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Research Report

The effect of adding Epigallocatechin-3-gallate on the porosity of calcium hydroxide

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ABSTRACT

Background: *Dental caries is a very common non-communicablae disease found in all age groups and found in 3.5 billion people in the world. Caries that harm the pulp tissue can be treated by direct or indirect pulp capping. One of the physical properties possessed by pulp capping is the porosity of material. The material most often used for pulp capping treatment of pulp perforations is calcium hydroxide* ($Ca(OH)_2$)*. However,* $Ca(OH)_2$ *has a high pH that caused the porosity to be low. High porosity can facilitate cell development and ion exchange. Thus, additional materials with a lower pH are needed to increase porosity such as Epigallocatechin-3-gallate (EGCG). However, the porosity of the combination of EGCG with* $Ca(OH)_2$ *is still unclear, so it needs to be researched.* ***Purpose:*** *To explain the differences of* $Ca(OH)_2$ *porosity after the addition of EGCG.* ***Methods:*** *This study used a laboratory experimental study with a post test only control group design. This research using 16 samples for each control* ($Ca(OH)_2$ *– aquadest) and treatment* ($Ca(OH)_2$ *–EGCG) group. Samples from each group were subjected to freeze drying, observed with SEM, analyzed with ImageJ software, and tested with independent T-test.* ***Results:*** *There was a significant difference* ($p<0.05$) *in the percentage of porosity between the addition of EGCG to* $Ca(OH)_2$ *namely 32,61% compared to* $Ca(OH)_2$ *with aquadest namely 23,38%.* ***Conclusion:*** *The porosity of the combination of calcium hydroxide with EGCG has a higher percentage compared to calcium hydroxide with aquadest*

Keywords*:* $Ca(OH)_2$*; Dentistry; EGCG; Porosity; SEM*

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INTRODUCTION

Dental caries is a very common non-communicablae disease found in all age groups and found in 3.5 billion people in the world. Caries in untreated permanent teeth is ranked number one out of 291 other diseases and injuries.[1,2] The development and morphology of caries lesions depend on the site of origin and the condition of the oral cavity. When the demineralization of the enamel reaches the Dentino Enamel Junction (DEJ), the previously formed cavity becomes a retentive habitat for cariogenic biofilms to accelerate their development leading to perforation. Bacterial invasion from the dentine to the pulp provides an opportunity for toxins and bacteria to enter the pulp, causing inflammation of the pulp tissue.[3]

In trauma or caries that harms the pulp tissue, pulp capping treatment can be performed either directly or indirectly. Biomaterials for pulp capping serve as a protective layer for exposed vital pulp at the base of deep cavities. An ideal material for pulp capping must have characteristics that can stimulate the formation of dentinal bridge from reparative dentine, maintain pulp vitality, release fluorine to prevent secondary caries formation, bactericidal or bacteriostatic. The selection of material or pulp capping material is one of the success factors of the treatment. One of the physical properties possessed by pulp capping materials is the porosity of the material.[4,5]

Porosity is an important parameter to characterize the microstructure of a material as a space that may contain liquid or air. Porosity determines the amount of leakage, yield of therapy, adsorption, permeability, strength, and density of the pulp capping material. Porosity with good size, percentage, and interconnection between pores can provide an environment to assist cell infiltration, migration, vascularization, flow of nutrients and oxygen.[6,7]

Calcium hydroxide ($Ca(OH)_2$) has been used for a long time in the field of dentistry, especially in endodontic treatment for pulp capping materials, pulpotomies, induction of dental hard tissue deposition, and sealer materials.[8] $Ca(OH)_2$ has the ability as an antibacterial and plays a role in stimulating hard tissue repair. However, it has several disadvantages, such as causing superficial coagulation necrosis in the pulp tissue, formation of tunnel defects, easy dissolution, very low bond to dentin, and degradation

