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Evaluating Bonding Agent's Effect in Microleakage of a Bioactive Restorative Material with Thermocycling and PH Challenge Test

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Abstract

Purpose: To evaluate microleakage of a bioactive restorative material with and without bonding agent, and a conventional nanocomposite. Method: Forty-eight Class V preparations were prepared on the buccal and lingual aspects of 24 human third molar teeth. Preps were restored with Activa with a bonding agent, Activa with no bonding agent, and Filtek Supreme Ultra with a bonding agent. Thirty-six specimens were thermocycled, while the remaining 12 non-thermocycled specimens served as controls. All groups underwent a 21 day cycle of acidic pH challenges followed by immersion in a saliva substitute. Specimens were immersed in methylene blue, sectioned, and evaluated under light microscopy. Microleakage was quantitatively assessed by measuring methylene blue penetration at the tooth-restoration interface. Results: Mean microleakage: Bonded Activa 0.40±0.12 mm, non-bonded Activa 0.04±0.02 mm, bonded Filtek Supreme Ultra 0.44±0.12 mm, and bonded Activa (non-thermocycled control) 0.49±0.07 mm. One-way ANOVA and Fisher LSD Post Hoc testing demonstrated significantly less microleakage for Activa restorations without bonding agent ($P<0.001$). Conclusions: Activa without a bonding agent had less microleakage when compared to Activa and Filtek Supreme with bonding agents ($P<0.001$).

Key words: Bioactive material, bonding agent, microleakage, thermocycling, PH challenge

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Introduction

New bioactive composites have recently been gaining attention in the field of dentistry due to their claimed ability to seal against microleakage. Activa, a bioactive restorative material, was introduced in February 2014, and is one of the first bioactive dental restorative materials that claims bioactive properties. Activa consists of the glass particles and polyacid components found in RMGIs incorporated with a new bioactive aids dynamic interaction with saliva, allowing it to recharge and release fluoride, calcium, phosphate, and fluoride ions.[1] Most importantly, bioactive restorative materials such as Activa claim to solve the issue of microleakage by stimulating apatite formation at the tooth restoration interface - filling gaps, sealing margins, and essentially rebuilding tooth structure.[1]

A restorative material that seals against microleakage would be considered a significant advantage to restorative dentistry. Microleakage is defined as the passage of bacteria, fluids, molecules, or ions between the tooth and the restorative material applied to it.[2] It is considered to be a major factor influencing the longevity of dental restorations, and is the main cause of restorative failure.[2,3] In a study that evaluated class I composite restorations, secondary caries was the cause in 113 out of 129 cases of failure.[3] Composite restoration failures are attributed to the composite-tooth interface which often forms microgaps allowing microleakage over time in vivo, and subsequent bacterial invasion. Microleakage also can lead to staining at the cavosurface margins, recurrent caries, hypersensitivity, and the development of pulpal pathology.[2] Unfortunately, conventional resin-based restorative materials produce dental restorations that do not always provide a complete marginal seal which frequently leads to restorative failure.

Numerous comparative studies on microleakage have been published on different dental restorative materials. In 2011, a study comparing glass ionomer, resin modified glass ionomer, compomer, microhybrid, and nanocomposite found that the nanocomposite (Filtek Supreme Ultra) used with a bonding agent had the least amount of microleakage out of all these restorative materials.[4] This finding was repeated in another comparative study of the same materials in 2017.[5] Additionally, there have been recent studies investigating the effects of bioactive materials. Composites with added bioactive glass fillers demonstrated greater resistance to bacterial penetration at restorative margins than conventional composite.[6] Multiple studies have investigated the bioactive effects of poly amido amine (PAMAM) combined with amorphous calcium phosphate (NACP). These studies have demonstrated that nanocomposites that have been treated with both PAMAM+NACP showed evidence of remineralization on dentin discs after a 21 day cycle in an artificial saliva/lactic acid environment.[7,8,9] These results suggest that bioactive materials have the potential to remineralize at the tooth restoration interface. These properties may allow Activa to offer a unique advantage over non-bioactive restorative resins.

