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The Effect of 2% Nanochitosan Oligosaccharide Gel as Cavity Cleanser on Dentin Resin Attachment to Ethanol-Water and Acetone Solvent Adhesives Using Dry and Wet Bonding Techniques

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ABSTRACT

Cavity cleanser is characterized by low toxicity, biocompatible, and antibacterial properties. It has the ability to remove the smear layer and inhibit the matrix metalloproteinase enzyme. Therefore, this study aimed to determine the effect of 2% nano-chitosan oligosaccharide gel as a cavity cleanser on dentin resin attachment using ethanol-water and acetone solvent adhesives with dry and wet-bonding techniques. A total of 48 upper premolars with class I restoration were divided into eight treatment groups. Four groups were treated with 2% nano-chitosan oligosaccharide gel combined with ethanol-water or acetone solvent adhesives, while the other four received 2% chlorhexidine digluconate with the same adhesives. In the process, both bonding techniques were applied. Microleakage was assessed by measuring the penetration of 2% methylene blue dye into samples cut mesiodistally through a stereomicroscope with 1x magnification and given a score of 0-3. The results showed that the 2% oligosaccharide nano-chitosan gel group with ethanol-water solvent adhesive using the wet-bonding technique (0.33 ± 0.516) had the lowest microleakage score. Additionally, the Kruskal-Wallis test presented significant differences between the four treatment groups ($p<0.05$).

Keywords: Cavity Cleanser, Chitosan, Microleakage, Ethanol, Acetone, Wet-Bonding, Dry-Bonding

ABSTRAK

Cavity cleanser yang ideal harus memiliki tingkat toksisitas yang rendah, biokompatibel, memiliki kemampuan antibakteri, mampu menghilangkan smear layer, dan dapat menghambat enzim matriks metalloproteinase. Tujuan penelitian ini untuk mengetahui pengaruh gel nanokitosan oligosakarida 2% sebagai cavity cleanser terhadap perlekatan resin dentin pada adhesif berpelarut etanol-air dan aseton dengan technique dry dan wet-bonding. Penelitian ini menggunakan 48 buah gigi premolar atas yang direstorasi klas I dan dibagi kedalam delapan kelompok perlakuan. Empat kelompok diaplikasikan gel nanokitosan oligosakarida 2% dengan adhesif berpelarut etanol-air dan aseton, kemudian empat kelompok diaplikasikan chlorhexidine digluconate 2% dengan adhesif berpelarut etanol-air dan aseton dengan technique dry-bonding dan wet-bonding. Pengamatan dan pengukuran celah mikro dilakukan dengan melihat penetrasi zat warna methylene blue 2% pada sampel yang dibelah secara mesio-distal melalui stereomikroskop dengan perbesaran 1x dan diberi skor 0-3. Skor celah mikro terendah ditunjukkan kelompok gel nanokitosan oligosakarida 2% dengan bahan adhesif berpelarut etanol-air dengan technique wet-bonding (0,33±0,516). Hasil uji Kruskal Wallis menunjukkan terdapat perbedaan yang signifikan antar keempat kelompok perlakuan ($p<0,05$).

Kata kunci: Cavity Cleanser, Kitosan, Celah Mikro, Ethanol, Aseton, *Wet-Bonding, Dry-Bonding*

1. Introduction

Caries is a progressive dental condition caused by the demineralization of tooth enamel due to acid produced by bacteria, making it the primary cause of tooth loss. Once a cavity forms, restorative treatment is required to regain the tooth's shape, chewing function, and aesthetic appearance.[1] The procedure begins with preparations, which include removing infected dentin's caries tissue and maintaining the remaining tooth structure. This process generates a smear layer consisting of organic and inorganic components. The thickness of the layer is approximately 40 µm in dentinal tubules. The organic fraction comprises heated coagulation proteins, odontoblast processes, saliva, blood cells, pulp tissue, and microorganisms. Meanwhile, the inorganic portion includes hydroxyapatite crystals and minerals from dentin tubules. A very thick smear layer can hinder the adhesion of the restoration material to cavity walls, necessitating its removal before applying a bonding agent.[2,3]

Cavity cleaning before restoration includes the application of a disinfecting agent to remove debris, bacteria, and microbes that colonize or increase inside the smear layer. An ideal cavity cleanser should possess a low level of toxicity, biocompatibility, and antibacterial properties. It is expected to have the ability to remove the smear layer and inhibit the matrix metalloproteinase enzyme. Commonly used commercial cavity cleansers include chlorhexidine digluconate (CHX), hydrogen peroxide (H_2O_2), and sodium hypochlorite (NaOCl). Chlorhexidine digluconate (CHX) at 2% concentration is considered the "gold standard" with an excellent antimicrobial effect. However, it has the disadvantage of not being able to dissolve organic and inorganic tissue in the smear layer, leading to lower shear bond strength, as reported by Mulyani et al.[4] In addition, according to a study by Boruziniat et al., it was stated that 2% chlorhexidine gluconate can interfere with the bonding procedure and reduce the bond strength of the adhesive system, because 2% chlorhexidine gluconate binds to the phosphate groups of apatite crystals in the smear layer or on the dentin surface, which can hinder resin infiltration.[5]

