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Comparative analysis of marginal microleakage of materials used as pit and fissure sealants: An *in vitro* study

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Abstract
Purpose: To compare the efficacy of different materials used as dental sealants by evaluating marginal microleakage and penetration capacity in pits and fissures.
Methods: Forty-five healthy premolars extracted for orthodontic reasons and meeting inclusion criteria were used. Teeth were cleaned, stored in saline solution up to 30 days, and randomly assigned to three groups: Clinpro, Ketac Molar Easymix, and Vitremer (3M ESPE). The materials were applied, and the samples underwent thermal cycling (5°C, 37°C, 55°C) in a distilled water solution. The apices were sealed with resin, surfaces coated with varnish, and samples immersed in 1% methylene blue stain for 24 hours. The teeth were sectioned buccolingually, and dye penetration was evaluated under a 40x stereoscopic microscope using Williams and Winters criteria.
Results: clinpro demonstrated superior performance, with 60% of samples scoring category 2 and 33.3% in category 4. Vitremer showed intermediate performance, with 33.3% in category 2. Ketac Molar Easymix had the lowest penetration, with 93.3% of samples scoring 1 or 2.
Conclusion: no statistically significant differences were found in the marginal penetration capacity among the evaluated materials. However, Clinpro demonstrated moderate to high performance, standing out for its penetration capacity and ease of handling.

Keywords: Dental sealant, pit and fissure sealant, Clinpro, microleakage, Ketac Molar Easymix, Vitremer

Introduction

Dental caries is one of the most common diseases globally affecting individuals of all ages and socioeconomic statuses, according to the Global Burden of Disease study, untreated caries in permanent teeth has been the most prevalent condition of the past decade, while in primary dentition, it ranks tenth in terms of morbidity [1]. To reduce the risk of caries, preventive measures such as the use of fluoridated toothpaste and topical fluoride applications have been implemented demonstrating limited efficacy; in contrast, physical preventive methods, such as dental sealants, have emerged as a significantly effective alternative [2]. Their mechanism of action prevents plaque accumulation and modifies the microenvironment of fissures, making it less conducive to the development of caries [3].

The first clinical trials were conducted in the 1960s and 1970s, introducing the use of fluid resin to seal fissures as an effective barrier against plaque accumulation and debris by hardening after application [4]. Additionally, alternative materials such as zinc chloride, potassium ferrocyanide, and amalgam were explored while these materials offered some protection, they did not achieve significant results [5]. Today, multiple materials are available for sealing pits and fissures: Resin-based sealants, primarily composed of a Bisphenol A glycidyl dimethacrylate (Bis GMA) matrix, incorporate monomers such as urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA) and may include fluoride particles or photoinitiators to enhance their functionality [6]. High-viscosity reinforced glass ionomer, characterized by its rapid setting and low solubility in the presence of oral fluids, is applied using the atraumatic restorative technique offering good marginal sealing and enhanced clinical durability [7, 8]. Resin-modified glass ionomer was developed to overcome the mechanical and aesthetic limitations of conventional glass ionomer, incorporating resins such as Bis-GMA, UDMA, TEGDMA and 2-hydroxyethyl methacrylate (HEMA), thereby providing greater durability [9].

The clinical efficacy of dental sealants largely depends on proper retention on the tooth [10], as various factors such as salivary pH, polymerization stress, thermocycling, and occlusal forces affect their durability, with an estimated annual volume loss of 5–10% [11]. However, microleakage is the primary cause of failure, as small gaps between the tooth and the sealant allow the infiltration of bacteria and oral fluids. This issue is exacerbated by thermal fluctuations in the oral cavity (ranging from 5°C to 55°C) and inadequate moisture control during application [12]. When proper sealing is not achieved, microleakage facilitates the development of caries beneath the restoration, thereby compromising its effectiveness [11].

Several studies agree on the effectiveness of sealants as a preventive strategy against caries, highlighting their key role in clinical practice in this case Ahovuo-Saloranta *et al.* [13] demonstrated that resin sealants significantly reduce the incidence of caries in first permanent molars, with 5.2% in treated surfaces compared to 16% in untreated surfaces, showing consistent results over time. Similarly, the meta-analysis by Wright *et al.* [14] reported a 76% reduction in caries incidence after two years and an 85% reduction after seven years compared to untreated surfaces. Hou *et al.* [15] confirmed the efficacy of resin sealants, demonstrating preventive effects lasting from 6 months to 5 years, particularly highlighting their usefulness in patients at high risk of caries. These studies consistently support the effectiveness of sealants, establishing them as a fundamental tool in caries prevention.

