

Impact of various pressures on fracture resistance and microleakage of amalgam and composite restorations

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Key words

Diving; Aviation; Dental; Barotrauma

Abstract

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Background: Pressure changes can influence dental restorations especially among divers. The aim of the current study was to evaluate the fracture resistance and microleakage of mesio-occluso-distal (MOD) amalgam and composite restorations undergoing pressure changes via diving and aviation simulation.

Methods: For the fracture resistance test, 60 sound maxillary premolar teeth were randomly allocated to two groups. Each group was then divided into three subgroups ($n = 10$) for simulating scuba-diving (pressure cycle to 203 kPa, 2 bar), flight (50.6 kPa, 0.5 bar), and control (atmospheric pressure). The teeth underwent pressure cycles for one month and then the fracture resistance test was conducted on them using the Instron testing machine. Microleakage scores were afterwards recorded by using a 2% methylene blue dye for 24 hours.

Results: Composite restorations showed significantly higher fracture resistance values compared to the amalgam group ($P < 0.05$). The control group had significantly higher fracture resistance values compared to the dive group, whereas there was no significant difference between the control group and the flight group ($P = 0.083$). No significant difference in the level of microleakage was observed between restoration materials or pressure cycles ($P > 0.05$).

Conclusions: Composite restorations showed promising fracture resistance compared to the amalgam group. Diving pressure cycles demonstrated adverse effects on the fracture resistance values of the two restorative materials.

Introduction

With the growing number of scuba divers and aircrew members, dentists will increasingly encounter oral conditions relating to pressure changes and these would require careful attention.^{1,2} These phenomena are mainly related to the law of Boyle–Mariotte, which states that at a constant temperature the volume and pressure of an ideal gas are inversely proportional.^{3,4} Among these oral conditions, barodontalgia is known as the toothache that is related to ambient pressure changes.^{5–7} In a diving environment, this pain is commonly called ‘tooth squeeze’. Although uncommon, in-diving or in-flight barodontalgia has been recognized as a potential cause of diver or aircrew-member vertigo and sudden incapacitation, jeopardizing the safety of diving or flight, respectively.⁸ Odontocrexia is another condition describing tooth or restoration structure destruction associated with pressure changes.⁹ Dental barotrauma describes the damage to tooth structure when pressure changes may occur with or without pain. All these

conditions potentially may cause incapacitation that could jeopardize the safety of diving or flight.⁸

Defective dental restorations, leakage and secondary caries are assumed to be the most important predisposing factors of dental barotraumas. In-flight bruxism in aircrew members was reported to be the main factor of amalgam restoration failures in World War II.¹⁰ Excessive bite forces were also proposed by the United States Air Force (USAF) symposium of aviation dentistry in 1946 as a predisposing factor for restoration dislodgment.¹¹ In divers, there is an argument about the effect of clenching on mouthpieces on the deterioration of dental restorations.¹² Based on our literature review, there appear to be no studies examining the effect of pressure changes on the properties of dental restorations.

This study aimed to assess the fracture resistance and microleakage of mesio-occluso-distal (MOD) amalgam and composite restorations undergoing pressure changes. The null hypothesis was that the pressure changes and dental

material used for restoring the tooth have no effect on the microleakage and fracture resistance of teeth via diving and aviation simulation.

Methods

SPECIMEN PREPARATION

A total of 60 sound single rooted mandibular premolars and 60 maxillary premolars, free of any microcracks and caries were extracted for orthodontic reasons within a three-month period and stored in normal saline solution at room temperature. Two weeks before use, all teeth were immersed in a 0.5% chloramine T trihydrate solution for infection control. Sixty maxillary premolar teeth of equal buccolingual dimension were used for the fracture resistance test and 60 mandibular premolars were used for the microleakage test. Care was taken to ensure that none of the teeth lost moisture. For each test, teeth were randomly divided into two groups, 30 teeth in each group, and treated as follows. Table 1 shows the groups and subgroups of this study.

Standard mesio-occluso-distal (MOD) cavities (a cavity on the mesial, occlusal, and distal surfaces of a tooth) were prepared using a coarse cylindrical flat-end diamond bur (MIC46078, Amalgadent, Australia). Each bur was changed after 10 preparations. The outline of the cavities was first drawn on the teeth using a digital calliper. The buccolingual widths of the cavities were considered half the inter-cuspal distance. The gingival margins of the cavities were placed 1mm above the cement-enamel junction (CEJ) with the pulpal floor 2 mm below the central groove. The depth of the axial wall was set at 1.5mm. The convergence of the buccal and lingual walls towards the occlusal was ensured. The cavosurface angle in all the walls was approximately 90 degrees.