A recent study has focused on chitosan, a hydrophilic biopolymer containing many free hydroxyl and amino groups. Chitosan features antioxidant, non-toxic, antifungal, and antimicrobial properties. It also shows high bioactivity, biodegradability, and selective permeability, allowing penetration into dentin tissue. Chitosan has been proven to strengthen dentin matrix and improve the biological and mechanical properties of collagen. This hydrophilic biopolymer has chelation capabilities that facilitate the removal of both inorganic and organic components of the smear layer. The role of chitosan as an inhibitor of metalloproteinase (MMP) enzymes is particularly significant. The enzyme is known to degrade exposed collagen beneath the hybrid layer, permitting dentinal fluid penetration [6–8]. The limitations of high molecular chitosan are difficulty in manipulation and solubility due to the presence of a long polymer chain structure. To address the limitations, a lower molecular weight derivative product was developed. This modification enhances water solubility, improves antibacterial properties, increases ease of manipulation, and maximizes smear layer dissolution. Preliminary studies showed that 2% chitosan oligosaccharide gel has the potential as the cavity cleaning agent, influencing adhesive restorations to cavity walls and producing low microleakage scores at the enamel and dentin junction.[7,8]

Physical modification of chitosan includes changing the particle size to smaller nanoparticles of 10 to 1000nm. The smaller the particle size, the greater the surface area, thereby increasing the ability of chitosan [9] Ururahy et al. stated that the application of chitosan on dentin promotes calcium phosphate layer formation during demineralization. Additionally, its nanoparticles show excellent anti-biofilm properties and inhibit bacterial attachment to dentin.[10]

Composite resin cannot chemically bond directly to the tooth surface, necessitating the use of an adhesive system. Bonding agents have amphophilic properties, containing both hydrophilic and hydrophobic components. The hydrophilic portion bonds to the moist dentin surface, while the hydrophobic components bond to the composite resin restoration.[1]

Rinsing and drying dentin after tooth preparation or certain etching procedures create a dehydrated superficial layer, causing collagen collapse. The degradation of the hybrid layer is influenced by factors such as enzymes, temperature, and functional stress, as well as insufficient resin infiltration into exposed collagen. This degradation leads to bond failure between adhesive material (resin) and dentin tissue, reducing bond strength.[11] The techniques adopted for drying dentin surface, include dry and wet bondings. In dry bonding, dentin is rinsed with water and then air-dried. In wet bonding, dentin is rinsed while maintaining a moist surface. Controlling moisture levels is critical, as excess water can hinder resin infiltration by preventing proper contact with collagen fibrils.[1,2]

Achieving ideal dentin hybridization relies on preventing the collapse of the demineralized dentin matrix and keeping the interfibrillar spaces open as infiltration pathways for adhesive resin monomers. However, adhesive monomers cannot remove water from the spaces, necessitating the use of solvents such as ethanol, acetone, or water.[2] Ethanol inhibits the release of water during monomer penetration, allowing better infiltration into demineralized dentin. It also reduces the diameter of collagen matrix fibrils, thereby increasing adhesion by forming more hybrid layers than water.[12] Based on observation, acetone, known as a water chaser, effectively removes water from dentin. Its high evaporation pressure further reduces surface water, facilitating monomer penetration into collagen.[13]

Numerous studies have been conducted on chitosan products, significantly to eliminate the smear layer in root canal dentin. However, there has been no investigation regarding the use of 2% oligosaccharide nano chitosan gel as cavity cleaner and its impact on adhesive restoration bonding. Several studies have also discussed the attachment of dentin resin after applying various bonding agents. In this context, none have focused on ethanol-water and acetone bonding solvents. Therefore, this study aimed to assess the effect of 2% nano chitosan oligosaccharide gel as a cavity cleanser on dentin resin adhesion when used with ethanol water and acetone solvents under dry and wet bonding techniques.

2. Materials and Methods

The study was a laboratory experiment with a posttest-only control group design approved by the Health Research Ethics Commission (KEPK) of the Universitas Sumatera Utara No: 85/KEPK/USU/2024.

A total of 48 premolars extracted for orthodontic purposes or due to mobility were obtained from the oral surgery clinic of RSGM USU. Each was cleaned with a scaler, soaked in saline solution, and divided equally into eight groups. The samples were then planted on plaster blocks to facilitate preparation and restoration.

The 2% chitosan oligosaccharide gel was produced by the Center for Innovative Excellence at the Universitas Sumatera Utara and formulated in the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences. The preparation process includes dissolving 1 gram of chitosan oligosaccharide powder in 50 ml of distilled water, followed by homogenization with a magnetic stirrer for 10 minutes. Separately, 1 gram of hyaluronic acid powder was dissolved into 50 ml of distilled water and stirred for 30 minutes to form a 2% hydrogel. Approximately 30 ml of this hydrogel was mixed with 2.6298 grams of NaCl. At the same time, 14 ml of 2% chitosan oligosaccharide solution was gradually added dropwise and stirred at 200 rpm until homogeneous for approximately 30 minutes to obtain a cloudy-colored gel. It was important to acknowledge that 0.1% CMC solution was added as a stabilizer. The cloudy solution was put in an Ultrasonic bath for 4 hours to break chitosan particles into nanoscale and obtain a 2% nano chitosan oligosaccharide gel. Particle measurements are conducted using tools and a Particle size analyzer.[6]

Sample preparation was conducted at the Conservative Dentistry Department, Faculty of Dentistry, Universitas Sumatera Utara A Class I cavity outline was drawn using a pencil and calipers, with dimensions of 3 mm in length, 3 mm in width, and 2 mm in depth. Cavity preparation was performed using a micro preparation bur, starting at the enamel surface and gradually deepening at medium speed until the desired depth was achieved. The prepared cavity was then washed, dried, and measured with a probe. A 2% oligosaccharide nano chitosan gel or 2% chlorhexidine digluconate solution was applied for 20 seconds using a micro brush, followed by rinsing and drying. Etching material was then applied for 15 seconds using the selective-etch technique, rinsed, and dried according to either the wet or dry bonding technique. Wet bonding was maintained by placing foam to keep the cavity moist, while dry bonding comprised air blowing until the surface was completely dry. In four groups, acetone solvent bonding was applied, and the remaining received an ethanol-water-based bonding agent. The adhesive was applied for 10 seconds using a micro brush to penetrate irregular structures, then illuminated with LED light curing for 20 seconds. Composite resin was incrementally applied to the cavity, with each layer illuminated for 20 seconds. The restored tooth was contoured and finished using fine and extra fine finishing burs to remove excess composite resin, followed by polishing with silicone rubber. This study included eight experimental groups, each consisting of six tooth samples:

Group I: 6 tooth samples were applied with 2% nanochitosan oligosaccharide gel and ethanol-water solvent adhesive using the wet bonding technique. Group II: 6 tooth samples were applied with 2% nanochitosan oligosaccharide gel and ethanol-water solvent adhesive using the dry bonding technique. Group III: 6 tooth samples applied with 2% chlorhexidine digluconate and ethanol-water solvent adhesive using the wet bonding technique. Group IV: 6 tooth samples applied with 2% chlorhexidine digluconate and ethanol-water solvent adhesive using the dry bonding technique. Group II: 6 tooth samples were applied with 2% nanochitosan oligosaccharide gel and acetone solvent adhesive using the wet bonding technique. Group I: 6 tooth samples were applied with 2% nanochitosan oligosaccharide gel and acetone solvent adhesive using the dry bonding technique. Group III: 6 tooth samples applied with 2% chlorhexidine digluconate and acetone solvent adhesive using the wet bonding technique. Group IV: 6 tooth samples applied with 2% chlorhexidine digluconate and acetone solvent adhesive using the dry bonding technique.

The restored samples were soaked in saline for 24 hours before undergoing thermocycling. The process included alternating immersion at 5°C and 55°C for 30 seconds each, with a 10-second transfer time, repeated 250 times. Samples were then soaked in aquabidest at 37°C for 24 hours.

Following thermocycling, the samples were immersed in a 2% methylene blue solution. To prevent unwanted dye penetration, the apex of each sample was sealed, and the tooth surface, except for the restoration area and a 1 mm margin, was coated with two layers of nail polish (acetone). The coating was allowed to dry in open air until it was no longer sticky. The samples were then immersed in the 2% methylene blue solution for 24 hours at room temperature. Subsequently, the teeth were rinsed under running water to remove excess dye before drying.

Each sample was placed on a support base and sectioned through the center of the restoration using a disc bur. Microleakage was assessed by examining methylene blue penetration along the restoration margins under a stereomicroscope with 1× magnification at the Basic Biology Laboratory of UPT PP LIDA USU. Observations were conducted by two independent observers using a double-blind technique to minimize subjectivity. The degree of microleakage was determined based on the extent of methylene blue penetration along the cavity wall, using a standardized scoring system (0–3) as described by Moosavi et al.[14]

Score 0: No dye penetration. Score 1: Dye penetration <1/2 the depth of cavity wall. Score2: Dye penetration >1/2 the depth of cavity wall. Score 3: The dye penetration has reached the axial wall of the cavity.

SEM examination was performed for each group with the lowest and highest microleakage scores to obtain an image of the microstructure. Samples were coated with a 5–20 nm layer of liquid gold and placed in a vacuum chamber at the calibrated height. The height of the sample must comply with the standard calibration. The SEM was operated at 20 kV, and samples were carefully positioned to capture the target area. Brightness, contrast, and focus were adjusted for optimal imaging, and photos were taken at 1K magnification.

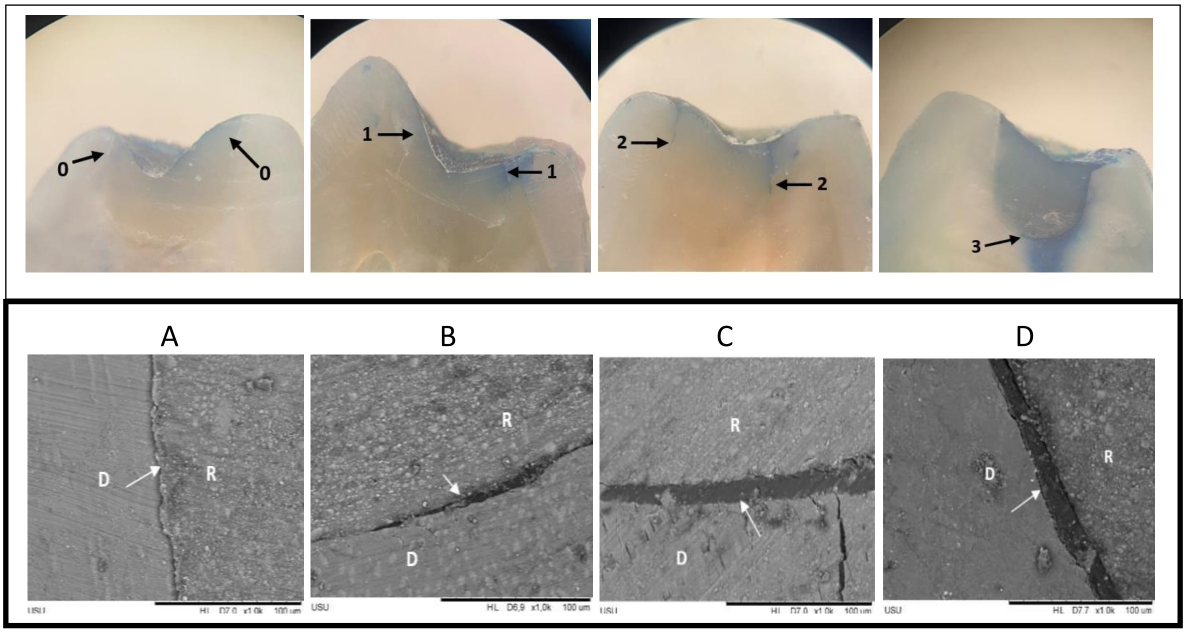

Figure 1. Stereomicroscope and SEM images of microleakage in the Ethanol-water group. A) 2%nano chitosan oligosaccharide gel with wet bonding technique, B) 2% nano chitosan oligosaccharide gel with dry bonding technique, C) chlorhexidine digluconate 2% with wet bonding technique, D) chlorhexidine digluconate 2% with dry bonding technique