Activa does not require a bonding agent,[1] eliminating a step which is useful for operators who need to work quickly such as pediatric dentists. Current literature shows good preliminary results for new bioactive restorative materials. In a laboratory study, Activa demonstrated greater sealing capabilities than zinc phosphate (the gold standard for crown cementation).[10] Furthermore, bioactive restorative materials have been shown to exhibit greater wear resistance in comparison to other RMGIs.[11] In addition, bioactive materials have also demonstrated acceptable results as a pulp capping material, albeit more cytotoxic compared to conventional liners such as Dycal and Theracal.[12]

More independent laboratory and clinical studies are needed to fully determine all properties and long-term clinical performance of the Activa materials. The purpose of this study was to evaluate microleakage of a bioactive material (Activa) relative to a conventional nanocomposite (3M Filtek Supreme Ultra) by analyzing dye penetration (methylene blue) at the tooth-restoration interface after thermocycling, pH challenge, and immersion in a saliva substitute solution.

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In addition, this study evaluated the efficacy of thermocycling by including a thermocycling control group.

Methods

Twenty-four extracted, caries- and restoration-free permanent molars were collected from oral surgery offices and stored at room temperature in deionized water for less than 6 months. The teeth were cleansed of soft tissue with a curette and stored in deionized water with 0.05% sodium azide until ready for use. Class V preparations were prepared on buccal and lingual surfaces coronal to the cementoenamel junction of the extracted molars. All preparations were placed by the same operator (the primary investigator) and measured approximately 6 mm long, 1.6 mm deep, and 3 mm wide. These dimensions were translated to the specimens by small marker holes punched in a Mylar strip designating the four corners of the preparation. A deionized-water cooled high-speed turbine handpiece (NL9000s, Brasseler USA Dental Instrumentation, Savannah, GA, USA) was used with a 330 carbide bur (Komet H24, Komet, Rock Hill, SC, USA). Each bur was replaced after every preparation. Specimens were allocated randomly into four different groups (see Figure 1).

