Dental Research Journal

Original Article

An *in vitro* micro-CT assessment of bioactive restorative materials interfacial adaptation to dentin

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Received: 06-Jul-2021
Revised: 11-Dec-2021
Accepted: 24-Jan-2022
Published: 18-Jul-2022

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ABSTRACT

Background: The background of this study was to improve the longevity of a restoration and optimal adaptation of restorative material to the prepared cavity walls is crucial. The study aimed to evaluate the interfacial adaptation of Activa, Micron, and Predicta bulk bioactive restorative materials to coronal dentin using micro-computed tomography (CT) analysis.

Materials and Methods: In this *in vitro* micro-CT assessment study, Class II mesio- and disto-occlusal cavities were prepared on 60 extracted human mandibular molar teeth. After etching and bonding procedures, all the mesial cavities (n = 60) were restored with Tetric N-Ceram and the disto-occlusal cavities with Activa or Micron or Predicta bioactive (n = 20 each) restoratives. Interfacial gap percentages were evaluated under micro-CT before (baseline) and after thermo-mechanical load cycling (TMC). Acquired data were analyzed statistically using one-way analysis of variance, Paired t-test, and Tukey's multiple *post hoc* procedures, at $P < 0.05$ level of significance.

Results: The interfacial gap percentages were lowest for Predicta bioactive and highest for the Micron group ($P < 0.05$). The number of gaps increased significantly after TMC in all the groups ($P < 0.05$). The adaptation of tested materials was inferior to axial wall and pulpal floor, whereas considerably better adaptation was observed on buccal and lingual walls.

Conclusion: Predicta bioactive followed by Activa bioactive has shown superior interfacial adaptation, whereas Micron bioactive demonstrated maximum microgaps compared to nanohybrid composite. Artificial aging with TMC has a negative influence on the internal adaptation of all tested materials.

Key Words: Activa bioactive, interfacial gaps, micro-computed tomography, micron bioactive, Predicta bioactive

INTRODUCTION

Due to the biocompatibility issues on mercury-containing products, restorative dental practice fosters an increased use of reliable materials other than amalgam.[1] Dental resin-based composites have evolved enormously in the recent past, offering esthetic and functional advantages with minimal preparation techniques.[2] Despite significant improvements, volumetric polymerization shrinkage during curing of resin composites ranges between 1% and 6%, and thus formation of interfacial gaps was the crucial issue

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How to cite this article: Angadala P, Mandava J, Ravi R, Hanumanthu KR, Penmatsa P, Pulidindi H. An *in vitro* micro-CT assessment of bioactive restorative materials interfacial adaptation to dentin. Dent Res J 2022;19:56.

with composite restorations.[1,3] A recent systematic review and meta-analysis reported the annual failure rate of 1%–3% for posterior composite restorations with bulk fracture and secondary caries being the main reasons for those failures.[2] Several studies stated that the bonded interface between the tooth and the restorative composite is a weak link by the formation of microgaps, allowing microleakage and bacterial invasion leading to secondary caries formation.[3,4]

With the improvements in technology, contemporary nanohybrid composites exhibited enhanced physical and mechanical properties with reduced shrinkage stress during polymerization compared to microhybrids.[5,6] However, a continuous, stable, 3-dimensional collagen-polymer network between adhesive and dentin substrate is hard to achieve. The disparity between the demineralized dentin depth and adhesive monomer infiltration activates the hydrolytic degradation of hybrid layer and endogenous collagenolytic degradation.[7,8] In addition, composite resin restorative materials accumulate more dental plaque biofilm, manifesting potential impact on recurrent caries formation.[9] To counteract these issues, several hybrid-type restoratives have been launched, and the manufacturers promoted them as "bioactive restoratives."

