International Journal of Contemporary Pediatrics
*Karthikeyan V et al. Int J Contemp Pediatr. 2026 Sep;13(9):1698-1703*
https://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20262958

**Original Research Article**

# An in vitro comparative assessment of microleakage among different pit and fissure sealants using dye penetration after thermocycling

**Vishaka Karthikeyan[1], Vinodh Selvaraj[1]*, Madhumitha Purushothaman[1], Manoharan Muthusamy[1], Kamatchi Mohanraj[1], Karthipriya Gurusamy[2]**

[1]Department of Pediatrics, and Preventive Dentistry, Vivekanandha Dental College for Women, Tiruchengode, Namakkal, Tamil Nadu, India
[2]Department of Dentistry and Endodontics, Vivekanandha Dental College for Women, Tiruchengode, Namakkal, Tamil Nadu, India

**Received:** 12 June 2026
**Revised:** 14 July 2026
**Accepted:** 18 August 2026

***Correspondence:**
Dr. Vinodh Selvaraj,
E-mail: vinodhmds@gmail.com



## ABSTRACT

**Background:** Dental caries is a highly prevalent condition among children and adolescents. Primary prevention is more cost-effective and lowers the risk of caries, especially with pit and fissure sealants. To evaluate and compare the microleakage of various pit and fissure sealants by dye penetration.

**Methods:** Eighty caries-free permanent premolars were randomly assigned into four groups (n=20): unfilled resin-based sealant (Clinpro; 3M), filled resin-based sealant (Helioseal F; Ivoclar), nano-filled flowable composite (Filtek Z350XT; 3M) and self-adhering composite (Constic; DMG). Groups 1-3 had their enamel etched and bonded before application whereas Group 4 received self-adhering composite directly. The specimens were thermocycled (1000 cycles, 5°C–55°C) and light-cured. After 24 hours, microleakage was assessed using 2% basic fuchsine dye penetration and evaluated under 50× magnification using a stereomicroscope. Data was analyzed using one-way ANOVA and post hoc tests.

**Results:** Filtek Z350XT had the lowest mean microleakage (0.45), whereas Clinpro had the highest (3.40). There was a statistically significant difference between the groups.

**Conclusions:** The nano-filled flowable composite demonstrated least microleakage, suggesting superior marginal sealing ability and alternative to conventional sealants in pediatric patients.

**Keywords:** Dental caries, Dye penetration, Microleakage, Pit and fissure sealant

## INTRODUCTION

Children and adolescents have an increased risk for dental caries, a highly prevalent intraoral illness that affects people. This risk can be minimized through primary caries prevention.[1] 50% of carious lesions form on the tooth's occlusal surface, though fluoride application has effectively minimized caries in the cementum and smooth surface, it has not been as successful in preventing occlusal caries.[2] Dental caries affects 58% of children aged 12 to 19 and 21% of children aged 6 to 11 in permanent teeth. It has been found that up to 66% of patients develop occlusal caries.[3] Inability of saliva to self-clean and complex anatomy in deep pits and fissures make them ideal environment for microbe retention. When compared to restorative cements, occlusal sealing is more affordable and lowers the risk of cavities.[4] The resin sealant's ability to completely fill in the pits and fissures and stay intact is directly responsible for the sealant therapy's effectiveness.[5] The resin-based sealant has shown better results compared to the glass ionomer-based sealant. The higher viscosity of the conventional composites made them unsuitable as pit and fissure sealants. Composite resins with improved flowability and mechanical properties have been developed.[6] A self- adhering flowable composite has also been developed recently. Hence, the purpose of this study is to assess the efficiency of the recent new flowable composites when employed as pit and Fissure sealants.

## METHODS

Institutional Ethics Committee approval was obtained prior to the commencement of the study. The study was conducted between February 2024 and June 2024 at Vivekanandha Dental College for Women, Tiruchengode, Tamil Nadu, India.

Eighty intact, caries-free permanent maxillary/ mandibular premolars which was extracted for orthodontic or periodontal reasons, teeth with deep groves requiring pits and fissure sealant were included. Teeth with caries, fractures, hypoplasia, restorations were excluded. To avoid dehydration and brittleness, the specimens were stored in saline at room temperature until they were subjected to the procedure.

### *Sample groups*

The samples were randomly divided into 4 groups (n=20).