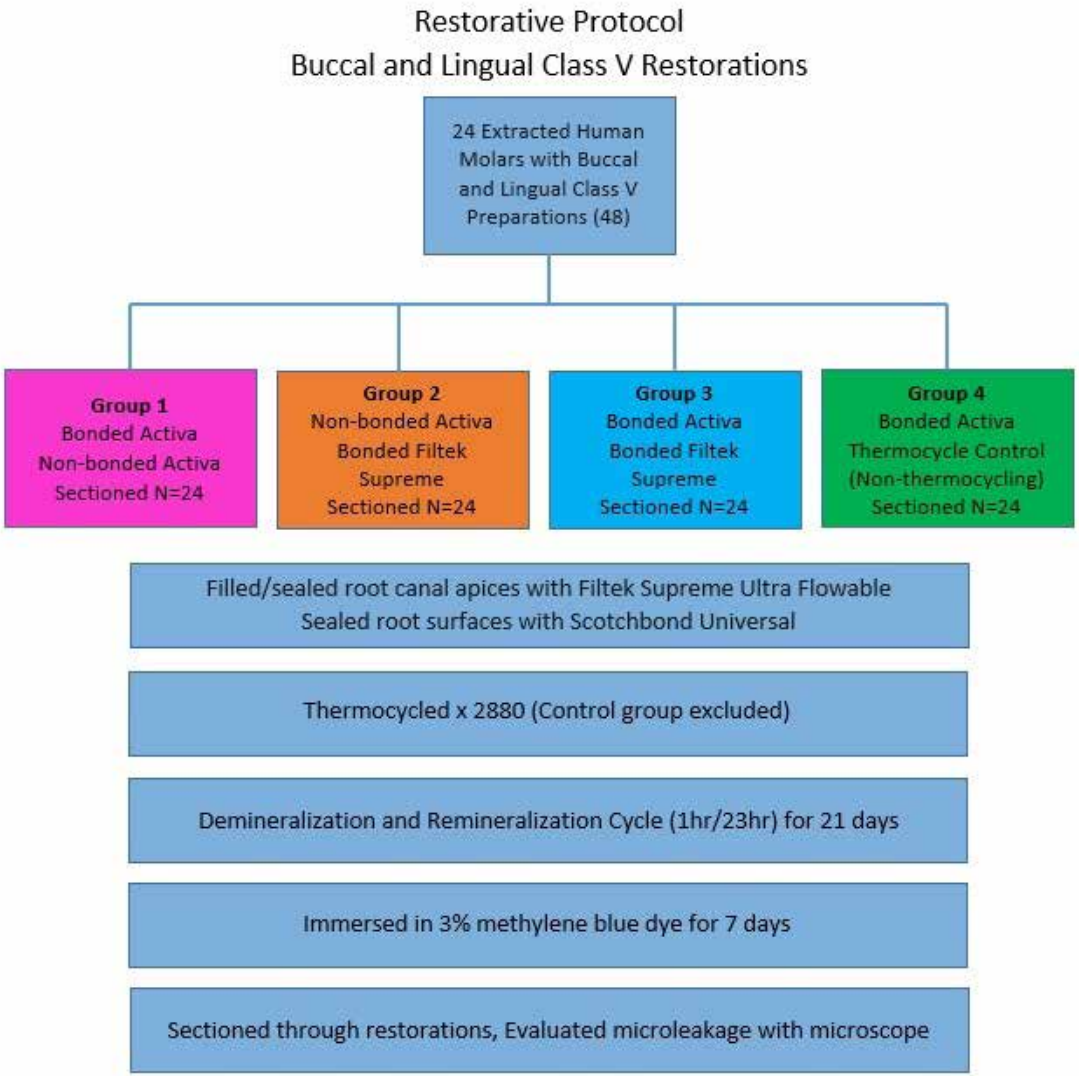

Figure 1. Flowchart of the research design

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In the following groups, all the preps were etched with 35% phosphoric acid (Ultra-Etch, Ultradent Products, Inc, South Jordan, UT, USA) for 15 seconds (per manufacturer guidelines). For the bonded Activa, 3M Scotchbond Universal Adhesive (Scotchbond Universal Adhesive Dose Pack, 3M ESPE, St. Paul, MN, USA) was applied (scrubbed in for 15 seconds) and air-dried prior to light curing for 10 seconds. For the Filtek Supreme restorations, 3M Scotchbond Universal Adhesive was applied (scrubbed in for 15 seconds), air-dried, and light cured for 10 seconds. Filtek Supreme Ultra placement followed manufacturer's guidelines. All Activa, Filtek Supreme and Filtek Supreme Ultra restorations were light cured for 20 seconds. Groups 1-3 underwent thermocycling from 5°C to 55°C for 5,000 cycles with 30-sec dwell time.

- Group 1 - Bonded Activa and Activa with no bonding agent: had one side restored with bonded Activa (Activa Bio-active Restorative, Pulpdent Inc., Watertown, MA, USA) and the other side restored with non-bonded Activa.
- Group 2 - Non-bonded Activa and bonded Filtek Supreme Ultra: The buccal and lingual preparations in this group were restored so that each tooth had one side restored with non-bonded Activa, and the other side restored with Filtek Supreme Ultra.
- Group 3 - Bonded Activa and bonded Filtek Supreme Ultra: The buccal and lingual preparations in this group were restored so that each tooth had one side restored with bonded Activa, and the other side restored with bonded Filtek Supreme Ultra.
- Group 4 – Non-thermocycled control with bonded Activa: The buccal and lingual preparations in this group were restored using Activa with bonding agent.

