In vitro study of marginal microleakage levels in restorations with Bulk Fill resins using the deproteinization technique prior to acid etching

Estudio in vitro de los niveles de microfiltración marginal en restauraciones con resinas Bulk Fill utilizando la técnica de desproteinización previo al grabado ácido.

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SUMMARY

Introduction:

Currently, the market offers Bulk Fill resins that are used with the monobloc or single-increment technique (up to 4 or 5 mm).

These resins are characterized by a lower filler content and larger filler particles, improved translucency, and a different chemical structure of the monomers in the filler compounds, which—when placed in a single block—reduce polymerization stress.

Objective:

To evaluate the level of marginal microleakage of two Bulk Fill resins using the deproteinization technique prior to acid etching.

Methods:

This is an in vitro study in which 64 extracted premolars underwent standardized cavity preparations. The teeth were randomly divided into four groups and subjected to a manual thermocycling process of 500 cycles.

All samples were immersed in a 2% methylene blue solution for 24 hours. Microleakage was then observed with a stereomicroscope.

Results:

For the Aura Bulk Fill resin, microleakage in enamel was observed in 25% of the deproteinized samples and in 18.8% of the non-deproteinized samples. For the Filtek Bulk Fill resin, 31.3% of the samples exhibited microleakage in enamel with the deproteinization technique and 25.0% without deproteinization. No statistically significant differences were found.

Conclusions:

All samples presented microleakage, with similar levels in both brands of Bulk Fill resins, and no significant differences were observed with the application of the deproteinization technique.

Keywords:

Marginal microleakage; Bulk Fill resins; deproteinization.

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INTRODUCTION

Currently, the vast majority of restorations in dental practice are performed using nanohybrid resins applied with the incremental technique, which involves the placement and photopolymerization of layers no thicker than 2 mm. In very deep cavities, this extends the restoration time and increases the risk of incorporating voids. Moreover, the repeated polymerization shrinkage from each increment may generate increased stress at the tooth–restoration interface, raising the probability of bond failure. This can lead to the formation of an interface between the tooth and the restoration, and eventually, bacterial and fluid infiltration, resulting in immediate sensitivity and long-term caries development (López, 2017; Rojas and Ríos, 2021).

Efforts in resin materials aim primarily to prevent microleakage, which is the main cause of failure in adhesive dentistry. Microleakage is defined as the passage of bacteria, fluids, molecules, or ions through the tooth–restoration interface, a persistent problem in resin restorations (Baltacioglu et al., 2024). It is considered a negative outcome that may occur due to the volumetric changes or contraction of the resin during photopolymerization, but it can also result from temperature fluctuations within the oral cavity, the type of resin, the adhesive system used, the restoration technique, or the design of the cavity preparation (Cáceres et al., 2021; Rojas and Ríos, 2021).

On the other hand, ongoing improvements in biomaterials are aiming to minimize the effects of polymerization contraction, thereby reducing the risk of bond loss and microleakage. This has led to the introduction of single-increment or Bulk Fill resins, which are intended to decrease working time, reduce the number of clinical steps, minimize the formation of voids or gaps, prevent interlayer contamination, and lessen polymerization shrinkage (Sampaio et al., 2024).

This new concept in restoration involves applying the resin in a single block of up to 4 or 5 mm, ensuring uniform polymerization, simplifying the procedure, reducing working time, lowering shrinkage during polymerization, and reducing voids within the increments (Behery et al., 2018; Rodríguez et al., 2018; Rojas and Ríos, 2021). Bulk Fill resins are based on modifications in chemical composition, increased translucency, the use of alternative resin monomers compared to conventional ones, and a different filler technology (Sampaio et al., 2024).

There are two classes of Bulk Fill resins available in the market:

- Low-viscosity (flowable) Bulk Fill resins: These have a lower filler content and a fluid consistency, making them suitable as a base in restorations.

- High-viscosity (condensable) Bulk Fill resins: These contain conventional fillers, are more condensable, and have higher viscosity, making them suitable for filling the entire cavity with the single-increment technique (Khairy et al., 2024; Sampaio et al., 2024).