*Group 1*

Unfilled resin- based pit and fissure sealant (Clinpro; 3M).

*Group 2*

Filled resin-based pit and fissure sealant (Helioseal F, Ivoclar).

*Group 3*

Nano filled flowable composite (Filtek Z350XT, 3M).

*Group 4*

Self-adhering composite (Constic, DMG).

In groups 1, 2 and 3, the occlusal surfaces were dried with cotton pellets and etched for 20 seconds with 37% phosphoric acid gel (Best Etch), followed by rinsing with water for 20 seconds. After etching, the samples were dried with oil-free compressed air and the frosty enamel appearance was assessed.

Two layers of bonding agent (Adper Single Bond 2; 3M) were then applied and light-cured for 20 seconds at 650 mW/cm² using an LED light-curing unit (Woodpecker, Beijing, China), following the manufacturer's instructions. The respective group sealant was subsequently applied from one side of the occlusal surface and carefully guided into each occlusal groove using a dental explorer to prevent void formation. Each specimen was light-cured for 60seconds (20 seconds from each of the occlusal, buccal and lingual).

In Group 4, the material was applied to the pits and fissures and uniformly distributed over the entire surface for 25 seconds, followed by light curing for 60 seconds. The specimens were then immersed in distilled water and incubated at 37°C for 24 hours. Thermocycling was subsequently performed for 1000 cycles between 5°C and 55°C, with a dwell time of 30 seconds in each bath and a transfer time of 15 seconds.

The root apex was sealed with sticky wax prior to the dye penetration test and the teeth was covered with two coats of nail polish, except for 1 mm margin around the sealant. To allow dye diffusion into potential spaces between the sealant and the tooth, the teeth were submerged in 2% basic fuchsine dye for 24 hours at room temperature. The roots were cut from the crown at the cementoenamel junction after the teeth were thoroughly washed with water. After drying, the samples were mounted in acrylic resin and a high-speed straight handpiece with diamond disk, was used to separate the samples buccolingually.

Sectioned teeth were evaluated below a stereomicroscope at 50X magnification with image analysis software. Two blinded observers assessed dye penetration qualitatively using the following criteria for microleakage,

The scores were 0: No dye penetration. 1: Dye penetration up to one-fourth of fissure. 2: Dye penetration up to one-half of fissure. 3: Dye penetration up to three-fourth of fissure. 4: Complete dye penetration.

### *Statistical analysis*

The statistical analysis was done using SPSS (Statistical Package for the Social Sciences, SPSS Inc., v.16). Intergroup comparison for microleakage of four different materials were assessed using one way ANOVA, followed by post hoc test. The level of significance for was p value of $<0.05$.

## RESULTS

The mean and standard deviation of all four groups is shown (Figure 1). Group 1- Clinpro; 3M (3.40) had the highest mean, while Group 3- Filtek Z350XT, 3M (0.45) had the lowest. Group 2- Helioseal F, Ivoclar (1.399) had the highest standard deviation, while Group 3- Filtek Z350XT (0.510) had the lowest. ANOVA test (Table 1) reveals there is a statistically significant difference when applied between and within the groups.

Tukey's HSD post hoc test (Table 2) for intergroup comparison of microleakage scores among the four groups revealed that the comparison between the unfilled resin-based pit and fissure sealant and both the nano-filled flowable composite and the self-adhering composite showed highly statistically significant differences (p=0.000).

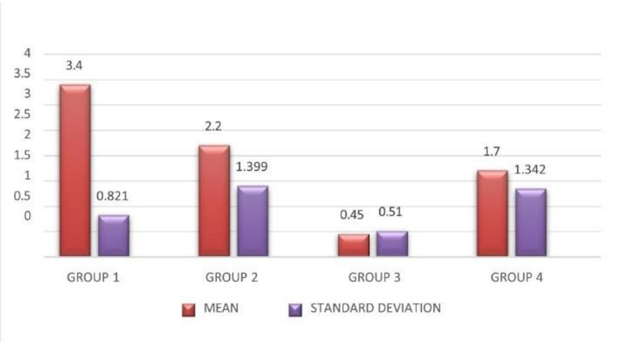


**Figure 1: Mean and standard deviation among four groups.**

A statistically significant difference was also observed between the unfilled resin-based pit and fissure sealant and the filled resin-based pit and fissure sealant (p=0.004).