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after application of acid etching.[9,10] Calcium hydroxide is widely used because it is a strong base resulting from the breakdown and release of hydroxyl ions.[11] Materials with a lower pH cause changes in the microstructural properties of a material thereby increasing porosity and affecting compressive strength. High pH causes a low porosity percentage of a material.[12] In another study, the results of $Ca(OH)_2$ porosity ranged from 7.49-9.04%. Meanwhile, the porosity of Mineral Trioxide Aggregate (MTA) ranges from 39.3-49.47% and bioceramic cement made from calcium silicate is 22.9%.[9]

Several natural ingredients are proposed as an alternative in pulp regenerative treatment. Epigallocatechin-3-gallate (EGCG) is an active polyphenolic catechin and is found in 59% of the total catechins in green tea. Other catechins in green tea include epigallocatechin (EGC) (19%), epicatechin-gallate (ECG) (13.6%), and epicatechin (EC) (6.4%). In another study, EGCG with a concentration of 0.0012 mg/ml combined with collagen scaffold had good cell viability, inhibit the growth *of S. mutans, F. nucleatum*, and *E. faecalis* bacteria, increasing proliferation and differentiation of human dental pulp cells (hDPCs). EGCG also helps improve the physical properties of the scaffold such as shortening the setting time, increasing the compressive strength and surface roughness.[13,14]

Many studies have been conducted with EGCG in combination with other ingredients. But, the addition of EGCG to $Ca(OH)_2$ has never been done before. This research will be carried out by combining $Ca(OH)_2$ and EGCG with concentrations of 0.0012 mg/ml.[13] Until now, the porosity of the combination of EGCG with $Ca(OH)_2$ is still unclear, so it needs to be investigated. Porosity examination was carried out using a Scanning Electron Microscopy (SEM) tool.[15]

MATERIALS AND METHODS

The Ethical Clearance which was prepared as a condition for conducting research has received approval from the Research Ethics Commission of the Faculty of Dental Medicine, Airlangga University (No. 603/HRECC.FODM/VIII/2022). This type of research is a laboratory experiment with a post-test only control group design. The data used is a primary data which is directly collected from research conducted by researchers.

The sample was divided into 2 groups, namely control and treatment consisting of 16 samples each with a total of 32 samples. The control group was a $Ca(OH)_2$ dissolved in aquadest with a w/p ratio of 0.1 g : 0.1 ml. The treatment group consisted of $Ca(OH)_2$ samples added with EGCG w/p ratio of 0.1 g : 0.1 ml. The EGCG solution was obtained by diluting 0.12 mg of EGCG powder with 100 ml of sterile aquadest to obtain a concentration of 0.0012 mg/ml or 10 µmol/L.[13]

Each group consists of 16 cylindrical samples with a diameter of 5 mm and a height of 2 mm (according to American Standard Testing and Materials (ASTM) E384). The sample mold is made of square acrylic and a cylindrical hole is placed in the center of the sample mold. The sample mold has a base and cover made of square acrylic. The sample is printed into the sample mold, then pressed so that the sample surface is flat. After setting, the samples were freeze dried at 48^{o}C for 2x24 hours.

The observing stage was carried out using SEM tools to see the morphology of the samples. Before testing, non-metallic samples must be coated first. Samples were observed with a magnification of 5,000 times and 50,000 times. Images generated from SEM were analyzed using Image-J software to see the porosity of the sample. Research data were analyzed using the SPSS (Statistical Package for Social Science) application. Data were tested for normality using the Shapiro-Wilk test and homogeneity was tested using the Levene test. Data that is normally distributed and homogeneous, then tested for differences in data variance using an independent T-test.

RESULTS

The results of the surface porosity test for $Ca(OH)_2$ – aquadest and $Ca(OH)_2$ - EGCG using SEM and analyzed with ImageJ software (Table 1) showed that the treatment group had a higher average result than the control group. The surface porosity values of the groups were tested for normality using the Shapiro-Wilk and showed that the data were normally distributed ($p>0.05$). Furthermore, the data was tested using the Independent T-test. Independent T-test (Table 1) showed that there was a significant difference between groups ($p<0.05$).