Fifth-generation adhesive systems are widely used in restorative dentistry to bond the restorative material to the dental tissue. These adhesive systems involve two steps: acid etching and the application of the adhesive.

The acid etching or conditioning of the cavity surface for restorations requires the use of 37% phosphoric acid for 15 seconds on enamel and 10 seconds on dentin. This treatment increases

No. 43, Vol 2, 07-15 2025 (23) | ISSN2215-5740

the surface area of the enamel by modifying the enamel prisms and their surrounding structure, thereby creating a high-energy surface conducive to adhesion. Essentially, a previously smooth surface is transformed into one with microporosities through which the low-viscosity adhesive system can penetrate (Licla and Albites, 2015; Zheng et al., 2022).

Furthermore, 5.25% sodium hypochlorite (NaClO) is used as a bactericidal and bacteriostatic agent, but more recently it has been employed as a deproteinizing agent to complement adhesive systems, being applied before acid etching (Gerardo et al., 2020).

Sodium hypochlorite is recognized for its ability to remove denatured proteins from the enamel and dentin surfaces, thereby obtaining a surface free of disorganized organic matrix. This allows the formation of three-dimensional channels that integrate optimally with the initial adhesive layer, resulting in better adhesion (Lang et al., 2020; Roque et al., 2021).

Deproteinization is a technique with significant potential for increasing the bond strength of adhesive materials, as it is noninvasive, convenient, and low-cost (Gurgel et al., 2011; López et al., 2019; Zheng et al., 2022).

Sodium hypochlorite acts as a deproteinizing substance on the tooth surface, removing the organic material (dentin smear layer and protein residues) present in the cavity. This can improve the effectiveness of acid etching and, consequently, the adhesive capability of bulk-fill resins, thereby reducing microleakage.

Therefore, the objective of the present study was to evaluate, in vitro, the levels of microleakage that occur in Bulk Fill resins when using conventional etching techniques with the additional application of sodium hypochlorite.

METHODS

This experimental in vitro study was approved by the Ethics Committee of Universidad Alas Peruanas. Sixty-four premolars, extracted for orthodontic reasons, were used. All study procedures were conducted in accordance with the principles of the Helsinki Declaration.

The 64 premolars were first cleaned using ultrasonic methods and then stored in distilled water until processing. Standardized simple occlusal cavity preparations with a depth of 4 mm were made on all selected teeth. The samples were randomly distributed into four groups (n = 16):

- Group 1: Restored with Aura Bulk Fill-SDI resin after applying 5.25% sodium hypochlorite for deproteinization prior to acid etching.
- Group 2: Restored with Aura Bulk Fill-SDI resin without the use of 5.25% sodium hypochlorite before acid etching.
- Group 3: Restored with Filtek Bulk Fill-3M resin after applying 5.25% sodium hypochlorite for deproteinization prior to acid etching.
- Group 4: Restored with Filtek Bulk Fill-3M resin without the use of 5.25% sodium hypochlorite before acid etching.

The application of 5.25% sodium hypochlorite for 60 seconds was performed to deproteinize the surface before applying 37% phosphoric acid (Del Valle et al., 2021). Fifth-generation adhesives were applied to all cavity preparations according to the manufacturers' protocols.

No. 43, Vol 2, 07-15 2025 (23) | ISSN:2215-5740

The restorations were performed using the single-increment (bulk) technique and were light-cured for 15 seconds at an intensity of 1000 mW (Mosharrafian et al., 2023).

After 24 hours, all samples underwent an artificial aging process through manual thermocycling for 500 cycles, with temperature changes from 5°C to 55°C. At each extreme, the immersion lasted 20 seconds, with 10-second intervals between temperature changes.

