20× magnification. Cervical microleakage was recorded following the ISO score system (ISO/TS 11405: 2003): score 0=no dye penetration, score 1=dye penetration into ½ of the cervical floor, score 2=dye penetration more than ½ of the gingival floor without reaching the axial wall, score 3=dye penetration into cervical and axial wall (Fig. 2). The extension of dye penetration was also measured in micrometres (μm) in each tooth section using the Leica DMS 1000® microscope measuring program (Fig. 3). In all cases, the highest dye penetration value of each section was considered as the value of the restoration.

All measurements were made by the same examiner. Kappa index was calculated to assess the intra-examiner agreement repeating randomly 10% of the measurements, and the level of agreement in the present study was 0.833.

Statistical analysis
The obtained results were statistically analyzed using the statistical software IBM SPSS V22.0 (IBM SPSS, New York, NY, USA). Differences were consired statistically significant for p<0.05.

Non-parametric tests were used for comparison between groups. Comparisons between the independent groups were made using the Mann-Whitney U test and the paired samples were compared using the Wilcoxon test. The data were submitted for the Chi-square test to compare the difference in microleakage scores among different composite insertion techniques.
RESULTS

Cervical microleakage score
The frequency of the different scores (ISO/TS 11405: 2003) of microleakage are shown in Fig. 4. All the groups showed microleakage. The statistical analysis showed that there was no significant difference between the restoration technique. But there was a significant difference at the gingival margins. The Chi-square test, ($p=0.468$), showed no statistical difference among the microleakage scores of the different composite insertion techniques. But there was a significant difference among the microleakage scores ($p<0.01$) when cervicals margins were located in cementum or in enamel. The mean microleakage of all the composites showed greater gingival leakage in the cementum margin than enamel margin ($p<0.01$). The results demonstrated no significant microleakage differences ($p=0.86$) between Group 1 and Group 2 (cervical margins in enamel). Both groups showed significantly higher frequency distribution of Score 0. In Group 1 microleakage did not occur in 55% of cases and in Group 2 microleakage did not occur in 50% of cases. Nevertheless, when the cervical margin was in cementum, Groups 3 and 4, microleakage was not found in 10 and 5% of the cases respectively, also without significant differences between the two groups ($p=0.26$).

Cervical microleakage in micrometers of dye penetration
Table 2 shows the average values and confidence intervals for dye penetration for each of the study groups. The two restoration techniques showed significantly greater microleakage in cementum than in enamel ($p=0.01$). The lowest leakage values were found in Group 2 (982.79 μm), without significant differences with Group 1 (1,066.49 μm). No significant differences were found between the average values of dye penetration in μm when the margins were in cementum independently of the material used. Group 3 obtained an average of 2,407.87 μm and the average of Group 4 was 1,807 μm, without differences between two groups, although the depth in μm was higher in the bulk fill group.

DISCUSSION
For a successful composite restoration, a good marginal
adaptation is essential. Microleakage seems to be an inherent shortcoming of dental restorations. If restoration’s margins are not completely sealed, fluids, bacteria, and debris can enter the cavity preparation. Leaky margins result in development of caries, pulpal irritation with tooth sensitivity and staining on the margins. Thus, microleakage is an important property that has been used in assessing the success of restorative material. Microleakage refers to microscopic openings between the margins of the resin filling and tooth structure so dye penetration tests are known to be valid tools for the determination of marginal gaps in vitro studies.

Several studies have examined microleakage of bulk-fill resins, but there is no uniformity in the methodology.

The sample size of 20 specimens for each group was decided for the present study. This size matches the one used by Campos et al. Many authors that analyze leakage of bulk-fill resins use a smaller sample size that ranges between 2 and 12. Some authors, such as Orlowski et al., use a larger sample (n=30).

Storage conditions affect the tooth structure for in vitro studies. Several storage solutions have been suggested in the published literature. The most commonly used solutions are 10% formalin-acetate, chloramine and 0.05–0.1% thymol. In the present study, thymol at 0.1% was used because it seems to alter less the conditions of dental structures for adhesion.