A 3M XL3000 visible-light polymerizing device (Model VCL 5530 BAW, 3M ESPE, St Paul, MN, USA) was selected for light polymerization of the restorations. Clear vinyl plastic tubing (clear vinyl tubing, 10 mm [3/8 inch], ID No. SVIG 20, Watts, North Andover, MA, USA) was cut approximately 25 mm long and adapted as a friction-fit sleeve over the light-guide tip. The tubing was adjusted until the tip of the light cure and the edge of the tubing was about 6mm apart. This distance was determined to provide adequate curing and resin poylmerization, based on an internal light meter that was built into the curing unit. Output was measured at ~750 mW/cm2 using a MARC Patient Simulator (MARC Patient Simulator, BlueLight Analytics, Inc., Halifax, Nova Scotia, Canada). Following subsequent full light polymerization, restorations were finished flush to the tooth surface with a 30-bladed finishing bur (Komet H274UF 016, Komet, Rock Hill, SC, USA) under water spray, and polished with medium-fine and fine Ultradent Jiffy Cups (Jiffy Cups, Ultradent Products, Inc., South Jordan, UT, USA). Finished specimens were stored in deionized water at 37°C with 100% humidity. The root apices of all specimens were sealed with prior to thermocycling. The root apex sealing procedure was accomplished by etching all root surfaces for 15 seconds, then applying 3M Scotchbond Universal Adhesive bonding agent over the etched surfaces, and light-polymerizing for 10 seconds. Finally, Filtek flowable composite resin (Filtek Supreme Ultra Flowable Restorative, 3M ESPE, St. Paul, MN, USA) resin was applied over the root canals ensuring adequate coverage of the apical opening, and light-polymerized for 20 seconds.

Thermocycling: Groups 1-3 were submitted to thermocycling between 5°C, and 55°C each with dwell times 30 seconds respectively in each water bath for a period of 2,880 cycles.15 These temperatures and dwell times represent exaggerated conditions of the upper and lower limits of temperature reached in the oral cavity.[2] Group 4 was the non-thermocycled control.

Acid Challenge and Artificial Saliva Immersion: To simulate the ongoing process of demineralization and remineralization in the oral cavity, all groups were immersed in a prepared artificial saliva at 37°C for 23 hours followed by

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immersion in a lactic acid solution at 37°C with a pH 4.5 for 1 hour. The artificial saliva was prepared by dissolving 0.795 g $CaCl_2 2H_2O$, 0.400 g KCl, 0.780 g $NaH_2PO_4 2H_2O$, 0.005 g $Na_2S_9H_2O$, 1.00 g CH_4N_2O, 0.795 g $CaCl_2 2H_2O$, and adjusted to pH 6.0 with NaOH (1 mmol/L) into 1 L distilled water.[13] The lactic acid solution was prepared by using a 0.1M lactic acid solution titrated to a pH of 4.5 with NaOH to simulate a cariogenic condition.[14] Specimens were kept upright in the solution baths, and were rinsed thoroughly with deionized water between transfers. The demineralization and remineralization cycle (1 hour demineralization/ 24 hour remineralization) was repeated for 21 days. This step was performed to utilize the bioactive nature of Activa - allowing for a period of remineralization in the saliva substitute and demineralization in the lactic acid solution. Dye Penetration: The root surfaces were coated with 3M Scotchbond Universal Adhesive to ensure that the roots remained sealed. This was accomplished by etching for 15 seconds, rinsing and drying the root surfaces, coating the root surface with 3M Scotchbond Universal Adhesive, and light-polymerizing for 10 seconds. The oxygen-inhibited layer was wiped off. To further seal all surfaces of the specimens, fingernail polish (Sally Hansen Hard as Nails Extreme Wear, Morris Plains, NJ, USA) was placed to within 1 mm of the restorations. Clear nail polish was placed on the root surfaces. Pink, orange, blue, and green fingernail polish colors were placed on the coronal portions of specimens with bonded Activa, non-bonded Activa, and bonded Filtek Supreme, and non-thermocycle control groups respectively. This step was performed to limit dye penetration to the tooth-material interface, and not be confounded by structural defects in the tooth itself. Specimens were immersed in a 3% solution of methylene blue dye (pH=9) at 37°C for 7 days in a sealed container (Deli Container 8 oz with Lid, Durahome, USA). Specimens were thoroughly rinsed with deionized water following methylene blue dye immersion.