Following the aging process, microleakage was evaluated by coating each tooth with two layers of nail varnish, leaving a 2-mm margin around the restoration free. The apices were sealed with a fast-curing acrylic resin. The teeth were then immersed in a 2% methylene blue solution for 24 hours at 37°C (Baracco et al., 2016; Navarrete, 2018). Finally, each tooth was sectioned in a bucco-palatal direction using a diamond disc and subsequently polished with rubber abrasives. A stereomicroscope was used to record the levels of microleakage according to the following criteria:

- Level 1: No microleakage observed.
- Level 2: Dye penetration at the enamel–restoration interface (microleakage in enamel).
- Level 3: Dye penetration reaching the dentin–restoration interface (microleakage in dentin).
- Level 4: Dye penetration reaching the pulpal floor of the restoration (microleakage at the floor).

RESULTS

Despite the manufacturers' efforts to minimize polymerization shrinkage stress with the introduction of Bulk Fill resins, the challenge remains significant. When evaluating the degree of microleakage in two commercially available resins subjected to the complementary deproteinization technique prior to acid etching, high levels of marginal microleakage were still observed. In all the samples restored with Aura Bulk Fill resin, microleakage was recorded. Comparison between the deproteinized and non-deproteinized techniques showed no statistically significant differences (P = 0.564) (see Table 1).

Table 1. Marginal Microleakage Levels in Aura Bulk Fill – SDI Resin Based on Phosphoric Acid Conditioning with or without a Deproteinizing Agent

Microleakage Level	With Deproteinization (N)	With Deproteinization (%)	Without Deproteinization (N)	Without Deproteinization (%)
No Microleakage	0	0%	0	0%
Microleakage in Enamel	4	25%	3	18.8%
Microleakage in Dentin	4	25%	3	18.8%
Microleakage at the Floor	8	50%	10	62.5%
TOTAL	**16**	**100%**	**16**	**100%**

a Non-parametric Mann-Whitney U Test

In restorations with Filtek Bulk Fill resin, all samples exhibited microleakage. When comparing based on the deproteinization technique, no statistically significant differences were found (p = 0.696) (Table 2).

Table 2. Marginal Microleakage Level in Filtek Bulk Fill – 3M Resin Based on the Etching Technique

Microleakage Level	With Deproteinization (N)	With Deproteinization (%)	Without Deproteinization (N)	Without Deproteinization (%)
No Microleakage	0	0%	0	0%
Microleakage in Enamel	5	31.3%	4	25.5%
Microleakage in Dentin	6	37.5%	6	37.5%
Microleakage at the Floor	5	31.3%	6	37.5%
Total	**16**	**100.0%**	**16**	**100.0%**

When comparing the levels of microleakage in the two types of resin without the deproteinization technique, no statistically significant differences were found (p = 0.287) (Table 3).

Table 3. Marginal Microleakage Level in Conditioning Without Deproteinization by Resin Brand

Microleakage Level	Filtek Bulk Fill - 3M (N)	Filtek Bulk Fill - 3M (%)	Aura Bulk Fill - SDI (N)	Aura Bulk Fill - SDI (%)
No Microleakage	0	0%	0	0%
Microleakage in Enamel	4	25.0%	3	18.8%
Microleakage in Dentin	6	37.5%	3	18.8%
Microleakage at the Floor	6	37.5%	10	62.5%
Total	**16**	**100.0%**	**16**	**100.0%**

When comparing the levels of microleakage in the two types of resin using the deproteinization technique, no statistically significant differences were found (p = 0.423) (Table 4).

Table 4. Marginal Microleakage Level in Conditioning with Deproteinization by Resin Brand

Microleakage Level	Filtek Bulk Fill - 3M (N)	Filtek Bulk Fill - 3M (%)	Aura Bulk Fill - SDI (N)	Aura Bulk Fill - SDI (%)
No Microleakage	0	0%	0	0%
Microleakage in Enamel	5	31.3%	4	25.0%
Microleakage in Dentin	6	37.5%	4	25.0%
Microleakage at the Floor	5	31.3%	8	50.0%
Total	**16**	**100.0%**	**16**	**100.0%**

No. 43, Vol 2, 07-15 2025 (23) | ISSN:2215-5740

DISCUSSION

In adhesive restorations, a high percentage of failure is recorded due to polymerization shrinkage and marginal microleakage. Additionally, when a restoration remains in the oral cavity for a long time, it is exposed to thermal dimensional changes, which could lead to the detachment of the adhesive restoration and thus be another factor contributing to marginal microleakage (Del Valle et al., 2021).

