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Comparative Evaluation of Marginal Microleakage of a Bioactive Composite Resin Using Three Different Bonding Agents in Non-Carious Cervical Lesions– An *In Vitro* Study

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Abstract

***Background and objectives*:** Increasing prevalence of non-carious cervical lesions makes it imperative to overcome challenges in tooth preparation, isolation and restoration. Bio-active restorative materials are relatively new concepts in dentistry having the potential to prevent secondary caries, a major cause of failure of restorations. Composite resins have the disadvantage of polymerization shrinkage leading to marginal gap formation. This study evaluated the marginal microleakage properties of Activa bio-active composite resin using three different adhesives in non- carious cervical lesions**.** ***Materials and method*:** Forty extracted maxillary & mandibular premolars for the study were collected. The specimens were divided into groups (n= 10). Box type class V cavities, 2mm inciso-gingivally, 3mm mesio-distally & 2mm in depth was prepared on the buccal surfaces. Group 1: Activa bio-active resin +No bonding agent, Group 2: Activa bio-active resin +Tetric N-bond, Group 3: Activa bio-active resin +Single bond Universal, Group 4: Activa bio-active resin + G Premio bond. Restorations were done and samples subjected to thermocycling. They were immersed in Rhodamine B dye solution for 24 hours. The samples were longitudinally sectioned in a bucco-lingual direction with diamond discs, examined under a stereo-microscope with 30x magnification and depth of penetration analyzed. The statistical analysis for microleakage was performed using the Kruskal Wallis test followed by the Mann Whitney U-tests with the Wilcoxon correction for pair-wise comparisons at a significance level of $p < 0.05$**.** ***Results*:** Activa bio-active resin + G Premio bond showed lesser micro-leakage compared to other groups and the difference was statistically significant**.** ***Conclusion*:** Activa bioactive resin with G Premio bond, 8th generation bonding agent showed lesser micro-leakage when compared with 5th and 7th generation bonding agents.

Keywords: Bioactive Restorative Material; G-Premio Bond; Microleakage;Stereomicroscope.

INTRODUCTION

Non-carious cervical lesions (NCCLs) include abrasion, erosion and abfraction. It includes tooth loss in various forms like shallow grooves, saucer shaped depressions, glassy smooth wedge shaped defects etc. Prevalence has been shown to range from 5-85% (Kaushik M *et al.*, 2017). The incidence of NCCLs is steadily increasing due to the increased life expectancy of the population. Based on the currently available literature, no single etiology can be identified, but there are indications that the condition is multifactorial (dos Perez C *et al.*, 2012).

Dental composites were developed as an aesthetic alternative to dental amalgams and were intended for the restoration of anterior teeth as they could not withstand the chewing loads of the posterior teeth. It is well known that polymerization shrinkage is the major cause of critical gap formation, subsequent microleakage and pulpal lesions. Despite improvements in modern dentin adhesion systems, the bond strength and marginal compatibility of composite resins to dentin remain less predictable than their bond strength to enamel. Difficulty in achieving proper sealing and hygiene increases marginal microleakage and subsequent caries recurrence, especially in deep cervical margins (Gerdolle DA *et al.*, 2008). Bioactive dental composites stimulate mineral apatite formation and has the potential to prevent secondary caries, a major cause of failure. This process forms a monoblock of the restoration and the tooth, penetrates and fills micro-gaps, reduces sensitivity, guards against secondary caries and seals margins against microleakage and failure. Activa (Pulpdent, MA, USA) is a highly esthetic, bioactive composite that delivers all the advantages of glass ionomers in a strong, resilient, resin matrix. Its formulation is based on amorphous calcium phosphate and bioactive glasses. The bioactive filler properties (composition, particle size, filler content, surface treatment) allow sufficient ion release in an aqueous environment without adversely affecting the basic properties of the composite like degree of conversion, polymerization shrinkage and related shrinkage stress, strength, hardness, modulus, degradation in water and biocompatibility (Song W *et al.*, 2021).

Based on their entry into the clinical scenario, the dentin bonding agents can be divided into various generations. The 5th generation agents were composed of the primer and adhesive in a single bottle. It is used along with the total etch technique where phosphoric acid is used to expose the collagen network and the agent will form a hybrid layer. These agents generally contain BIS-GMA, HEMA, GPDM, ethanol, Barium, Aluminium borosilicate glass, fumed silica, sodium, hexafluorosilicate and camphoroquinone. 7th generation bonding agents combine etching, priming and bonding in a single bottle. They are considered as 'All-in-one' preparations. The eighth-generation bonding agent uses nanosized fillers which increase the penetration of the resin monomers and the hybrid layer thickness, thus improving the mechanical properties of the adhesive (Ganesh AS 2020, Arisu HD *et al.*, 2008).