Sectioning: Appropriate length pieces of 0.030" stainless steel wire (Retainer Wire 14" 0.030 Medium, Henry Schein, Melville, NY, USA) were fixed in position across the occlusal surfaces and centered over the buccal and lingual restorations using dental sticky wax (Stick Wax, Light Amber Sticks, Kerr Restorative, Orange, CA, USA) (Figure 2). The specimens

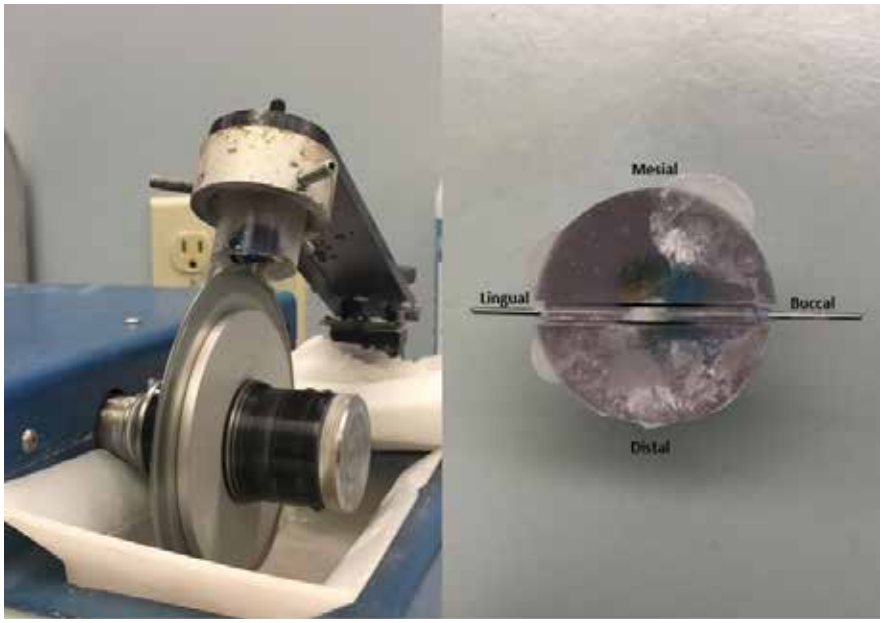

Figure 2. Slide preparation with tooth embedded in acrylic.

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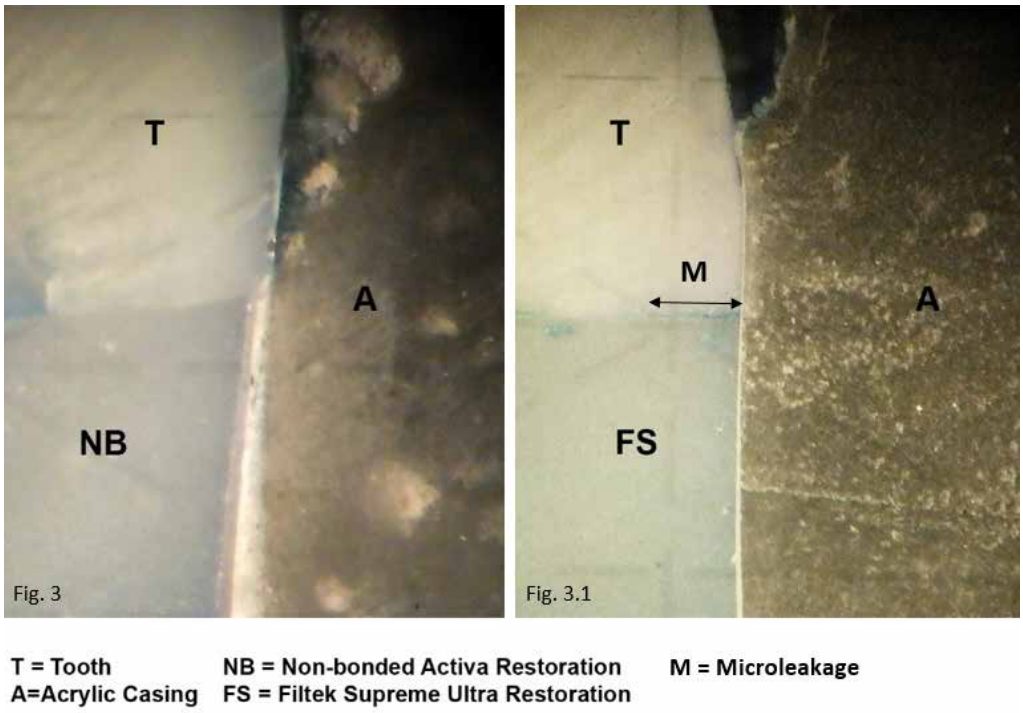