This loss of adhesion between the restorative material and the tooth allows the ingress of bacteria and fluids, which can penetrate the dentin-pulp complex, leading to negative consequences such as postoperative sensitivity and, in the long term, recurrent caries (Mosharrafian et al., 2023).

Polymerization shrinkage in conventional resins is minimized through the incremental technique, where the material is layered obliquely to achieve better peripheral sealing and, consequently, less peripheral microleakage (Elgendy et al., 2019). Bulk Fill resins have emerged as an alternative to reduce polymerization stress at the adhesive interface. A case report demonstrated that Bulk Fill resins maintain proper anatomical conservation and good marginal sealing, with no observed failures over time and acceptable clinical performance, despite encouraging results from studies with two or three years of follow-up (Grandon et al., 2020; Kury et al., 2020).

In an in vitro study, the degree of microleakage and adhesive strength of Bulk Fill resins was evaluated by applying 18% EDTA gel compared to 37% phosphoric acid. Microleakage was observed at the enamel level with 37% phosphoric acid, and microleakage at both enamel and dentin levels was noted when EDTA was used for 60 seconds. When EDTA was applied for 90 seconds, microleakage was recorded only at the enamel level, demonstrating that microleakage remains highly prevalent in single-increment restorations (Cayo et al., 2019). When comparing the levels of microleakage between conventional resins and Bulk Fill resins, no statistically significant differences were found. However, high levels of microleakage were observed in Bulk Fill resins, similar to the results obtained in this study (Lorca et al., 2023).

Despite being marketed as an alternative to counteract the negative effects of polymerization shrinkage—using 4 to 5 mm deep increments, theoretically leading to minimal or no shrinkage upon polymerization (Del Valle et al., 2021)—an in vitro study on primary dentition found high levels of microleakage, results similar to ours (Rojas & Ríos, 2021). Furthermore, when evaluating microleakage in Class II cavities in primary molars restored with Bulk Fill resins and conventional resins, no significant differences in microleakage were observed between the two groups. Thus, it was determined that Bulk Fill resins serve as an alternative to conventional resins, primarily due to their ease and speed of application (Mosharrafian et al., 2023).

Enamel proteins interfere with the establishment of proper acid etching patterns, making deproteinization a promising technique for improving adhesive mechanisms. Deproteinization involves removing proteins from the enamel surface, which can be achieved with sodium hypochlorite, capable of degrading defective proteins and dissolving the smear layer and residual contaminated tissue (López et al., 2019). Applying sodium hypochlorite before acid etching enhances the amount

No. 43, Vol 2, 07-15 2025 (23) | ISSN2215-5740

and quality of the etched surface, thereby potentially optimizing adhesive capability and improving resin bonding strength on enamel (López et al., 2019). Despite theoretical evidence, Cayo and Carrillo found high levels of marginal microleakage even when using 10% sodium hypochlorite, findings that align with our results. While sodium hypochlorite is an effective conditioning agent, it did not produce the desired effect when applied before single-increment resin restorations (Cayo & Carrillo, 2020).

Many findings from both in vitro and clinical studies suggest that Bulk Fill resins may slightly increase the failure rate of restorations in the medium term. However, no increased risk of postoperative sensitivity has been reported compared to other resin materials (Larraechea et al., 2020).

CONCLUSIONS

Despite the use of sodium hypochlorite, which has demonstrated its ability to remove organic material from the tooth surface and enhance acid etching effectiveness and adhesion, microleakage was present in all samples. This indicates that regardless of the brand of Bulk Fill resin or the use of the deproteinization technique, the levels of microleakage remained high and similar. Therefore, it must be considered that microleakage can lead to the premature failure of restorations, as it allows the penetration of bacteria and fluids into the interface between the tooth and restoration, potentially causing postoperative sensitivity or secondary caries over time.

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No. 43, Vol 2, 07-15 2025 (23) | ISSN:2215-5740

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