**Effect of different gingival margin restorations of class II cavities on microleakage.**

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Purpose:

This study was directed to evaluate the influence of the location of the gingival margin (enamel, dentin or cementum) on nanohybrid composite resin on the microleakage of Class II posterior restoration.

Materials and Methods:

A total number of eighty one human molars were randomly divided into three main groups (27 each) according to the location of the gingival margin. Group 1: the location of the gingival margin was in enamel. Group 2: the location of the gingival margin was in the cemento-enamel junction. Group 3: the location of the gingival margin was in cementum. Each main group was divided into three subgroups (9 each) according to storage time. Subgroup A: storage time was 24 hours. Subgroup B: storage time was 1 month. Subgroup C: storage time was 3 months. All samples were restored by nanohybrid resin composite (Tetric EvoCeram. Class II cavity was prepared in one proximal surface for each molar following the general principles of cavity preparation. The depth of the gingival floor was placed according to each group. (group1: in enamel, group2: in CEJ, group3: in cementum). The cavo-surface margins for all walls were without bevels. Nanohybrid composite was placed incrementally. Specimens were stored in distilled water at 37oC and humidity 100% in an incubator for one day, one month and three months. After sealing, samples were immersed in 2.5% methylene blue dye at 37oC for 24 hours, removed from the dye, cleaned under tap water then left to dry for another 24 hours then were sectioned mesio-distally. Each sample was examined microscopically by a stereomicroscope using a computerized image analyzing system.

Results:

It was observed that the value of dye penetration increased remarkably from enamel, CEJ to cementum and this was statistically very high significant (p < 0.001).

Conclusion:

The least dye penetration was detected at the enamel gingival margin followed by CEJ then cementum.

Keyword:

class II restoration, gingival margins, microleakage, nanohybrid compsite.

Introduction:

Dental composite are used as restorative material since early 1960. The demand for posterior resin composite restorations has dramatically increased in recent years, because of their ability to match tooth color, absence of mercury, biocombitability and bond to tooth structure (1). However, like all dental materials, composites have their own limitations, such as the gap formation caused by polymerization contraction during setting, leading to marginal discoloration and leakage (2). The literature has shown that major marginal microleakage occurs on the gingival surfaces located in dentin or cementum. This is because these two structures do not show the same conditions for adhesion to resin composites, of which enamel has better results. In addition, difficulty in accessing the proximal boxes of the preparations and the control of contamination are complicating factors for restorative technique. In addition, dentin bonding is more difficult because the heterogeneous nature of the tissue requires the bonding system to accommodate simultaneously the properties of the hydroxyapatite, collagen, smear layer, dentinal tubules and fluids. (3)

Materials and Methods:

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Results:

Table(1): Statistical analysis of dye penetration results (µm) recorded in nanohybrid groups at different locations and storage times.

|  |  |  |  |
| --- | --- | --- | --- |
| Nanohybrid | | | |
| Margin | Mean ± SD | ANOVA | |
| Enamel |  | F | P |
| 24 Hrs | 1774± 361.1 | 19.70035 | 0.002308 |
| 1 M | 514 ± 145.7 |  | \*\* |
| 3 M | 973 ± 185 |  |  |
| CEJ |  | F | P |
| 24 Hrs | 2032 ± 368.3 | 2.381746 | 0.173218 |
| 1 M | 1297 ± 389.1 |  | ns |
| 3 M | 1691 ± 473.48 |  |  |
| Cementum |  | F | P |
| 24 Hrs | 2138 ± 641 | 2.362088 | 0.175131 |
| 1 M | 1321 ± 383 |  | ns |
| 3 M | 1901 ± 340 |  |  |

ns; non-significant \*; significant \*\*; high significant \*\*\*; very high significant

Dye penetration mean ± standard deviation values (µm) at enamel after 24 hrs, 1 month and 3 months were 1774 ± 361.1, 514 ± 145.7 and 973 ± 185 respectively. It was observed that 24 Hrs recorded a higher leakage rate as recorded by dye penetration, 3 months recorded an intermediate leakage rate while 1 month recorded a lower leakage rate and this was statistically high significant (p<0.01).

Dye penetration mean ± standard deviation values(µm) at CEJ after 24 hrs, 1 month and 3 months were 2032 ± 368.3 , 1297 ± 389.1 and 1691 ± 473.48 respectively. It was observed that 24 Hrs recorded a higher leakage rate as recorded by dye penetration; 3 months recorded an intermediate leakage rate while 1 month recorded a lower leakage rate and this was statistically non-significant (p > 0.05).

