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THE EFFECT OF LACTIC ACID AND ARTIFICIAL SALIVA SOLUTION IMMERSION TO THE RELEASE OF CALCIUM IONS OF BIOACTIVE RESIN

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ABSTRACT

Background: Bioactive resin can release calcium ions when contact with solution media, even in acid condition. In the oral cavity, pH may change into acid condition due to the metabolic results of *Streptococcus mutans*. The bacteria metabolize carbohydrates into organic acids, one of which is lactic acid. **Purpose:** Analyze the effect of lactic acid solution and artificial saliva on the number of the release of calcium ions of bioactive resin. **Methods:** Forty-two specimens (diameter 15 mm x thickness 1 mm; n=7/group) fabricated with Aactiva™ Bioactive Restorative (Pulpdent). The specimens that meet the criteria are divided into 6 groups. The specimen was immersed for 1 and 7 days in the incubator at 37°C. The number of calcium ion release is measured using titration method. **Results:** Two Way Anova test and Post Hoc Bonferonni test show there are significant differences among all group for lactic acid 1 day (4.040 ± 0.360), artificial saliva 1 day (0.640 ± 0.338), distilled water 1 day (1.040 ± 0.504), lactic acid 7 days (5.400 ± 0.312), artificial saliva 7 days (1.640 ± 0.215), distilled water 7 days (3.520 ± 0.356). **Conclusion:** There is an influence of lactic acid and artificial saliva on the number of releases of bioactive composite calcium resin ions.

Keywords: artificial saliva, bioactive resin, lactic acid, calcium ion release.

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INTRODUCTION

Dental caries is the most common dental disease and multifactorial disease, caused by the presence of cariogenic bacteria, diet, and the host factor.¹ Dental caries treatment goals are to restore teeth to a proper form, function, and esthetic appearance, to prevent the recurrence of caries and part of the treatments are to apply restorative materials.² One of the most reported causes of restoration failure is secondary caries.³ The carious lesion located at marginal of restoration or caries bound to the restoration or sealant.⁴ Secondary caries may form due to the entry of bacteria into the margin of the restoration.⁵ *Streptococcus mutans* is bacteria that commonly responsible for dental cavities, it can metabolize carbohydrates into organic acids, namely lactic acid, which results in demineralization of tooth structure and marginal of restoration.⁶ This acid may decrease pH of the oral cavity characterized by the increase in hydrogen ion concentration. Hydrogen ions may damage tooth enamel hydroxyapatite by diffusing throughout the enamel and dentin pores, thereby triggering demineralization.^{7,8} The demineralization process may occur when the pH is less than 5.5 and the pH of the oral cavity after food consuming is range of 5.2-5.5. The pH of the

oral cavity will return to normal after 20-30 minutes and the demineralization process in this normal cycle is followed by a remineralization process.⁹ Remineralization is the process of returning calcium and phosphate mineral ions to form hydroxyapatite crystals on the enamel. The use of restorative materials that can release fluor ions, calcium ions, and phosphate ions may contribute to the remineralization of dental tissue, protection against caries, and other dental health problems.^{10,11} One of restorative materials that can release fluor, calcium, and phosphate ions is bioactive resin.^{12,13} The release of ions in bioactive composites through the reaction of dimethylacrylic acid-base phosphate and polyacrylic acid.¹⁴ This material releases more phosphate, fluor and calcium ions than glass ionomer.¹⁵ Restorative materials that can release calcium ions can protect the hard tooth tissue from the effects of acidic foods or drinks consumed and also promote the remineralization.⁵ The release of ions are affected by changes in pH.¹⁶ One of the causes of the pH of the oral cavity to decrease to acid is due to the metabolism of *Streptococcus mutans*. The average remineralization process occurs in the span of 7-21 days.¹⁷

The purpose of the research is to analyze the effect of lactic acid solution and artificial saliva on the number of release calcium ion bioactive composite resin.

