

Potential of chitosan oligosaccharide gel as a cavity cleanser against adhesive restoration adhesive on the cavity wall

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ABSTRACT. Cavity cleanser is a cavity cleaner with ideal properties such as being biocompatible, antibacterial, removing smear layers, and not interfering with the adhesive bond. This study aims to examine the potential of gel chitosan oligosaccharide (COS) as a cavity cleanser on the adhesion of the restoration to the cavity wall. Thirty-two mandibular premolars with class V restorations are divided into two groups with 2% COS gel and 2% chlorhexidine digluconate (CHX). Samples were immersed in 2% methylene blue, cut longitudinally, observed under a stereomicroscope with 1x magnification, and scored 0-3. The Mann-Whitney test was used to see the potential of gel COS as a cavity cleanser. The results showed a significant difference between the two groups at the edge of the enamel junction restoration ($p=0.038$) and the dentin junction restoration ($p=0.027$). The mean microleakage score in group 1 showed better results at the enamel junction (1.19 ± 1.328) and the dentin junction (2.00 ± 1.211). It shows that 2% COS gel has the potential as a cavity cleanser. There was a significant difference between the two groups as a cavity cleanser on the adhesion of adhesive restorations to the cavity wall.

KEYWORDS: cavity cleanser; chitosan oligosaccharide; chlorhexidine digluconate; microleakage

INTRODUCTION

Caries is a multifactorial disease mainly caused by an imbalance of oral flora (biofilm).¹ The development of caries can be prevented by restorative treatment. Restoration of class V cavities is a challenge in dentistry because frequent failures caused microleakage in the restoration, especially at the gingival margin close to the cemento-enamel junction (CEJ). Factors that can influence the occurrence of this microleakage are that at the cervical margin, there is little or no enamel which makes adhesive more difficult because dentin contains more organic components and water content than enamel. It prevents restorative materials and adhesives from adequately penetrating the dentin.^{2,3}

Before filling the dental restorative material in the cavity, preparation was carried out.⁴ During the preparation process, the dentin was covered by bacteria, debris, and a smear layer which later

became a significant problem in restoration. The smear layer comprises organic and non-organic components with a thickness of 5-10 μ m. The smear layer may prevent the adhesion of cavity wall adhesive restorations.^{5,6}

Cavity cleanser is a cavity cleanser before dental restorative procedures that can clean, moisten, and disinfect the smear layer remaining on the dentin-enamel junction after cavity preparation. The ideal cavity cleanser should be of low or no toxicity to pulp cells while not interfering with the adhesive bond of the restorative material.^{6,7}

Chlorhexidine digluconate (CHX) 2% is the most widely used cavity cleanser in dentistry because it is considered the "gold standard" and is the most commonly used antimicrobial agent. CHX 2% has high antibacterial effectiveness against gram-positive and negative bacteria. CHX 2% has drawbacks, namely a lower value because it cannot

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dissolve organic tissue in the smear layer, has disadvantages in long-term use, which causes discoloration of teeth and tongue, has an unpleasant odor, and can be allergenic in continuous contact.^{8,9,10,11}

Previous research used chitosan as an irrigant to remove the smear layer on the dentin of the root canals. Chitosan is a natural material resulting from the deacetylation of chitin through chemical and biochemical reactions in the shells of shrimp, crabs, and crabs. Chitosan has biodegradable, biocompatible, non-toxic properties, broad antimicrobial effect, and chelation ability which can remove smear layers with inorganic components. In the study of Ayu and Trimurni et al. (2013) regarding the ultrastructural description of the root canal wall using SEM, it was found that 0.2% chitosan solution was able to lift the smear layer, while the combination of 2.5% NaOCl plus 0.2% chitosan, there was more smear layer in the dentinal tubules.^{9,12}

Research on chitosan has been modified with the development of chitosan oligosaccharides.

MATERIALS AND METHODS

This experimental study was carried out using a posttest-only control group design. Ethical clearance was approved by the Ethical Research Committee Faculty of Medicine Universitas Sumatera Utara (USU). It was conducted in the Laboratory of Analytical Chemistry FMIPA, USU, Department of Dental Conservation FKG USU, Department of Prosthodontics FKG USU, Basic Biology Laboratory UPT PP LIDA USU

A sample of 32 premolars that had been extracted was cleaned and immersed in saline solution. Then, they were randomly grouped into two groups, each with 16 illustrations, and planted on plaster beams.

COS powder was obtained from the Center for Innovative Excellence, University of North Sumatra. The making of 2% COS gel was done by dissolving 1 gram of chitosan oligosaccharide powder into 50 ml of distilled water and then stirring until homogeneous with a magnetic stirrer for 10 minutes. Next, 2% hyaluronic acid was made by dissolving 1 gram of hyaluronic acid powder into 50 ml of distilled water and then stirring until homogeneous with a magnetic bar for 30 minutes to form a hydrogel preparation. Then, 30 ml of 2% hyaluronic acid was mixed with 2.6298 g of NaCl while dropping 14 ml of COS 2% solution with a dropper gradually and stirred until homogeneous for 24 hours, then refrigerated for 24 hours.