However, the comparison between the nano-filled flowable composite and the self-adhering composite did not show a statistically significant difference (p=0.467). A statistically significant difference was noted between the filled resin-based pit and fissure sealant and the self-adhering composite (p=0.003). Overall, microleakage was highest in Clinpro (unfilled resin-based pit and fissure sealant) and lowest in Filtek Z350XT (nano-filled flowable composite) (Figure 2).

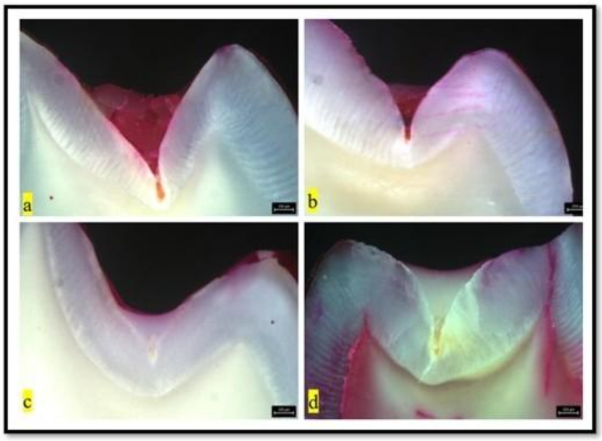


**Figure 2 (a-d): Microleakage of unfilled resin- based pit and fissure sealant, filled resin-based pit and fissure sealant, nano filled flowable composite, self-adhering composite.**

**Table 1: Comparison for microleakage of four different materials.**

| Source of variation | Sum of Squares | Degree of freedom | Mean squares | F ratio | P value |
|---|---|---|---|---|---|
| **Between groups** | 89.538 | 3 | 29.846 | 25.443 | 0.000* |
| **Within groups** | 89.150 | 76 | 1.173 | | |

*p<0.05 level is statistically significant.

**Table 2: Intergroup comparison for microleakage of four different materials.**

| Group (I) | GROUP (J) | Mean Difference | Std. Error | P value |
|---|---|---|---|---|
| **Group 1** | Group 2 | 1.200* | 0.342 | 0.004* |
| | Group 3 | 2.950* | 0.342 | 0.000* |
| | Group 4 | 1.700* | 0.342 | 0.000* |
| **Group 2** | Group 1 | -2.950* | 0.342 | 0.000* |
| | Group 3 | -1.750* | 0.342 | 0.000* |
| | Group 4 | -1.250* | 0.342 | 0.003* |
| **Group 3** | Group 1 | -1.200* | 0.342 | 0.004* |
| | Group 2 | 1.750* | 0.342 | 0.000* |
| | Group 4 | 0.500 | 0.342 | 0.467* |
| **Group 4** | Group 1 | -1.700* | 0.342 | 0.000* |
| | Group 2 | -0.500 | 0.342 | 0.467* |
| | Group 3 | 1.250* | 0.342 | 0.003* |

*p<0.05 level is statistically significant.

## DISCUSSION

Dental caries is a common disease affecting individuals across all geographic regions, irrespective of age or socioeconomic status.[7] According to Safwat et al nano-flowable composites performed significantly better and showed promising results, demonstrating improved microleakage and interfacial morphology compared to conventional pit and fissure sealants.[8] Nearly 50% of all carious lesions in children aged 6–14 years occur on occlusal surfaces, as reported by Spolsky et al.[9] Nagono et al observed that approximately 42% of fissures have narrow occlusal openings that change shape as they extend deeper into the tooth, making it difficult for an explorer to reach the base of the fissure. Since penetration is directly related to viscosity, the primary clinical characteristic of pit and fissure sealants that determines effective sealing in deep and narrow fissures is flowability. Recently developed sealant systems demonstrate improved viscosity and enhanced penetration depth. Newer low-viscosity composites, such as flowable composites and nanocomposites, exhibit superior flow properties compared to conventional high-viscosity composites.[10] Nanocomposites have been reported to provide better marginal sealing ability than conventional pit and fissure sealants.