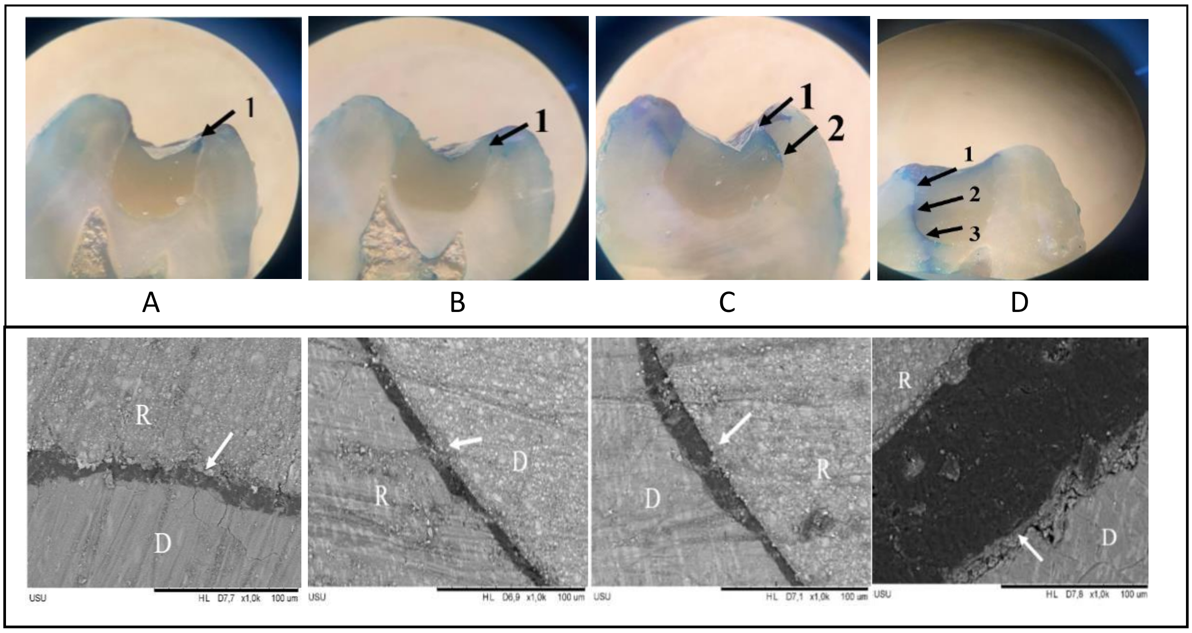

Figure 2. Stereomicroscope and SEM images of microleakage in Acetone Group. A) 2% nano chitosan oligosaccharide gel groups with wet bonding technique, B) 2% nano chitosan oligosaccharide gel with dry bonding, C) chlorhexidine digluconate 2% with wet bondingtechnique, D) chlorhexidine digluconate 2% with dry bonding technique

In Figure 1, Group A, which used 2% nano chitosan oligosaccharide gel with the wet bonding technique, showed good marginal adaptation and minimal microleakage, indicating effective penetration and bonding capability on moist dentin surfaces. Meanwhile, Group B, with the dry bonding technique, exhibited increased microleakage, possibly due to dentin dehydration that hindered adhesive penetration. Group C, which used 2% chlorhexidine digluconate with the wet bonding technique, showed microleakage involving more than half of the cavity wall. In Group D, which used 2% chlorhexidine digluconate with the dry bonding technique, the images revealed the most significant microleakage, with large gaps at the restoration interface extending to the axial wall of the cavity, indicating weak adhesive interaction.

In Figure 2, which represents the group using acetone-based adhesive, Group A showed minimal microleakage involving less than half of the cavity wall. Group B also exhibited microleakage, with the presence of small gaps along the restoration margin, indicating reduced bonding effectiveness due to the dry dentin surface. Group C showed increased microleakage extending to more than half of the cavity wall. Group D displayed the most prominent microleakage, with wide and irregular gaps at the interface, indicating the limitations of the dry bonding technique, especially when using a chlorhexidine-based cavity cleanser.

The kappa statistic indicated strong agreement between the two observers, and there was no statistically significant difference in their responses. The microgap score was selected based on the observations of Observer 1. The microgap scores obtained through observations using a stereomicroscope at 1x magnification were analyzed using the non-parametric Kruskal-Wallis test to determine differences in microgaps among all treatment groups. The statistical analysis was continued using the Mann-Whitney test to examine differences between two treatment groups.

3. Results

The results of the average micro gap score and standard deviation for ethanol-water and acetone solvent adhesive materials in each sample were obtained through the stereomicroscope examination.

