Scripta Scientifica Medicinae Dentalis, 2023;9(2):27-34
DOI: 10.14748/ssmd.v9i2.9364

ISSN 2367-7236 (Print)
ISSN 2367-7244 (Online)

REVIEWS

MICROLEAKAGE IN COMPOSITE AND CERAMIC RESTORATIONS—A REVIEW OF STAINING PROTOCOLS

Sabina Keremedchieva, Stefan Peev

Department of Periodontology and Dental Implantology, Faculty of Dental Medicine, Medical University of Varna, Bulgaria

ABSTRACT

INTRODUCTION: Various kinds of dye infiltration protocols for the inspection of microleakage in composite and ceramic restorations have been described in the literature.

AIM: The aim of this article is to briefly present the different staining methods used to inspect microleakage in composite and ceramic restorations.

MATERIALS AND METHODS: This review includes articles searched without date restriction in the Medline/PubMed database. A variety of keywords and their combinations were used: "microleakage", "staining methods", "ceramic restorations", "fuchsine", "methylene blue", "silver nitrate", "composite restorations", "dye infiltration".

RESULTS: The review was based upon 71 references. The literature provided information regarding the different staining protocols used for the inspection of microleakage in composite and ceramic restorations.

CONCLUSION: Numerous types of protocols have been reported. The most common staining agents used for research purposes are fuchsine, methylene blue, and silver nitrate. In order to make the results between different experimental studies comparable, it will be beneficial to have standardized methodologies.

Keywords: *microleakage, direct restorations, indirect restorations, staining methods, fuchsine, methylene blue, silver nitrate*

INTRODUCTION

Microleakage is recognized as a phenomenon which has many different aspects and features (1). The definition for microleakage described in the literature is "the clinically undetectable passing of fluids, bacteria, molecules and ions between the tooth preparation walls and the restorative material" (2).

Microleakage around the restorative margins may lead to the failure of the restoration. The formation of a marginal gap is linked to caries and pulpal pathology (3,4). Microleakage may also cause sensitivity and poor aesthetics (5).

Adequate sealing of the interface between the cavity walls and the restoration is essential in order to minimize microleakage (6). The reduction of microleakage to the greatest extent possible guarantees the long-term success of the treatment (7). No restorative material is able to fully eliminate microleakage, especially at the dentine-cementum margins (8). Larger areas of exposed dentine during tooth preparation increase the prospects of microleakage. (9) Enamel margins allow greater marginal sealing than dentine-cementum margins (10). One of the techniques, which aim to reduce microleakage, is *immediate dentin sealing* by Pashley et al. (11). It is also called *prehy-*

Address for correspondence:
Sabina Keremedchieva
Faculty of Dental Medicine
Medical University of Varna
84 Tzar Osvoboditel Blvd
9002 Varna, Bulgaria
e-mail: sabina.keremedchieva@gmail.com

Received: August 18, 2023
Accepted: September 30, 2023

bridization or *dual bonding technique* (12). Immediate dentine sealing is a method which seals exposed dentin tubules immediately after tooth preparation, but some aspects of the technique need further investigation (13,14). Unfortunately, despite the innovations in dental materials and techniques, complete marginal sealing is yet to be achieved (15).

The sealing properties of restorations can be tested both in vivo and in vitro. A wide variety of testing protocols and methodologies is reported in the literature (16,17). No standardization of microleakage inspection protocols has been established yet (18). Dye infiltration using different staining agents is one of the most popular techniques for the assessment of marginal sealing (19,20,21). It visualizes in color the degree of microleakage at the tooth-restoration interface without using any specific chemical reactions or hazardous substances (22,23). The most common staining solutions used in experimental studies are fuchsine, methylene blue, and silver nitrate (18).

Specimens usually undergo thermocycling and mechanical loading before executing the staining protocol. Thermocycling refers to the exposure of the dental materials and teeth to fluctuations in temperature (24) in order to imitate the conditions in the oral cavity while eating and drinking (25–30). On the other hand, in vitro mechanical loading attempts to mimic the forces applied to the teeth during masticatory function or grinding. This may lead to deterioration of the restorative margins and materials, which corresponds to a prolonged exposure of the teeth to these forces in the mouth (31–34).