The configuration of the cavity significantly influences the polymerization shrinkage and therefore the in vitro results. There is no consensus about cavity dimensions. In the current study, we performed cavities of 2 mm mesio-distally width×4 mm bucco-lingually width and 4 mm occluso-cervically depth, like those in the study of Roggendorf et al. and in the study of Miletic et al.

Tooth preparation of a bevel is recommended to improve marginal quality of a composite restoration. The occlusal cavosurface margin is not bevelled, since the composite in small thicknesses and subjected to load would fracture. With regard to the gingival cavosurface margin, in the present study we decided to bevel the enamel using gingival margin trimmers, to remove the unsupported enamel rods and to improve the disposition of enamel prisms against acid etching. This cavo-surface margin bevelling is carried out by various authors in their studies such as Campos et al. and Poggio et al.

On the other hand, microleakage can be aggravated by changes in temperature that occur in the mouth, due to the different coefficients of thermal expansion of dental tissues and composite resins. Campos et al. Agarwal et al. reported a satisfactory marginal adaptation values before thermo-mechanical loading, but these values were not maintained after subjecting the samples to thermocycling and load. In the current study we used a thermocycler (SD Mechatronik Chewing Simulator CS-4) that accelerated the aging process of the teeth restorations. This mechanism increases the permeability of the hybrid layer and the porosity of the restorative material. This way, the stress that a restoration presents over the years can predict in vitro the longevity of a restoration in vivo. In the present study, the restored teeth were subjected to 500 cycles of thermocycling, immersed alternately in cold water baths at a temperature of 5°C (±5°C) and hot water at a temperature of 55°C (±5°C) with a dwell time of 30 s in each bath for 500 cycles. There is also no consensus about the number of thermocycling cycles, although many studies perform 500 cycles, in accordance with the International Organization for Standardization Technical specification n°11405.

The degree of microleakage between tooth-restoration can be monitored by the penetration of tracers and staining agents. Despite the large use of microleakage tests, several different methodological approaches can influence the test. As with the adhesion tests, there are also large variations in the leakage data between different laboratories. It depends on the adhesive technique and the manipulation variables. Several dyes have been proposed to be used in leakage test, used by the different authors to monitor the microleakage of the fillings. The most common are 0.2 or 0.5% basic fuchsin, 1% methylene blue and silver nitrate, which allow to evaluate and quantify microfiltration of restorations made with composite resins.

As we have seen, composite marginal integrity might be affected by several factors, including the cavity size and geometry, the composite resin physico-mechanical properties, the layering protocol and the polymerization technique. In the present study, we standardized the cavities size; the same adhesive technique and polymerization technique was used for both resins, so that only the resin variable will influence the results.

Table 2  Microleakage in micrometers for each group

| Group | Mean | 95% confidence interval |
|-------|------|------------------------|
|       |      | Min. | Max.       |
| G1: Filtek™ Bulk Fill Enamel | 1,066.49<sub>a,b</sub> | 322.94 | 1,810.04 |
| G2: Filtek™ Suprem XTE Enamel | 982.79<sub>c,d</sub> | 272.83 | 1,692.76 |
| G3: Filtek™ Bulk Fill Dentin | 2,407.87<sub>a</sub> | 1,679.17 | 3,136.58 |
| G4: Filtek™ Suprem XTE Dentin | 1,807.00<sub>d</sub> | 1,119.43 | 2,494.56 |

Values with the same letter in the subscript are significantly different.
Also the dental structure factor can play an important role; therefore, in the present study, we eliminated the variability dependent on the tooth by performing the mesial filling with the block technique and the distal restoration with the incremental technique, using different specimens for the cervical margins in enamel and dentin.

Polymerization stress is considered one of the biggest drawbacks of direct composite restorations\(^1\). One of the undesirable effects of the contraction tension manifests itself when the tensile forces are transferred to the tooth-restoration interface. If the bond strength is not sufficient to withstand these forces, the marginal integrity of the restoration is damaged causing loss of retention or the formation of a marginal gap\(^5\).

Many in vitro studies show leakage and shrinkage stress for different composites, providing a physically observable result of polymerization shrinkage. As previously observed by some authors, cervical marginal integrity below the cementum-enamel junction was inferior to that observed in enamel margins, since dentine has a more vulnerable adhesion\(^2\).