Dye penetration mean ± standard deviation values(µm) at cementum after 24 hrs, 1 month and 3 month were 2138 ± 641, 1321 ± 383 and 1901 ± 340 respectively. It was observed that 24 Hrs recorded a higher leakage rate as recorded by dye penetration; 3 months recorded an intermediate leakage rate while 1 month recorded a lower leakage rate and this was statistically non-significant (p > 0.05).

It was observed that the value of dye penetration increased remarkably from enamel, CEJ to cementum and this was statistically very high significant (p < 0.001).

**Fig.(1)**Abar chart comparison of dye penetration mean values (µm) of hybrid groups at enamel margin.

**Fig. (2)** A bar chart comparison of dye penetration mean values (µm) of hybrid groups at CEJ.

**Fig. (3);** A bar chart comparison of dye penetration mean values (µm) of hybrid groups at cementum margin.

Two way ANOVA revealed a very high significant difference between different locations (P < 0.001). Also storage time revealed high significant difference (P < 0.01).

**Table (2);** Two way ANOVA test comparing dye penetration mean values(µm) of hybrid groups at different locations and storage times.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Source of Variation* | *Df* | *Sum-of-squares* | *Mean square* | *F* | *P value* | *Sig.?* |
| Margin site | 2 | 2538000 | 1269000 | 8.335 | 0.0027 | Yes |
| Storage time | 2 | 3954000 | 1977000 | 12.98 | 0.0003 | Yes |
| Residual | 18 | 2741000 | 152300 |  |  |  |

**Fig. (4);** A bar chart comparing dye penetration mean values (µm) for hybrid groups at different locations as a function of storage time.

Discussion:

Microleakage is one of the major disadvantages of resin composite restorations. It results from failure of the material to adapt to dental structure, usually at the gingival margin. When a layer of resin composite is packed in the cavity and cured, a competition between shrinkage of the composite and adhesion to the substrate begins. Stresses produced by polymerization shrinkage are critical to adhesion between the resin composite and the tooth structure. If shrinkage stresses are stronger than the bond strength between the resin and adhesive system, the tooth-restoration interface can break, forming a gap that will allow marginal microleakage. This shrinkage depends on factors, such as cavity size and shape, substrate type and location of the margins, restorative material and technique of placement and polymerization. (4)

In this study, statistically significant difference was observed when the enamel and dentin margins were compared. All dentin margins had inferior results compared to enamel margins. These variations may be explained by structural differences in the substrate. Bonding to enamel is relatively simple process, without major technical requirements or difficulties. Bonding to dentin, on the other hand, presents a much greater challenge. Several factors account for this difference between enamel and dentin bonding. Whereas enamel is a highly mineralized tissue composed of more than 90% (by volume) hydroxyapatite, dentin contains a substantial proportion of water and organic material, primarily Type I collagen. Dentin also contains a dense network of tubules that connect the pulp with dentin-enamel junction. The tubules are lined by a cuff of hypermineralized dentin called peri-tubular dentin. The less mineralized inter-tubular dentin contains collagen fibrils with the characteristic collagen banding. The inter-tubular dentin is penetrated by sub-micron channels, which allow the passage of tubular liquid and fibers between neighboring tubules, forming inter-tubular anastomoses.

Dentin is an intrinsically hydrated tissue, penetrated by a maze of 1 to 2.5 m diameter fluid filled dentin tubules. Movement of fluid from the pulp to the dentin-enamel junction is a result of a slight but constant pulpal pressure.(5).pulpal pressure has a magnitude of 25 to 30 mm Hg or 34 to 40 cm H2O.(6) Dentinal tubules enclose cellular extensions from the odontoblasts and therefore are in direct communication with the pulp. Inside the tubules lumen, other fibrous organic structures (the lamina limitans) can be observed; these substantially decrease the functional radius of the tubule. The relative area occupied by dentin tubules decrease with increasing distance from the pulp. The number of tubules decrease from about 45,000 per mm2 close to the pulp to about 20,000 per mm2 near the dentin-enamel junction. (7) The tubules occupy an area of only 1% of the total surface near the dentin-enamel junction, whereas they comprise 22% of the surface close to the pulp.(8) The average tubule diameter ranges from 0.63 m at the periphery to 2.37 m near the pulp.(9) Adhesion can be affected by the remaining dentin thickness after tooth preparation. Bond strengths are generally less in deep dentin than in superficial dentin. (10)