AIM

The main objective of this literature review is to present a variety of staining protocols for the inspection of microleakage in composite and ceramic restorations used in experimental studies.

MATERIALS AND METHODS

This literature review includes data from scientific articles. Articles searched without date restriction in the Medline/PubMed database were taken into consideration. A variety of keywords and their combinations were used: “microleakage”, “staining methods”, “ceramic restorations”, “fuchsine”, “methylene blue”, “silver nitrate”, “composite restorations”, “dye infiltration”.

RESULTS

The review is based upon 71 references. The collected data provided information on the different staining protocols used in experimental research studies inspecting the microleakage of composite or ceramic restorations.

Protocols Featuring Fuchsine Staining

Fuchsine is an aniline dye with purplish-red color, when dissolved in water. It has a wide variety of uses such as: industrial dye, disinfectant, and staining agent of biological tissues (35,36).

According to the literature, one of the most frequently applied protocols in fuchsine staining is the use of 0.5% fuchsine solution for a total of 24 h.

Brian J Kenyon et al. researched microleakage in indirect composite restorations. The specimens were subjected to thermocycling for a total of 1000 cycles with a temperature range from 5°C to 55°C. The samples were covered with a layer of nail varnish and submerged in 0.5% basic fuchsine solution for 24 h. After thorough irrigation with water, the teeth were separated and investigated under a stereomicroscope with 10x magnification to register staining penetration (37).

Bulent Uludag et al. observed microleakage scores in ceramic inlays. Their samples were subjected to 100,000 cycles of mechanical loading of 50N at 1.6 Hz in 37°C water. The prepared teeth were then stained with 0.5% basic fuchsine solution for 24h. The samples were separated using a low-speed diamond blade and dye penetration was evaluated (38).

Kumbuloglu Ovul et al. inspected microleakage in indirect restorations using the following protocol: the samples were subjected to thermal cycling for a total of 6000 cycles with temperature range between 5°C and 55°C for 30 sec each. The specimens were then stained with 0.5% basic fuchsine solution for 24 h. Microleakage scores were evaluated using a stereomicroscope with 40x magnification (39).

Esra Uzer Celik et al. subjected their samples to thermocycling for a total of 1000 cycles with temperature ranging between 5°C and 55°C for 30 sec each. An application of 1 layer of nail varnish followed, leaving 1 mm around the margins of the restorations. The specimens were immersed in 0.5% basic fuchsine solution for 24 h. Microleakage was evaluated under a stereomicroscope at 30x magnification (40).

P. Hahn et al. did an experimental study on microleakage in porcelain inserts. Their samples were subjected to thermocycling for a total of 1000 cycles with temperature range between 5°C and 55°C for 30 sec each. Then the prepared teeth were immersed in 0.5% basic fuchsine solution for 24 h. After sectioning the samples, microleakage scores were evaluated under a stereomicroscope at 40x magnification (41).

Ali Eslambol Nassaj et al. prepared their specimens in the following way: the teeth were exposed to 500 thermal cycles with temperature ranging between 5°C and 55 °C. They sealed the apex area with composite and applied 2 layers of varnish on the teeth, avoiding the restorative margins. Then the specimens were submerged in 0.5 % fuchsine solution for 24 h and mounted in a polyester resin mold (42).

Other fuchsine protocols feature the studies described below.

Rita de Cássia Silva Duquia et al. inspected microleakage in indirect composite inlays in their experimental study. Their samples were submitted to thermolcycling and a layer of nail varnish was applied afterwards. The specimens were then stained with 2% basic fuchsine dye for 24 h. A precise sectioning of the samples was followed by a microscopic inspection under 40x magnification (43).

Saturnino Calabrez-Filho et al. subjected their sample teeth restored with indirect composite inlays to thermocycling. A total of 1000 thermal cycles were executed with temperature ranging between 5°C and 55°C for 15 sec each. After that, the teeth were dyed with 0.5% basic fuchsine solution for 12 h, washed under running water and separated through the center of the inlays. Microleakage inspection was performed afterwards (44).

D. Ziskind et al. thermocycled 750 times their sample teeth which were restored with indirect composite restorations. The specimens were stained with 2% basic fuchsine solution and then separated in 3 planes. Penetration scores were microscopically evaluated (45).

Martin Rosentritt et al. inspected microleakage in teeth restored with ceramic inlays.