Chitosan oligosaccharide (COS) is a hydrolysis of chitosan consisting of 2-10 D-glucosamine, which can be made by chemical and enzymatic hydrolysis. However, enzymatic hydrolysis of chitosan is considered more effective and easier to control. In the study of Ernani et al. (2015), COS has a higher solubility and is easier to apply.¹³ Several studies have reported that COS is also proven to be a promising antimicrobial, immune stimulant, and antitumor. In the study of Suzuki et al. (2014), it was confirmed that COS dissolved in citric acid was able to remove the smear layer significantly with minimal erosion after soaking for five minutes.¹⁴⁻¹⁶

Currently, research on chitosan has been widely used in root canal treatment to remove the smear layer. However, the effect of COS in the form of a gel as a cavity cleanser is not yet known, especially its impact on restoration adhesion. Therefore, researchers are interested in seeing the potential of COS as a gel as a cavity cleanser on the bonding of adhesive restorations to the cavity wall by looking at the microleakage.

Sample preparation was carried out by making an outline form for a Class V cavity design of 3 mm x 2 mm x 2 mm with the cervical edge 1 mm above the CEJ using a diamond bur (round bur and tapered fissure bur). Wash and dry the prepared cavity surface. Restoration of the sample was carried out in 2 groups. In group 1, a cavity cleanser (2% COS gel) was applied for 20 seconds. In group 2, a cavity cleanser (CHX 2% (Bisco, USA) was used for 20 seconds with a micro brush, then washed and dried. Next, both groups were applied with an adhesive system (total-etch two steps) by applying etching material with a micro brush for 15 seconds, then rinsed. Then use bonding (Esbond, Korea) for 15 seconds, lightly blow for 10 seconds, and cure for 20 seconds. Apply a flowable composite resin (Esflow, Korea) with a bulk-filled insertion technique in the cavity, then cure for 20 seconds. The restored teeth were contoured and finished using a finishing bur and polished using rubber silicone on the surface of the restoration.

The sample was soaked in a saline water container for 24 hours. Then a thermocycling process was carried out using a water bath (Mommert, Japan) by inserting the sample into a glass beaker containing ice at a temperature of 5°C, left for 30 seconds, and then transferring within a transfer time of 10 seconds into a water bath at 55°C, went for 30 seconds and repeated for 250 seconds. Round times. Next, the apex of the sample was

covered with wax and the tooth surface was coated with 2 coats of nail polish (acetone) except the

restoration surface and 1 mm around the edge of the restoration and then allowed to dry in the open air.

RESULTS

The mean microleakage score at the edges of enamel junction restorations showed that the mean microleakage score in group 1 (1.19 ± 1.328) was better

than in group 2 (2.13 ± 1.258). The same thing was also seen at the edge of the dentin junction restoration, which showed the microleakage's mean score.

Table 1. Microleakage score mean

Edge	Groups	N	Microleakage score (Mean)
Enamel Junction	1	16	1.19 ± 1.328
	2	16	2.13 ± 1.258
	Total	32	
Dentin Junction	1	16	2.00 ± 2.00
	2	16	2.75 ± 0.775
	Total	32	

Information: Group 1: COS gel 2%; Group 2: CHX 2%

Then, it was soaked in a 2% methylene blue solution for 24 hours at room temperature. Next, all teeth were cleaned until dye are removed in running water and dried. The sample was split longitudinally using a disc bur. Microleakage observations were carried out by observing the penetration of 2% methylene blue dye through a stereomicroscope (Olympus, Japan) with 1x magnification.

The sample was then assessed using a standard scoring system with a score of 0-3 as in the study conducted by Moosavi et al. (2013) as follows: Score 0: No dye penetration Score 1: dyes penetration

<1/2 cavity wall depth Score 2: Penetration of dye >1/2 the depth of cavity wall Score 3: Penetration of dye has reached the axial cavity wall

The data obtained will be processed and analyzed using the Statistical Package for the Social Sciences (SPSS). The Shapiro-Wilk test was conducted to determine whether the data were normally distributed. The data obtained were tested for normality with the Shapiro-Wilk test p-value <0.05. Because the data were not normally distributed, the analysis was carried out using a non-parametric statistical test, namely the Mann-Whitney test.

Table 2. Statistic test result with *Mann Whitney*

Edge	Groups	Microleakage score			P
		N	Microleakage score (Mean)		
Enamel Junction	1	16	1.19 ± 1.328		0,038*
	2	16	2.13 ± 1.258		
	Total	32			
Dentin Junction	1	16	2.00 ± 2.00		0,027*
	2	16	2.75 ± 0.775		
	Total 32	32			

Table 1 shows that group 1 (2.00 ± 1.211) was better than group 2 (2.75 ± 0.775) and Table 2 shows that there were significant differences between the COS

2% gel and CHX 2% groups at the edges of the enamel junction restoration ($p=0.038$) and dentin junction restoration ($p=0.027$).