In the present study, 80 non-carious premolars were selected considering they are frequently extracted for orthodontic purposes and their occlusal morphology remains intact. The collected samples underwent pumice prophylaxis, as Ansari et al reported that pumice prophylaxis improves sealant retention and reduces microleakage.[12] Sample selection criteria were based on studies by Stritikus et al and Burrow et al.[12,13] Sealants were applied without enameloplasty, similar to studies conducted by Prabhakar et al and Bahrololoomi et al.[14,15]

The samples were stored in saline to prevent dehydration.[16] In Groups 1, 2 and 3, acid etching was performed for 20 seconds using 37% phosphoric acid, which enhances sealant bonding, as reported by Hannig et al.[17] Resin-based sealants are hydrophobic in nature; therefore, acid etching produces a honeycomb-like pattern on enamel, increasing surface roughness and allowing deeper resin penetration to establish micromechanical retention. The durability of the sealant primarily depends on this mechanical bonding, as it does not form a hydrolytically stable bond. Waggoner et al recommended etching for 15–20 seconds to improve sealant retention.[18]

Following etching, bonding agents were applied to the enamel surface. Studies by Bahrololoomi et al and Dukic and Glavina reported that the application of a dentin bonding agent prior to sealant placement did not significantly influence microleakage.[15,19] However, Tulunoğlu et al and Pérez-Lajarín et al found that bonding agents improved fissure sealant retention and reduced microleakage.[20,21]

Sealants were then applied according to the manufacturers' instructions and light-cured for 60 seconds (20 seconds each from occlusal, buccal and lingual directions). Stritikus et al and Owens et al concluded that conventional light curing is an appropriate method for Class I composite restorations.[12] Wadhwa et al suggested that self-adhering composites can serve as alternatives to traditional pit and fissure sealants when time constraints exist.[22] Gomes-Silva et al reported that self-adhering composites and adhesives reduce the risk of saliva contamination.[23] In Group 4, the self-adhering composite was placed and light-cured for 60 seconds, similar with Rahimian-Imam et al methodology.[24]

Sealants are continuously exposed to thermal and pH fluctuations in the oral environment.[25] Marginal gaps may form due to differences in the coefficients of thermal expansion between resin materials and tooth structure.[26] Based on investigations by Penugonda et al and Styner et al specimens in the present study were thermocycled for 1000 cycles between 5°C and 55°C, with a dwell time of 30 seconds and a transfer time of 15 seconds.[27,28] The coefficient of thermal expansion ranges from 25–60 ppm/°C for resin materials, compared to 11.4 ppm/°C for enamel and 8 ppm/°C for dentin. Therefore, thermocycling is an effective method for simulating long-term thermal stress on restorative materials.[29]

The specimens were immersed in 2% basic fuchsin dye for 24 hours. Dye penetration is a simple, cost-effective and widely used method for evaluating microleakage.[30] The dye diffuses into any gaps between the tooth and sealant interface. This method was employed as described by Arastroo et al.[3]

In the present study, nanocomposites exhibited the least microleakage, followed by the self-adhering composite and filled resin-based sealant, while the unfilled resin-based sealant showed the highest microleakage. All materials demonstrated some degree of microleakage, consistent with the findings of Theodoridou et al who reported that all restorative materials exhibit marginal leakage to some extent.[31] Nano-filled composites exhibit lower polymerization shrinkage and higher microhardness. Reduced polymerization shrinkage may contribute to improved marginal adaptation and decreased microleakage.[32]

Constic, a self-adhering flowable composite, contains the adhesive monomer glycerol phosphate dimethacrylate (GPDM), which facilitates simultaneous etching and bonding.[33] Oz et al reported that self-adhering flowable composites combined with adhesive resins demonstrate improved dentin bond strength and marginal sealing.[34] However, Oz et al in a two-year follow-up study, concluded although self-adhesive flowable composites performed satisfactorily, they exhibited inferior marginal adaptation compared to highly filled conventional resin composites.[35] The stereomicroscope was used to evaluate the enamel-sealant interface, as it is a simple and cost-effective method for assessing resin tag formation and dye penetration.[36] Similar methodologies were adopted by Dixit et al and Singh et al.[1,7]

The limitations of this study include it's in vitro design, which does not completely simulate clinical conditions. Sealant performance may vary depending on fissure morphology and intraoral factors such as saliva contamination. No enameloplasty was performed, which may have influenced microleakage outcomes, as suggested by Zervou et al.[37] Additionally, moisture control is easier to maintain under laboratory conditions than in clinical settings. Therefore, further in vivo studies are required to evaluate long-term clinical performance under variable oral conditions.

