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The Capability of Cocoa Pulp Juice (Theobroma cacao L.) on Crown Dentin Smear Layer Removal

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ABSTRACT

Introduction: smear layer is a thin layer formed from tooth structure instrumentation which can prevent the restoration material bonds the tooth structure. Partial smear layer should be removed using dentin conditioner. One of dentin conditioners that is often used is 10% polyacrylic acid. The natural ingredient that is thought to be an alternative to dentin conditioner is cocoa pulp juice because it contains many acidic compounds.

Methods: This research is a laboratory experimental study to determine the ability of 100% cocoa pulp juice (Theobroma cacao L.) to remove the crown dentin smear layer and compare it to 10% polyacrylic acid. Eight dental elements were prepared for class I cavities on the middle 1/3 of buccal surface to reach 3 mm in diameter and 2 mm in depth, enamel was prepared until 1 mm in depth of cavity left. The samples were irrigated with sterile aquades and dried. Furthermore, 4 samples were applied 10% polyacrylic acid and 4 samples used cocoa pulp juice, left for 20 seconds, irrigated, dried, and put in the oven for 2x24 hours. The samples were examined for their cavity cleanliness score using Scanning Electron Microscope (SEM) photo with 2000x of magnification.

Result: both groups have the same score and statistical test shows that there is no significant difference between them.

Conclusion: the 100% cocoa pulp juice was able to clean the smear layer of crown dentin and its ability equal to 10% polyacrylic acid.

KEYWORDS: cacao pulp juice, dentin conditioner, smear layer.

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INTRODUCTION

Smear layer is a layer of amorphous debris that covers the underlying structure, formed iatrogenic from the results of tooth preparation [(1)]. The smear layer consists of organic components in the form of protein coagulation due to heating by the bur during preparation, necrotic tissue, blood cells, saliva, and microorganisms, as well as inorganic components including tooth structure and contamination of other non-specific inorganic components [(2)]. The superficial smear layer has a thickness of 0.5–5.0 µm with a smear plug depth of up to 40 µm [(3,4,5)].

Smear layers can reduce the permeability of the dentinal tubules and create a dry area. Decreased permeability helps reduce the infiltration of harmful substances or microorganisms into the dentinal tubules[(6)]. Its presence also has a negative impact because it prevents the restorative material from adhering to the tooth structure.

Humairah et al stated that the cleaning of the 0.5 µm thick smear layer by polyacrylic acid was better than the cleaning of 2-5 µm by phosphoric acid. This was due to the lower microleakage produced in the RMGIC restoration and the better bonding strength of the tooth with the resulting restorative material. Partial cleaning of the smear layer using acid (dentin conditioner) has become a routine procedure in dental restorations [(6,7)]. In addition to using commercial

dentin conditioners, a natural ingredient that has the potential as an alternative is cocoa pulp juice.

Cocoa pulp juice is acidic with a pH of 3.75. This acidic nature is caused by several acidic compounds contained in it[8,9]. Cocoa pulp juice is also suspected to contain saponins in its pectin which helps in cleaning the smear layer [10].

The existence of cocoa pods in Indonesia is very easy to find because it is one of the largest plantation products spread across several regions of Indonesia[11]. The use of cocoa pods is still limited to seeds and skins. Most of the cocoa pulp becomes waste and does not have good economic value [12]. This study hopes that cocoa pulp juice can be effective in cleaning the smear layer on crown dentin.

MATERIALS AND METHODS

This research is a laboratory experimental research with post test only control group design. The research was conducted from January 2021 to February 2021 at the Plant Laboratory of the Jember State Polytechnic Faculty of Agriculture, the Bioscience Laboratory of the RSGM Jember University, the Conservation Clinic of the RSGM Jember University, and the Pharmacy Laboratory of the Faculty of Pharmacy, University of Jember. The number of samples in this study were 4 elements of human teeth for each treatment group according to the results of calculations using the Daniel formula [13].