The aim of this study was to evaluate the marginal microleakage properties of Activa bio-active composite resin using 5th, 7th and 8th generation adhesives in non- carious cervical lesions.

METHODOLOGY

Forty freshly extracted non-carious, intact human premolar teeth indicated for orthodontic extraction were collected from the Department of Oral and Maxillofacial Surgery. Surface deposits were cleaned with an ultrasonic scaler tip and the samples were stored in normal saline. Box-type Class V cavities of dimension, 2x3x2 (2mm inciso- gingivally, 3mm mesio-distally & 2mm in depth) were prepared on the buccal surface of the premolars using No $33_{1/2}$ inverted cone bur (Mani, Japan). This has a head diameter of 0.6mm, head length of 0.5mm and a total bur length of 19mm. Occlusal part of the cavity was in enamel while the cervical part was in cementum. The dimensions of the prepared cavities were standardized using Vernier caliper. The prepared cavities were rinsed thoroughly with air/water spray.

The samples were randomly divided into 4 groups (n=10), depending on the type of restorative material and bonding agent. Group I - Activa without bonding agent, Group II – Activa + 5th generation bonding agent (Tetric N-bond, Ivoclar Vivadent India, Gurugram, India), Group III- Activa + 7th generation bonding agent (Single Bond Universal adhesive, 3M ESPE, MN, USA), Group IV – Activa + 8th generation bonding agent (G-Premio bond, GC Corp., Japan).

The cavities were restored with Activa Bioactive composite A2 shade using incremental technique and light cured for 20 sec (Bluephase, Ivoclar, Schaan, Liechtenstein). The restorations were finished and polished using Shofu finishing kit (Shofu Dental Asia-Pacific, Singapore), after which the specimens were stored in 100% humidity for 24 hours.

The samples were subjected to thermocycling in a water bath for 500 cycles between 5° and 55°C with a dwell time of 25s followed by subjecting them to cyclic loading for 10,000 cycles for another 25s. All the surfaces of the samples were triple coated with nail varnish, except a 1 mm cavosurface margin around the restoration and their apices were sealed with wax. All the specimens were immersed in 2% rhodamine B dye solution (Nitin Dye Chem, Valsad, India) for 24hrs after which they were rinsed with isotonic saline.

The samples were longitudinally sectioned in a buccolingual direction using a diamond disc. The sectioned restorations were examined under a stereomicroscope at 30X magnification. The depth of dye penetration was analyzed based on a scoring system suggested by Silveira de Araújo *et al.,* (2022). Score 0 - No dye penetration, Score 1 – Penetration involving half the occlusal/gingival wall, Score 2 – Penetration involving more than half the occlusal/gingival wall, Score 3 – Penetration involving the axial wall. The statistical analysis for microleakage was performed using the Kruskal Wallis test followed by the Mann Whitney U-tests with the Wilcoxon correction for pair-wise comparisons at a significance level of $p < 0.05$.

RESULTS

A total of 40 teeth were restored during this study with Activa Bioactive composite resin restorative material. The tooth- resin interface at the restorative margins was assessed for microleakage. The depth of penetration in Activa Bioactive restorative material was assessed with three different adhesives (Table 1). Group IV (Activa Bioactive + G Premio bond) showed least microleakage as compared to group II (Activa + Tetric N bond), Group III (Activa + Single bond Universal) and Group I (Activa + no bonding agent). Maximum leakage was observed at cervical margins of 7th generation agent compared to 8th and 5th generation bonding agents.

The dye penetration was observed to be higher in cervical margins among all the groups as shown in Table no 2. However, when comparisons were made by the Kruskal Wallis test, Group 4 (Activa Bioactive + 8th generation bonding agent) showed lesser dye leakage than the rest of the groups at cervical margins but this difference was not statistically significant.

Table 1: Distribution of dye penetration scores at the cervical margins in all the groups

Groups	Frequency (n)	Scores*			
		0	1	2	3
Activa bioactive + No bonding agent	10	0	2	5	3
Activa bioactive + 5th generation bonding agent	10	3	5	2	0
Activa bioactive + 7th generation bonding agent	10	1	4	4	1
Activa bioactive + 8th generation bonding agent	10	5	3	2	0
Total (N)	40	9	14	13	4

*Dye penetration scores
0 – No dye penetration
1– Penetration involving half the occlusal/ gingival wall
2– Penetration involving more than half the occlusal/ gingival wall
3 – Penetration involving axial wall

The results for dye leakage are as follows: Activa Bioactive + 8th generation bonding agent < Activa Bioactive + 5th generation bonding agent < Activa Bioactive + 7th generation bonding agent < Activa Bioactive + No bonding agent.