The research team prepared their specimens using the following protocol: the samples were loaded with an axial force of 50N 106 times and thermocycled 6000 times with temperature ranging between 5°C and 55°C for 2 min each. Then the teeth were covered with nail varnish excluding the preparation margins and immersed in 0.5% basic fuchsine solution for 16h. Microleakage was inspected microscopically under 12x magnification (46).

G. Schmalz et al. researched microleakage in ceramic inlays using the following protocol: the specimens were subjected to thermocycling 5000 times with temperature range between 5°C and 60°C for 60 sec each and mechanical loading 500000 times with 72N force. All of the teeth were covered with a layer of nail varnish, except for the area of the preparation margins. Then they were immersed in 0.5% basic fuchsine solution for 16 h. After thorough irrigations with water, they were sectioned in mesiodistal direction and microleakage scores were microscopically evaluated (47).

A. N. Ozturk et al. inspected microleakage in teeth restored with ceramic inlays. The samples were thermocycled 100 times with temperature range between 5°C and 55°C for 30 sec each. Then they were coated with nail varnish, leaving 1 mm around the edges of the restoration. The specimens were immersed in 0.2% basic fuchsine solution for 24 h, water irrigated and sectioned in vestibulolingual direction. Microleakage scores were evaluated using a stereomicroscope (48).

Alireza Keshvad et al. inspected microleakage in leucite-reinforced ceramic inlays using the following methodology: the samples were thermocycled 5000 times with temperature range between 5°C and 55°C for 60 sec each. Nail varnish was applied all over the teeth except for 2 mm around the preparation margins. The specimens were then immersed in 0.2% basic fuchsine solution for 24 h. Microleakage was registered using a stereomicroscope at 40x magnification (49).

U. Hasanreĭsoğlu et al. inspected microleakage in indirect composite inlays using the following protocol: the samples were thermocycled and submerged in a basic fuchsine solution for 24 h. Then they were sectioned in mesiodistal direction. The penetration of the staining agent was evaluated using a stereomicroscope (50).

L. Karaağaclioğlu et al. inspected microleakage in indirect composite inlays. The prepared samples were subjected to thermal cycling, followed by an immersion in basic fuchsine solution for 24 h. Microleakage score was evaluated using a stereomicroscope (51).

M. Thordrup et al. used sample teeth restored with ceramic and composite inlays in their experimental study. The samples were subjected to thermocycling for 2500 cycles with temperature range between 12°C and 62°C. After nail varnish application excluding the 1 mm area around the restoration margins, the teeth were immersed in basic fuchsine solution for 24 h. Then they were irrigated under running water and sectioned. Microleakage was microscopically assessed at 125x or 250x magnification (52).

Protocols Featuring Methylene Blue Staining

Methylene blue is a dye and a drug used in medicine (53, 54). When dissolved in water, it has a deep blue color and is widely used as a staining agent and an indicator (55).

Aguir Mabrouk Najet et al. used the following protocol to prepare their samples for microleakage inspection in composite restorations: the specimens were subjected to thermocycling with temperature range from 6°C to 60°C. A daily cycle of 45 min at 6°C, followed by 45 min at 60°C was executed 4 times in a row for a total of 5 consecutive days. Afterwards the specimens were covered with 2 coats of varnish and submerged in 2% methylene blue solution for 48 h. In the end, the samples were rinsed with water for 15 min. Microleakage was evaluated under a stereomicroscope 6.5x zoom by 2 observers (56).

David A. Gerdolle et al. inspected microleakage in indirect composite inlays using the following staining protocol: half of the total number of inlays included in the study underwent 2000 thermal cycles with temperature range from 5°C to 55°C. The other half was not subjected to thermocycling. All the specimens were stained with 1% methylene blue solution for 48 hours and inspected microscopically (57).

Fereshteh Shafiei et al. used the following methodology for microleakage evaluation in indirect restorations. The samples were immersed in 1% methylene blue solution for 24 h, followed by precise sectioning and inspection under a stereomicroscope with 20x magnification (58).

For the inspection of microleakage in indirect restorations Everton Ribeiro Dos Santos and Adair Luis Busato thermally cycled their samples for 30 sec at 5°C and 30 sec at 55°C, executing a total of 500 cycles. Afterwards, the samples were placed in methylene blue solution for 24 h and inspected under stereomicroscope by 2 blinded observers (59).

