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**Organoleptic of Pulp Capping Paste-Based Casein and Lactoferrin Cow's Milk**

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**ABSTRACT**

**Introduction:** Pulp capping is a caries treatment, so teeth withstand for a long time in the oral cavity. The pulp capping material is expected to not only stimulate the mineralization of the pulp tissue to form reparative dentin in the various area, but it might be able to adhere and stay for a long time in dental tissues. Casein Cow's milk has a protein content that can stimulate the remineralization of dentin. **Purpose:** Testing the properties of a paste formulation based on casein and cow's milk lactoferrin. **Methodology/Approach:** Evaluation of dental fillings in the form of pastes made from casein (20.450 gram/liter) and cow's milk lactoferrin (160.370 gram/liter) using the organoleptic test, homogeneity test, spread-ability test, and adhesion test. The ratio used between lactoferrin and casein is 8: 1. The formulations used include F1 (5% Carbopol, 40% ZnO, 2% TiO<sub>2</sub>, and 20% silica, Ph buffer 6.8), F2 (5% Carbopol, 40% ZnO, 2% TiO<sub>2</sub>, and 20% silica, 10% PVP, Ph buffer 6.8), F3 (5% Carbopol, 40% ZnO, 2% TiO<sub>2</sub>, and 20% silica, Eydragit RL 100 10%, Ph 6.8 buffer), F4 ( Carbopol 5%, ZnO 40%, TiO<sub>2</sub> 2%, and silica 40%, Eydragit RL 100 10%, Dapar Ph 6.8), F5 (Carbopol 5%, ZnO 20%, TiO<sub>2</sub> 2%, and silica 40%, PVP 10%, Buffer Ph 6.8). **Findings:** This research shows that there are 5 (five) formulations of pulp-capping paste ingredients. Each formulation of pulp capping paste had different average adhesion, spread, pH, and viscosity (p<0.05). Formulation V has the highest adhesion and spread among other formulations. FI, FIV, and FV toothpaste preparations had the lowest pH among the other groups. However, formulation II showed the best viscosity compared to the other groups. **Conclusion:** Formulations based on comparing the basic ingredients of casein-lactoferrin in cow's milk with additives having different physicochemical properties.

**Keywords:** Casein, Lactoferrin, Pulp Capping Paste First Section

**INTRODUCTION**

Dental caries is a dental hard tissue disease in which almost the entire Indonesian population in all age groups experience caries. According to the 2018 Basic Health Research Results (Riskesdas) data, the largest proportion of dental problems in Indonesia is dental caries (45.3%).[1] Dental caries that are not treated will cause a decrease in a person's quality of life because it causes toothache and tooth loss.[2] Nearly 83.5% of caries cases do not receive treatment, causing

acute tooth pain and periapical tissue abnormalities. [3]

Pulp capping treatment is one of the treatments for dental caries. The success of treatment is influenced by the ability of the pulp capping material to maintain tooth vitality by increasing the regeneration and remineralization of cells in the pulp tissue. [4] The development of natural-based pulp capping materials is currently rife, including casein and lactoferrin-based. Casein and lactoferrin in cow's milk are proven to have growth factors and calcium,

which can trigger remineralization and reparative dentin formation. [5]

Based on previous research, lactoferrin and cow's milk casein could stimulate pulp tissue regeneration and form reparative dentin. *Lactoferrin* is an anabolic peptide that can trigger fibroblasts and osteoblasts' proliferation, differentiation, and maturation. Lactoferrin also plays a role in bone remodeling by triggering collagen matrix formation, increasing alkaline phosphatase activity, and osteocalcin production through the mitogen-activated protein kinase (MAPK) pathway. Lactoferrin also regulates the expression of transforming growth factor beta (TGF- $\beta$ ), stimulating the extracellular matrix's deposition and increasing fibronectin expression. Meanwhile, casein will form calcium phosphate compounds as micellular complexes to transport calcium and phosphate in osteoblast mineralization and calcification. Casein forms calcium-phosphate nanoclusters as a phosphate reservoir for crystal growth or tissue remineralization. The research showed that with a concentration of 7.5 g/l, casein affected the mass of the precipitate in the growth process of carbonate apatite crystals.

However, as a pulp capping material, casein and lactoferrin are in cow's milk. It should not only trigger dentine regeneration and remineralization, but the pulp capping material must also have organoleptic and rheological properties acceptable to the dentine tissue not to irritate the dental pulp. In order to meet these requirements, the development of bioactive pulp capping materials was added with gelling ingredients and other ingredients that improve the physical properties of casein-based pulp capping paste and cow's milk lactoferrin.