The SEM image show the particles in the control group sample with a magnification of 5,000x (Figure 1a) and 50,000x (Figure 2a), treatment group with a magnification of 5,000x (Figure 3a) and 50,000x (Figure 4a). All of the sample surfaces show a sharp and clear image quality with crystalline particle shapes. Porosity measurements were carried out at a magnification of 5,000x (Figure 2b and 4b). At the magnification by 5,000x (Figure 1b and 3b) it can be seen that there is agglomeration that appears due to the particles coalescing to form clumps. The agglomeration occurs in both groups but not as much as in the control group.

Table 1. The mean and standard deviation of surface porosity in all groups

Groups	N	Porosity (%) $\bar{x} \pm SD$	P
$Ca(OH)_2$+ Aq	16	23.38±2.5	0.001*
$Ca(OH)_2$+ EGCG	16	32.61±3.12	

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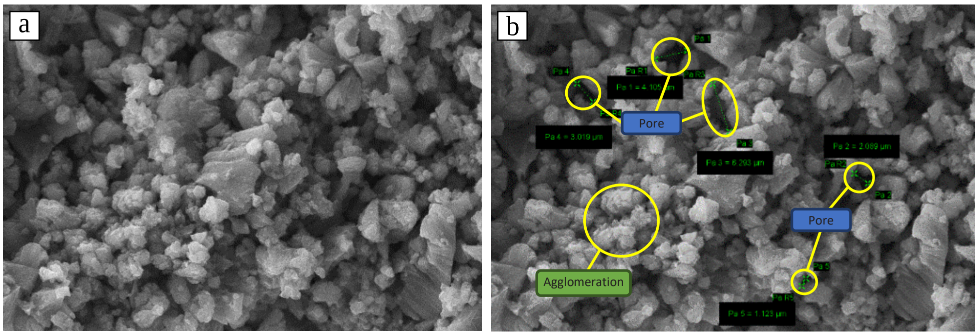

Figure 1. a) SEM characterization of a mixture of $Ca(OH)_2$ and aquadest at 5,000x magnification. b) Measurement of sample pores at 5,000x magnification.

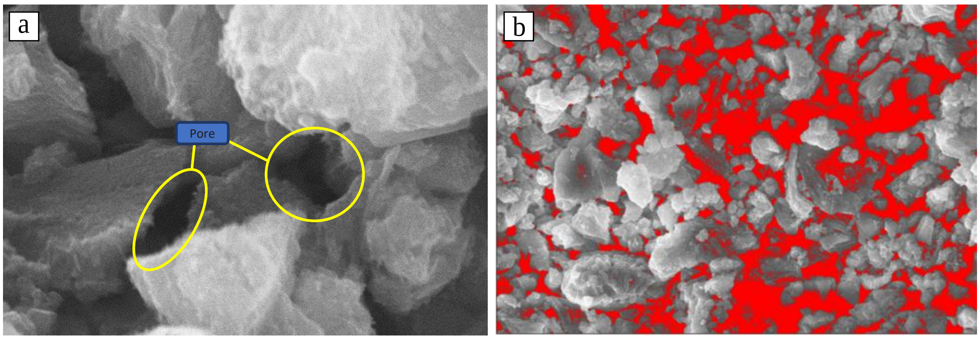

Figure 2. a) SEM characterization results of a mixture of $Ca(OH)_2$ and aquadest at 50,000x magnification. b) Porosity (%) measurement at 5,000x magnification.

