Comparative Evaluation of Sealing Ability of Biodentine, Smart Dentine Replacement (SDR) and Resin Modified Glass Ionomer Cement (RMGIC) in Cervical Lining Restorations by Dye Extraction Method: An in-Vitro Study

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Running Title: In vitro microleakage study of cervical lining restorations by dye extraction method

Abstract: ***Introduction**: The increasing demand for aesthetic procedures, along with the advantage of minimal invasive procedure and bonding to tooth structure has drastically increased the popularity of composite resin restorations.The major disadvantage of resin based materials is polymerization shrinkage that causes microleakage leading to bacterial penetration which results in post-operative sensitivity, marginal discolouration, secondary caries and even restoration loss.To overcome the demerits of Glass ionomer cementsand resin modified GIC, use of flowable resin composite&tricalcium based bioactive restorative material (Biodentine) as an intermediate material in the gingival seat has been suggested to reduce polymerization shrinkage. **Aim**: To evaluate the sealing efficacy of Biodentine, Smart dentine replacement (SDR) and Resin modified Glass Ionomer cement (RMGIC) at the cervical margins of approximal cavities of posterior teeth. **Material and methodology**: 30 approximal cavities were prepared on 30 extracted human molars. Teeth were randomly assigned into 3 groups of 10 cavities each: (G1) - Biodentine as lining material+ Filtek Z350 XT (light cured composite) as restorative material, (G2) – Smart dentine replacement as lining material+ Filtek Z350 XT (light cured composite) as restorative material, (G3) – Resin modified Glass Ionomer cement+ Filtek Z350 XT (light cured composite) as restorative material as positive control. The material was used according to manufacturer's instructions. The teeth were thermocycled (5°C to 55°C).The specimens were then sealed with a 1-mm window around the marginal interface with red nail varnish and were immersed in a methylene blue solution for 24 hours. Samples were then immersed in a 69% w/v nitric acid solution for dye extraction test. The amount of dye extracted was directly measured using spectrophotometer. The results were expressed as Absorbance that is amount of dye extracted from each specimen. The data were analysed with the parametric One-way ANOVA test (p<0.05). **Results**: Statistically significant difference was found between the groups. Group (G1) showed best sealing efficacy than Group (G2 and G3) when used as cervical lining restoration. **Conclusion**: Within the limits of this in vitro study, microleakage scores were least in those teeth where biodentine was used as cervical lining restorations. Biodentine when used as cervical lining restoration showed good sealing ability as compared to smart dentine replacement (SDR) and Light cured composite.***

Keywords: Microleakage; methylene blue dye; dye extraction method; spectrophotometer

1. Introduction

The increasing demand for esthetic procedures, along with the advantage of minimal invasive procedure and bonding to tooth structure has drastically increased the popularity of composite resin restorations. Incorporation of new monomers, initiation systems and filler technologies have significantly improved the physical properties of materials and expanding their use as direct and indirect restorations. [1]

An ideal restorative material should create a permanent and tight seal between the restorative margin and the tooth structure. [2] However a major disadvantage of resin based materials is polymerization shrinkage that causes microleakge leading to bacterial penetration which results in post-operative sensitivity, marginaldiscoloration, secondary caries and even restoration loss. Therefore, marginal adaptation remains a most important challenge in composite restorations, especially at the gingival wall of a class II restoration. [3], [4], [5]

One of the methods to decrease polymerization shrinkage is sandwich technique, which is less technique sensitive to that of composite restorations and shows a higher value of gap free interfacial adaptation to dentin. [6]

Earlier Glass ionomer cements and resin modified glass ionomer cements have been used as intermediate restorative material but has its own demerits. To overcome that, the use of flowable resin composite as an intermediate material in the gingival seat has been suggested to reduce polymerization shrinkage. Flowable resin composite used as liners may also act as a flexible intermediate layer and also helps to relieve stresses during polymerization shrinkage of the overlying restorative resin hence are called as stress – absorber. [7]

Biodentine, tricalcium silicate-based bioactive restorative cement was claimed to havesufficient mechanical properties to withstand occlusal load when used as lining material under composite resin material. It is suggested as a good option as a dentin substitute in class II sandwich restoration technique, where the interface with dentin and lining material is a contributing factor for microleakage. [8]

Ability of dye to penetrate into dentinal tubules resulting in an area of stained dentin which can be measured using image analysis is suggested to be one of the best techniques to check microleakage. Dye extraction method is used to measure the microleakage in restoration-dentin interface. [9]

The clinical success of the restorative materials largely depends good adhesion with the dentinal surface which in turn resists various dislodging forces acting on them. Hence the purpose of this in-vitro study was to compare marginal microleakage in posterior open sandwich restorationsby using recently available tricalcium silicate-based restorative material (Biodentine), flowable composite that is smart dentine replacement (SDR) and Resin Modified Glass Ionomer Cementin standard class II cavities as lining material on permanent teeth by using dye extraction method.