MATERIALS AND METHODS

The study has approved by the ethical clearance test published by the Faculty of Dentistry, Lambung Mangkurat University No.010/KEPKG-FKGULM/EC/II/2021. The specimens were fabricated using bioactive resin (Activa™ Bioactive Restorative, Pulpdent), a total of forty-two specimens, with six treatment groups, each group consisted of seven specimens were prepared using plastic mold with 1 mm diameter and 15 mm thickness according to the ISO 4049 (2000).

The bioactive resin from syringe with two compartments were mixed by extrusion through a spiral nozzle or gun. Each resin was applied on the mold, covered with celluloid strip and glass slide to the top of the mould, then gently pressed to obtain a smooth surface and to remove the excess material. Specimens were light cured with a LED Light Curing Unit with an output of 800 mW/cm² for 20s on the top of mould surfaces with the tip touching the glass, subsequently stored in incubator for 24 h at 37°C and were prepared for immersion based on the group treatment. The groups are described as follows: (1) immersion on lactic acid solution for 1 day, (2) artificial saliva for 1 day, (3) distilled water for 1 day, (4) lactic acid solution for 7 days, (5) artificial saliva for 7 days, (6) distilled water for 7 days; all immersion media are treated with pH measurement, replaced every day during the research process and stored in incubator with the temperature of 37° C. The pH measurement exhibits lactic acid solution pH 5.2, artificial saliva pH 6.7 and distilled water pH 7.

The number of calcium ion release measurement was conducted by using titration method. Firstly, the specimen was grinded using mortar to get fine particle and put in a 250 ml beaker which contains of 100 ml distilled water and 3 drops of red methyl then heated until boil. The solution of NH₄ -oxalate 0.75 grams was added to 12.5 ml of distilled water and then slowly put into a beaker. the solution is heated at a temperature of 70-80° C for 15 minutes. Subsequently, 3 drops of ammonia solution (1:1) are inserted while stirring slowly and the solution is left in heated condition for 1 hour. The precipitate is filtered using filter paper and deposits are washed using distilled water until free from oxalate, subsequently the sediment is rinsed with a solution of sulfuric acid (1:8) into another erlenmeyer. Filter paper is washed with hot distilled water to a volume of 50 ml then the solution is titrated with KMnO₄ 0.1 N until the color turns pink. Calculation of Ca weight are conducted based on the formula:

$$0,7056 \times \text{vol. KMnO}_4 \times 2,8 \text{ mg CaO}$$

Data analysis was carried out with a two-way test ANOVA with a confidence level of 95% ($\alpha = 0.05$) and continued using the Post Hoc Bonferroni test to determine the value of significance.

RESULTS

The mean values of the amount of calcium ions release in the bioactive resin are summarized in table 1.

The mean and standard deviation of the number of calcium ion release of bioactive resin.

Immersion media and time	Mean ± SD (µg)
Lactic acid 1 d	4.040 ± 0.360 ^A
Artificial saliva 1 d	0.640 ± 0.338 ^B
Distilled water 1 d	1.040 ± 0.504 ^C
Lactic acid 7 d	5.400 ± 0.312 ^D
Artificial saliva 7 d	1.640 ± 0.215 ^E
Distilled water 7 d	3.520 ± 0.356 ^F

Two Way Anova test

*Different superscript letters indicate significant differences.

Based on table 1, it can be seen that the highest mean value of calcium ion release was in the group of bioactive resin specimens immersed in lactic acid solution for 7 days, while the group with the lowest mean value was the group of artificial saliva immersion for 1 day.

The results of the Two Way Anova statistical test showed that the significance value of the immersion media group was $p = 0.000$ ($p < 0.05$), which means that there was a significant difference in the number of calcium ion release of bioactive resin immersed in lactic acid, artificial saliva and distilled water. The significance value of the immersion time group was $p = 0.000$ ($p < 0.05$), which means that there was a significant difference in the amount of calcium ion release in the bioactive resin that was immersed for 1 and 7 days. The significance value of the immersion media and time was $p = 0.138$ ($p > 0.05$), which means there was no interaction between the immersion media and time. Post Hoc Bonferroni test show that there are significant differences among all group.