In the amalgam groups, amalgam (SDI Ltd) was used according to the manufacturer's instructions to restore the teeth using the tofflemire (DS-DI-1166, Delmaks Surgio, Pakistan) retainer and a stainless steel (SS) matrix band. Filtek Z250 (3M ESPE, USA) was used to restore the teeth in the composite groups in accordance with the manufacturer's instructions. The teeth were etched for 15 seconds using 37% phosphoric acid; they were then rinsed for 10 seconds with water after which they were air dried until a shiny hydrated surface of moist dentin was achieved. Adper single bond II (3M ESPE, USA) was applied in two layers with disposable applicators, each layer was air dried for 5 seconds to ensure solvent evaporation and then light cured for 20 seconds with a light-emitting diode (LED) (650mW/cm²) (Optilux 501, Kerr, Danbury, CT, USA). Then, using the SS matrix bands and tofflemire, the teeth were restored with A2 shade composite resin. The oblique layering technique was performed with the first layer not thicker than 1 mm in gingival and pulpal floors. Following this, increments were placed in 2 mm thicknesses having contact with only

two walls of the cavity. Each increment was light cured for 40 seconds from the occlusal surface. After removing the matrix band, additional curing was performed from buccal and lingual planes for 40 seconds each. Moreover, 24 hours after restoration, both amalgam and composite groups were finished and polished according to standard methods. All the procedures were performed by a single dentist (ARB) trained and experienced in the cavity preparation and filling procedures described above and blinded to which group teeth were to be allocated.

PRESSURE CHANGE SIMULATION

To simulate pressure changes during dives and flights, an experimental chamber was designed with an external manometer. Compressed air was used to increase the chamber pressure and a vacuum pump to decrease pressure. The speed of pressure change was set to 1 bar (101.3 kPa) per minute. The diving descent was simulated by increasing the pressure to 203 kPa (2 bar) roughly equivalent to a depth of 10 metres under water. Decreasing the pressure to 0.5 bar (50.6 kPa) is equivalent to 5,500 m above sea level. Each tooth underwent 30 simulated dives (D) or flights (F) according to their subgroups. For the dive subgroups, the teeth were maintained in 203 kPa pressure for 45 minutes each day before returning to ambient pressure. For the flight subgroups, the teeth were de-pressurised to 50.6 kPa for 45 minutes in the same manner. The control subgroups were stored at ambient pressure for one month.

FRACTURE RESISTANCE TEST

All specimens were mounted in self-cure acrylic resin up to 2 mm below the CEJ. A dental surveyor was employed to ensure uniform alignment of all specimens parallel to the analysing rod. All specimens were then placed in a jig, which allowed loading at the central fossa parallel to the long axis of the teeth. The Instron universal testing machine (Z010, Zwick GmbH, Ulm, Germany) was used to deliver compressive load at a crosshead speed of 1 mm·min⁻¹ until fracture. The fracture resistance scores were recorded in Newtons (N).

MICROLEAKAGE TEST

The entire tooth surface was covered with two layers of nail polish, except for the restoration and a 1 mm margin around it on the tooth surface. The root apices were sealed with sticky wax. The specimens were then immersed in 2% methylene blue for 24 hours and rinsed under running water to remove excessive dye. The teeth were subsequently sectioned mesio-distally with a water-cooled low-speed saw (TC-3000, Vafaei Industrial Co., Tehran, Iran). Two sections of each specimen were examined under the stereomicroscope at 16X magnification. Dye penetration was quantified in gingival margins of the restoration using a 0–3 scale system, where 0 – no dye penetration, 1 – dye penetration limited

Table 1
Experimental groups and subgroups

| Groups (n) | Subgroups (abbreviation) (n) |
|-----------------------------|------------------------------|
| Amalgam restorations (30) | Flight (FAF) (10) |
| | Diving (FAD) (10) |
| | Control (FAC) (10) |
| Composite restorations (30) | Flight (FCF) (10) |
| | Diving (FCD) (10) |
| | Control (FCC) (10) |
| Amalgam restorations (30) | Flight (MAF) (10) |
| | Diving (MAD) (10) |
| | Control (MAC) (10) |
| Composite restorations (30) | Flight (MCF) (10) |
| | Diving (MCD) (10) |
| | Control (MCC) (10) |