2. Material and Methodology

The present study was conducted in the Department of Conservative Dentistry and Endodontics. It is an in-vitro study which includes 30 intact extracted permanent human molars. The 30 extracted teeth were free from caries, restoration, hypoplasia or distinctly visible fractures.

2.1 Methodology

30 extracted and sound human molars were collected and cleaned of calculus, soft tissue and debris and stored in thymol solution for 1 month.

All 30 extracted teeth used in the study were equally divided into 3 groups: G1, G2, and G3.

Group G1: Biodentine (Biodentine™Bioactive Dentin substitute Septodont) as lining material+ light cured composite (3M ESPE Filtek™ Z350 XT) as a restorative material

Group G2: Smart dentine replacement (Smart Dentin Replacement-Compules Tips Gun) as lining material+ light cured composite (3M ESPE Filtek™ Z350 XT) as a restorative material

Group G3: Resin Modified Glass Ionomer Cement ((Light-Cured Universal Restorative-GC Gold Label Glass ionomer) + light cured composite (3M ESPE Filtek™ Z350 XT) as a restorative material as the positive control

Cavity preparation are done on the proximal surface with specific dimensions using high speed contra angle hand piece and No. 245 carbide bur. (**Figure 1**)

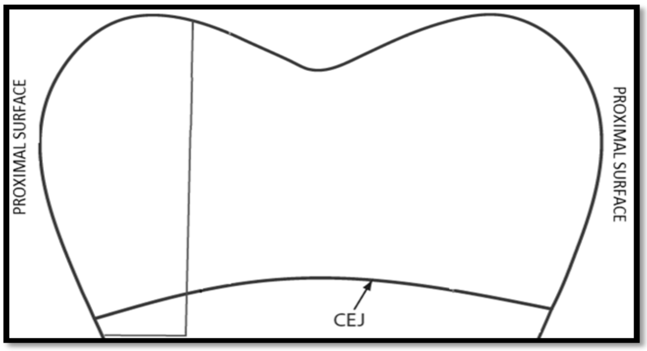


Figure 1: Schematic picture of cavity preparation

Restorative Procedure

The materials were applied according to the manufacturer's instructions. The cavities of all Groups were etched with 37%phosphoric acid (3M ESPE Scotchbond™ Multi-Purpose Etchant) for 15 seconds, thoroughly washed with water for 15 seconds and blot dried .The dentin was kept moist. Bonding agent (3M ESPE Adper™ Single Bond 3) was applied with applicator tip and light cured for 20 seconds.

Samples of each group were restored with biodentine, smart dentine replacement and Resin Modified Glass Ionomer Cement respectively as cervical lining restoration followed by increments of light cure composite as restorative material. Each increment was cured for 40 seconds. (**Figure 2, 3, 4**)

Figure 2: Group 1 Biodentine as lining material+ light cured composite (Filtek Z350 XT) as restorative material

Figure 3: Group 2 Smart dentine replacement as lining material+ light cured composite (Filtek Z350 XT) as restorative material.

Figure 4: Group 3 Resin modified Glass Ionomer cement as lining material +light cured composite (Filtek Z350 XT) as restorative material

Preparation for Microleakage Test:

After the restorations were complete finishing and polishing of the samples was done. The surfaces of the teeth were covered with 2 layers of nail varnish, except for the restoration and 1-mm around it. The apical foramens were sealed with acrylic resin. Samples were then stored in saline at 37˚ C and 100 % humidity for 24 hours.

The specimens were then thermocycled for 2500 cycles at 5-/+1˚C and 55+/- 1˚C with 30 seconds dwell time and were then immersed in 2% methylene blue dye for 24 hours at 37 degree C. The teeth were then thoroughly washed with water and acetone to remove the nail varnish.

Then, for use of spectrophotometer, the samples were placed in vial containing 69% nitric acid (69% EMPLURA Merck Life Science Private Limited) for 3 days to let methylene blue within restoration dentin interface dilute in nitric acid. There was 1000 µl acid volume in each vial.

The vials were then centrifuged at 14,000 rpm for 5 minutes and after that, 100 µl of the supernatant from each were transferred to a cuvette. The dye absorption was measured by an automatic double beam spectrophotometer at 550 nm wavelength using concentrated nitric acid as the blank.

The values on the spectrophotometer indicated the absorbance that is light absorption of the methylene blue in the resin-dentin interface which explains the microleakage of the restoration.