Over the years, various studies have sought to identify the ideal sealant for pits and fissures, but no definitive consensus has been reached. A comparative study evaluated four sealants (Embrace Wetbond®, UltraSeal XT®, Clinpro™, Helioseal®) applied using conventional and induced techniques, finding that the induced technique significantly improves sealant penetration and reduces microleakage. UltraSeal XT® was highlighted as the most efficient material. The methodological rigor of the study supports these findings, guiding future research toward the improvement of existing sealants [16]. On the other hand, clinical studies comparing sealant retention have shown that unfilled sealants (Clinpro 3M ESPE) exhibit greater retention (64.29% vs. 53.57%) 12 months after application. This result is attributed to their lower viscosity, which enhances penetration into fissures [17]. Similarly, a 10-year follow-up study compared the survival and clinical efficacy of resin-based sealants (RBS) (Clinpro 3M ESPE) and glass ionomer cement (GIC) (Fuji IX) in second permanent molars. The results showed higher retention rates for RBS (90% at 1 year and 6.7% at 10 years) compared to GIC (43.3% at 1 year and 0% at 10 years). However, no significant differences were found in caries prevention between the two materials. While GIC represents a viable alternative in conditions with challenging moisture control, RBS stands out for its long-term durability [18]. These studies emphasize the need for regular follow-ups and timely reapplications to maximize the efficacy of sealants, particularly in newly erupted teeth and patients at high risk of caries [19].

Current evidence highlights the importance of ongoing evaluation and refinement of both sealant materials and application techniques to ensure their maximum clinical effectiveness.

The aim of this study is to compare the efficacy of different materials used as dental sealants by evaluating their marginal microleakage and penetration capacity in pits and fissures. It is hypothesized that the resin-based sealant will demonstrate superior penetration capacity and reduced microleakage compared to the other materials assessed.

Materials and Methods

After obtaining approval from the ethics committee of Universidad Hemisferios on September 9th 2024.

Search strategies: A comprehensive search was conducted in scientific databases such as PubMed, Scopus, Web of Science, and Cochrane Library, focusing on publications written in English between 2015 and 2024. The selection included recent and relevant literature, particularly from the last five years, complemented by previous studies that provided historical context or fundamental background. Priority was given to *in vitro* studies, systematic reviews, and meta-analyses related to the evaluation of dental sealants, marginal microleakage, and penetration capacity in pits and fissures.

45 healthy premolars were collected, these teeth were extracted for orthodontic purposes and met the inclusion criteria: no caries, restorations, or morphological defects. The samples were donated by a specialized dental clinic.

They were clinically evaluated under standard illumination using an explorer, and residual tissues were removed using a prophylactic brush on a low-speed handpiece with pumice and distilled water irrigation [20]. Subsequently, the samples were stored in saline solution at room temperature for a maximum of 30 days [21]. The samples were then randomly divided into three groups: G1= Clinpro-3M Oral Care Solutions Division (St. Paul, MN, USA)

G2= Ketac Molar Easymix - 3M Oral Care Solutions Division (St. Paul, MN, USA)

G3= Vitremer -3M Oral Care Solutions Division (St. Paul, MN, USA)

After applying the materials, the samples were placed in sterilization pouches and transported and the Termociclagen OMC 350 TS (Odeme Dental Research). Each group was subjected to thermal cycling in a distilled water solution, with temperature oscillations of 5°C, 37°C, and 55°C. The tooth apices were sealed with composite resin, and the surfaces were coated with two layers of nail varnish, leaving a 1 mm margin around the tooth-sealant interface exposed. Subsequently, the samples were immersed in a 1% methylene blue solution (pH 7) for 24 hours, rinsed, and cleaned with running water for 10 minutes. Finally, the samples were sectioned in a buccolingual direction [22].

The dye penetration in the tooth sections was observed under a stereoscopic microscope with a 40x magnification lens. Each section was photographed and evaluated using the Williams and Winters microleakage criterio [23]:

Score 0: No dye penetration.

Score 1: Dye penetration up to one-third of the total fissure height.

Score 2: Dye penetration between one-third and two-thirds of the total fissure height.

Score 3: Dye penetration between two-thirds and the full fissure height.

