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THE EFFECT OF *Streptococcus sp* **AND** *Lactobacillus sp* **ATTACHMENT ON THE LEVEL OF FLUORIDE ION SOLUBILITY IN GIC RESTORATION**

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ABSTRACT

Background: Glass ionomer cement (GIC) is a restoration material that has the ability to release Fluoride ion. The Extrinsic factor that affect the solubility of fluoride is low or acidic pH. Streptococcus sp and Lactobacillus sp are asiduric and acidogenic bacteria which are resistant to acidic conditions and may produce acid. A lower pH (acid) causes higher level of fluoride to be released. Objective: This study was to analyze the effect of Streptococcus sp and Lactobacillus sp attachment on solubility of fluoride level on GIC restoration Method: Twenty-one specimens (5mm diameter x 2mm thickness; n = 7 / group) were fabricated with GIC. The treatment group (Streptococcus sp and Lactobacillus sp) while the control group (Artificial Saliva) was immersion for 7 days in an incubator 37^oC. Solubility of fluoride was measured using a pH meter Lutron (208) TL Lutron (Taiwan). Result: One Way Anova and Post Hoc Bonferonni showed that there were significant differences between the treatment groups of Streptococcus sp (5.13 ± 0.86ppm) and Lactobacillus sp (6, 02 ± 0.79ppm) with artificial Saliva group controls (3.03±0,56ppm). Conclusion: There was an effect of Streptococcus sp and Lactobacillus sp bacteria attachment on the solubility of fluoride from the GIC restoration.

Keywords: Fluor solubility, GIC, Lactobacillus sp, Streptococcus sp.

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INTRODUCTION

Glass ionomer cement (GIC) is one of the dental filling materials that is often used by clinicians in dentistry. Kent and Wilson introduced GIC for the first time in 1972 .¹They combine the advantages of translucency and release of fluoride ion from silicate cement and the biocompatibility and adhesive property of polycarboxylic cement. GIC is a restoration material that has anticariogenicpropertiy because this restoration material has the ability to release fluoride ion which may increase remineralization around the restoration.^{1,2,3}

According to the research of Neelakantan (2011), it showed that GIC has the highest average value of fluoride solubility among other restoration materials. Solubility of fluoride ion from restorative materials is influenced by intrinsic and extrinsic factors, one of the extrinsic factors is low pH or acid.⁴A lower pH (acid) causes higher level of fluoride ion to be released.⁵ One of the causes of decreased pH in the oral cavity is increased bacterial activity in the oral cavity.⁶

*Streptococcus sp and Lactobacillus sp*are flora normal in the oral cavity. These bacteria may turn into pathogen if there are predisposing factors, one of which is decreased pH in the oral cavity. Mantuil is the outermost area of the city of Banjarmasin, where the community still uses peatland water for MCK (bath, wash, toilet), because of the Perusahaan Daerah Air Minum (PDAM) pipes are not installed in the area.⁷ Based on Febriyanti's research (2018) , the number of *Streptococcus sp*and *Lactobacillus sp* colonies in children saliva who gargle with peatland water is more than children who rinse with PDAM water. These bacteria are acidic (asiduric) and can produce acid (acidogenic). It is known that Streptococcus sp bacteria can produce acid up to pH 4.5 while Lactobacillus sp bacteria can produce acid up to pH $4⁶$

One characteristic of bacteria is the ability to attach to all locations in the oral cavity, so it does not rule out the possibility of bacteria attached to the surface of the GIC restoration.⁸Increased colonies of bacteria will increase the acidity of the oral cavity, so that many hydrogen ion is released in the oral cavity. The hydrogen ion then dissolves in the liquid and diffuses into the GIC restoration material. This will cause the release of fluoride ion in GIC, then the fluoride ion will diffuse out of GIC and will dissolve in the water. $9,10$

Based on the description above and there have been no studies yet that prove directly that bacterial attachment can affect the levels of fluoride ion solubility of GIC restoration material, it is necessary to do the research on the effect of bacterial attachment on fluoride ion solubility in GIC.

MATERIALSAND METHODS

This research has through ethical feasibility test No.176 / KEPKG-FKGULM / EC / I / 2019 published by the Faculty of Dentistry, LambungMangkurat University. This study was a true experimental study with post-test only with control group design consisting of three groups; two groups which treated with soaking *Streptococcus sp*and group bacteria by immersing *Lactobacillus sp*, and a control group only soaked with artificial saliva.Samples were made with Glass Ionomer Cement Fuji IX in cylindrical shaped with a diameter of 5mm x thickness 2mm based on specifications (ISO) 9917-1: 2003, then soaked for 7 days at 37° C. GIC samples was made as many as 21 specimens by stirring a mixture of powder and liquid (1: 1) then put into a mold then closed with celluloid strip until it set*.* The hardened sample was stored in a petri dish and placed in an $37 \degree$ C incubator for 24 hours to give a perfect reaction.

The bacterial suspension was made from dental plaque of patients who had caries in the Mantuil area of South Kalimantan. Bacteria was obtained and then bred by taking Colony of *Streptococcus sp* and *Lactobacillus sp* bacteria on blood agar media as much as 1 ose then dissolved into 100 ml of aqudes in test tubes by standardization using McFarland 3 solution (number of colonies 3×10^8). *Streptococcus sp* and *Lactobacillus sp* bacteria were taken with spreaders reviewed on BHI-B media so that the planting was carried out in an anaerobic atmosphere at 37 ° C for 24 hours. The 5 colonies taken from *Streptococcus sp* and *Lactobacillus sp* bacteria were taken and then planted in 100 ml of BHIB in the beaker.