## CONCLUSION

The nano-flowable composite exhibited the lowest microleakage, while the unfilled resin-based pit and fissure sealant demonstrated the highest microleakage. The superior marginal sealing ability demonstrated by the nano-flowable composite suggests its potential as a reliable alternative to conventional pit and fissure sealants in pediatric patients.

*Funding: ICMR funded*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

1. Singh S, Pandey RK. An evaluation of nanocomposites as pit and fissure sealants in child patients. J Indian Soc Pedo Preventive Dentistry. 2011;29(4):294.
2. Tehrani MH, Birjandi N, Nasr E, Shahtusi M. Comparison of microleakage of two materials used as fissure sealants with different methods: an in vitro study. Int J Prevent Med. 2014;5(2):171.
3. Arastoo S, Behbudi A, Rakhshan V. In vitro microleakage comparison of flowable nanocomposites and conventional materials used in pit and fissure sealant therapy. Front Dentist. 2019;16(1):21.
4. Ratnaditya A, Kumar MM, Jogendra SS, Zabirunnisa M, chowdhary Kopuri RK. Clinical evaluation of hydrophobic and hydrophillic pit and fissure sealants-A two year follow-up study. J Young Pharma. 2015;7(3):171.
5. Bahrololoomi Z, Soleymani A, Heydari Z. In vitro comparison of microleakage of two materials used as pit and fissure sealants. J Dental Res, Den Clin, Dent Prosp. 2011;5(3):83.
6. Shafik O. Comparison between nanocomposites and conventional pit and fissure sealants an in vitro study. J Orofacial Res. 2022;8:34-7.
7. Dixit A, Awasthi N, Jha S, Suprakasam S, Penumatsa NV, Vijayan AA. Assessment of penetration depth and microleakage of different pit and fissure sealants using dye penetration method: an in vitro study. J Contemporary Dental Practice. 2021;22(8):890-3.
8. Smales RJ, Gao W, Ho FT. In vitro evaluation of sealing pits and fissures with newer glass-ionomer cements developed for the ART technique. J Clin Pediatr Dent. 1997;21(4):321-3.
9. Spolsky VW. Epedemiology of dental caries: The impact of sealants viewpoints on preventive dentistry, the role of pit and fissure sealants. Johnson and Johnson Company, Woodbridge, Massachusetts, Medical Dynamics. 1978
10. Nagano T. Relation between the form of pit and fissure and the primary lesion of caries. Shika Gakuho. 1960;60:80-90.
11. Ansari G, Oloomi K, Eslami B. Microleakage assessment of pit and fissure sealant with and without the use of pumice prophylaxis. Int J Paediat Dent. 2004;14(4):272-8.
12. Stritikus J, Owens B. An in vitro study of microleakage of occlusal composite restorations polymerized by a conventional curing light and a PAC curing light. J Clin Pediatr Dent 2000;24(3):221–7.
13. Burrow MF, Burrow JF, Makinson OF. Pits and fissures; Relative space contribution in fissures from sealants, prophylaxis pastes and organic remnants. Aust Dent J. 2003;48(3):1779.
14. Prabhakar AR, Murthy SA, Sugandhan S. Comparative evaluation of the length of resin tags, viscosity and microleakage of pit and fissure sealants–an in vitro scanning electron microscope study. Contemporary Clin Dent. 2011;2(4):324-30.
15. Bahrololoomi Z, Soleymani A, Heydari Z. In vitro comparison of microleakage of two materials used as pit and fissure sealants. J Dental Res, Dental Clin, Dental Prosp. 2011;5(3):83.
16. Butail A, Dua P, Mangla R, Saini S, Chauhan A, Rana S. Evaluation of marginal microleakage and depth of penetration of different materials used as pit and fissure sealants: an in vitro study. Int J Clin Pediat Dent. 2020;13(1):38.
17. Hannig M, Gräfe A, Atalay S, Bott B. Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents. J Dent. 2004;32(1):75-81.
18. Waggoner WF, Siegal M. Pit and fissure sealant application: updating the technique. J American D Association. 1996;127(3):351-61.
19. Dukić W, Glavina D. Clinical evaluation of three different materials for fissure sealing after 12 months. Acta medica Croatica: casopis Hravatske akademije medicinskih znanosti. 2006;60(3):209-14.
20. Tulunoğlu Ö, Bodur H, Üçtaşli M, Alacam AL. The effect of bonding agents on the microleakage and bond strength of sealant in primary teeth. J Oral Rehabil. 1999;26(5):436-41.
21. Pérez-Lajarín L, Cortés-Lillo O, García-Ballesta C, Cózar-Hidalgo A. Marginal microleakage of two fissure sealants: a comparative study. J Dentist Children. 2003;70(1):24-8.
22. Wadhwa S, Nayak UA, Kappadi D, Prajapati D, Sharma R, Pawar A. Comparative clinical evaluation of resin-based pit and fissure sealant and self-adhering flowable composite: an in vivo study. Int J Clin Pediat Dentist. 2018;11(5):430.
23. Gomes-Silva JM, Torres CP, Contente MM, Oliveira MA, Palma-Dibb RG, Borsatto MC. Bond strength of a pit-and-fissure sealant associated to etch-and-rinse and self-etching adhesive systems to saliva-contaminated enamel: individual vs.