3. Statistical Analysis

Null Hypothesis: There is no significant difference between the three groups with respect to micro-leakage i.e. $\eta_1 = \eta_2 = \eta_3$

Alternate Hypothesis: There is a significant difference between the three groups with respect to micro-leakage i.e. $\eta_1 \neq \eta_2 \neq \eta_3$

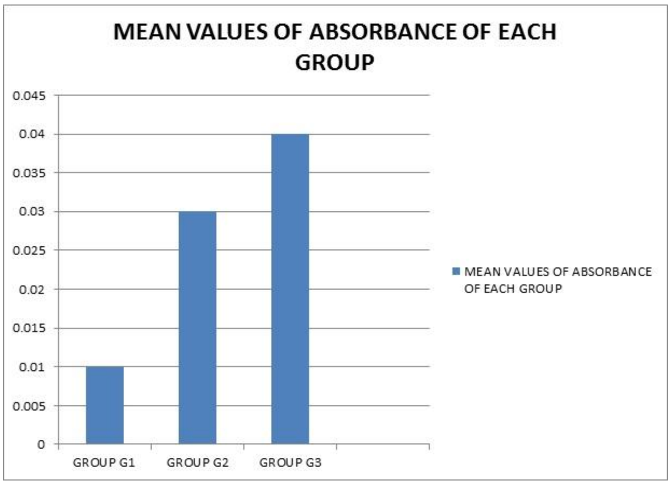
Level of Significance: α=0.05

Statistical Technique Used: One way ANOVA (analysis of variance).

Decision Criterion: We compare the p-Value with the level of significance. So, if $p<0.05$, we reject the null hypothesis and accept the alternate hypothesis, if $p \geq 0.05$, we accept the null hypothesis and if there is a significant difference, we carry out multiple comparisons using Tukey's HSD test.

4. Results

The mean score was found to be highest in Group G3 then Group G2 and Group 1. (**Graph 1**)



Graph 1: Graph Showing Comparison of Mean Values Of Absorbance of Each Groups

The difference in scores between the groups is found to be statistically significant (p<0.01). (**Table 1**)

Group	Mean	StdDev	ANOVA	p-Value
G1	0.01	0.01	F= 74.4	p<0.000001*
G2	0.03	0.01		
G3	0.04	0.01		

*denotes significant difference
Mean- mean value of absorbance of each group, Std Dev- is standard deviation

Table 1: The tables give us the various computations and the p-Value

In order to find out significant difference, we carried out multiple comparisons using Tukey's HSD test.We observed that there was a significant difference between all the groups (p<0.001). (**Table 2**)

Groups Compared	t-value	p-value	Q-value
[GROUP G1]vs[GROUP G2]	10.42	p< 0.0001	11.8212**
[GROUP G1]vs[GROUP G3]	12.76	p< 0.0001	16.7962**
[GROUP G2]vs[GROUP G3]	2.88	p< 0.0099	4.9750**

[Q=TukeyHSD: *=p<0.05 **=p<0.01]

Table 2: The tables give us multiple comparisons using Tukey's HSD test

5. Discussion

Microleakage is a dynamic phenomenon which results into compromised marginal seal. It causes hydrodynamic fluid movement through a degraded smear layer into the patent dentinal tubules underneath to result in hypersensitivity to thermal and osmotic stimuli and is referred to as sensory component of microleakage. Penetration of bacteria and their products through such potential gap accounts for pathologic component of microleakage those results in recurrent caries and subsequent pulpal pathosis. Clinically it is seen as staining of the marginsaround the restoration, post-operative sensitivity, secondary caries formation, restoration failure, pulpal inflammation and even pulpal necrosis. [10]

Amongst various methods to reduce polymerization shrinkage and microleakage, one of the methods is to apply an intermediate material as cervical lining restorations.

In our study standard class II restorations were made on posterior teeth with biodentine and smart dentin replacement as lining material followed by composite restoration and microleakge was compared with positive control light cured composite group. Biodentine group showed least microleakage followed by smart dentine replacement group and light cured composite group.

Biodentine is a two component material; tricalcium silicate (3CaO.SiO2) is the main component of the powder. It regulates the setting reaction, Dicalcium silicate (2CaO.SiO2): It acts as second main core material, Calcium carbonate (CaCO3): It acts as filler, Zirconium dioxide (ZrO2): It is added to provide the radio-opacity to the cement, Calcium chloride (CaCl2.2H2O): It is an accelerator, Water reducing agent (Superplasticiser): It is based onpolycarboxylate but modified to obtain a high short-term resistance.It reduces the amount of water required by the mix (water / cement),decreases viscosity and improves handling of cement. Liquid part is an aqueous solution containing calcium chloride which accelerates the system and partially modified polycarboxylate act as a super plasticizing agent to reduce the water content, which decreases the setting time and sets completely within 9 to 12 minutes. This material exhibits the same excellent biological properties as that of MTA and can be placed in direct contact with dental pulp. Thus, Biodentine is both a dentin substitute base and cement for maintaining pulp vitality and stimulating hard tissue formation, (i.e.) formation of both reactive and reparative dentin.[13]