Table 2: Group wise mean leakage scores

Groups	N	Mean	Std. Deviation	Min	Max	x2	df	p value
1. Activa bio-active + No bonding agent	10	2.10	0.738	1	3	3	3	0.392*
2. Activa bio-active + 5th Generation bonding agent	10	0.90	0.738	0	2			
3. Activa bio-active + 7th Generation bonding agent	10	1.50	0.850	0	3			
4. Activa bio-active + 8th Generation bonding agent	10	0.70	0.823	0	2			

a. Kruskal Wallis Test
* Non-significant, (P value<0.05)

DISCUSSION

The longevity of composite resin restorations are compromised if the bond between the resin and the tooth surface fails to prevent microleakage. This can result in a compromised marginal seal, allowing bacteria, oral fluids, molecules and ions to enter the preparation walls/restorative material interface (Hegde N *et al.*, 2020). Dentin and enamel are different adhesive substrates. Dentin is low in minerals but high in organic matter and water content when compared with enamel. Hence, the bonding of resin to dentin is much more difficult and less predictable compared to enamel (Hegde N *et al.*, 2020). In the present study, class V cavities were prepared so that the coronal region was present in enamel and cervical region present in dentin. This simulated the clinical appearance of NCCLs (Arisu HD *et al.*, 2008).

When restorative materials are placed in the oral environment, they are constantly subjected to thermal changes that may compromise their long term function due to the intake of food and fluids at changing temperatures. To simulate the oral temperature, the specimens were subjected to thermocycling procedure in a water bath for 500 cycles of 5-55ºC with a dwell time of 25 sec. The samples were subjected to a further loading of 10,000 cycles with a dwell time of 25 sec to simulate occlusal forces produced in the cervical region of a tooth during normal function and parafunction (Zhao XY *et al.*, 2014).

Microleakage is caused by a variety of factors including dimensional changes due to polymerization shrinkage, thermal contraction, water absorption, mechanical stress and dimensional changes in tooth structure. Polymerization shrinkage can result in forces of contraction that may disrupt the bond to the cavity wall (Owens BM *et al.*, 2007, Santini A *et al.*, 2004). Microleakage is the basis for predicting the performance of any restorative material. There are several methods available for microleakage detection including scanning electron microscopy, dyes, chemical and radioactive tracers, neutron 5 activation analysis, fluid filtration etc. In the present study, dye leakage method was used as it is simple, reliable and cost-effective (Sooraparaju SG *et al.,* 2014). Rhodamine B dye was chosen for this investigation above other dyes because it diffuses more readily on human dentin than methylene blue and has molecular size of 1nm, which is less than the diameter of a dentinal tubule. The depth of dye penetration reveals any gaps between the restorative material and tooth interface that could allow bacteria and their byproducts to enter the area (Bortoluzzi EA *et al.,* 2006).

The Activa Bioactive - Restorative is a 'smart restorative' material releasing fluoride, calcium, sodium and silicon ions. According to the manufacturer, it is the first bioactive resin composite material which stimulates mineralization at tooth-resin interface with a resilient ionic resin matrix. It combines the hydrophilic properties, release and recharge of calcium, phosphate and fluoride ions which is more than glass ionomers with the durability and improved physical properties of composite resins (Amairah EI *et al.,* 2019, Tahidkheh S *et al.,* 2022).

Bioglasses have been used in Orthopaedics mainly due to their ability to naturally precipitate hydroxyapatite (HA) and facilitate bony integration. In dental composite resins, this ability has made BAG a source of remineralized ions. The surface of the BAG initially recharges Na and Ca ions with the H ions from the solution. This forms Si-OH groups with a Si rich coating by Na/Ca depletion. As the local pH increases, Si-O-Si bonds are attacked by OH^- ions forming SiOH. This precipitate coalesce thereby attracting Ca and PO_4 ions which in turn forms amorphous calcium phosphate (Tahidkheh S *et al.,* 2022). During the dissolution of BAG, there is an increase in pH. This along with the addition of trace elements like Silver imparts an antibacterial effect. The presence of hydrated Si provides a nucleation site for the hydroxyapatite hence inducing its precipitation immediately following dissolution. This has a beneficial effect in the reduction of marginal gap and nanoleakage at the resin-dentin interface. Additionally, the increase in local pH inhibits the MMP activity thereby protecting the hybrid layer from hydrolysis (Tahidkheh S *et al.,* 2022).