simultaneous light curing. Brazilian Dental J. 2008;19:341-7.
24. Rahimian-Imam S, Ramazani N, Fayazi MR. Marginal microleakage of conventional fissure sealants and self-adhering flowable composite as fissure sealant in permanent teeth. J Dentist (Tehran, Iran). 2015;12(6):430.
25. Kern M, Thompson VP. Influence of prolonged thermal cycling and water storage on the tensile bond strength of composite to NiCr alloy. Dental Materials. 1994;1;10(1):19-25.
26. Versluis A, Douglas WH, Sakaguchi RL. Thermal expansion coefficient of dental composites measured with strain gauges. Dental Materials. 1996;12(5-6):290-4.
27. Penugonda B, Scherer W, Cooper H, Kokoletsos N, Koifman V. Bonding Ni-Cr Alloy to Tooth Structure with Adhesive Resin Cements. J Esthetic Restorative Dentistry. 1992;4:26-9.
28. Styner D, Scherer W, LoPresti J, Penugonda B. Bonding composite to glass lonomer with adhesive resin cements. J Esthetic Restorative Dentistry. 1992;4:13-5.
29. McCabe JF, Walls AW, editors. Applied dental materials. John Wiley & Sons. 2013.
30. Shingare P, Chaugule V. An in vitro microleakage study for comparative analysis of two types of resin-based sealants placed by using three different types of techniques of enamel preparation. Int J Clin Ped Dent. 2021;14(4):475.
31. Theodoridou-Pahini S, Tolidis K, Papadogiannis Y. Degree of microleakage of some pit and fissure sealants: an in vitro study. Int J Paediat Dentist. 1996;6(3):173-6.
32. Ersoy M, Civelek A, L'hotelier E, Say EC, Soyman M. Physical properties of different composites. Dental Mat J. 2004;23(3):278-83.
33. Pruthi T, Pandit IK, Gugnani N, Gupta M. Bonding technologies in young permanent molars: a case series. Int J Clin Pediat Dentis. 2023;16(1):159.
34. Ozel Bektas O, Eren D, Akin EG, Akin H. Evaluation of a self-adhering flowable composite in terms of micro-shear bond strength and microleakage. Acta Odontologica Scandinavica. 2013;71(3-4):541-6.
35. Oz FD, Meral E, Gurgan S. Does a self-adhesive flowable resin composite perform similarly to highly filled and conventional flowable resin composites in occlusal cavities. A 2-year Follow-up Study. J Adhesive Dentist. 2021;1;23(6):587.
36. Rastogi A, Kamble V. Comparative analysis of the clinical techniques used in evaluation of marginal accuracy of cast restoration using stereomicroscopy as gold standard. J Advan Prosthodont. 2011;3(2):69.
37. Zervou C, Kugel G, Leone C, Zavras A, Doherty E, White G. Enameloplasty effects on microleakage of pit and fissure sealants under load: an in vitro study. J Clin Pediat Dent. 2000;24(4):279-85.

**Cite this article as:** Karthikeyan V, Selvaraj V, Purushothaman M, Muthusamy M, Mohanraj K, Gurusamy K. An in vitro comparative assessment of microleakage among different pit and fissure sealants using dye penetration after thermocycling. Int J Contemp Pediatr 2026;13:1698-703.