Figure 1

Boxplots of fracture resistance (Newtons) in hypobaric, normobaric and hyperbaric pressures based on the material group (amalgam/composite). Differences in the Amalgam group not significant, whereas in the composite group, the most fracture resistance was observed in the normobaric group ($P = 0.034$, ANOVA)

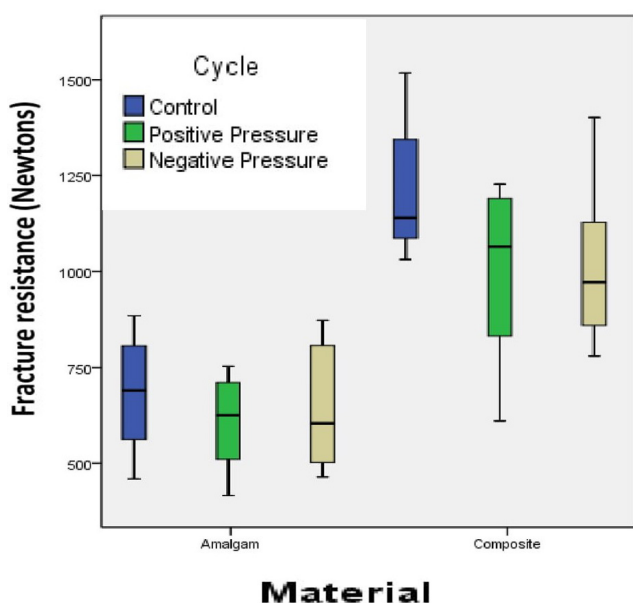


Table 2

* The abbreviations represented in Table 1; ** Microleakage scores refer to a dye penetration scale; 0 – no dye penetration; 1 – dye penetration limited to the enamel of the gingival wall; 2 – dye penetration into the dentin in the gingival wall; 3 – dye penetration past the gingival wall and involving the axial wall

| Subgroups (n) | Microleakage scores** (n) | | | |
|---------------|---------------------------|---|---|---|
| | 0 | 1 | 2 | 3 |
| MAF* (10) | 2 | 2 | 6 | 0 |
| MAD (10) | 1 | 0 | 8 | 1 |
| MAC (10) | 2 | 1 | 6 | 1 |
| MCF (10) | 2 | 6 | 1 | 1 |
| MCD (10) | 1 | 4 | 3 | 2 |
| MCC (10) | 3 | 5 | 2 | 0 |

to the enamel of the gingival wall, 2 – dye penetration into the dentin in the gingival wall, 3 – dye penetration past the gingival wall involving the axial wall. The highest scores were recorded.

STATISTICAL ANALYSIS:

Data analysis was carried out using SPSS software (SPSS version 18.0, SPSS, Chicago, IL, USA). In order to compare the fracture resistance and microleakage amounts between groups Kruskal–Wallis and Mann–Whitney tests were used.

Results

FRACTURE RESISTANCE

The normality of distribution of the data was confirmed using Shapiro–Wilk test ($P > 0.05$). The mean values of fracture resistance of the groups are shown in Figure 1. Both the materials used ($P < 0.001$) and pressure change cycles ($P = 0.027$) had significant effects on fracture resistance amounts. Composite restorations showed significantly higher fracture resistance values compared to the amalgam group ($P < 0.001$). A pairwise comparison of the cycles demonstrated that the control group had significantly higher fracture resistance values ($P = 0.034$) in comparison with the dive groups, whereas there was no significant difference between the control group and the flight group ($P = 0.083$).

MICROLEAKAGE

The microleakage scores of different groups are shown in Table 2. There were no significant differences in the microleakage amounts among the six subgroups (Kruskal–Wallis test, $P = 0.076$; Figure 1). In both the amalgam and composite groups there were no statistically significant differences ($P = 0.341$ for amalgam groups and $P = 0.228$

for composite groups, respectively). The Mann-Whitney test also revealed no significant differences between the C, D, and F groups.