The "hybrid" restorative materials by incorporating bioactive agents such as bioglass, hydroxyapatite, calcium aluminate, or phosphate in the polymer matrix can preserve the interfacial adhesive integrity by promoting remineralization.[10] In addition, resin-modified glass-ionomer-based bioactive restoratives exhibited minimum polymerization shrinkage, i.e., <1.7%, and thus can reduce microgap formation and leakage at dentin restorative interface.[11]

The Activa bioactive (Pulpdent, Watertown, USA) is the first marketed "smart dental restorative" material, claimed to stimulate remineralization by releasing fluoride, calcium, sodium, and silicon ions. It is a dual-cure, hydrophilic resin-modified glass-ionomer containing bioglass and a patented polybutadiene diurethane dimethacrylate (Embrace) resin.[12] Earlier *in vitro* studies demonstrated enhanced wear and fracture resistance with high flexural strength for Activa bioactive, and they advocated its use for restoring occlusal stress-bearing areas.[13,14] Nevertheless, a recent randomized *in vivo* assessment of Class-II restorations with Activa reported a high failure rate and unacceptable clinical performance.[15,16]

Hydroxyapatite modified glass-ionomer restorative cement, the Micron bioactive (Prevest DenPro, Jammu, India), was recently introduced into the market. This bioactive restorative has shown excellent adhesion to the tooth, prolonged fluoride release, and exhibited high compressive strength with the mineralizing potential property.[17] The patented "alkasite" filler is responsible for the increased release of hydroxyl ions from the micron material, which regulates the pH during scathing attacks, thereby preventing demineralization.[12]

The Predicta Bioactive (Parkell, NY, USA), a bulk-fill dual-cure resin composite, has high compressive, tensile, and flexural strengths and is suitable for direct and indirect restorations. According to manufacturer claim, Predicta with its bioactive property provides a strong bond with the tooth by sealing the interfacial microgaps and protecting against microleakage.[18]

Internal adaptation refers to restorative material's ability to adapt to the dental substrate internally. Poor interfacial adaptation of a restorative material may result in plaque accumulation, microleakage, marginal discoloration, secondary caries, fracture, and restoration loss.[4,16,19] Hence, a longitudinal assessment of internal adhesive defects is required to gauge the newly introduced restorative material's clinical applications. For evaluating internal adaptation of the restorations, nondestructive micro-computed tomography (micro-computed tomography [CT]) imaging is a more authentic technique. This technique allows 3-dimensional evaluation of the entire prepared tooth effectively, irrespective of the shape or dimensions of a specimen. In addition, repeated evaluations on the same specimen can be done quantitatively at baseline and after the aging process.[20]

Considering the potential advantages of bioactive materials, this study investigated the internal adaptation of Activa, Micron, and Predicta-bulk bioactive restoratives compared to a nanohybrid composite in Class II cavities. The null hypothesis tested was (i) the internal adaptation of Class II restoration is not dependent on the type of restorative or different tooth-restorative interfacial locations. (ii) Aging with thermo-mechanical cyclic loading application did not have a detrimental influence on the integrity of tooth-restorative interface.

MATERIALS AND METHODS

In this *in vitro* micro-CT assessment study, sixty noncarious, fully formed mandibular molar teeth with approximal mesiodistal and buccolingual dimensions (±1 mm) were collected for the study following the protocols approved by the Dr. NTR University of Health Sciences (D188601021).Teeth were stored in saline till the experimental period that was not more than 3 months after extraction. Sample size estimation was done depending on the data acquired from the pilot study using G*power 3.1 software (version 2.0) Aichach, Bavaria Germany. Based on power analysis with a Type I error of 5% and confidence interval of 95%, the minimal sample size calculated was 12 teeth per group. Thus, 20 specimens for each group were considered to assess the internal adaptation of restorative materials.

Restorative procedure

Standardized Class II mesio and disto-occlusal (MO and DO) cavities with 3.5 mm depth and 3 mm wide occlusally, 5 mm deep proximally from cervical to occlusal marginal ridge area with a gingival seat width of 1.5 mm were prepared in all 60 teeth samples. Cavities were prepared using # Ex 41 straight fissure diamond abrasive (Mani, Tochigi, Japan) and #245 carbide burs (SS White, New Jersey, USA) and discarded each bur after every five tooth preparations.