(Figures 1- 4)

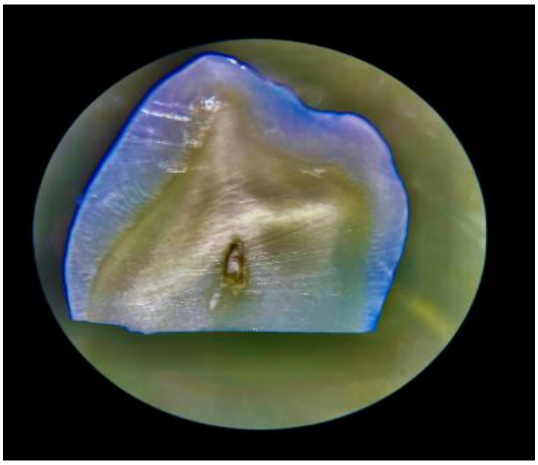

Fig 1: Score 0

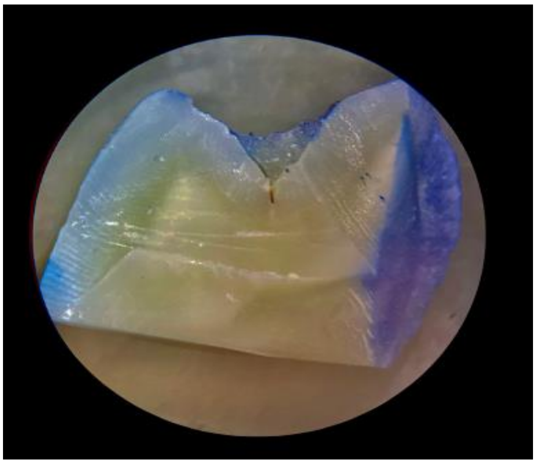

Fig 2: Score 1

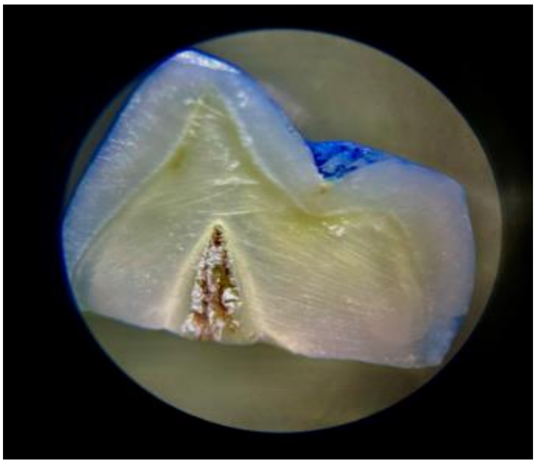

Fig 3: Score 2

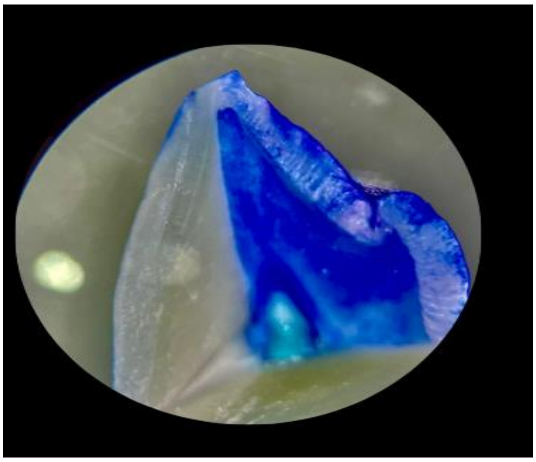

Fig 4: Score 3

Statistical Analysis: the data were analyzed using SPSS version 25.0. (IBM)

- **Kolmogorov-Smirnov test:** Used to assess the normality of the means of dye penetration data for each material.
- **Kruskal-Wallis test:** Used to compare differences in dye penetration among the studied materials.

Results

The distribution of penetration capacity for the Clinpro (3M ESPE) material. A score of 2 predominated, with a frequency of 60%, suggesting that in most cases, the material exhibited moderate to high penetration capacity. A smaller proportion of samples achieved higher scores of 4, with 33.3%, indicating a high penetration capacity. The overall trend highlights the moderate efficacy of the material in terms of penetration, reflecting a variable but acceptable performance of the evaluated material (Table 1)

Table 1: Distribution of Penetration Scores for Clinpro Material (3M ESPE)

Valid score	Frequency	Percentage	Cumulative percentage
2	9	60	60
3	1	6,7	66,7
4	5	33,3	100
Total	15	100	

The of penetration scores for Ketac Molar Easymix (3M ESPE), highlighting its lower penetration capacity compared to the other materials studied. The lowest score (1) predominated, accounting for 53.3% of the cases, followed by a score of 2 in 40% of the cases, reflecting limited penetration. Only 6.7% of the samples achieved a score of 4 (Table 2)

Table 2: Distribution of Penetration Scores for Ketac Molar Easymix Material (3M ESPE)