Herbal products are gaining popularity in dental and medical practice

nowadays due to their biocompatibility, higher antimicrobial activity, antioxidant and anti-inflammatory properties, especially for endodontic treatment. However, herbal formulation ingredients must follow all testing procedures, from testing pure ingredients to forming the formulation, such as making pulp capping paste. This process is done to avoid side effects on tissues and organs, thereby endangering the individual's life.

Paste preparation uses this herbal ingredient to make it easier to use and modern. This study used Carbopol as a semi-solid paste-forming agent, where Carbopol functions as a gelling agent, binder, stabilizer, suspending agent, and film former. [6] One of the ingredients in the toothpaste formulation contains zinc oxide, silica, PVP, eudragit RL-100,[7]–[9] which of these ingredients may influence the organoleptic pulp capping paste made from casein and lactoferrin. This study aimed to examine the properties of a pulp-capping paste formula made from casein and cow's milk lactoferrin.

## RESEARCH METHOD

This study used 20 grams/liter of casein in cow's milk and 160 grams/liter of lactoferrin. The paste preparation was started by sifting the basic ingredients, namely ZnO, TiO<sub>2</sub>, and Silica TLC GF 24, with mesh numbers 100. After that, the gelling agent, namely Carbopol 940, is weighed, and the ingredients are mixed into a homogeneous dry mixture. PVP and Eudragit RL 100 each binder dissolved in ethanol and allowed to stand until dissolved. The two mixtures of base and binder were mixed with the addition of buffer pH 6.8 to form a toothpaste mass with the desired viscosity [6]–[8].

**Table 1.** Formulation of Dental Pulp Capping Paste

Ingredients	Function	F1	F2	F3	F4	F5
Carbopol	Gelling agent	5 %	5 %	5 %	5 %	5 %
ZnO	Adsorben	40 %	40 %	40 %	20 %	20 %
TiO <sub>2</sub>	Covering agent	2 %	2 %	2 %	2 %	2 %

Silika GF 24	Covering agent	20 %	20 %	20 %	40 %	40 %
Eudragit RL 100	Bond agent	-	-	10 %	10 %	-
PVP	Bond agent	-	10 %	-	-	10 %
Buffer pH 6,8	Solvent	100 %	100 %	100 %	100 %	100 %

Evaluation of pulp capping paste preparations

### Organoleptic

An organoleptic examination was carried out by objectively observing the dental fillings' shape, color, and smell.

### Homogeneity Test

The preparations were tested using two glass slides, where the sample was placed on one of the slides and placed evenly, then seen as homogeneous or not by visual observation. A good preparation must be homogeneous and free from clumping particles.

### Spread-ability Test

The sample was weighed 0.5 grams and placed in the middle of the glass plate, then the top of the glass plate (the weight is known) was placed on the filling and left for 1 minute. The width of the sample in the glass plate was measured in diameter by adding a load of 50, 100, 150, 200, to 500 grams placed on the glass successively. Each additional load was incubated for a minute, and the spread diameter was recorded.

### Stickiness Test

The sample was weighed 0.5 grams, placed on the object glass, connected with the right and left clamps, and a 50-gram weight was hung to release the two object glasses. The time required until the two glass objects were released and recorded.

### Viscosity

Viscosity measurements were carried out using a Brookfield DV-I Prime viscometer.

The paste was put in the provided container, and the ON button, and the auto range or enter were pressed. Spindle 64 was installed, and the Spindle was lowered to the center limit. The select Spindle was pressed until the letter S flashed and was set according to the Spindle installed. If it is appropriate, press selects Spindle again. The speed set button was pressed, and the desired rpm was set. The ON button was pressed, and the display could read cP (centipoise) and % torque. This viscosity test used a 64 spindle with rpm 2. If you want to turn off the Brookfield viscometer, press the OFFmotor button and raise the Spindle.

### pH test

Calibrate the pH meter; The electrodes were washed with distilled water and dried; the pH meter was set at 25°C. The pH meter for testing dental fillings was calibrated with standard buffer solutions, namely 0.05 M potassium hydroxy phthalate (KH phthalate) and 0.05 M equimolar phosphate.

### Method of measuring the pH of the preparation

The electrode was immersed in the tooth filling preparation, and the pH of the preparation produced during the test was observed and recorded.