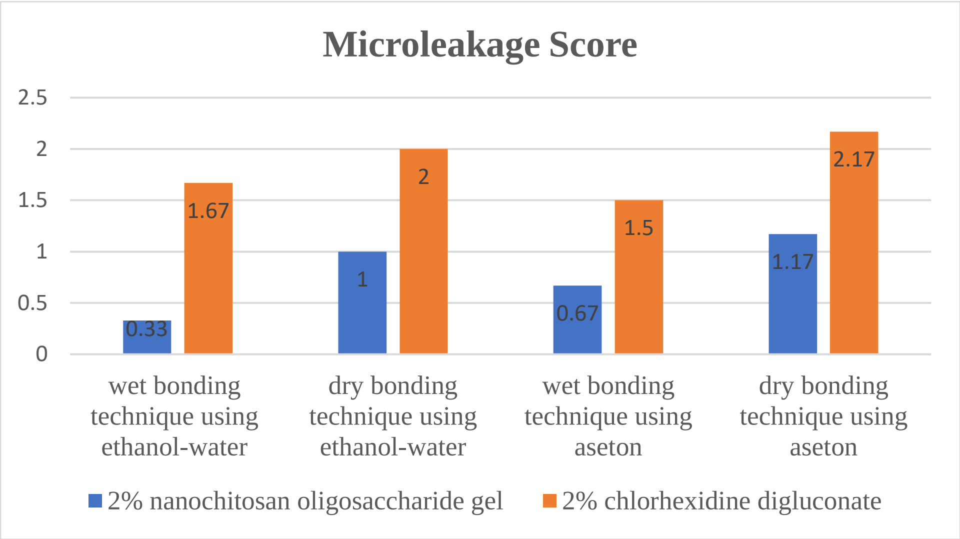

Figure 3. Graph of the average microleakage score for adhesive materials with ethanol-water and acetone solvents.

Table 1. Kruskal Wallis test results

AdhesiveSolvents	**Group**	**Mean±SD**	***p*-value**
Ethanol-water	2% nanochitosan oligosaccharide gel wet-bonding technique	0.33±0.516	0.034
	2% nanochitosan oligosaccharide gel dry-bonding technique	1.00±1.095	
	2% Chlorhexidine digluconate technique wet-bonding	1.67±1.211	
	2% Chlorhexidine digluconate technique dry-bonding	2.00±0.894	

Acetone	2% nanochitosan oligosaccharide gel wet-bonding technique	0.67±0.516	0.036
	2% nanochitosan oligosaccharide gel dry-bonding technique	1.17±0.753	
	2% Chlorhexidine digluconate wet-bonding technique	1.50±1.049	
	2% Chlorhexidine digluconate dry-bonding technique	2.17±0.753	

* Significant ($p<0.05$)

The graph in figure 3 showed the smallest value in the group treated with 2% nano-chitosan oligosaccharide gel using the wet-bonding technique. This was followed by the 2% oligosaccharide nano chitosan gel group using the dry-bonding technique, the 2% chlorhexidine digluconate group with wet-bonding, and the 2% chlorhexidine digluconate group with dry-bonding. Similarly, for acetone-based solvent adhesives, the lowest average micro-gap score was recorded in the 2% oligosaccharide nano chitosan gel group using wet-bonding, followed by the same material applied with dry-bonding. The next lowest scores were observed in the 2% chlorhexidine digluconate group using wet-bonding, followed by the 2% chlorhexidine digluconate group with dry-bonding.

Statistical analysis was conducted using the Kruskal-Wallis Test to determine significant differences in each group. The results presented in Table 1, showed a significant difference in microcrack scores ($p<0.05$) between all treatment groups for both ethanol-water and acetone solvent adhesives. Further tests were conducted using Mann Whitney to determine differences between treatment groups on the same adhesive material, with the results being summarized in Table 2.

Based on the results of the test Mann Whitney test, a significant difference was observed between the 2% oligosaccharide nano chitosan gel group with wet-bonding and both the 2% chlorhexidine digluconate group with wet-bonding and the 2% chlorhexidine digluconate group with dry-bonding in ethanol-water solvent adhesives. Additionally, for acetone-based solvent adhesives, the 2% oligosaccharide nano chitosan gel group with wet bonding showed a significant difference compared to the 2% chlorhexidine digluconate group using wet bonding.

Table 2. Mann Whitney test result

Adhesive Solvents	**Treatment Group**		***p*-value**	**Mean Differences**
Ethanol-Water	2% Nanochitosan oligosaccharide gel wet-bonding technique	2% Nanochitosan oligosaccharide gel dry-bonding technique	0.212	2.34
		2% Chlorhexidine digluconate technique wet-bonding	0.042*	4.00
		2% Chlorhexidine digluconate technique dry-bonding	0.008	5.34
	2% Nanochitosan oligosaccharide gel dry-bonding technique	2% Chlorhexidine digluconate technique wet-bonding	0.312	2.00
		2% Chlorhexidine digluconate technique dry-bonding	0.093	3.34
	2% Chlorhexidine digluconate wet-bonding technique	2% Chlorhexidine digluconate technique dry-bonding	0.616	1.00
Acetone	2% Nanochitosan oligosaccharide gel wet-bonding technique	2% Nanochitosan oligosaccharide gel dry-bonding technique	0.206	2.34
		2% Chlorhexidine digluconate technique wet-bonding	0.120	3.00
		2% Chlorhexidine digluconate	0.007*	5.34

		technique dry-bonding		
	2% Nanochitosan oligosaccharide gel dry-bonding technique	2%Chlorhexidine digluconate technique wet-bonding	0.553	1.16
		2% Chlorhexidine digluconate technique dry-bonding	0.051	3.84
	2% Chlorhexidine digluconate wet-bonding technique	2% Chlorhexidine digluconate technique dry-bonding	0.238	2.34