DISCUSSION

In the present study, all groups of bioactive resin immersed in a solution of lactic acid, artificial saliva and distilled water exhibits the release of calcium ions with varying number. The immersion in lactic acid solution group had the highest mean value of calcium ion release among all group. In addition, 7 days immersion group had a higher calcium ion

release number than 1 day immersion group. Lactic acid is a carboxylic acid with the molecular formula ($\text{CH}_3\text{CHOHCOOH}$). Lactic acid may dissolve in water and releases protons (H^+) to form lactate ions. The ion (H^+) will increase in acidic conditions, thereby (OH^-) from the material will be released due to binding with H^+ in acid and forms water by the reaction of $\text{H}^+ + \text{OH}^-$ to become H_2O . Subsequently, the release of OH^- from material which will be present in the forms of, namely H_2PO_4 , H_2PO_4^- , HPO_4^{2-} , PO_4^{3-} , H_3PO_4 , H_2PO_4^- , and HPO_4^{2-} . These compounds cause the resin material to dissolve and calcium release from it.⁹

One of the factors that play a role in the release of ions is the degree of acidity or pH.¹⁸ Bioactive resins may release more ions at an acidic pH, furthermore, bioactive resins are able to release calcium, fluorine and phosphate ions when in contact with saliva. In the present study, lactic acid solution (pH 5.2) is classified as more acidic than artificial saliva (pH 6.7) and distilled water (pH 7). This is result in the number of calcium ion release in the lactic acid immersion group has a higher amount than the artificial saliva and aquadest immersion group. This is in accordance with research conducted by Irpansyah et al (2020) which states that the lower the pH, the greater the number of ions released.¹³

The group with the lowest number of calcium ion release was the group that was immersed in artificial saliva, even though the immersion group had a more acidic pH than the pH of distilled water. Artificial saliva was fabricated from a mixture of mineral salts using the McDougall method with a mixed composition of NaHCO_3 (58.80g), $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (48 g), KCl (3.42 g), NaCl (2.82 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.72 g) and CaCl_2 (0.24 g) in 6 liters of distilled water. Artificial saliva with a normal pH, due to unperfect ionization process the solution does not produce H^+ and OH^- ions, therefore fewer calcium ions are released rather than when the acid state has amount of H^+ ions, furthermore in a neutral pH state, the bioactive glass state becomes more stable than other conditions. furthermore, solutions with high viscosity are difficult to penetrate the bioactive glass particles in the bioactive composite resin.¹⁸

The bioactive resin contains of bioactive glass which is composed of silica (SiO_2), phosphorus pentoxide (P_2O_5) and boron trioxide (B_2O_3). The silica network in bioactive glass is more open, therefore water molecules may penetrate to the resin more easily. Water molecules that penetrating may contact with the silica glass, subsequently an ion exchange process occurs between hydronium ions (H^+) with modifier ions Na^+ and Ca^{2+} to form silanol or $\text{Si}(\text{OH})_4$ caused by hydrolyzed silica. Furthermore, silanol undergoes a condensation process to form a gel layer on the silica surface. The gel layer formed

does not function as a protector due to the open silica network allowing deeper penetration of water, causing degradation of the bioactive resin which result in the higher release of calcium ions in the bioactive glass.¹⁹

The immersion time also has a role in the ion release process. The longer the immersion is carried out, the greater the ion will be released.²⁰ Therefore, in this study the number of ions released during the 1-day immersion time was less than the 7-days immersion time.

Within the limitations of this study, the following conclusions can be drawn there is an effect of lactic acid solution and artificial saliva on the amount of calcium ion release in bioactive resins. This study attempted to approach the actual situation, which was put in an incubator with a temperature of 37°C to adjust to the actual state of the oral cavity.

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