Rogéli Tibúrcio Ribeiro Peixoto et al. inspected microleakage in indirect composite inlays in their experimental study. The samples were thermocycled 500 times with temperature range from 5°C and 55°C. Afterwards they were stained with 2% methylene blue dye for 24 h. Microleakage score was microscopically evaluated (60).

Cristiane Soares Mota et al. prepared their samples in the following manner: they applied varnish over teeth restored with ceramic inlays. The samples were then dyed with 2% methylene blue for 8 h and washed under running water. The research team made 3 sections through the restorations and microleakage was inspected microscopically (61).

S. Deliperi et al. did the following: the restored teeth were thermocycled 500 times at temperatures of 5°C and 55°C. The apex area of the teeth was covered with wax and the crown was coated with varnish, except for the restoration margins. The samples were embedded in acrylic resin. All specimens were immersed in 0.5% methylene blue solution for 24 hours. The teeth were rinsed with water and sectioned. Dye penetration was examined by two specialists under a stereomicroscope at 20x magnification (62).

Tapan Satish Yeolekar et al. prepared their sample teeth in the following way: 200 thermal cycles with a temperature range between 5° to 55°C were executed. Then the teeth were coated with 2 layers of varnish leaving a 2 mm border around the restorative margins. The specimens were submerged in 1% methylene blue solution for 24 hours. After washing under running water, the teeth were sectioned in mesiodistal direction and microleakage was evaluated under a stereomicroscope at 10x magnification (63).

Protocols Featuring Silver Nitrate Staining

Silver nitrate is an inorganic compound used as an astringent, disinfectant, a precursor in the making of other silver-containing compounds, in the making of photographic films and as a biological staining agent (64,65). It becomes black, when exposed to light or organic material.

In order to evaluate marginal microleakage in indirect onlay restorations, Isabel Sinche-Ccahuana et al. used the following protocol for their in vitro study: the samples were subjected to thermocycling 10000 times with temperature range between 5°C and 55°C. The restored teeth were stained with a silver nitrate solution for 24 h in darkness, rinsed for 5 min, and placed in a photoreflective solution under fluorescent light for 8 h. Afterwards the specimens were precisely cut mesiodistally and observed under a stereomicroscope with 20x magnification to inspect the microleakage in the area of the cervix (66).

Terry J. Fruits et al. inspected microleakage using the following methodology: their samples were covered in nail polish, except for 1 mm around the restorative margins. The specimens were stained with silver nitrate solution for 2 h in darkness, irrigated with water for 1 min and then immersed in radiographic developing solution for 6 h under fluorescent lighting. After thorough rinse and sectioning of the samples, dye penetration was evaluated microscopically (67).

E. A. Hasegawa et al. did an experimental study on microleakage in indirect composite inlays. Their samples were subjected to thermal cycling 300 times with temperature ranging between 5°C and 50°C. The specimens were then immersed in silver nitrate for 2 h with the absence of light, rinsed with running water and placed in photo-developing solution for 6 h under fluorescent lighting. The teeth were precisely sectioned and investigated for microleakage using a microscope with 100x magnification (68).

W. Romão Jr. et al. used teeth with ceramic inlays in their experimental study. The samples underwent mechanical loading of 100000 cycles with 78N force and 700 thermal cycles with temperature ranging from 5°C to 55°C. Then they were placed in silver nitrate in darkness, precisely sectioned in mesiodistal direction and inspected for dye penetration with 40x magnification on an optical microscope (69).

Ella A. Naumova et al. inspected microleakage in teeth restored with ceramic inlays.

The samples were thermocycled 5000 times with temperature ranging from 5°C to 55°C. They were covered with 2 layers of superglue excluding a 0.5 mm border around the restorative margins. The specimens were immersed in silver nitrate for 6 h, illuminated and developed. Then they were embedded in Technovit 9100, sectioned and microleakage was evaluated under a scanning electron microscope at 500x magnification (70).