*significant

4. Discussion

Cavity wall preparation influences restorative success, making cavity cleanser essential for removing the smear layer, which contains debris and mycobacteria. This process disinfects the cavity by eliminating bacteria and improving wettability. Chlorhexidine digluconate (CHX 2%) has broad-spectrum antimicrobial properties, inhibiting gram-positive and gram-negative bacteria, with effects lasting up to 72 hours.[4] However, Mulyani et al. reported that CHX 2% lowers shear strength since it does not dissolve organic components of the smear layer.[15]

This study examined the application of 2% nano chitosan oligosaccharide gel as a natural cavity cleanser before etching. It led to the lowest mean micro-gap scores compared to CHX 2% in both ethanol-water and acetone-based adhesives. Chitosan has antimicrobial activity, strengthens dentin, enhances collagen properties, and chelates smear layer components.[6] Madya et al., reported that 2% chitosan oligosaccharide has potential as cavity cleaning agent when developed in gel form. It influences the adhesion of restorative materials to cavity walls and achieves the lowest microleakage score at the enamel and dentin junctions.[7] Similarly, Varshneya et al., presented that chitosan served as an effective disinfectant before restoration, preventing microleakage without disrupting adhesive bonds.[16] Kishen et al. stated that the application of chitosan nanoparticles before the restorative procedure enhanced collagen resistance on to bacterial degradation due to the non-specific interaction with the collagen matrix and collagenase enzymes.[17]

Material bonding enhances the adhesion between the tooth surface and composite resin, improving the quality of composite resin as a restorative material. The solvent content in bonding agents forms hydrogen bonds with collagen fibril peptides, maintaining demineralized dentin in an expanded state during resin infiltration.[2] This study incorporated etching techniques, such as selective etching and resin bonding, using ethanol-water and acetone solvents. Ethanol inhibits water release during monomer penetration due to its low vapor pressure, allowing monomers to infiltrate demineralized dentin.[12] Acetone, known as a water chaser, effectively removes water from dentin.[13] Its high evaporation pressure reduces residual moisture on the dentin surface. According to Graph 1, the ethanol-water adhesive group exhibited the lowest mean micro-gap score compared to the acetone-based group. This suggests superior marginal adaptation, which is critical in preventing microleakage and secondary caries. In line with this, Balkenhol et al. recommend the use of a three-step ethanol-water-based adhesive due to its lower technique sensitivity and slower compositional degradation compared to acetone-based systems. Clinically, this indicates that ethanol-water adhesives may provide more consistent and durable outcomes, especially in conditions where moisture control is challenging.[18]

Two bonding techniques—dry bonding and wet bonding—are used to achieve adequate hybridization. Dry bonding leads to dentin dehydration, causing the collagen matrix to collapse, weakening the resin-dentin bond and preventing proper hybrid layer formation. In contrast, wet bonding preserves dentin moisture, ensuring the collagen matrix remains hydrated.[1,2] In this study, the wet-bonding group exhibited lower average microcrack scores than the dry-bonding group. Dwiyanti et al. support the result, stating that wet bonding enhances the bond strength of composite resin nanohybrids by maintaining collagen interfibrillar spaces, leading to improved adhesion.[19]

The restorative material used in this study is nanohybrid composite resin where the distribution of filler materials within the matrix is enhanced by combining nanoparticles, leading to improved mechanical, chemical, and optical properties. A study by Lin et al. showed that the microhardness of microhybrid composite resin with larger particle sizes showed lower hardness values compared to nanofill and nanohybrid composites. This suggested that smaller filler particles allow for higher dispersion concentrations and lead to more compatible molecules to be incorporated with resin polymer during the polymerization process.[20]

This study showed that nano chitosan oligosaccharide gel has potential as an alternative cavity cleanser, providing better adhesion between composite resin and dentin compared to the commercial cavity cleanser, 2% chlorhexidine digluconate. According to Sennel et al., chitosan has a broad-spectrum antibacterial activity, effectively eliminating gram-positive and harmful bacteria by altering the cell permeability, leading to cell death.[21] It functions as a chelating agent for calcium ions in dentin and facilitates smear layer removal. The chelating ability of chitosan dissolves inorganic components from the smear layer and support calcium ion loss.[22] The limitation of this study is the absence of a flow rate test. This is because the teeth used were nonvital, where the fluid in the dentinal tubules has gradually decreased over time, thus not affecting the balance of this fluid, which could lead to changes in osmotic pressure that may impact the integrity of the dentin tissue.

5. Conclusion

In conclusion, 2% oligosaccharide nano chitosan gel as a cavity cleanser showed good properties by increasing the adhesion of the composite resin to dentin. Furthermore, it produced the lowest microcrack score with the wet-bonding technique using ethanol-water and acetone solvent adhesives.

In future studies, it is recommended to evaluate resin–dentin adhesion after the application of 2% nanochitosan oligosaccharide gel using both dry and wet bonding techniques, employing SEM-EDX to observe more detailed microstructural features such as resin tags and the hybrid layer.

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7. Conflict of Interest

The authors declare no conflicts of interest.