Sterilization Stage

All glass and metal tools were washed and sterilized by autoclaving for 15 minutes at 121°C. Tools made of plastic are washed, dried, and smeared with 70% alcohol.

Preparation Stage of 100% Cocoa Pulp Juice

Cocoa pods were washed, dried, and disinfected with 70% alcohol on the cocoa shell. The cocoa pods are crushed with a stainless-steel knife and the entire pulp which includes the beans is removed. Cocoa pulp is squeezed on a sieve and collected in a glass beaker, then filtered again gradually using sterile gauze, starting from 1 layer of cotton, 2 piles of gauze, and 3 piles of gauze. The pH of the cocoa pulp juice was measured using a pH meter and obtained a pH of 3.20. Cocoa pulp juice was sterilized with 20-watt UV light at laminar flow for 2 hours. 100% cocoa pulp juice is ready to use.

Dental Sample Preparation Stage

Eight elements of the maxillary first premolar were implanted in the night beam. Elements were made as an outline of a class I cavity preparation in the form of a circle in the middle 1/3 of the buccal surface of the tooth with a diameter of 3 mm. Elements were prepared at 180,000 RPM using a highspeed handpiece. Round burs are used to make cavities and cavity widening using flat end cylindrical fissure burs up to 3 mm in diameter and 2 mm in depth. The enamel surface was prepared using a flat end cylindrical fissure bur of 1 mm to leave a cavity depth of 1 mm. The tooth element was cut into a 7 x 7 x 4 mm beam using a diamond disk wheel with the cavity positioned right in the middle. Each tooth beam was implanted with the cavity facing up on plasticine with a size of 3 x 3 x 1 cm according to the color of the treatment group.

Treatment Stage

Samples that were ready to be treated were irrigated once with 0.5 ml sterile distilled water using a disposable syringe and then dried with water spray. Applying the material to the sample cavity according to the treatment group:

1) Group K: 2 L of 10% polyacrylic acid dripped into the sample cavity with an eppendrof pipette, smoothed with a microbrush, and waited 20 seconds.

2) Group P: dripping 2 L of 100% cocoa pulp juice on the sample cavity with an eppendrof pipette, flattened with a microbrush, and waited 20 seconds.

The cavity was irrigated once with 0.5 ml of sterile distilled water and then dried with water spray. The sample is removed from the plasticine and placed into a petridish. The sample was put in an incubator at 37˚C for 2 x 24 hours to dry the sample. After 2 x 24 hours, petridish wrapped in aluminum foil to prevent contamination.

Scanning Electron Microscope (SEM) Shooting Stage

The sample is glued to the holder using double-sided tape. Holders and samples are inserted into the SEM. Observations were made with a magnification of 2000 times. Photographs were taken after obtaining a clear picture of the dentinal tubules on the surface of the cavity.

Cavity Cleanliness Assessment Stage

The transparent sheet is divided into 10 equal squares using a marker and then pasted on the photo shoot. Each box is given a score for its level of cleanliness. The assessment was carried out by 3 observers with reference to the scoring system7 (Figure 1):

1 = no smear layer, the entire dentinal tubular orifice is exposed

2 = slight smear layer, partially exposed dentin tubular orifice

3 = homogeneous smear layer covering most of the surface, no or only a few exposed dentinal tubular orifices

4 = the entire surface is covered with smear layer, no exposed dentinal tubular orifices

5 = the surface is covered by a thick and inhomogeneous smear layer (heavy smear layer).

Each observer determines the mode of the 10 boxes. The result of this mode is the value of the presence of the smear layer of the sample. The lower the value, the cleaner the cavity in the sample.