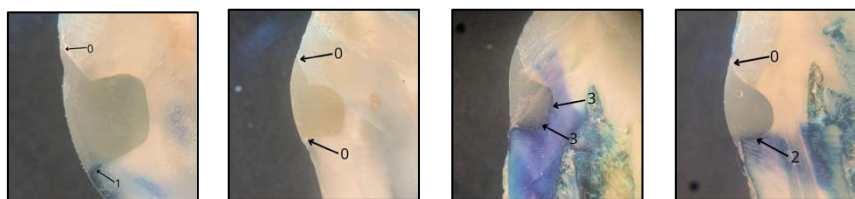


Figure 1. Stereomicroscope photos in group 1



Figure 2. Stereomicroscope photos in group 2

DISCUSSION

This study used 2% COS gel as an alternative to cavity cleansers derived from natural ingredients, which are expected to have the potential as cavity cleansers and affect the adhesion of adhesive restorations to cavity walls that were applied before adhesives were used. Table 2, obtained $p < 0.05$, which proves that 2% COS gel has the potential as a cavity cleanser that can affect the adhesion of adhesive restorations to cavity walls.

COS is a hydrolysis of chitosan that has a shorter polymer structure, so it has a lower molecular weight and higher solubility than high molecular chitosan.^{17,18} COS above, it can be seen that chitosan oligosaccharide has the potential as a cavity cleanser because it has the ideal properties that a cavity cleanser should possess. Research done by Varshneya et al. (2017) shows that chitosan has the potential as an effective disinfectant before restoration because it can prevent microleakage and does not interfere with existing adhesive bonds.¹⁹ The same result was also found by Paschoini et al. (2021), showing that chitosan can increase the adhesive strength of the material better than the untreated control.²⁰

This study used a chemical modification of COS in the gel preparation. The gel is a polymeric bond that is physically/chemically bonded to each other, which traps the liquid phase so that it is a viscoelastic semisolid. The water contained in the hydrogel is a type of imbibition water that can enter a material and will increase the volume of the material. Guibal et al. (1997) research show that the chemical modification of chitosan into chitosan gel can increase the absorption power because the gel form has a larger pore volume than the flake form.²¹ In addition Paschoini et al. (2021) research

shows that chitosan in the form of a gel can also increase and maintain adhesion bonds.²⁰

The results of statistical tests with Mann Whitney in Table 5 show that there is a significant difference ($p < 0.05$) between the COS 2% gel and CHX 2% groups at the edges of the enamel junction and dentin junction restorations ($p = 0.027$) as a cavity cleanser against adhesion of adhesive restorations to cavity walls so that the hybridization layer formation on composite resin restorations is well established. Silva et al. (2017) show that the chelating properties of chitosan solution removed the inorganic smear layer in root canal treatment. Although the exact mechanism is unknown, it is believed that this is due to the adsorption properties, ion exchange effects, and chelating properties that form complexes of chitosan substances with metal ions. The same results were also found by.²² Besides being able to remove the smear layer, as a cavity cleanser, it must also have ideal antibacterial properties. Kaur et al. (2020) also proved that the combination group of COS with COS solution was the highest in removing the smear layer from other groups and had high antibacterial effectiveness.¹⁷

The microleakage scores in Table 1 show that the restoration at the dentinal margin was higher than that of the enamel in all treatment groups, with an average value of groups 1 and 2. It was because, at the cervical margin, there was little or no enamel in the Class V cavity, so bonding was achieved. Dentin also has a smaller hydroxyapatite structure and is arranged in a criss-cross pattern making it more challenging to form micromechanical bonds with dentin.² In addition, it can be caused by heat caused by friction of the bur with the teeth during cavity preparation which causes the interprismatic enamel to break and the

collagen in the dentin to collapse, polishing after filling the cavity.²³ The thermocycling process can also cause a high microleakage score. The extreme temperature changes in the oral cavity will affect the difference in expansion and contraction between the restorative material and the tooth structure, which causes the restoration surface to

become weak.^{24,25} The occurrence of microleakage is also related to shrinkage during polymerization caused by the C-factor. Class V cavities have a C-factor with a value of 5. The higher the C-factor, the higher the potential for polymerization shrinkage.²⁶

CONCLUSION

Chitosan oligosaccharide 2% has the potential as a cavity cleanser material when developed as a gel and affects the adhesion of adhesive restorations to cavity walls. The 2% chitosan oligosaccharide could be a cavity cleanser material resulting in the lowest mean microleakage score both at the edge of the enamel junction restoration and at the edge of the dentin junction restoration.

The results showed a significant difference between 2% oligosaccharide chitosan gel and 2% chlorhexidine digluconate as a cavity cleanser material on the adhesion of adhesive restorations to cavity walls so that the formation of hybridization layers on composite resin restorations was well formed.

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