Figure 3. Shows no microleakage
Figure 3.1. Shows the sample with microleakage separate

were then encased in acrylic resin (Neocryl Orthodontic Acrylic Resin, Keystone Industries Bosworth, Gibbstown, NJ, USA). Restorations were sectioned twice vertically in a buccal-lingual direction using a slow-speed diamond wheel saw (Model 650, South Bay Technologies Inc, San Clemente, CA, USA). The stainless steel wire served as a guide to ensure that each cut passed properly through the buccal and lingual restorations (see Figure 2). A horizontal cut with distilled water coolant using a dental model trimmer (Model MT12, Ray Foster Dental Equipment, Huntington Beach, CA, USA) was used to subsequently separate the sectioned restorations from the apical half of the roots. All surfaces of each specimen and acrylic cylinder were dried using Kimwipes absorbent tissues and placed in a dry storage container to prevent further dye penetration.

Light microscopy: The vertical depth of dye penetration at the tooth-restorative interface was recorded using a 3D traveling light microscope (SKU 38025, Opto-metric Tools, Inc., Rockleigh New Jersey) at X20 magnification (see Figure 3). The measurements in this study were all taken from the margins of the restorations. Each buccal and lingual class V preparation were evaluated and recorded (see Figure 3; M=microleakage). Each of the cut sections were recorded on both mesial and distal sides.

Statistical Analysis

This study protocol offered 0.99 statistical power to detect a dye penetration, assuming two-sided tests with P=0.05. Power was calculated with G*Power (Version 3.9.1.2., G*Power, Universit t Düsseldorf, Germany) using data from a previous microleakage study. [15] Statistical Package for the Social Sciences (SPSS) 25.0 computer software (IBM, Armonk, New York) was used for descriptive and inferential statistics. Descriptive statistics for the dye penetration included means, standard deviations, frequencies, and graphs. Inferential statistics included one way ANOVA, and Fisher least significant difference (LSD) post hoc tests, at significance level P <0.05.

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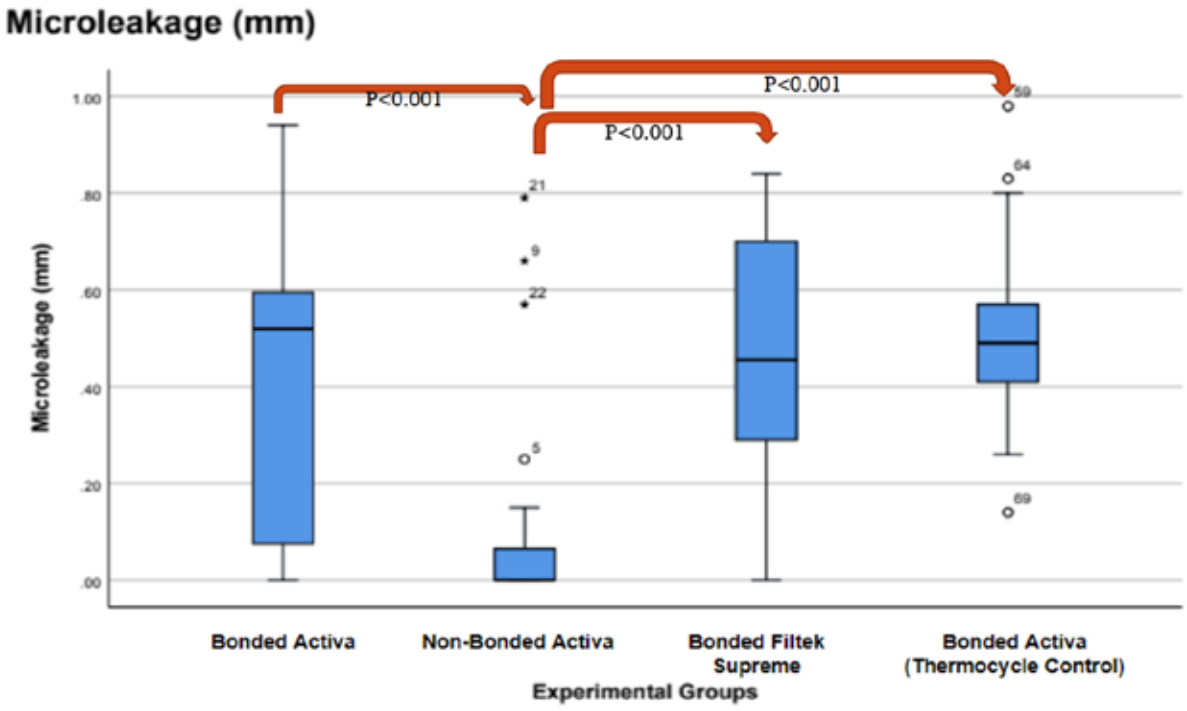