Several studies evaluate the marginal adaptation of bulk-fill resins. Although the composition varies considerably among them\(^9\), most studies do not find significant differences in the marginal integrity of bulk-fill resins\(^1,2,12,23-25,29,31,34,37,38\). Some authors claim that flowable bulk-fill composites improve this marginal adaptation\(^3,10,11,39\). However, Miletic et al.\(^10\) found in their experiment that, with the exception of the Z250 composite, stratified composites showed less gingival leakage than bulk-fill resins. Benetti et al.\(^19\), in their 2015 study reported no differences in most bulk-fill resins with respect to the conventional resins, but found larger gaps in two of the low-viscosity bulk-fill resins (X-tra base and Venus Bulk Fill).

Based on the results of the present study, the null hypothesis cannot be rejected since no differences were found in microleakage between bulk-fill resins and conventional resins, regardless of whether the cervical margin was in enamel or in cement.

The results of this study demonstrated that all materials under investigation (Filtek™ Supreme XTE A2 and Filtek™ Bulk Fill A2) showed gingival microleakage after thermocycling, not being able to guarantee a perfect gingival seal. This was indeed observed in several in vitro studies that evaluate the leakage of different bulk-fill resins compared with conventional composite resins\(^2,23-25,27,29,34\). In the current study, although when the margin was placed in enamel, for both groups 70% of the samples showed a filtration ≤1. In cervical cementum margin, in Group 4 (Filtek™ Supreme XTE) the 55% of the samples showed filtration ≤1, whereas in Group 3 (Filtek™ Bulk Fill) only this assessment was obtained in 35% of the samples. This same relationship was found when the dye penetration in μm was evaluated, which could be due to a greater shrinkage of the bulk-fill resin that becomes more evident in the most critical areas. The most critical area of Class II restorations is the gingival floor, where detachments and microleakage are more frequent, especially when the margins are not in enamel\(^2,12,23\). The results of the current study are in agreement with what has been published so far, since we observed that the restorations with margins in enamel filtered significantly less than the fillings with gingival margins in cement. In general, the results are especially worse if the gingival margins are below the cementum-enamel junction. The most commonly adduced explanation for this fact is the scarcity or absence of enamel and the weakest adhesion to dentine. Numerous authors confirm this fact in their studies. Campos et al.\(^12\), conclude in their study that the margins in dentin show greater discontinuity than those located in enamel. Webber et al.\(^29\), evaluated in vitro microleakage using flowable bulk-fill or not, also obtaining greater microleakage in dentin than in enamel. Poggio et al.\(^9\) evaluated the microfiltration of different composite resins in Classes II with gingival cavosurface margin in cement, obtaining microleakage in all groups. Agarwal et al.\(^23\), obtain lower gingival marginal leakage in Classes II with margins in enamel than in cement, concluding that the viscosity of the bulk-fill resin influences the internal adaptation in the dentine. These results support the study by Gamarra et al.\(^36\) that found more severe microleakage of SonicFill composite at the cervical margins located in dentin than at the cervical margins located in enamel regardless of the photopolymerization technique employed.

In our study, only regular bulk-fill composite was tested. Numerous authors have investigated the marginal adaptation of the flowable bulk-fill with respect to the regular ones\(^2,23\). Orlowski et al.\(^32\) evaluated the marginal sealing of four bulk-fill composites: SDR, SonicFill, TetricEvoCeram BF, Filtek BF, showing that flowable bulk-fill resins had better marginal adaptation than regular ones. Kim et al.\(^10\) confirm the results, since they obtained an improvement in terms of polymerization shrinkage and marginal integrity with flowable bulk-fill composites (SDR and Filtek Bulk-Fill). However, other authors do not find differences in the microleakage between flowable bulk-fill and the conventional incremental technique, thus Webber et al.\(^29\) do not find differences between SDR and incremental technique.

**CONCLUSION**

With the limitations of the present study, it can be concluded that microleakage was significantly lower in enamel with both materials. And neither in the enamel nor in the cement were differences found between the two materials analyzed.

However, clinical success is only discovered after long periods of use, so clinical studies are needed to assess the success and longevity of bulk-fill resins.

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