Statistic Analysis

The research data were analyzed using the Statistical Package for the Social Science (SPSS) 24 software for windows. The normality test used the Shapiro-Wilk Test to see the distribution of the data. Homogeneity test was

carried out using Levene Test. If the test results obtained data that is normally distributed and homogeneous, then the test can be continued with parametric statistical tests, namely the Independent Samples T-Test. If the data obtained are not normally distributed and not homogeneous, then the Mann-Whitney Test non-parametric statistical test is carried out to see the differences between groups.

RESULTS

The results of observations through Scanning Electron Microscope shooting with a magnification of 2000x obtained a similar picture of the cleanliness of the basic surface of the cavity in each group in the form of the formation of several dentinal tubular orifices which were still filled with a smear plug and there was a small portion of the smear layer on the surface (Figure 2).

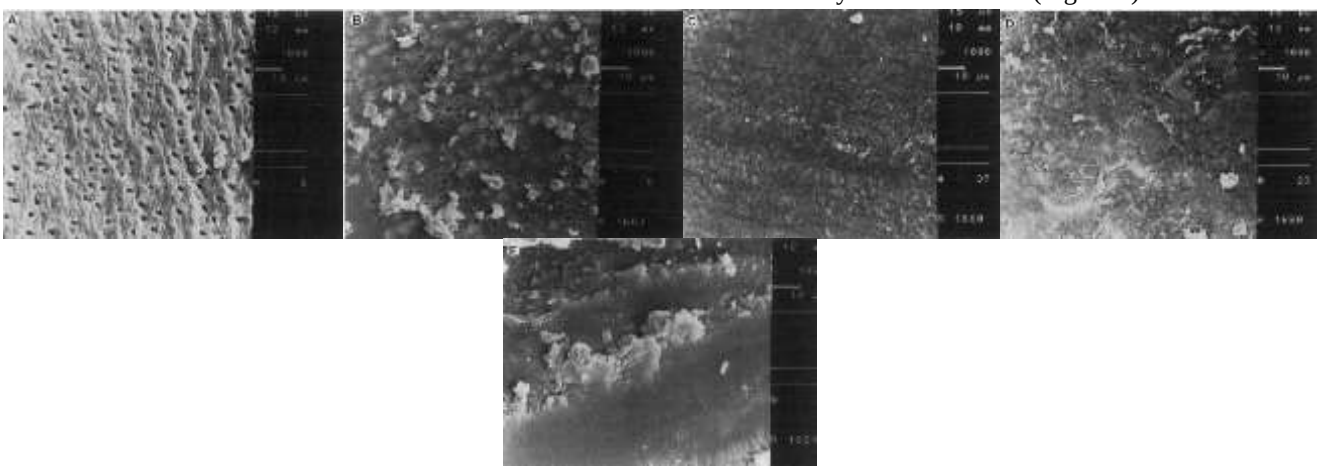
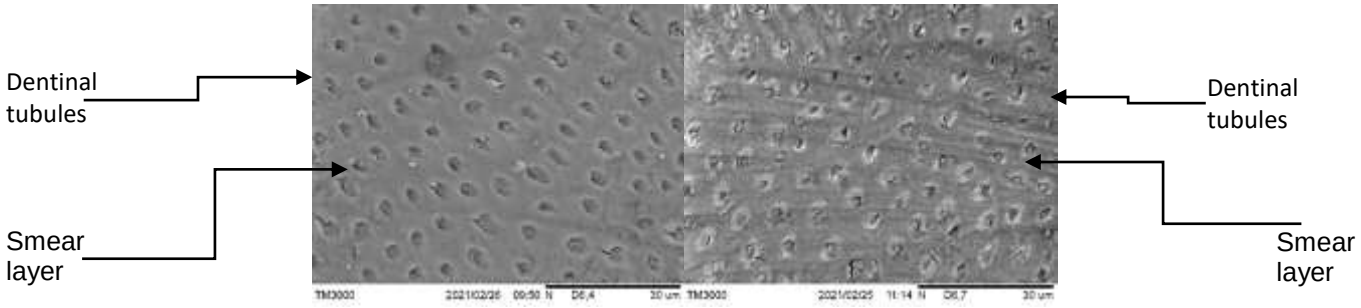


Figure 1. Reference for scoring. (A) score 1; (B) score 2; (C) score 3; (D) score 4; (E) score 5 [(14)].