The materials used, their composition, and application methods are presented in Table 1. After cleaning with distilled water, all the prepared cavities were randomly assigned into three groups (n = 20 each). The mesio-occlusal cavities of all 60 teeth were etched with N-etch (Ivoclar, Liechtenstein, Europe) and after Tetric N-Bond adhesive application, restored with Tetric N-Ceram nanohybrid composite (Ivoclar, Schaan, Europe) using a horizontal incremental layering technique. Each increment was allowed to cure for about 20 s using Bluephase C8 light-emitting diode unit (Ivoclar, Schaan, USA) having an irradiance of 800 mW/cm^2.

Disto-occlusal cavities were restored with one of the three tested bioactive restorative materials (n = 20 each). Activa material was available as a two-paste system that can be dispensed through the spiral nozzle by co-extrusion. After conditioning the disto-occlusal cavities of 20 teeth with 37% phosphoric acid for about 15 s, the Tetric N-Bond adhesive was light cured for 10 s. The flowable Activa was dispensed first into the proximal cavity with slow pressure allowing the material to flow ahead of the nozzle tip. The cavities were filled with two increments and each increment was allowed to self-cure for about 30 s followed by light curing for 20 s, based on manufacturer's instructions.

In the Micron group, disto-occlusal cavities (n = 20) were conditioned with 10% polyacrylic acid for 20 s and thoroughly cleansed with water. Two scoops of Micron powder along with two drops of liquid were dispensed and mixed vigorously for about 30 s. The material was deposited proximally, then in the occlusal cavity, and was condensed by Teflon coated plastic filling instruments-1102-T2 and 1208T2 (Addley, Germany) to circumvent void formation. The restoration was contoured and allowed to self-cure for 2 min by following the manufacturer's instructions.

In disto-occlusal cavities (n = 20) of Predicta bioactive group samples, after etching and Tetric N-bond application, the material was expressed into the cavity with slow pressure, filling the deepest portion to occlusal surface. To reduce the shrinkage stresses, the material was allowed to self-cure for 2 min, followed by light curing for 20 s. Using flame-shaped TR-25EF (Mani, Tochigi, Japan), diamond abrasives, marginal overhangs, and gross irregularities of the restorations were removed. Sof-Lex medium to fine-grit flexible disks (3M ESPE, MN, USA) and polishing cups (Shofu, San Marcos, USA) were used at slow speed to finish and polish the restorations.

All the restored teeth samples were stored in artificial saliva (Jiana Lifescience, Mumbai, India) to mimic intraoral conditions during the whole experimental period till the post TMC evaluations. The baseline inter-facial defects were digitized using a micro-CT scanner (X-Radia Versa 500, Zeiss, Germany) within 2 weeks after the completion of restorations.

Thermo-mechanical cyclic loading

After recording the baseline values of interfacial microgaps, teeth were loaded mechanically and thermically cycled. Thermocycling was done at 5°C to 55°C for 10,000 cycles with 5 s transfer time and 30 s dwell time in the cold and hot cycle.[21] The restorations were simultaneously subjected to 100,000 mechanical load cycles of 50 Newtons using a round piston of 5 mm diameter at 1 Hz frequency.[22]