Activa bio-active restorative material have showed improved properties like prevention of secondary caries, marginal gap sealing, improving the longevity of hybrid layer and reducing post-operative sensitivity, remineralizing effect along with anti-bacterial property. Studies have shown that Activa bio-Active material when used in Class V cavities have good adaptation to the tooth surface and a low gap % (Abdel-Maksoud HB *et al.,* 2021).

The type and age of the teeth, the degree of dentin mineralization, the dentin surface being bonded, the type of bond strength test (shear or tensile), the storage medium, the relative humidity of the environment in the substrate, the complexity of the testing procedures, the sensitivity of handling and manipulating these systems and the composite material being used are some of the factors that affect *In Vitro* bond strength to dentin (Meshram P *et al.,* 2019). Due to the need for the adhesive to penetrate the micropores of the dentin, the hydrophilic resin thinner and solvent needs to be incorporated. For the liquid to be uniformly distributed on the substrate, the surface tension of the liquid must be less than the surface of the substrate. The low viscosity of the primer and/or adhesive resin is affected by the solubility of the monomer in the solvent, thereby improving the diffusion to the micro retained tooth surface. The hydrophilic nature of HEMA helps to stabilize the collagen framework in the hybrid layer. A good penetration and diffusion of resin to the dentin matrix is required to achieve high adhesion strength to dentin (Chandra PR *et al.,* 2013). When the inorganic part of dentin is removed, resin infiltration and tubular penetration will occur. However, in addition to removing the inorganic portion, the acidic conditioner alters the peptide charge, extracts non-collagenous proteins and protonates the collagen. The higher bond strength after application of HEMA may be due to the demineralized collagen being kept moist (Chandra PR *et al.,* 2013).

Two-step 5th generation bonding agents using the total etch concept have shown favorable clinical efficacy. Tetric N bond is composed of 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), HEMA (hydrophilic monomer), MCAP (methacrylated carboxylic acid polymer; hydrophilic agent which bonds with hydroxyapatite) and D3MA (decanediol dimethacrylate; hydrophobic linking monomer which bonds to the composite resin) (Ganesh AS 2020).

The next generation in dentin bonding agents has been the introduction of acid-based adhesives which allow simultaneous application of acid etching, priming and bonding steps as seen in the 6th and 7th generation adhesive systems. The mechanisms of action of these involve the demineralization of the dentin surface and the simultaneous penetration of monomers into the pores. The one-step system shortens the bonding protocol and has fewer chances of contamination during the clinical procedure (Santini A *et al.,* 2004). The seventh generation simplifies it further into a single component, one bottle system.

Adper Single Bond Universal (SBU) is a 7^{th} generation bonding agent having MDP which helps in chemical bonding to hydroxyapatite through nano-layering. It also has a polyalkenoic acid copolymer which forms MDP- Ca salts with hydroxyapatite, thereby promoting chemical bonding and stability in aqueous medium. These have a low pH of 2 thus demineralizing dentin and allowing penetration of resin monomers. The rehydrating monomer here is the Vitrebond copolymer which stabilizes the collagen framework in the hybrid layer thereby increasing the bond strength (Ganesh AS 2020). In our study, Activa in combination with Adper SBU showed more microleakage than Activa with 5th and 8th generation agents. This could be due to the instability of the collagen framework thereby reducing the strength of the hybrid layer (hegde N *et al.*, 2020).

GC G-Premio BOND is a universal, 8th generation bonding agent that is compatible with total-etch, self-etch and selective etch techniques. It can also be used to repair indirect restorations without the use of primer. It can be used in combination with a silane when repairing glass or hybrid ceramics and is also ideal in the treatment of hypersensitivity. The initial low pH helps in better adhesion thereby reducing post-operative pain (Ganesh AS 2020). A unique combination of three functional monomers (4-MET, MDP and MDTP) except HEMA ensures excellent stability and high bond strengths to tooth tissue and all indirect substrates, including composites, precious and non-precious metals, zirconia and alumina (Ganesh AS 2020). The smear layer acts as a bonding substrate, leaving smear plugs that reduce dentin fluid flow. These adhesives leave hydroxyapatite crystals available for chemical bonding of functional monomers to calcium thereby contributing to interface stability (Ganesh AS 2020).

CONCLUSION

Activa bioactive composite resin showed lesser microleakage when used with 8th generation bonding agents in non-carious cervical lesions. Activa bioactive composite resin with 5th generation bonding agent showed lesser microleakage when compared with 7th generation bonding agents. However, this difference was not statistically significant.

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