(a) (b)

Figure 2. Results of sample shooting with SEM: (a) the bottom surface of the cavity in the 10% polyacrylic acid group; (b) the bottom surface of the cavity in the 100% cocoa pulp juice group

The assessment of the cleanliness of the smear layer on the basis of the sample cavity was carried out by 3 observers by calculating the score for the presence of the smear layer in each box and looking for the score that appeared most frequently (mode). The results of the assessment of the cleanliness of the smear layer at the base of the cavity are presented in Table 1 and Figure 3.

Table 1. Value of the mode of presence of smear layer in each group

10% polyacrylic acid					100% Cocoa Pulp Juice				
Sample	Observer			Modus	Sample	Observer			Modus
	P1	P2	P3			P1	P2	P3	
Sample 1	2	2	2	2	Sample 1	3	2	2	2
Sample 2	2	2	2	2	Sample 2	3	3	2	3
Sample 3	3	3	2	3	Sample 3	2	2	3	2
Sample 4	2	2	2	2	Sample 4	2	2	2	2
Modus				2	Modus				2

The results of the assessment showed that the cleanliness of the smear layer in the 10% polyacrylic acid group and the 100% cocoa pulp juice group had the same mode, namely 2, meaning that there was a small amount of smear layer, part of the dentinal tubular orifice was open.

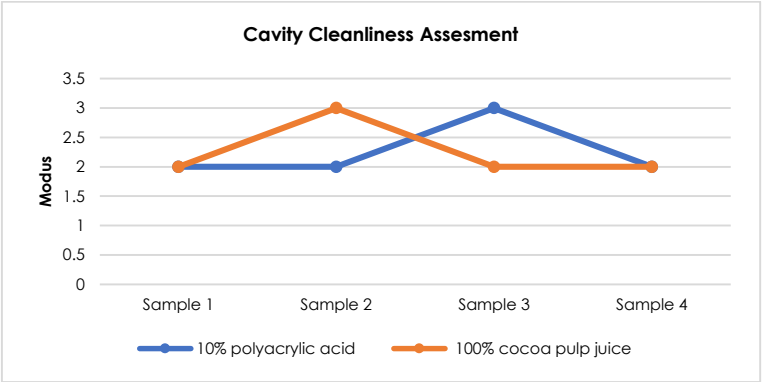


Figure 3. Line diagram of the mode of presence of smear layer on the bottom surface of the cavity after application of 10% polyacrylic acid and 100% cocoa pulp juice.

Figure 3 shows that the mode values of each sample in the two groups are mostly at the same coordinates. It can be interpreted that 100% cocoa pulp juice has the ability to clean the smear layer on crown dentin equivalent to 10% polyacrylic acid.

The results of the normality test using the Shapiro Wilk test showed a significant data value of 0.001 ($p < 0.05$), which means that the data is not normally distributed. The results of the homogeneity test using Levene's test showed a significant data value of 1,000 ($p > 0.05$), which means the data is homogeneous. The results of the different test using the Mann-Whitney test showed a significant data value of 1,000 ($p > 0.05$), which means that there was no significant difference between the mode values of the 10% polyacrylic acid sample group and the 100% cocoa pulp juice sample group.

DISCUSSION

The results of the study based on the search mode from 3 observers, it was found that the 10% polyacrylic acid group with a pH of 1.65 had the ability to clean the smear layer with a score of 2, meaning that the smear layer was small and part of the dentinal tubular orifice was open. This score was obtained because polyacrylic acid has the ability to clean the smear layer only about 0.5 µm so that it still leaves smear plugs. This ability will reduce the level of microleakage in the RMGIC restoration and the bond strength of the tooth with the restorative material is better because only a small amount of demineralized dentin remains, leaving hydroxyapatite around the collagen fibers which is useful for chemical bonding with the restorative material[7,15]. This residual smear layer is also needed for composite restorations with a self-etch bonding system that modifies it so that it is more permeable to monomer penetration [16].