Interfacial adaptation evaluation with micro-computed tomography

To analyze the internal adaptation of the materials,

Table 1: Composition and application modes of the tested dental materials

Name of the product	Composition	Manufacturer	Mode of application
N-etch etching gel	37 wt.% phosphoric acid in water, thickeners, and pigments	Ivoclar Vivadent Liechtenstein, Europe.	Etchant was applied first on enamel for 20 s, then on dentin for 10 s rinsing was done thoroughly with water for 10 s, and air-dried gently for 2 s
Tetric N-bond adhesive	Methacrylates, ethanol, water, highly dispersed silicon dioxide, and initiators	Ivoclar Vivadent Liechtenstein, Europe	With an applicator tip, adhesive was applied for 10 s with a gentle scrubbing motion. With a stream of air, excess adhesive was removed and polymerized for 10 s with light curing
Tetric N-Ceram composite	Resin: BisGMA, BisEMA, UDMA, polyacrylate. Filler: 50% wt barium glass, 5%wt Ba-Al-fluorosilicate, 5% wt mixed oxide, 1% wt highly dispersed silica, 17% wt ytterbium trifluoride	Ivoclar Vivadent AG, Schaan, Liechtenstein, Europe	Tetric resin composite was placed in 2 mm increments and light-cured for 20 s
Activa bioactive restorative	Blend of diurethane and other methacrylates with modified polyacrylic acid (44.6%), reactive glass filler (21.8 wt%), inorganic filler (56 wt%), sodium fluoride (0.75%), patented rubberized resin (Embrace), water	Pulpdent, Watertown, MA, USA	The material was dispensed into the cavity through a spiral nozzle, first in the proximal cavity and then occlusally. Each increment was allowed to self-cure for 20 s and then light-cured for 20 s
Predicta bulk bioactive dual-cure restorative	Di-benzoyl peroxide, diphenylphosphine oxide, poly (oxy-1,2-ethanediyl), 2-propionic acid, 2-methyl 1,6-hexanedyl ester, bicyclo (2,2,1) heptane, 2-hydroxy ethyl methacrylate, 4-methyl phenyl acrylate, nanofillers, titanium dioxide	PBD, Parkell, Edgewood, NY, USA	The material was dispensed into the cavity through a spiral nozzle and placed proximally in bulk and then occlusally. The material was allowed to self-cure for 20 s and then light-cured for 20 s
Micron bioactive	Powder: Fluoroaluminosilicate glass powder, hydroxyapatite powder Liquid: Polyacrylic acid	Prevest Denpro, Jammu, India	Dispensing of 2 scoops of powder and two drops of liquid was done. Initially, one-half of the powder was mixed in liquid and slowly remaining powder was mixed with liquid for 20 s
Micron conditioner	10% polyacrylic acid	Prevest Denpro, Jammu, India	The cavity was conditioned for 20 s by applying polyacrylic acid using cotton pellet and then cleansed with water. Then using damp cotton pellet, excess moisture was removed

BisGMA: bisphenol A-glycidyl methacrylate, BisEMA: bisphenol A diglycidyl methacrylate ethoxylated, UDMA: Urethane dimethacrylate

teeth were mounted in acrylic blocks and placed in the micro-CT scanner. The parameters were set as follows; a rotation of 360° vertically, a 0.5° rotational step, 100 kV voltage, and an exposure time of 20 min with 100 mA° beam current using a 0.5 mm aluminum filter. Three-dimensional images were generated and reconstructed using Scout and Scan TM control system 10.7.3679.13921 (Zeiss, Germany) software [Figure 1]. The images were assessed for microgaps, and data was analyzed using AVIZO software (FEI, Thermo scientific, Germany). All the values obtained in micrometers were converted into percentages by dividing the observed microgaps length by the total length of that particular wall/floor.[20]

$$\%\ \text{Interfacial defects} = \frac{\text{Gap length}}{\text{Wall / floor length}} \times 100$$

Statistical analysis

The interfacial gap percentages obtained were subjected to statistical analysis using SPSS software version 22.0 (IBM, Armonk, NY, USA). Shapiro–Wilk test observations revealed that the variable is normally distributed, and thus to compare the mean total interfacial gaps among the groups, one-way analysis of variance (ANOVA) was used. Tukey's multiple *post hoc* procedure was applied among different groups to compare the microgap percentages at different locations. Paired *t*-test was used to differentiate microgap percentages before and after thermomechanical cyclic loading. The entire assessment was done at a 95%

confidence level, hence $P \leq 0.05$ was considered significant statistically.

RESULTS

The outcome obtained indicates lower the percentage of microgaps; the better will be the adaptation of material to the cavity walls. One-way ANOVA results revealed significantly different mean microgap percentages between the groups at baseline and after artificial aging ($P \leq 0.001$) [Figure 2 and Table 2]. The mean interfacial gap percentage was significantly high for Micron bioactive and minimum for Predicta bioactive material. Paired *t*-test results revealed a significant increase in the interfacial mean gap percentages among the groups after thermomechanical cyclic loading ($P = 0.005$), and the increase in microgaps was not different among the groups ($P = 0.4256$).