Discussion

To the best of our knowledge, this is the first investigation that has assessed the effect of pressure changes on the mechanical properties of teeth restored with different restorative materials. Amalgam and composite materials were selected because they are the most frequently used. It is reported by various authors that among a variety of predisposing factors for barodontalgia and odontocrexia, leaking restorations rather than caries are of great importance.^{6,11} On the other hand, excessive bite forces applied to teeth from clenching and bruxism during flights and dives are also reported to be a crucial factor in tooth destruction.^{13–15} Therefore, the effects of pressure changes and restorative materials were examined in this study by means of fracture resistance and microleakage.

Regarding the fracture resistance test, the results revealed that teeth restored with amalgam in all groups were significantly more prone to fracture compared to composite groups. This is in accordance with previous studies which reported on the effect of cusp reinforcement by means of adhesive dentistry.^{16–18} On the other hand, the fracture resistance of teeth restored with composite restorations is still significantly lower than intact teeth and composite restorations are not able to fully restore the mechanical properties of teeth.^{18,19} Also, it is worth mentioning that different clinical conditions like thermocycling can have adverse effects on the reinforcement impact of adhesive restorations.^{20,21}

The normal biting force on maxillary premolars has been observed to be 100–300 N.²¹ In our study, none of the groups have shown fracture resistance values lower than these amounts, and even the lowest group (amalgam in diving simulation) showed a mean fracture resistance value of 622 N. Although the clinical conditions and the forces applied to the teeth in the oral cavity are different from the design of this study, these numbers do have clinical relevance.

There remain concerns regarding the fact that patients are having clenching or bruxism, especially in amalgam restorations, as occlusal forces have been reported to be as high as 520–800 N.²¹ The weakening effect of clenching on tooth structures in pilots and divers has been noted by different studies.^{12,13} Researchers have reported aircrew members and divers to have a higher prevalence of jaw parafunctional activity.^{6,11} It was estimated that 60–70% of pilots in World War II had suffered from bruxism, whilst more recently, the prevalence of clinically important bruxism in a military environment occurred in 69% of aircrew members.¹³ In scuba divers, there is an argument that clenching on the mouthpiece during diving increases in cold water and with

stress and this may contribute to the deterioration of dental restorations.¹² Higher prevalence of clenching in scuba divers has been reported in other studies.^{6,14} Owing to the higher prevalence of jaw parafunctional activities in aircrew members and divers and its subsequent weakening effect on tooth structures, the use of amalgam restorations seems to be controversial regarding fracture resistance.

In both divers and pilots the air void trapped in a dental restoration expands according to Boyle's law during each ascent owing to the decrease in pressure and weakens the restoration structure.^{9,22} This explains the fracture resistance decrease in both flight and dive groups compared to control groups in the current study. However, this reduction was only statistically significant in dive groups, not in the flight groups, probably related to the greater range of pressure change in the dive groups compared to the flight groups (1 bar versus 0.5 bar pressure changes).

The microleakage test showed no statistically significant differences among the different groups, whereas in a previous study, higher microleakage was reported with amalgam than with posterior composite resin.²³ Similarly, in another study, the microleakage of amalgam restorations in primary molars had more leakage compared to the composite restorations at the occlusal margins. On the other hand, the same study revealed no significant difference between amalgam and composite restorations in cervical margins, which is consistent with the present results.²⁴ In contrast, composite restorations demonstrated higher microleakage than amalgam restorations in a study which evaluated microleakage in both *in vivo* and *in vitro* around Class I restorations, though our findings in control groups were similar to their control results.²⁵

Among different techniques for microleakage test, dye penetration is the most widely used method to assess microleakage because of its sensitivity, ease of use, and convenience,²⁶ and stereomicroscopic examination at 16 × magnification was chosen for this study as this provides a well-magnified two-dimensional view of the surface to be examined.

These contradictory results may be because of the variations in leakage evaluation techniques, test conditions, cavity design and dimensions, restorative materials, type of teeth and observation time and underline the obvious importance of standardized testing parameters for leakage studies.²⁵ The use of composite restoration is suggested for divers and aircrew members as it showed superior fracture resistance values compared to amalgam.