José Roberto de Oliveira Bauer et al. prepared their samples Subsequently, the crowns of the specimens from all groups were coated with three layers in the following way: the specimens were coated with 3 layers of varnish, excluding 1 mm area around the restorative margins. Then they were immersed in a 50% silver nitrate solution in darkness for 2 hours. After rinsing under running water, they were immersed in a photo-developing solution and exposed to fluorescent light for 8 hours. The samples were sectioned and microscopically evaluated (71).

DISCUSSION

Staining protocols for microleakage inspection are not standardized (18). The research team decides which protocol to use in their experimental study. There is a wide variety of methodologies to choose from.

CONCLUSION

Numerous types of staining protocols for microleakage evaluation have been reported in the literature. The most common dyes used for research purposes are fuchsine, methylene blue, and silver nitrate. In order to make the results between different studies comparable, it will be beneficial to have standardized protocols (18).

REFERENCES

1. Cox CF. Microleakage related to restorative procedures. Proc Finn Dent Soc. 1992;88 Suppl 1:83-93.
2. Rathi SD, Nikhade P, Chandak M, Motwani N, Rathi C, Chandak M. Microleakage in composite resin restoration-a review article. J Evol Med Dent Sci. 2020;9(12):1006-11. doi:10.14260/jemds/2020/216.
3. Roulet JF. Marginal integrity: clinical significance. J Dent. 1994;22 Suppl 1:S9-12. doi: 10.1016/0300-5712(94)90164-3.
4. Ferracane JL. Resin composite--state of the art. Dent Mater. 2011;27(1):29-38. doi: 10.1016/j.dental.2010.10.020.

5. Bajabaa S, Balbaid S, Taleb M, Islam L, Elharazeen S, Alagha E. Microleakage evaluation in class v cavities restored with five different resin composites: in vitro dye leakage study. Clin Cosmet Investig Dent. 2021;13:405-11. doi: 10.2147/CCIDE.S331426.

6. Cox CF. Microlekkage: evaluatie en behandeling [Microleakage: evaluation and treatment]. Ned Tijdschr Tandheelkd. 1990;97(3):98-100.

7. Fabianelli A, Pollington S, Davidson CL, Cagidiaco MC, Goracci C. The relevance of microleakage studies. Int Dent SA. 2007; 9(3):64-74.

8. Majeed A, Osman YI, Al-Omari T. Microleakage of four composite resin systems in class II restorations. SADJ. 2009;64(10):484-8.

9. Pashley DH. Clinical considerations of microleakage. J Endod. 1990;16(2):70-7. doi: 10.1016/S0099-2399(06)81567-0.

10. Arisu HD, Uçtasli MB, Eligüzeloglu E, Ozcan S, Omürlü H. The effect of occlusal loading on the microleakage of class V restorations. Oper Dent. 2008;33(2):135-41. doi: 10.2341/07-49.

11. Pashley EL, Comer RW, Simpson MD, Horner JA, Pashley DH, Caughman WF. Dentin permeability: sealing the dentin in crown preparations. Oper Dent. 1992;17(1):13–20.

12. Helvey GA. Adhesive dentistry: the development of immediate dentin sealing/selective etching bonding technique. Compend Contin Educ Dent. 2011;32(9):22,24–32, 34–5; quiz 36, 38.

13. Magne P, Kim TH, Cascione D, Donovan TE. Immediate dentin sealing improves bond strength of indirect restorations. J Prosthet Dent. 2005;94(6):511–9. doi:10.1016/j.prosdent.2005.10.010

14. Samartzi TK, Papalexopoulos D, Sarafianou A, Kourtis S. Immediate dentin sealing: a literature review. Clin Cosmet Investig Dent. 2021;13:233-256. doi: 10.2147/CCIDE.S307939.

15. Gladys S, Van Meerbeek B, Lambrechts P, Vanherle G. Microleakage of adhesive restorative materials. Am J Dent. 2001;14(3):170-6.

16. Taylor MJ, Lynch E. Microleakage. J Dent. 1992;20(1):3-10. doi: 10.1016/0300-5712(92)90002-t.

17. Shaikh A, Hegde V, Shanmugasundaram S. A new approach to microleakage assessement: review. Int J Curr Adv Res. 2017;6(5):3635-8.

18. Raskin A, D'Hoore W, Gonthier S, Degrange M, Déjou J. Reliability of in vitro microleakage tests: a literature review. J Adhes Dent. 2001;3(4):295-308.