As stated by One-way ANOVA and Tukey's *post hoc* test, the mean microgap percentages at different tooth regions were significantly high for axial and pulpal walls ($P = 0.003$)[Figure 3 and Table 3]. The microgap percentage values in descending order were as follows: Axial >Pulpal >Gingival >Buccal and lingual walls. All tested materials exhibited an increase in gap percentages after thermal and mechanical cyclic loading at all cavity locations ($P = 0.001$) without exhibiting any statistical difference among them ($P = 0.2741$).

Table 2: Interfacial gap percentages comparison between groups at different time periods by Tukey's *post hoc* test

Variables	Tetric N-Ceram	Activa	Micron	Predicta
Baseline	22.38^{Aa} (2.23)	18.32^{Ab} (2.45)	26.36^{Ac} (2.83)	11.24^{Ad} (1.90)
After TMC	25.18^{Ba} (2.10)	21.57^{Bb} (1.54)	29.14^{Bc} (2.10)	14.63^{Bd} (1.61)

TMC: Thermomechanical load cycling. A,a,B,b,c,d.values in paranthesis are standard deviations different lowercase letters in vertical coloumn indicates statistical difference among groups at each wall *P*=<0.05*.Different uppercase letters in a horizontal row indicates statistical difference among walls in each group *P*=<0.05*. identical letters indicates no difference among them

Table 3: Comparison of interfacial gap percentages at pulpal, axial, gingival, buccal, and lingual walls by ANOVA test, and pairwise multiple comparisons by Tukey's *post hoc* procedures

Groups	Pulpal	Axial	Gingival	Buccal and lingual
Tetric	24.75^{Aa} (4.63)	30.05^{Ba} (4.00)	20.79^{Ca} (4.03)	13.95^{Da} (2.65)
Activa	19.46^{Ab} (3.35)	27.49^{Ba} (4.00)	16.84^{Ab} (3.08)	12.49^{Ca} (3.56)
Micron	28.71^{Ac} (5.59)	29.71^{Aa} (4.19)	24.96^{Bc} (3.89)	22.07^{Bb} (3.67)
Predicta	12.80^{Ad} (2.82)	14.93^{Bb} (2.70)	11.61^{Ad} (2.15)	9.05^{Cc} (1.82)

A,a,B,b,C,c,D,d values in paranthesis are standard deviations.different lowercase letters in a vertical coloumn indicates statistical difference among groups at eachwall *P*=<0.05*.Different uppercase letters in a horizontal row indicates statistical difference among walls in each group *P*=<0.05*.identical letters indicates no difference among them

DISCUSSION

Microgap formation due to localized failure of a bond at the tooth-restorative interface is one of the most

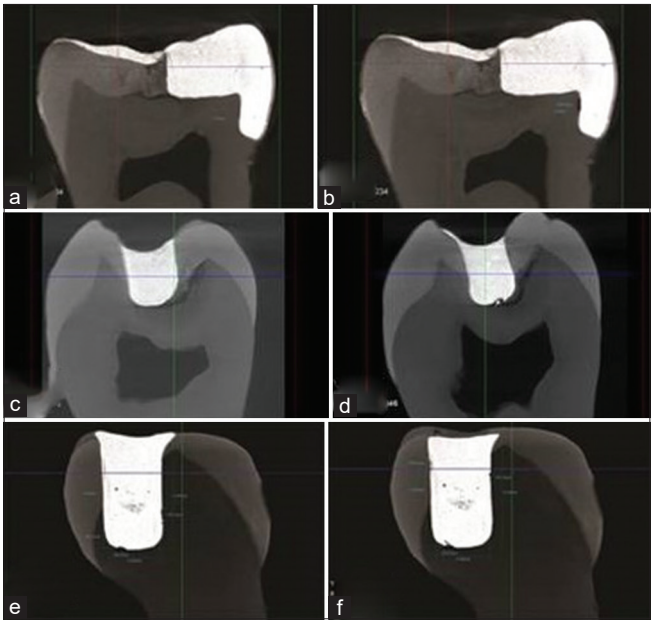

Figure 1: Micro-computed tomography images of Predicta group: (a) Axial wall before TMC, (b) Axial wall after TMC, (c) Pulpal wall before TMC, (d) Pulpal wall after TMC, (e) Buccal, lingual, and gingival walls before TMC, (f) Buccal, lingual, and gingival walls after TMC.