## RESULT AND DISCUSSION

**Table 2.** Organoleptic Of Pulp Capping Paste Formulation

Homogeneity	Organoleptic			Stickiness (s)	Spreading (cm)	pH	Viscosity (cPs)
	Texture	Color	Aroma				
F1 Homogen	Rough	grayish white	No	1.273	1,862	6.6	1385
F2 Homogen	Rough	grayish white	No	1.33	1,738	6.9	4691.33
F3 Homogen	Rough	grayish white	No	1.433	1,905	6.8	2351.33

F4	Homogen	Smooth	grayish white	No	1.523	2,026	6.6	5122.67
F5	Homogen	Smooth	grayish white	No	2.26	2,347	6.6	2435

Organoleptic tests in this study included homogeneity, texture, color, and odor tests. The homogeneity test aims to determine whether the active ingredients from cow's milk are mixed perfectly with other additives while making pulp capping paste. The material for the formulation of pulp capping paste containing casein lactoferrin in cow's milk showed the same homogeneity during the storage period. In the test, no coarse grains were found on the object glass. Although based on the homogeneity test results, the pulp capping paste formula presented a different texture. Formulas 4 and 5 were smooth textures, while formulas 1, 2, and 3 were rough. Formulas 4 and 5 are smoother than formulas 1,2,3 because formula 4 contains 10% Eudragit RL 100 and formula 5 contains 10% PVP. Apart from being an adhesive agent, these two materials can also increase the solubility of other materials, so that the size is smaller and the texture is more elastic. This difference in texture was indicated by whether the pasta dough was sandy (coarse) or not. As for color and smell, the five formulas have the same color and smell.

The adhesiveness test aims to determine the ability of the pulp capping paste to adhere to the cavity surface. The five formulas presented an adhesive power of less than 2 minutes. The pulp capping paste formula presented a dense and elastic consistency, making it easy to adhere to the dental cavity.

The spreadability test aims to determine how widely the pulp capping paste was spread when it was used. The five formulas were spread less than 2.5 cm. The consistency of the five pulp capping paste formulas was dense or thick, especially at F5.

The pH test aims to determine the acidity of the pulp capping paste. All pulp

capping paste formulas presented a pH below normal, an average of 6. F2 was a formula with a pH closest to normal, namely 6.9.

This study used five pulp capping paste formulas because the paste preparations are physically more stable and do not melt at body temperature, so they are good for use as pulp capping pastes to seal and protect pulp tissue.[10] The advantages of pastes compared to other topical preparations are that pastes bind to liquid secretions so that they are better for acute wounds than unguentum, medicinal ingredients in paste preparations are more attached to the skin, thereby increasing their local effectiveness, the concentration of pastes is thicker than ointments, and the absorption power of pastes is greater and less fatty than ointment preparations.[11]

Making the formula is distinguished by the percentage of ZnO and silica. The difference in the percentage of ZnO and silica affects the texture of the pulp capping paste material. The addition of silica will affect the rheological materials of cement and semi-solid pastes. In this study, the addition of the percentage of silica can refine the texture of the pulp capping paste material.[8] ZnO is a mild astringent with pharmacological studies as a local antiseptic. The mild astringent in question is to shrink the pores of the tissue so that the addition of ZnO can protect the vital tissue under the dentin.[11],[12]

Combining Silica and ZnO can improve semi-solid preparations' physical and chemical properties. In addition, the silica and ZnO bonds will increase the stability of the chemical bonds of the ingredients in the pulp capping paste formula, except in acidic or alkaline solutions. This combination of minerals with oxygen is very effective for protein synthesis, cell proliferation, and tissue

repair. In addition, this combination of ingredients will strengthen the activity of casein and lactoferrin in cow's milk in improving dental pulp tissue repair.[11]–[13]

The physical properties of the formulation are also influenced by the introductory materials, eudragit RL-100 and PVP. PVP and eudragit RL-100 in this study functioned as binders or adhesives. PVP is a stable adhesive in water or alcohol solvents. PVP is good for granulating; granules dry quickly, have good flow properties, have a minimum angle of repose, and produce better compatibility. Adding PVP also affects the spreading time and adhesion of pulp capping paste preparations.[8],[14]

Apart from being an adhesive, Eudragit RL-100 can maintain the stability of the pH of semi-solid preparations. The pH of the preparation must be maintained neutral to maintain the stability of the preparation during storage and protect the pulp tissue/not irritate the tissue. Adding Eudragit RL-100 can also reduce the particle size of the pulp capping paste.[7],[15]

## CONCLUSION AND RECOMMENDATION

Based on the results of the research that has been done, casein and lactoferrin can be formulated into pulp capping pastes that have good organoleptic properties. Endodontic paste made from casein and lactoferrin is a herbal product that has several advantages, including safety, ease of use, increased shelf life and low cost. However, this research is still basic research, so comprehensive *in vitro*, *in vivo* and preclinical testing is still needed to scientifically prove that this lactoferrin casein paste formulation is safe for use in humans and must undergo comprehensive *in vitro* and *in vivo* testing. Its biocompatibility and safety must be evaluated in preclinical and clinical studies before it can be definitively recommended for use in endodontics. Herbs are generally

not dangerous when used correctly, but can be dangerous if consumed incorrectly.

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