19. Kidd EA. Microleakage: a review. J Dent. 1976;4(5):199-206. doi: 10.1016/0300-5712(76)90048-8.

20. Alani AH, Toh CG. Detection of microleakage around dental restorations: a review. Oper Dent. 1997;22(4):173–85.

21. Heintze S, Forjanic M, Cavalleri A. Microleakage of Class II restorations with different tracers-comparison with SeM quantitative analysis. J Adhes Dent. 2008;10(4):259–67.

22. Wu W, Cobb EN. A silver staining technique for investigating wear of restorative dental composites. J Biomed Mater Res. 1981;15(3):343–8.

23. Wu W, Cobb EN, Dermann K, Rupp NW. Detecting margin leakage of dental composite restorations. J Biomed Mater Res. 1983;17(1):37–43.

24. Rodrigues SA, Jr, Ferracane JL, Della Bona A. Flexural strength and Weibull analysis of a microhybrid and a nanofill composite evaluated by 3- and 4-point bending tests. Dent Mater. 2008;24:426–31.

25. Peterson EA 2nd, Phillips RW, Swartz ML.. A comparison of the physical properties of four restorative resins. J Am Dent Assoc. 1966;73(6):1324–36.

26. Crabtree MG, Atkinson HG. A preliminary report on the solubility of decalcified dentine in water. Austral J Dentistry. 1955;55:340-2.

27. Palmer DS, Barco MT, Billy EJ. Temperature extremes produced orally by hot and cold liquids. J Prosthet Dent. 1992;67(3):325-7. doi: 10.1016/0022-3913(92)90239-7.

28. Eliasson ST, Dahl JE. Effect of thermal cycling on temperature changes and bond strength in different test specimens. Biomater Investig Dent. 2020;7(1):16-24. doi: 10.1080/26415275.2019.1709470.

29. Plant CG, Jones DW, Darvell BW. The heat evolved and temperatures attained during setting of restorative materials. Br Dent J. 1974;137(6):233-8. doi: 10.1038/sj.bdj.4803293.

30. Nelsen RJ, Wolcott RB, Paffenbarger GC. Fluid exchange at the margins of dental restorations. J Am Dent Assoc. 1952;44(3):288-95. doi: 10.1016/s0002-8177(52)43006-8.

31. Campos PE, Barceleiro Mde O, Sampaio-Filho HR, Martins LR. Evaluation of the cervical integrity during occlusal loading of Class II restorations. Oper Dent. 2008;33(1):59-64. doi: 10.2341/07-35.

32. Aggarwal V, Logani A, Jain V, Shah N. Effect of cyclic loading on marginal adaptation and bond strength in direct vs. indirect class II MO composite restorations. Oper Dent. 2008;33(5):587-92. doi: 10.2341/07-152.

33. Pongprueksa P, Kuphasuk W, Senawongse P. Effect of elastic cavity wall and occlusal loading on microleakage and dentin bond strength. Oper Dent. 2007;32(5):466-75. doi: 10.2341/06-132.

34. Khvostenko D, Salehi S, Naleway SE, Hilton TJ, Ferracane JL, Mitchell JC, et al. Cyclic mechanical loading promotes bacterial penetration along composite restoration marginal gaps. Dent Mater. 2015;31(6):702-10. doi: 10.1016/j.dental.2015.03.011.

35. Cooksey C, Dronsfield A. Fuchsine or magenta: the second most famous aniline dye. A short memoir on the 150th anniversary of the first commercial production of this well known dye. Biotech Histochem. 2009;84(4):179-83. doi: 10.1080/10520290903081401.

36. Ng CH, Ohlin CA, Winther-Jensen B. Characterisation of a series of triarylmethane dyes as light harvesters for photo-electrochemical systems. Dyes Pigm. 2015;115:96-101. doi:10.1016/j.dyepig.2014.12.016.

37. Kenyon BJ, Frederickson D, Hagge MS. Gingival seal of deep Class II direct and indirect composite restorations. Am J Dent. 2007;20(1):3-6.

38. Uludag B, Yucedag E, Sahin V. Microleakage of inlay ceramic systems luted with self-adhesive resin cements. J Adhes Dent. 2014;16(6):523-9. doi: 10.3290/j.jad.a32811.