Less microleakage in group G1 (biodentine) might be due to the ability to form hydroxyapatite crystals at the surface. Whencalcium silicate materials formed at the interface between the restorative material and the dentin walls, these crystals may contribute to the sealing of the material and tooth interface. Along with formation of apatite crystals the ultrastructure of calcium silicate hydrate may also explain the good sealing ability of the calcium silicate cement.[8]Thus, it is considered that this layer prevented the degradation of the cement and kept the marginal seal intact.[14]

Study by Han and Okiji[15] on the ultrastructure of the Biodentine-dentine interface following phosphatebuffered saline immersion (PBS) has demonstrated the formation of a "mineral tag" and confirmed that the formation of tag-like structures alongside an interfacial layer called as "mineral infiltration zone" by intertubular diffusion of carbonate from calcium silicate cement into the dentin.[16]

Similar results were shown by studies concluded by Koubi S [20], Solomon RV [21], Aggarwal V [22], Raju VG [8] when biodentine was used as intermediate material as cervical lining restorations.

Smart dentin replacement (SDR) is incorporated with photoactive group in a modified urethane dimethacrylate resin. Activated resin has demonstrated a relatively slow radical polymerization rate, suggesting that the photo initiator incorporated into the resin affects the polymerization process; moreover, the incorporation of activated resin results in 60-70% less shrinkage stress when compared to conventional methacrylate-based resins.[8]

SDR was initially available as a fluid composite resin whose reduced polymerization stress allowed it to be used as bulk in a single layer up to 4 mm thick, followed by a mandatory 2-mm cover layer of conventional composite resin.[12]

The use of flowable materials as a liner underneath the composite resin may also reduce the effects of C-factor. The use of fluid layer lowers the C-factor, hence lowering the internal stresses.[11] When the internal stresses are low, there is less competition between the contraction forces arising from monomer conversion and the adhesive agent to keep the composite bonded to the surface. Hence there is less microleakage.[11]

Similar results were shown by studies concluded by Atali PY [19], Sadeghi M [11]when smart dentine replacement was used as intermediate material as cervical lining restorations.

Various techniques have been advocated for detection and evaluation of microleakage around restoration margins such as dye penetration, chemical tracers, radioactive tracers, scanning electron microscope, air pressure, neutron activation analysis, electrical conductivity, yet the use of dyes as tracers is one of the oldest and most common method of detecting microleakage in vitro as it is inexpensive, nontoxic and leakage can be easily detected by various concentrations and also includes precision in evaluation of marginal seal. Its capability to give data on linear penetration and direct reading of the penetrated markers by the microscope, spectrophotometer, are the main advantages of this method. [17], [18]

The present study concluded that using biodentine as lining material reduces microleakage at the gingival margin as compared to a class II restoration with smart dentine replacement (flowable composite) and light cured composite.

Marginal integrity and adaptationto evaluate microleakage are performed to evaluate the effects of the different lining materials and techniques on the quality of the margins in composite restorations. This study was performed in vitro, which can be a screening procedure for ensuing in vivo studies. Previous studies indicated that results obtained from in vitro microleakage studies may be useful, but not always necessarily reproducible and completely reliable in clinical in vivo settings. However long term in-vivo studies are required before routine clinical usage.

6. Conclusion

Light cured composite showed greatest absorbance followed by smart dentin replacement andbiodentine which showed significant difference in absorbance. Higher the value of absorbance more is the microleakage. Within the limitations of this in- vitro study, it can be concluded that – Microleakage scores were less in those teeth where biodentine was used as cervical lining restorations. Yet none of the materials were able to completely eliminate microleakage when used as cervical lining restoration. Further in vivo and in vitro studies are required to determine the clinical validity of these techniques.

7. Acknowledgement

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8. Declaration

Funding: None
Conflict of interest: None declared
Ethical approval: Not required

We certify that we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have our name listed as a contributor. We believe the manuscript represents valid work. Neither this manuscript nor one with substantially similar content under my/our authorship has been published or is being considered for publication elsewhere, except as described in the covering letter. We certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately. We attest that, if requested by the editors, we will provide the data/information or will cooperate fully in obtaining and providing the data/information on which the manuscript is based, for examination by the editors or their assignees. Financial interests, direct or indirect, that exist or may be perceived to exist for individual contributors in connection with the content of this paper have been disclosed in the cover letter. Sources of outside support of the project are named in the cover letter.

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