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Appendix

1. Microleakage Test Result

Group 1: 2% Nanochitosan Oligosaccharide Gel with Ethanol-Water Solvent using Wet Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	0	0
2	1	2
3	0	0
4	1	1
5	0	0
6	0	0

Group 2: 2% Nanochitosan Oligosaccharide Gel with Ethanol-Water Solvent using Dry Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	1	0
2	0	0
3	3	2
4	0	0
5	1	1
6	1	1

Group 3: 2% Chlorhexidine Digluconate with Ethanol-Water Solvent using Wet Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	1	1
2	0	1
3	3	3
4	1	1
5	2	2
6	3	2

Group 4: 2% Chlorhexidine Digluconate with Ethanol-Water Solvent using Dry Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	1	1
2	1	0
3	3	3
4	2	1
5	3	3
6	2	2

Group 5: 2% Nanochitosan Oligosaccharide Gel with Acetone Solvent using Wet Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	0	1
2	0	0
3	1	1
4	1	0
5	1	2
6	1	1

Group 6: 2% Nanochitosan Oligosaccharide Gel with Acetone Solvent using Dry Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	0	1
2	1	2
3	1	1
4	1	0
5	2	2
6	2	2

Group 7: 2% Chlorhexidine Digluconate with Acetone Solvent using Wet Bonding Technique

Sample	Microleakage Score	
	Observer 1	Observer 2
1	0	0
2	1	1
3	1	2
4	2	1
5	2	1
6	3	3

Group 8: 2% Chlorhexidine Digluconate with Acetone Solvent using Dry Bonding Technique

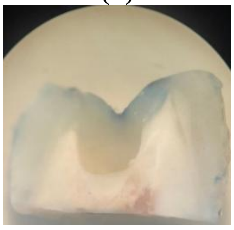
Sample	Microleakage Score	
	Observer 1	Observer 2
1	1	2
2	2	2
3	2	2
4	2	3
5	3	2
6	3	3

2. Stereomicroscope Image of Microleakage

Group 1: 2% Nanochitosan Oligosaccharide Gel with Ethanol-Water Solvent using Wet Bonding Technique

(1)

(2)



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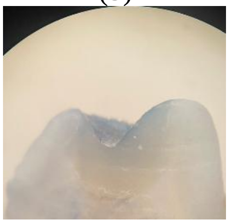

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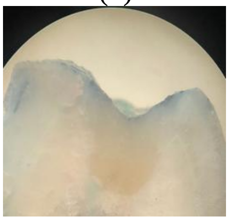

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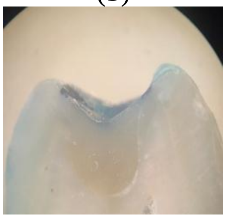

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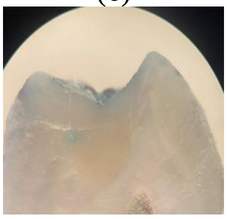

Group 2: 2% Nanochitosan Oligosaccharide Gel with Ethanol-Water Solvent using Dry Bonding Technique

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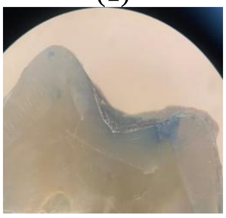

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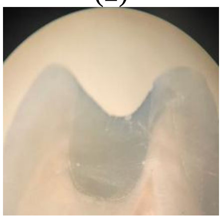

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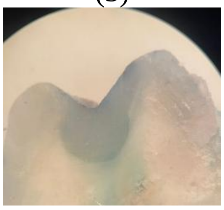

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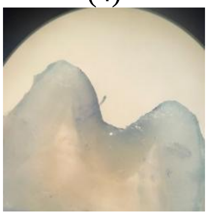

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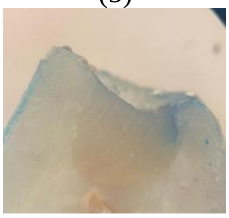

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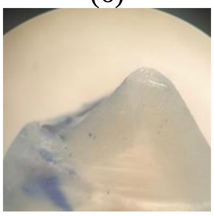
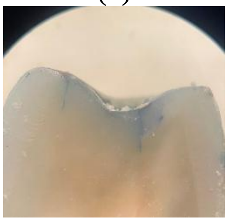


Group 3: 2% Chlorhexidine Digluconate with Ethanol-Water Solvent using Wet Bonding Technique

(1)



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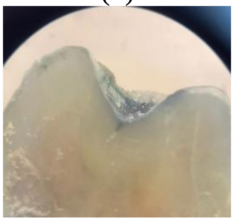

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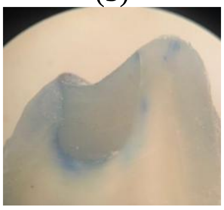

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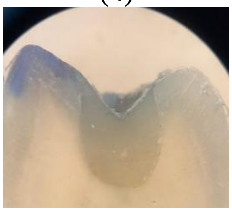

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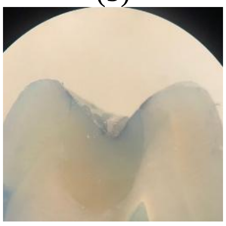

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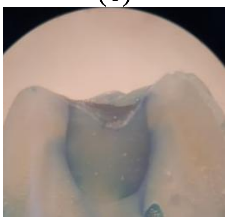

Group 4: 2% Chlorhexidine Digluconate with Ethanol-Water Solvent using Dry Bonding Technique

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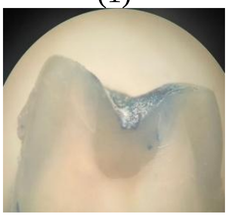

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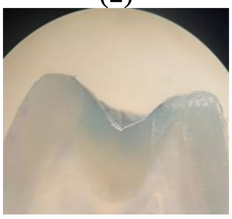

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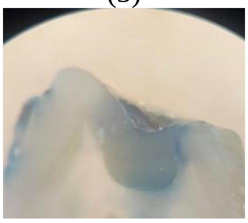

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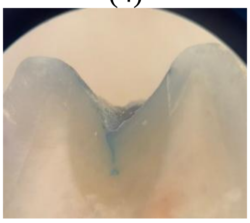