39. Ovul K, Arzu TM, Ahmet S, Lippo Vj L, Pekka K V. Marginal adaptation and microleakeage of directly and indirectly made fiber reinforced composite inlays. Open Dent J. 2011;5:33-8. doi: 10.2174/1874210601105010033.

40. Celik EU, Kumbaraci N, Cal E, Turkun M. Influence of two desensitizer agents on the microleakage of adhesively luted ceramic inlays. Eur J Dent. 2011;5(1):77-83.

41. Hahn P, Schaller HG, Müllner U, Hellwig E. Marginal leakage in class II-restorations after use of ceramic-inserts luted with different materials. J Oral Rehabil. 1998;25(8):567-74. doi: 10.1046/j.1365-2842.1998.00281.x.

42. Nassaj AE, Ghadimi S, Seraj B, Chiniforush N. Effect of photodynamic therapy on microleakage of class V composite restorations in primary teeth. Photodiagnosis Photodyn Ther. 2020;32:101964. doi: 10.1016/j.pdpdt.2020.101964.

43. Duquia Rde C, Osinaga PW, Demarco FF, de V Habekost L, Conceição EN. Cervical microleakage in MOD restorations: in vitro comparison of indirect and direct composite. Oper Dent. 2006;31(6):682-7. doi: 10.2341/05-132.

44. Calabrez-Filho S, Calabrez VC, Reston EG, de Andrade MF, Borges LH. Influence of the internal conditioning of indirect restorations of resin composite in relation to microleakage using LEDs and QTH units. Oper Dent. 2009;34(3):293-8. doi: 10.2341/08-83.

45. Ziskind D, Elbaz B, Hirschfeld Z, Rosen L. Amalgam alternatives-microleakage evaluation of clinical procedures. Part II: direct/indirect composite inlay systems. J Oral Rehabil. 1998;25(7):502-6. doi: 10.1046/j.1365-2842.1998.00272.x.

46. Rosentritt M, Behr M, Lang R, Handel G. Influence of cement type on the marginal adaptation of all-ceramic MOD inlays. Dent Mater. 2004;20(5):463-9. doi: 10.1016/j.dental.2003.05.004.

47. Schmalz G, Federlin M, Reich E. Effect of dimension of luting space and luting composite on marginal adaptation of a class II ceramic inlay. J Prosthet Dent. 1995 Apr;73(4):392-9. doi: 10.1016/s0022-3913(05)80337-3. PMID: 7783020.

48. Ozturk AN, Ozturk B, Aykent F. Microleakage of different cementation techniques in Class V ceramic inlays. J Oral Rehabil. 2004;31(12):1192-6. doi: 10.1111/j.1365-2842.2004.01361.x.

49. Keshvad A, Hooshmand T, Asefzadeh F, Khalilinejad F, Alihemmati M, Van Noort R. Marginal gap, internal fit, and fracture load of leucite-reinforced ceramic inlays fabricated by CEREC inLab and hot-pressed techniques. J Prosthodont. 2011;20(7):535-40. doi: 10.1111/j.1532-849X.2011.00745.x.

50. Hasanreĭsoğlu U, Sönmez H, Uçtaşli S, Wilson HJ. Microleakage of direct and indirect inlay/onlay systems. J Oral Rehabil. 1996;23(1):66-71. doi: 10.1111/j.1365-2842.1996.tb00814.x.

51. Karaağaclioğlu L, Zaimoğlu A, Akören AC. Microleakage of indirect inlays placed on different kinds of glass ionomer cement linings. J Oral Rehabil. 1992;19(5):457-69. doi: 10.1111/j.1365-2842.1992.tb01110.x.

52. Thordrup M, Isidor F, Hörsted-Bindslev P. Comparison of marginal fit and microleakage of ceramic and composite inlays: an in vitro study. J Dent. 1994;22(3):147-53. doi: 10.1016/0300-5712(94)90198-8.

53. Tucker D, Lu Y, Zhang Q. From mitochondrial function to neuroprotection-an emerging role for methylene blue. Mol Neurobiol. 2018;55(6):5137-5153. doi: 10.1007/s12035-017-0712-2.

54. Xue H, Thaivalappil A, Cao K. The potentials of methylene blue as an anti-aging drug. Cells. 2021;10(12):3379. doi: 10.3390/cells10123379.