The results of the study on the 100% cocoa pulp juice group with a pH of 3.20 also had a score of 2. The ability to clean the smear layer was due to the content of various acid compounds in the 100% cocoa pulp juice including citric acid, acetic acid, maltic acid, oxalic acid, lactic acid, malic acid, fumaric acid, and ascorbic acid (vitamin C) [9].

The acid contained in the juice of 100% cocoa pulp and 10% polyacrylic acid acts as a chelating agent that can chemically bind metal ions such as calcium and form dissolved calcium [17]. The acid will also release hydrogen ions that are able to bind and decompose the hydroxyapatite matrix so that a demineralization process occurs with the release of water-soluble Ca2+ and HPO42- ions. This process is described in the following chemical reaction [18,19]:

$$Ca10(PO4)6(OH)2 + 8H^{+} \longrightarrow 10Ca^{2+} + 6(HPO4)^{2-} + 2H2O$$

The juice of 100% cocoa pulp is also suspected to contain saponins in pectin. Saponins are compounds that function as emulsifiers (detergents), are able to dissolve the smear layer and reduce surface tension so as to facilitate the penetration of materials [19]. Based on research by Wana and Pagarra (2018), it was found that the pectin extract contains saponins which are characterized by the formation of foam when shaken with water[10]. Pectin contained in cocoa pulp is 5% [9] , this strengthens the potential for saponin content in cocoa pulp juice.

Meanwhile, although 100% cocoa pulp juice has advantages in its content, its cleaning ability is equivalent to 10% polyacrylic acid. This equivalence is due to the possibility of decreasing the concentration or ability of the compounds affected by the research procedure by filtering and sterilizing with UV light. The pH level of 100% cocoa pulp juice in this study has been shown to remain unchanged from filtration to UV sterilization. Saponin levels are not known for sure whether affected or not. Further research is needed on the impact of filtering and sterilizing 20 watt UV light for 2 hours on the water content of 100% cocoa pulp.

The main factors in smear layer cleaning are the pH level and exposure time of the acid [1(17)]. 100% cocoa pulp juice is superior because it has a higher pH, but is able to clean the smear layer equivalent to 10% polyacrylic acid due to the presence of supporting compounds such as saponins.

Commercial dentin conditioners usually contain other chemicals such as glutaraldehyde which stabilize the collagen structure, but cause tissue necrosis in other areas [(20)]. Polyacrylic acid concentrations above 1% have also been said to be cytotoxic in tissue culture studies [(6)]. 100% cocoa pulp juice is thought to be better than 10% polyacrylic acid because it is a natural ingredient with minimal side effects, but further research is needed on its toxicity.

This dosage form of 100% pure cocoa pulp juice is still a weakness of its own as an alternative material for dentin conditioner. The short shelf life causes 100% cocoa pulp juice to be used as soon as possible to maintain its content. This preparation is also not practical in the manufacturing process so that other research is needed for the manufacture of extracts or preservation of this 100% cocoa pulp juice.

The suggestion that can be conveyed is that there is a need for further research on the toxicity of 100% cocoa pulp juice (Theobroma cacao L.) as an alternative dentin conditioner to cells in vitro and in vivo, as well as to tissues around teeth in animals. There is a need for further research on the antibacterial effect, the content of saponins and the manufacture of extracts in the juice of 100% cocoa pulp (Theobroma cacao L.).

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the juice of 100% cocoa pulp (Theobroma cacao L.) is able to clean the smear layer of crown dentin and its ability is equivalent to 10% polyacrylic acid.

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