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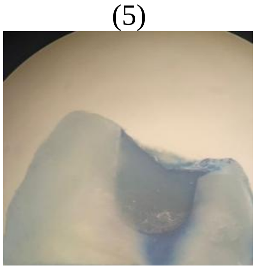

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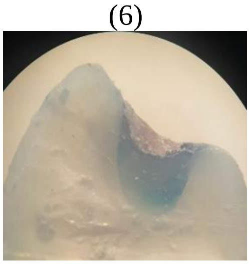

Group 5: 2% Nanochitosan Oligosaccharide Gel with Acetone Solvent using Wet Bonding Technique

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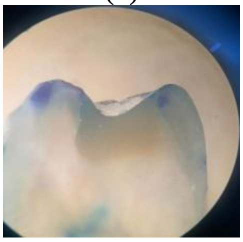

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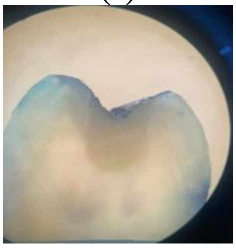

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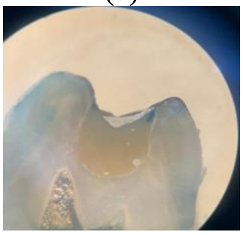

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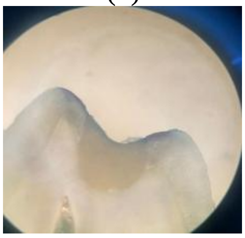

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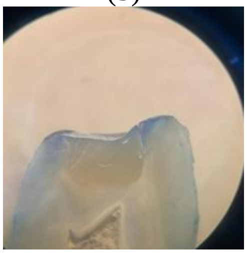

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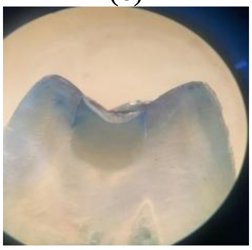

Group 6: 2% Nanochitosan Oligosaccharide Gel with Acetone Solvent using Dry Bonding Technique

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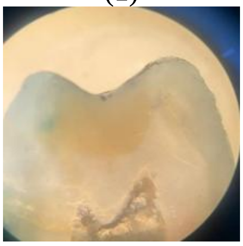

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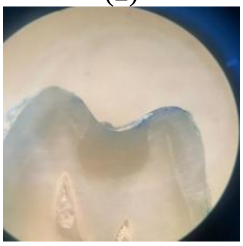

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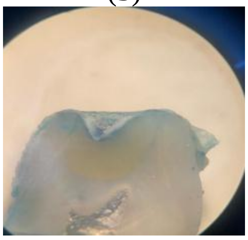

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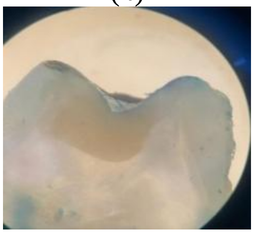

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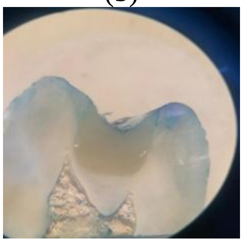

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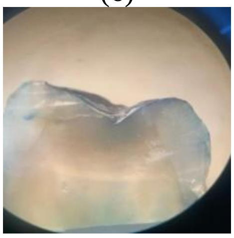

Group 7: 2% Chlorhexidine Digluconate with Acetone Solvent using Wet Bonding Technique

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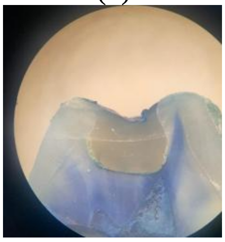
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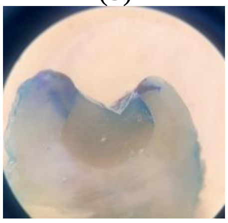
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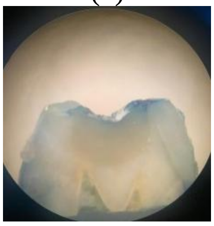
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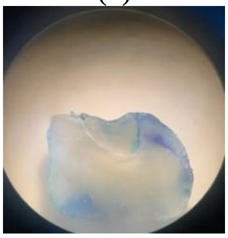
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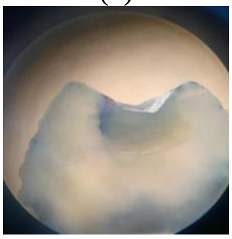

Group 8: 2% Chlorhexidine Digluconate with Acetone Solvent using Dry Bonding Technique

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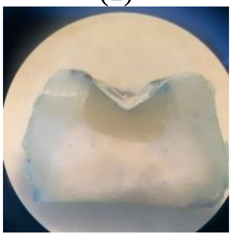
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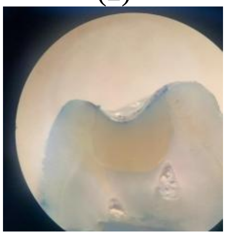
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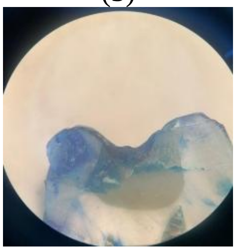
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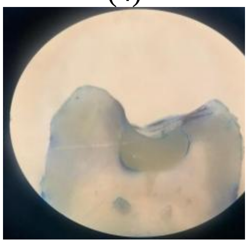
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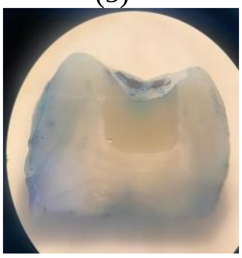
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