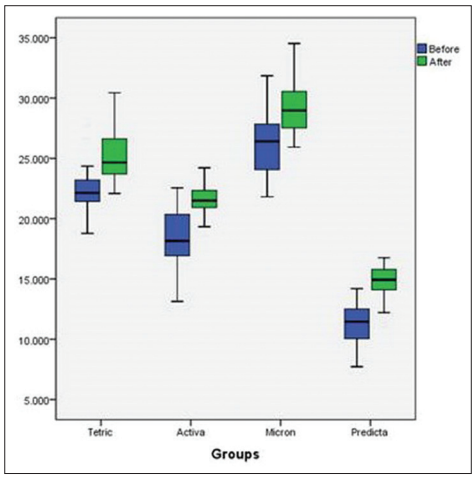

Figure 2: Box-plot diagram showing comparison of interfacial gaps percentages among four groups before and after thermomechanical cyclic loading.

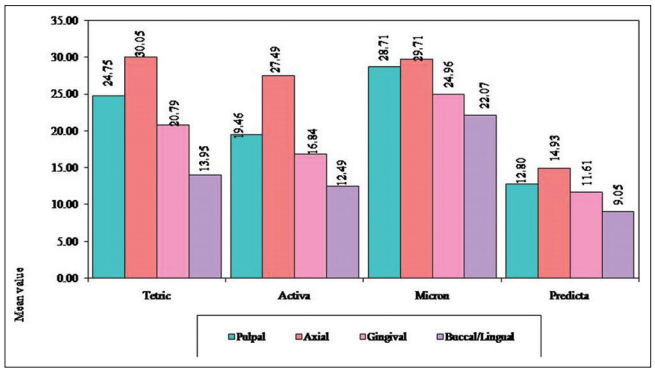

Figure 3: Comparison of interfacial gap percentages at different tooth regions.

critical aspects, while it may affect the strength and longevity of a restoration.[23] The bioactive restoratives containing bioglass may facilitate the dissemination of fluoride, calcium, and phosphate ions into dental hard tissues, motivating remineralization by forming a hydroxyapatite layer along the tooth-restorative interface.[24] Deposition of minerals around denuded collagen prevents enzymatic and hydrolytic degradation of hybrid layer by minimizing matrix metalloproteinase (MMPs) and cathepsin activity and thus improves the dynamic tissue behavior besides restoring dentin mineral content.[25]

At the time of remineralization, glass particle dissolution depends on the type of aqueous medium present. To simulate the intraoral conditions, restored teeth samples in the study were immersed in artificial saliva having ionic constituents similar to plasma throughout the experimental period.[26,27] Intraorally, restorations get exposed to occlusal masticatory and parafunctional forces along with intermittent temperature changes. These thermal changes bring about contraction and expansion of restorative materials, permit water and oral fluids to penetrate through resin matrix, and accelerate hydrolysis at the interface leading to adhesive bond failure.[28] Among the different techniques available, thermomechanical load cycling is the most effective method to mimic artificial aging.[21] Hence, the teeth were subjected to 10,000 thermal cycles and one lakh mechanical load cycles with an intermittent occlusal load of 50 Newtons in the study to simulate intraoral functioning for 1-year period.[22]

Different teeth may have dissimilar mineral content properties and are subjected to varying types of age-related changes. To avoid bias and achieve better standardization, restorations were executed on the same tooth for test and control materials in the study. Though large Class I occlusal preparations might have high C-factor by generating maximum polymerization shrinkage stresses, microleakage and secondary caries occurrence were noted most commonly at the cervical margins of proximal restorations.[29] In consequence, occluso-proximal restorations were done in the study to assess the internal adaptation of the tested materials.