Whenever tooth structure is prepared with a bur or other instrument, residual organic and inorganic components form a smear layer of debris on the surface. (11) The smear layer fills the orifices of dentin tubules forming smear plugs and decrease dentin permeability by up to 86%.(12) The composition of the smear layer is basically hydroxyapatite and altered de-natured collagen. This altered collagen may even acquire a gelatinized consistency as a result of the friction and heat created by the preparation procedure. (13) Submicron porosity of the smear layer still allows for diffusion of dentinal fluid. (14) The removal of the smear layer and smear plugs with acidic solutions may result in an increase of the fluid flow on to the exposed dentin surface. This fluid may interfere with adhesion because hydrophobic resins do not adhere to hydrophilic substrates even if resin tags are formed in the dentin tubules. (15) Several additional factors affect dentin permeability. Besides the use of vasoconstrictor in local anesthesia which decrees pulpal pressure and fluid flow in the tubules, other factors such as the radius and length of the tubules, the viscosity of dentin fluid, the pressure gradient, the molecular size of the substances dissolved in the tubular fluid, and the rate of removal of substances by the blood vessels in the pulp affect permeability.(16) All of these variables make dentine a dynamic substrate and consequently a very difficult substrate for bonding..(17) It has been shown that when the gingival margin is placed below the cemento-enamel junction, an outer layer of cementum provides a hypo-mineralized and hyper-organic substrate for bonding. This tissue even after etching does not provide the adequate conditions for the micro-mechanical retention of an adhesive material. (18) This was in agreement with the present study.

In this study least dye penetration was found at 1 month storage time then at 3 months then at 1 day. This may be explained by the water sorption potential of composite resins. Storage of the specimens for 1 month would allow some water sorption by the resin and subsequent hygroscopic expansion of the restoration. This expansion would not establish a perfect marginal seal but could contribute to less dye penetration. Conversely, 24 hours would not permit the time necessary for this phenomenon to occur. (19) This was in agreement with YAP and Wang HB (20), they found a significant decrease in marginal gaps between 1 day and 1 week and they found that all materials showed a decrease in gap width within 1 week storage in water. This result appear to support the results obtained with Momal and McCabe.(21) who concluded that expansion caused by water sorption is able to rapidly compensate the effects of polymerization shrinkage. On the other hand, Davidson and Feilzer (22) are of the opinion that the water sorption is a slow process and its compensatory effects for polymerization shrinkage often come too late. It is important to note that the amount or rate of water sorption and compensation is a product specific and may be dependent on the chemistry of the resin matrix.(23) Li HP and Burrow ME(24) found that a minimum of 1 year of storage in water is necessary to correctly evaluate its effect on microleakage. A significant improvement of the marginal sealing was observed by Carlos Torres and Maria de Araujo (25) at 6 months in comparison to base line. This may be attributed to the water sorption by composite resin which expand hygroscopically and contribute to closing marginal gaps. Youngson CC and Jones JC (26) noticed that marginal microleakage decreased after the second or fourth week in water storage. These results were in agreement with the present study.

Composites shrink as they polymerize, creating stresses of up to 7 MPa within the composite mass depending on the configuration of the preparation.(27) When the composite is bonded to one surface only such as in the case of a direct facial veneer, stresses within the composite are relieved by flow from the un-bonded surface. However, stress relief within a three-dimensional bonded restoration is limited by its configuration factor or C-factor. (28) In this study, stress relief was limited because flow can occur only from two surfaces. Unrelieved stresses in the composites may cause internal bond disruption as well as marginal gaps around the restorations that increase microleakage. (29)

References:

1. **Anand V.S, Kavitha C,and Subbarao C.V.** Effect of cavity design on strength of direct posterior composite restorations: An empirical and FEA analysis. Int. J of Dentistry 1-6 (2011).
2. **Bagheri M and Ghavamnasiri M.** Effect of cavo-surface margin configuration of class V cavity preparations on microleakage of composite resin restorations. J of Contem Dent Pract 9(2): 122-9 (2008).
3. **Anusavice KJ.** Philips science of dental materials. 10th ed. Philadelphia VVB. Saunders Company1996.
4. **Davidson CL, De Gee AJ & Feilzer A**. The competition between the composite-dentin bond strength and the polymerization contraction stress. Journal of Dental Research 63(12) 1396-1399 (1984).
5. **Brannstrom M, Linden LA, Johnson G.** Movement of dentinal and pulpal fluid caused by clinical procedures. J Dent Res 47: 679-682 (1968).
6. **Terka LG, Van Hassel HJ.** Testing sealing properties of restorative materials against moist dentin. J Dent Rest 66: 1758-1764 (1987).
7. **Garberoglio R, Brannstrom M,** Scanning electrom microscope investigation of human dentinal tubules, Arch Oral Biol 21: 355-362 (1976).
8. **Pashley DH,** Dentin: a dynamic substrate- a review, scanning microscope 3:161-176 (1989).
9. **Marchetti C, Piacenttini C, Menghini P**, Morphometric computerized analysis on the dentinal tubules and the collagen fibers in the dentin of human permanent teeth, Bull Group Int Rech Sci Stomatol Odontol 35: 125-129 (1992).
10. **Mitchem JC, Gronas DG.** Effects of time after extraction and depth of dentin on resin dentin adhesives. JADA 113: 285-287 (1986).
11. **Ishioka S, Caputo AA.** Interaction between the dentinal smear layer and composite bond strengths. J Prosthet Dent 61: 180-185 (1989).
12. **Pashley DH, Livingstone MJ, Greenhill JD**. Regional resistances to fluid flow in human dentin in vitro. Arch Oral Biol 23: 807-810 (1978).
13. **Eick JD**. The dentinal surface: its influence on dentinal adhesion. Quintessence Int 22: 967-977 (1991).
14. **Pashley DH.** The effects of acid etching on pulpodentinal complex. Oper Dent 17: 229-242 (1992).
15. **Torney D**. The restorative ability of acid-etched dentin. J Prosthet Dent 39: 169-172 (1978).
16. **Reeder OW.** Dentin permeabity determinants by hydraulic conductance. J Dent Res 57: 187-193 (1978).
17. **Soderholm K, JM**. Correlation of in vivo and in vitro performance of adhesive restorative materials, a report of the ASC MD156 Task Group on test methods for the adhesion of restorative materials. Dent Mater 7: 74-83 (1991).
18. **Cagidaco MC, Vichi A, Ferrari M.** SEM evaluation of outside dentin-cementum layer of cervical margins of class II restorations. J Dent Rest 75: 1220 (1996).
19. **A. U. J. YAP, K. C. SHAH & C. L. CHEW.** Marginal gap formation of composites in dentine: effect of water storage. Journal of oral rehabilitation 30; 236–242 (2003).
20. **YAP, AUJ, Wang HB, Siow KS & Gan LM.** Polymerization shrinkage of visible light cured composites. J Oper Dent 25 98 (2000).
21. **Momal Y & Mc Cabe JF.** Hygroscopic expansion of resin based composites during 6 months of water storage. British Dental Journal 5 91 (1994).
22. **Davidson CL, Feilzer AJ**. Polymerization shrinkage and polymerization shrinkage stress in polymer based restoratives. Journal of Dentistry 6, 435 (1997).
23. **AUJ, Yap KC Shahand Cl Chew.** Marginal gap formation of composites in dentin, effect of water storage. Journal of Oral Rehabilitation. 30, 236-242 (2003).
24. **Li HP, Burrow ME, Tyas MJ.** The effect of long term storage on nanoleakage. J Oper Dent 26: 609-616 (2001).
25. **Carlos Torres, Maria de Araujo, Adriana de Mella Torres**. Effect of dentin collagen removal on microleakage and bonded restorations. J Aesth Dent 6: 33-42 (2004).
26. **Youngson CC, Jones JCG, Fox K, Smith IS, Gale M**. A fluid filtration and clearing technique to asses microleakage associated with three time bonding systems. J Dent 27: 223-233 (1999).
27. **Hegdahl T, Gjerdet NR.** Contraction stresses of composite filling materials. Acta Odontol Scand 35: 191-195 (1977).
28. **Feilzer A, De Gee AJ, Davidson CL**. Setting stress in composite resin in relation to configuration of the restoration. J Dent Res 66: 1636-1639 (1987).
29. **Perdigao J**. The interaction of adhesive systems with dentin. Am J Dent 9: 167-173 (1996).