Figure 4

Table 1

Technique	Microleakage (mm)					
	Mean	SD	Min	Max	95% Confidence Interval Lower	Upper
Bonded Activa (N=24)	0.40	0.12	0	0.87	0.29	0.56
Non-bonded Activa (N=24)	0.04	0.02	0	0.26	0.01	0.06
Bonded Filtek Supreme (N=24)	0.44	0.12	0	0.83	0.32	0.56
Bonded Activa (N-TC*) (N=24)	0.49	0.07	0.14	0.84	0.41	0.56

*Abbreviation N-TC = Non-Thermocycle Control

Results

Of the anticipated 96 data points, all 96 were used to conduct the statistical analysis. Table 1 and box plot (see Figure 4) summarize the mean microleakage values and standard deviations for the sample restorations organized by restoration technique and use of finishing varnish.

The mean microleakage for the bonded Activa was 0.40±0.12 mm, compared to 0.04±0.02 mm, for non-bonded Activa, and 0.44±0.12 mm, for bonded Filtek Supreme. The mean microleakage for the bonded Activa non-thermocycle control was 0.49±0.07 mm.

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One-way ANOVA revealed significant difference in microleakage observed in the different restorative materials used (P<0.001, Figure 4). Experimental groups were compared using the Fisher LSD post hoc test, shown in Figure 4. There was a significant decrease in microleakage in the non-bonded Activa group compared to bonded Activa (P<0.001) and bonded Filtek Supreme Ultra (P<0.001). There was no significant difference between the bonded Activa and bonded Filtek Supreme Ultra groups (P=0.512). There was also no significant difference between the bonded Activa and bonded Activa non-thermocycled control groups (P=0.194)

Discussion

Traditional dental composites and bonding agents replace the missing volume of teeth after cavity preparation, but are typically bioinert - resulting in microleakage between the tooth-restoration interface. Bioactive restorative materials have recently been introduced as a solution against microleakage, which could potentially decrease restorative failure. The term bioactive refers to the ability of a substance to elicit a response from a host tissue for future formation (reformation) of a new specific substance or material.[16] Activa, a bioactive restorative material, is advertised to facilitate apatite formation at the tooth-restoration interface, thereby effectively sealing against microleakage. This is reportedly accomplished by the ability of Activa to extract fluoride, calcium, and phosphate ions from the saliva and release these ions to promote remineralization around the tooth-restoration interface.[1]

The results of this study demonstrated that non-bonded Activa yielded significantly less microleakage than bonded Activa and bonded Filtek Supreme (Figure 4). In addition, the use of bonding agent with Activa had comparable microleakage results with Filtek Supreme. These findings suggest that remineralization does occur around the tooth-restoration interface, and that the use of bonding agent interferes with this process. One of the reasons may be that the bonding agent does not allow for intimate contact of the Activa with the tooth surface. Therefore, the ionic exchange between the Activa restorative material and tooth structure does not allow remineralization to occur. Conversely, using Activa without a bonding agent allows for remineralization to take place at the tooth-restoration interface, and develop a seal against microleakage.