Conclusions

Composite restorations showed promising fracture resistance compared to amalgam. Diving pressure cycles demonstrated adverse effects on the fracture resistance values of both

restorative materials. Despite the experimental conditions of this study not fully mimicking the conditions of the oral cavity during diving or flight experiences, as teeth are subjected to a mixture of different factors outside the laboratory setting, some important clinical relevance can still be inferred. This is a pioneer study and further studies with different pressure cycles, longer durations, different restorative materials and different tests are required to fully understand the effect of ambient pressure changes on tooth structures and their restoration.

References

- Zadik Y. Aviation dentistry: current concepts and practice. *Br Dent J.* 2009;206:11–6. doi: [10.1038/sj.bdj.2008.1121](https://doi.org/10.1038/sj.bdj.2008.1121). PMID: [19132029](https://pubmed.ncbi.nlm.nih.gov/19132029/).
- Zadik Y. Dental barotrauma. *Int J Prosthodont.* 2009;22:354–7. PMID: [19639071](https://pubmed.ncbi.nlm.nih.gov/19639071/).
- Von See C, Rucker M, Koch A, Kokemueller H, Schumann P, Ziebolz D, et al. The influence of pressure changes on endodontically treated teeth during simulated dives. *Int Endod J.* 2012;45:57–62. doi: [10.1111/j.1365-2591.2011.01947.x](https://doi.org/10.1111/j.1365-2591.2011.01947.x). PMID: [21899567](https://pubmed.ncbi.nlm.nih.gov/21899567/).
- Peker I, Erten H, Kayaoglu G. Dental restoration dislodgment and fracture during scuba diving: a case of barotrauma. *J Am Dent Assoc.* 2009;140:1118–21. PMID: [19723944](https://pubmed.ncbi.nlm.nih.gov/19723944/).
- Jagger RG, Shah CA, Weerapperuma ID, Jagger DC. The prevalence of orofacial pain and tooth fracture (odontocrexia) associated with scuba diving. *Prim Dent Care.* 2009;16:75–8. doi: [10.1308/135576109787909463](https://doi.org/10.1308/135576109787909463). PMID: [19366523](https://pubmed.ncbi.nlm.nih.gov/19366523/).
- Zadik Y, Drucker S. Diving dentistry: a review of the dental implications of scuba diving. *Aust Dent J.* 2011;56:265–71. doi: [10.1111/j.1834-7819.2011.01340.x](https://doi.org/10.1111/j.1834-7819.2011.01340.x). PMID: [21884141](https://pubmed.ncbi.nlm.nih.gov/21884141/).
- Kollmann W. Incidence and possible causes of dental pain during simulated high altitude flights. *J Endod.* 1993;19:154–9. doi: [10.1016/S0099-2399\(06\)80512-1](https://doi.org/10.1016/S0099-2399(06)80512-1). PMID: [8509756](https://pubmed.ncbi.nlm.nih.gov/8509756/).
- Zadik Y. Barodontalgia: what have we learned in the past decade? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010;109:e65–9. doi: [10.1016/j.tripleo.2009.12.001](https://doi.org/10.1016/j.tripleo.2009.12.001). PMID: [20303049](https://pubmed.ncbi.nlm.nih.gov/20303049/).
- Calder IM, Ramsey JD. Ondontocrexia - the effects of rapid decompression on restored teeth. *J Dent.* 1983;11:318–23. PMID: [6142064](https://pubmed.ncbi.nlm.nih.gov/6142064/).
- Sognaes RF. Further studies of aviation dentistry. *Acta Odontol Scand.* 1946;7:165–73.
- Zadik Y, Einy S, Pokroy R, Dayan YB, Goldstein L. Dental fractures on acute exposure to high altitude. *Aviat Space Environ Med.* 2006;77:654–7. PMID: [16780246](https://pubmed.ncbi.nlm.nih.gov/16780246/).
- Hobson RS, Newton JP. Dental evaluation of scuba diving mouthpieces using a subject assessment index and radiological analysis of jaw position. *Br J Sports Med.* 2001;35:84–8. PMID: [11273967](https://pubmed.ncbi.nlm.nih.gov/11273967/). PMID: [11273967](https://pubmed.ncbi.nlm.nih.gov/11273967/). PMID: [11273967](https://pubmed.ncbi.nlm.nih.gov/11273967/).