Valid score	Frequency	Percentage	Cumulative percentage
1	8	53,3	53,3
2	6	40	93,3
4	1	6,7	100
Total	15	100	

Vitremer (3M ESPE) exhibits intermediate performance with a relatively balanced distribution. Score 2 was the most frequent, representing 33.3%, followed by score 4 at 26.7%. Scores 1 and 3 also had notable occurrences, each accounting for 20% of the results. This indicates a variable performance, with penetration capacity ranging from low to high but showing a slight tendency toward intermediate to high values (Table 3)

Table 3: Distribution of Penetration Scores for Vitremer Material (3M ESPE)

Valid Score	Frequency	Percentage	Cumulative Percentage
1	3	20	20
2	5	33,3	53,3
3	3	20	73,3
4	4	26,7	100
Total	15	100	

The results of the Kolmogorov-Smirnov test to assess the normality of the data for Clinpro and Ketac Molar Easymix showed p-values < 0.05 (0.000 and 0.001, respectively) Therefore non-parametric test were used to assess statistical significance for this test.

For the Kruskal-Wallis test Clinpro was used as a gold standard for dye penetration [24, 25]. Results indicate that there are no significant differences in dye penetration between Ketac Molar Easymix and Clinpro (significance value=0.528) or between Vitremer and Clinpro (significance value= 0.332) (Table 4)

Table 4: Results of the Kruskal-Wallis Test for Comparing Materials in Dye Penetration

Null Hypothesis	Test	Sig	Decision
The distribution of Ketak is the same across Clinpro categories	Kruskal-Wallis test for independent samples	0,528	Retain the null hypothesis
The distribution of Vitremeris the same across Clinpro categories	Kruskal-Wallis test for independent samples	0,332	Retain the null hypothesis

Discussion

The evolution and significance of dental sealants in preventive dentistry have demonstrated their effectiveness in both preventing caries on occlusal surfaces and managing initial lesions in proximal areas [21]. However, their implementation still faces significant challenges, with microleakage being one of the primary causes of failure in these treatments this underscores the need to optimize materials and application techniques to ensure a durable and clinically effective seal [26]. Although scientific evidence supports the use of dental sealants, the variability in results regarding the effectiveness of different materials highlights the complexity of the issue and the need for more rigorous and standardized research [27].

In this study, the results revealed a heterogeneous penetration capacity among the evaluated materials. Clinpro (3M ESPE) demonstrated moderate performance, with the majority of samples classified in intermediate penetration categories (score 1), although some cases exhibited maximum effectiveness (score 3). Ketac Molar Easymix (3M ESPE) showed limited performance, with over 90% of the samples in the lowest scores (0 and 1). Vitremer (3M ESPE) displayed a more balanced distribution across all categories, indicating greater clinical adaptability.

Practical differences between the materials are also noteworthy. For instance, Clinpro not only demonstrates good penetration but also offers ease of handling and fluoride release, making it suitable for procedures requiring speed and practicality. In contrast, the study by Juntavee *et al*. [16] highlights UltraSeal XT as a preferred option due to its greater durability, reduced microleakage, and superior sealing ability, though it demands more precise application.

Garg *et al*. [22] found that the application of a self-etching sealant results in lower porosity and better adaptation compared to Clinpro. However, our analysis suggests that Clinpro excels in scenarios requiring the sealing of deep pits and fissures with intact enamel. On the other hand, while Arastoo *et al*. [28] identified Filtek Z350 as one of the most effective materials for reducing microleakage, our data indicate that Clinpro and Vitremer could also be considered as viable options.

Khan *et al*. [29] demonstrated that Vitremer's adaptability to various fissure morphologies highlights its utility in situations where anatomical variability demands good adaptability. This aligns with our analysis, which suggests that, despite not excelling in terms of adhesion, its intermediate and consistent performance positions it as a reliable alternative.

The absence of significant differences in penetration capacity among the evaluated materials may be attributed to the inherent conditions of an *in vitro* study. These include temperature fluctuations, the standardized material application technique, and operator handling differences.

Additionally, the relatively limited sample size (45 teeth) may have constrained the study's ability to detect differences between the materials. While sufficient for a preliminary analysis, a larger sample size could provide greater statistical power and enable the identification of additional variables.

Moreover, the limitations of this *in vitro* study underscore the need to validate these findings through *in vivo* research. Incorporating the analyzed variables, adding shear strength tests, and conducting long-term follow-ups would contribute to more informed material selection and a better understanding of their clinical performance.

Conclusion

No statistically significant differences were found in the marginal penetration capacity among the evaluated materials. However, Clinpro demonstrated moderate to high performance, standing out for its penetration capacity and ease of handling.

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