It was observed that interfacial microgap formation significantly differed among the tested materials. Moreover, thermomechanical cyclic loading significantly increased the percentage of microgaps interfacially for all the tested materials. These results suggest that both the first and second null hypotheses were rejected. Predicta bulk bioactive material has shown superior interfacial adaptation with minimal microgap formation. With dual-cure ability, Predicta restorative can attain high compressive, tensile, and flexural strengths with bulk placement.[30] Besides, it releases calcium, phosphate, and fluoride ions to stimulate apatite mineral formation and might block the microgaps formed.[31] In addition, these bioactive restoratives have shown minimal shrinkage during polymerization facilitating better interfacial adaptation of the Predicta restorative. With its remineralizing ability, Predicta bioactive can reduce postoperative sensitivity, prevent secondary caries occurrence, seals the margins of a restoration better against microleakage, and improves the durability of the restoration.[18]

Activa bioactive has shown less percentage of interfacial gaps compared to Tetric N-Ceram and Micron bioactive restorative materials. Activa is a hybrid resin material having physical properties similar to composite resins and biological properties simulating that of glass ionomers.[30] However, some earlier studies reported less amount of fluoride release and more amount of microleakage with Activa bioactive.[12,27] A recent *in vitro* study recommended combining mechanical grinding of the substrate and chemical adhesive application to obtain optimal bonding outcomes with Activa restorative.[32] The better adaptability of Activa to the tooth surface in the present study can be attributed to the Tetric N-bond adhesive ability to provide a strong bond. It was manifested earlier that in contrast to G-Bond, 5th generation bonding agents can provide better adaptability with less microleakage for Activa restorative.[31]

Despite the manufacturer's claim that Micron bioactive has excellent adhesion to tooth and antibacterial property, it produced more percentage of interfacial microgaps among the tested bioactive restoratives in this study. Insufficient smear layer removal and lack of adhesive agent application might have caused defective adhesion of Micron to the prepared walls. The Tetric N Ceram composite resin disclosed a significantly higher percentage of microgap formation than Predicta and Activa bioactive restoratives in the study. Lack of chemical interaction with the tooth substrate and passive bonding of adhesive components of the Tetric N-Ceram might have caused the formation of microgaps maximum.

Bond degradation with increased interfacial gap percentages was noticed in all the tested teeth samples after thermomechanical cyclic loading. It was hypothesized that the bioactive materials could promote ion diffusion through the bonded interface and thereby increase the matrix to mineral ratio and minimize the nanoleakage.[10,12,14] The released fluoride ions inhibit pro- and active MMP-2 and MMP-9 activity and prevent bond degradation by diffusing phosphate and calcium ions through hybrid layers. These ions may precipitate and crystallize with the formation of Ca-PO4/MMP complexes inhibiting the MMP's activity.[33] During functional, occlusal activity, the generated stresses get transmitted through rigid, brittle filler particles to the flexible resin matrix. High interfacial stresses may be generated due to the larger interfacial area between the matrix and filler particles during thermal changes and mechanical stresses intraorally.[34] In spite of their proven ion-releasing ability, artificial aging has negatively influenced the interfacial adaptation of bioactive restoratives in the study.

In agreement with the outcome of previous studies, the interfacial gaps observed were maximum on the pulpal and axial walls compared to gingival, buccal, and lingual walls.[20,35] Dentinal walls of pulpal and axial walls are closer to the pulp. These walls, due to increased volume of fluid-filled dentinal tubules and elevated dentin permeability, impart suboptimal conditions for bonding. Compared to buccal and lingual walls, gingival interfacial gap percentages were more for all the tested materials. In the proximal cavity, gingival-occlusal height was longer than the buccolingual width and thus created more polymerization shrinkage stresses at the gingival seat area. Furthermore, insufficiency of enamel for bonding at gingival seat area makes this region less favorable for bonding.[36]

The study results indicate the use of bioactive restoratives as they exhibited less interfacial microgap formation compared to nanohybrid composites. Though *in vitro* testing of restorative materials is done for preclinical investigations, the outcome wouldn't necessarily translate the clinical presentation. Moreover, none of these new bioactive restoratives revealed good evidence regarding their long-term clinical performance, though very few *in vivo* studies were conducted till now.

CONCLUSION

The following conclusions can be drawn from the study results:

I. Predicta and Activa bioactive restoratives presented less percentage of interfacial microgaps compared to nanohybrid composite
II. Interfacial gap percentages on pulpal and axial walls were maximum and minimal on buccal, lingual walls
III. Thermal and mechanical cyclic loading has a negative impact on the internal adaptation of bioactive restorative materials.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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