- Lurie O, Zadik Y, Einy S, Tarrasch R, Raviv G, Goldstein L. Bruxism in military pilots and non-pilots: tooth wear and psychological stress. *Aviat Space Environ Med.* 2007;78:137–9. PMID: [17310886](https://pubmed.ncbi.nlm.nih.gov/17310886/).
- Lobbezoo F, van Wijk AJ, Klingler MC, Ruiz Vicente E, van Dijk CJ, Eijkman MA. Predictors for the development of temporomandibular disorders in scuba divers. *J Oral Rehabil.* 2014;41:573–80. doi: [10.1111/joor.12178](https://doi.org/10.1111/joor.12178). PMID: [24766672](https://pubmed.ncbi.nlm.nih.gov/24766672/).
- Gunepin M, Derache F, Audoual T. Fracture of a sound tooth in a pilot under hypobaric conditions. *Aviat Space Environ Med.* 2010;81:691–3. PMID: [20597251](https://pubmed.ncbi.nlm.nih.gov/20597251/).
- Molinario JD, Diefenderfer KE, Strother JM. The influence of a packable resin composite, conventional resin composite and amalgam on molar cuspal stiffness. *Oper Dent.* 2002;27:516–24. PMID: [12216572](https://pubmed.ncbi.nlm.nih.gov/12216572/).
- Morin D, DeLong R, Douglas WH. Cusp reinforcement by the acid-etch technique. *J Dent Res.* 1984;63:1075–8. doi: [10.1177/00220345840630081401](https://doi.org/10.1177/00220345840630081401). PMID: [6379008](https://pubmed.ncbi.nlm.nih.gov/6379008/).
- Denehy GE, Torney DL. Internal enamel reinforcement through micromechanical bonding. *J Prosthet Dent.* 1976;36:171–5. PMID: [789864](https://pubmed.ncbi.nlm.nih.gov/789864/).
- Yamada Y, Tsubota Y, Fukushima S. Effect of restoration method on fracture resistance of endodontically treated maxillary premolars. *Int J Prosthodont.* 2004;17:94–8. PMID: [15008239](https://pubmed.ncbi.nlm.nih.gov/15008239/).
- Eakle WS. Fracture resistance of teeth restored with class II bonded composite resin. *J Dent Res.* 1986;65:149–53. doi: [10.1177/00220345860650021201](https://doi.org/10.1177/00220345860650021201). PMID: [3511111](https://pubmed.ncbi.nlm.nih.gov/3511111/).
- Shahrbaf S, Mirzakouchaki B, Oskoui SS, Kahnemoui MA. The effect of marginal ridge thickness on the fracture resistance of endodontically-treated, composite restored maxillary premolars. *Oper Dent.* 2007;32:285–90. doi: [10.2341/06-83](https://doi.org/10.2341/06-83). PMID: [17555181](https://pubmed.ncbi.nlm.nih.gov/17555181/).
- Zanotta C, Dagassan-Berndt D, Nussberger P, Waltimo T, Filippi A. Barodontalgias, dental and orofacial barotraumas: a survey in Swiss divers and caisson workers. *Swiss Dent J.* 2014;124:510–9. PMID: [24853026](https://pubmed.ncbi.nlm.nih.gov/24853026/).
- Hersek N, Canay S, Akça K, Ciftçi Y. Comparison of microleakage properties of three different filling materials. An autoradiographic study. *J Oral Rehabil.* 2002;29:1212–7. PMID: [12472859](https://pubmed.ncbi.nlm.nih.gov/12472859/).
- Shih W-Y. Microleakage in different primary tooth restorations. *J Chin Med Assoc.* 2016;79:228–34. doi: [10.1016/j.jcma.2015.10.007](https://doi.org/10.1016/j.jcma.2015.10.007). PMID: [26839288](https://pubmed.ncbi.nlm.nih.gov/26839288/).
- Alptekin T, Ozer F, Unlu N, Cobanoglu N, Blatz M. In vivo and in vitro evaluations of microleakage around Class I amalgam and composite restorations. *Oper Dent.* 2010;35:641–8. doi: [10.2341/10-065-L](https://doi.org/10.2341/10-065-L). PMID: [21180003](https://pubmed.ncbi.nlm.nih.gov/21180003/).
- Chandra PR, Harikumar V, Ramkiran D, Krishna MJ, Gouda MV. Microleakage of class V resin composites using various self-etching adhesives: an in vitro study. *J Contemp Dent Pract.* 2013;14:51–5. PMID: [23579893](https://pubmed.ncbi.nlm.nih.gov/23579893/).

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