The level of fluoride ion solubility was measured by grinding GIC until smooth and put in a beaker. The crushed GIC was diluted with 10 ml of sodium fluoride buffer and added 10 ml of distilled water, followed by immersing the electrode from the

pH meter (Lutron pH-208) (Taiwan) device into the sample solution for 3 minutes. The obtained data was deducted from fluoride ion level from the baseline*.*

The data obtained were analyzed statistically using One-way ANOVA with a confidence level of 95% ($\alpha = 0.05$), followed by Post Hoc Bonferroni analysis to determine the value of significance.

RESULTS

Statistical results showed that the data of all groups were normally distributed and homogeneous $(p> 0.05)$. The results of the research on the rate of release of fluoride GIC ion obtained by the average value presented in Figure 1.

Figure 1.Average diagram of the solubility of GIC fluoride ions after soaking in suspension of *Streptococcus sp* bacteria, suspension of *Lactobacillus sp* and artificial saliva bacteria

Based on Figure 1, the highest level of fluoride ion solubility was the highest in the group that soaked in the suspension of *Lactobacillus sp* (6.02 ± 0.79ppm), while the average level of solubility of fluoride ion was the the lowest in the group immersed in artificial saliva $(3.03 \pm 0.56$ ppm). When three groups were compared, the average level of fluoride ion solubility that soaked in *Streptococcus sp* $(5.13 \pm 0.86$ ppm) was higher than the artificial saliva group $(3.03 \pm 0.56$ ppm). The mean solubility of fluoride ion which were immersed in *Lactobacillus sp* $(6.02 \pm 0.79$ ppm) was higher than the group that soaked in suspension of *Streptococcus sp* (3.03 ± 0.56ppm).

DISCUSSION

From the results of the study, the solubility of GIC restoration materials may occur both in soaking with the suspension of *Streptococcus sp* and *Lactobacillus sp* bacteria as a treatment group or with artificial saliva as a control group, because the behavior of fluoride ion solubility is similar for all ionomer-based materials.²

In the measurement results, fluoride ion from GIC undergo solubility after immersion in artificial saliva. Artificial saliva has water as its main content which is around 99%. Artificial saliva that has a neutral pH does not experience perfect ionization, so

that it produces H^+ and OH $\bar{ }$ ions. The concentration of H $+$ ion in artificial saliva can break ionic bond and carboxyl chain so that they dissolve fluoride ion from type II GIC.^{11,12} This is also supported by the sensitivity of GIC. The nature of GIC sensitivity to water may affect microstructure, solubility, and adhesion to restoration materials. GIC contains silica hydrogel which forms around glass particles during the setting process that has a hydrophilic nature that is easily bound or absorbed by water. Absorption of water into GIC restoration material involves penetration of liquid molecule into the structure of the restoration material with diffusion. Water absorption may increase the volume of restoration material, causing damage to the matrix structure and the solubility of components from GIC type II restoration material. $1,9,13$

Solubility of fluoride ions from GIC restoration material soaked in the suspension of *Streptococcus sp* and *Lactobacillus sp* bacteria occurs because these bacteria may produce a pH about 4- 4,5.8,9Where bacteria *Streptococcus sp* and *Lactobacillus sp* are able to produce lactic acid due to the metabolic activity of bacteria that produce energy, so that bacteria continue to grow and develop. Lactic acid, produced by bacteria, will affect the condition in the bacterial environment. The more bacteria that are in the environment, the more acid will be produced so that the acidity of the solution will occur. The acidity of this solution will cause more fluoride ion solubility.^{9,14}Moreaou and Hockin (2010) prove that immersion of restoration material containing fluoride in a solution with pH 4 will lose more fluoride ion and reduce the number of fluoride ion reservoirs that are close to the specimen surface, compared to the same material if immersed in a solution with pH $7.^{10}$

When a solution has low pH, the hydrogen ion contained in the solution will be ionized, so that there will be many hydrogen ions released.¹⁵ A free hydrogen (H^+) ion will break the chain link of the polycarboxylic salt (RCOO⁻) which is nucleophilic from GIC thos it will be released and formed a single chain. ¹⁶ The detachment of the chain will cause free fluoride ions contained in the GIC to undergo solubility by diffusing out causing solubility in the restoration material.^{9,16,17}

The measurement results of the average levels of fluoride ion solubility in GIC after immersion in the suspension of *Lactobacillus sp* bacteria experienced a higher solubility than the solubility in the suspension of *Streptococcus sp* bacteria. This difference is caused by differences in the characteristics of the two bacteria where the metabolism of the *Lactobacillus sp* bacteria which may produce lower pH than *Streptococcus sp.* It is known that Streptococcus sp bacteria can produce acid up to pH 4.5 while Lactobacillus sp bacteria can produce acid up to pH $4⁶$ Also supported by the characteristics of bacteria, where the higher pH, the

bacteria that grow well are *Streptococcus sp* whereas in the lower pH, the *Lactobacillus sp*will grow better. The results of the Ochsner study (2006) showed that *Lactobacillus sp* bacteria had good growth at pH 4 while at pH 6.5, it grows with small amount. On the other hand,the*Streptococcus sp* bacteria had good growth at pH 6.5 while decrease at pH 4.8

BHIB media, used for bacterial breeding, contains dextrose which function as a source of nutrition for bacteria. When bacteria are exposed to dextrose, it will be able to form extracellular and intracellular polysaccharides that play a role in the growth and attachment of bacteria. Extracellular polysaccharides serve to keep the plaque firmly attached to the surface of the GIC and maintain the integrity of the plaque. Intracellular polysaccharides serve as a metabolic material for bacteria that will produce acid. This acid from metabolic results will cause low pH. It can be concluded that there is an effect of the attachment of *Streptococcus sp* and *Lactobacillus sp* bacteria to the levels of fluoride ion solubility in GIC restoration.

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