55. Cwalinski T, Polom W, Marano L, Roviello G, D'Angelo A, Cwalina N, et al. Methylene Blue-Current Knowledge, Fluorescent Properties, and Its Future Use. J Clin Med. 2020;9(11):3538. doi: 10.3390/jcm9113538.

56. Najet AM, Sawsan K, Saida Z, Kamel BS. In vitro comparison of Biodentine and Riva LC interfaces with cervical dentin and Filtek Z350 in posterior class II open sandwich restorations. SRM J Res Dent Sci. 2021;11:178-84.

57. Gerdolle DA, Mortier E, Loos-Ayav C, Jacquot B, Panighi MM. In vitro evaluation of microleakage of indirect composite inlays cemented with four luting agents. J Prosthet Dent. 2005;93(6):563-70. doi: 10.1016/j.prosdent.2005.04.004.

58. Shafiei F, Doozandeh M, Alavi AA. Effect of resin coating and chlorhexidine on the microleakage of two resin cements after storage. J Prosthodont. 2011;20(2):106-12. doi: 10.1111/j.1532-849X.2010.00670.x.

59. Santos ERD, Busato AL. Microleakage in posterior teeth with different materials and different types of cavities. Indian J Dent Res. 2019;30(5):783-787. doi: 10.4103/ijdr.IJDR_113_15.

60. Peixoto RT, Poletto LT, Lanza MD, Buono VT. The influence of occlusal finish line configuration on microleakage of indirect composite inlays. J Adhes Dent. 2002;4(2):145-50.

61. Mota CS, Demarco FF, Camacho GB, Powers JM. Microleakage in ceramic inlays luted with different resin cements. J Adhes Dent. 2003;5(1):63-70.

62. Deliperi S, Bardwell DN, Wegley C. Restoration interface microleakage using one total-etch and three self-etch adhesives. Oper Dent. 2007;32(2):179-84. doi: 10.2341/06-54.

63. Yeolekar TS, Chowdhary NR, Mukunda KS, Kiran NK. Evaluation of microleakage and marginal ridge fracture resistance of primary molars restored with three restorative materials: a comparative in vitro study. Int J Clin Pediatr Dent. 2015;8(2):108-13. doi: 10.5005/jp-journals-10005-1294.

64. Gao SS, Zhao IS, Duffin S, Duangthip D, Lo ECM, Chu CH. Revitalising silver nitrate for caries management. Int J Environ Res Public Health. 2018;15(1):80. doi: 10.3390/ijerph15010080.

65. Chevallet M, Luche S, Rabilloud T. Silver staining of proteins in polyacrylamide gels. Nat Protoc. 2006;1(4):1852-8. doi: 10.1038/nprot.2006.288.

66. Sinche-Ccahuana I, Ladera-Castañeda M, Paucar-Rodríguez E, Aliaga-Mariñas A, Dapello-Zevallos G, Cervantes-Ganoza L, et al. Microleakage in indirect onlay restorations cemented with three different types of adhesives: An in vitro study. J Clin Exp Dent. 2023;15(8):e641-8. doi: 10.4317/jced.60725.

67. Fruits TJ, Knapp JA, Khajotia SS. Microleakage in the proximal walls of direct and indirect posterior resin slot restorations. Oper Dent. 2006;31(6):719-27. doi: 10.2341/05-148.

68. Hasegawa EA, Boyer DB, Chan DC. Microleakage of indirect composite inlays. Dent Mater. 1989;5(6):388-91. doi: 10.1016/0109-5641(89)90106-1.

69. Romão W Jr, Miranda WG Jr, Cesar PF, Braga RR. Correlation between microleakage and cement thickness in three Class II inlay ceramic systems. Oper Dent. 2004;29(2):212-8.

70. Naumova EA, Schiml F, Arnold WH, Piwowarczyk A. Marginal quality of ceramic inlays after three different instrumental cavity preparation methods of the proximal boxes. Clin Oral Investig. 2019;23(2):793-803. doi: 10.1007/s00784-018-2492-0.

71. Bauer JR, Reis A, Loguercio AD, Barroso LP, Grande RH. Effects of aging methods on microleakage of an adhesive system used as a sealant on contaminated surfaces. J Appl Oral Sci. 2005;13(4):377-81.