The findings from this study deviate from those that have been conducted in the past. Previous published reports on Activa indicated no significant differences in microleakage between Activa, RMGIs, and nanocomposite.[17,18] However, these studies failed to take into account the bioactive nature of bioactive materials in that a step that would simulate the oral environment and promote hydroxyapatite formation at the tooth restoration interface was not included. To accomplish this in the present study, a pH challenge and artificial saliva immersion cycle was introduced following thermocycling. The incorporation of a saliva immersion and pH challenge cycle induced remineralization and demineralization and is necessary, since the dynamic interaction between the ions in saliva seems critical to elicit the bioactive properties of Activa.[1] Previous studies evaluating bioactive materials have shown remineralizing capability after 21 days of saliva immersion and pH challenge.[7,8,9]

The method of microleakage evaluation in this present study also differed from previous studies, which could also explain the difference in the results. Cannavo et al. (2014) immersed specimens in ammoniacal silver nitrate solution for 3 hours, followed by immersion in a photo developer for 6 hours.[17] In Owens et al. (2017), specimens were immersed in methylene blue for a total 8 hours.[18] In these two studies, there was no reported difference in microleakage between Activa, RMGI, or composite. In this study, specimens were immersed in methylene blue for a total of 7 days. A significant difference was found between non-bonded Activa compared to bonded Activa and Filtek Supreme. Time of immersion for microleakage studies have a wide variation, ranging from 4 hours to as much as 30 days.[2] While the use of 7 days for

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this investigation falls within this range, it would be more pertinent for future Activa studies to reduce the immersion time to no more than 24 hours in order to compare results across studies.

The use of thermocycling has been used repeatedly in the dental literature for in vitro microleakage studies.[4,15] The purpose of thermocycling is to induce marginal percolation at the tooth-restoration interface due to differences between the thermal coefficients of expansion of the restorative material and tooth.[19] However, the necessity of including thermocycling in microleakage studies has yet to be determined. Previous investigators evaluated Class V and Class II resin restorations and concluded that there was no difference in microleakage scores for the group that was not thermocycled versus the groups that were thermocycled.[20,21,22] Therefore, a non-thermocycled control was added to the present study to evaluate the efficacy of thermocycling and its role in microleakage studies. Interestingly, the group that was not thermocycled exhibited greater microleakage than the thermocycled group, however, this result was not statistically significant (P=0.194). This finding suggests that meta-analyses are needed to draw any definitive conclusions.

This investigation is one of the first to demonstrate that the use of Activa without bonding agent may result in less microleakage than the use of a nanocomposite. This finding deviates from some previous in-vitro Activa studies, perhaps due to the addition of a saliva immersion and pH challenge cycle to this study. The introduction of the saliva immersion plus pH challenge could have taken advantage of the bioactive nature of Activa, resulting in a superior seal against microleakage when used without a bonding agent. In addition, the present investigation demonstrated that Activa used with bonding agent compared favorably with Filtek Supreme Ultra. Clinical studies may be able to show if these properties provide greater restorative longevity and lower failure rate.[23]

Conclusions

Within the limitations of the present study, the results support that:

1. The use of a bioactive restorative material without bonding agent exhibits significantly less marginal microleakage than a nanocomposite material placed using a bonding agent (P<0.001).
2. The use of a bioactive restorative material without bonding agent exhibited significantly less marginal microleakage than its use with a bonding agent (P<0.001)
3. When placed using a bonding agent, the microleakage of a bioactive restorative material is comparable to that of a nanocomposite. (P<0.512)
4. The addition of a non-thermocycle control group also suggests that thermocycling does not have a statistically significant effect on the microleakage. (P<0.194)

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