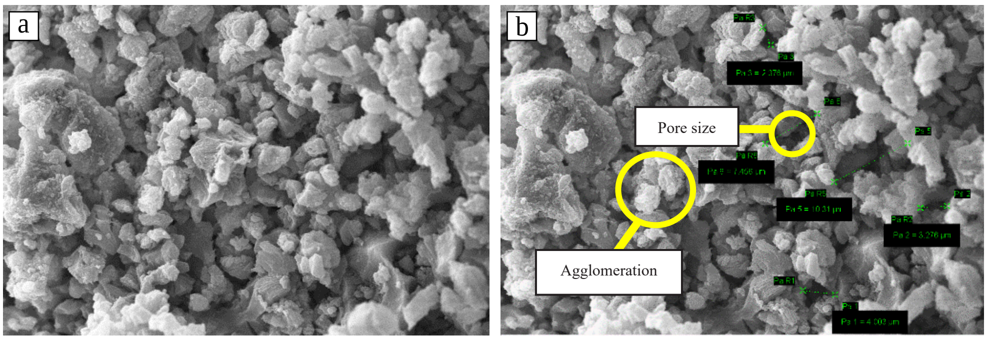

Figure 3. a) SEM characterization results of $Ca(OH)_2$ dissolved with EGCG at 5,000x magnification. b) Measurement of sample pores at 5,000x magnification.

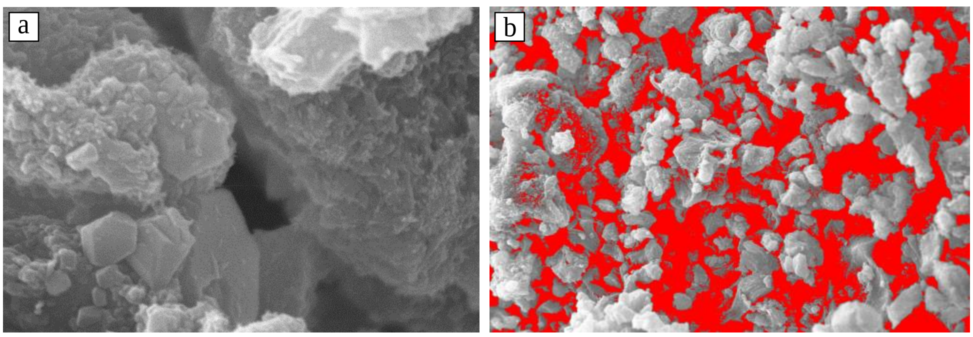

Figure 4. a) SEM characterization results of $Ca(OH)_2$ dissolved with EGCG at 50,000x magnification. b) Porosity measurement (%) at 50,000x magnification.

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DISCUSSION

Calcium hydroxide ($Ca(OH)_2$) is one of the standard materials for pulp capping, but this material has some disadvantages, which can cause pulp inflammation, pulp necrosis, and has properties that dissolve easily in dentinal tubule fluid and cause tunnel defects.[16,17] Porosity is one of the physical properties of a tissue regeneration treatment material. Porosity properties are necessary for bone cell regeneration, increasing cell infiltration, cell migration, vascularization, and the transfer of nutrients and oxygen.[18,19]

The addition of EGCG to $Ca(OH)_2$ aims to improve biological properties. EGCG as the largest polyphenol component that has anti-inflammatory and antioxidant capabilities can bind free radical hydroxyl ions (OH-) originating from $Ca(OH)_2$. In addition, EGCG has a role in the pulp healing process because of its ability to reduce the inflammatory response by binding to Nitric Oxide (NO) so that cell membrane damage can be prevented and an increase in the number of fibroblast cells occurs.[20,21]

There are several studies showing that the results of porosity testing on hydroxyapatite materials (75%), $Ca(OH)_2$ (7.49-9.04%), Mineral Trioxide Aggregate (MTA) (39.3 -49.47%), and bioceramic cement made from calcium silicate (22.9%). MTA that have a fairly high porosity are associated with a large enough amount of Ca release to encourage ion exchange. Ion release depends on the properties of the mineral particles and their ability to absorb water, solubility, and permeability present in the porosity. The higher the porosity of the material, the easier and more abundant the release of ions will occur.[9]

In this study, the pore size was included in the macroporous classification. This porosity is due to the coalescence of microscopic air bubbles during mixing. Adequate porosity and pore size (5-10 μm) can facilitate the delivery of nutrients to cells and diffusion through all structures of cells to achieve tissue reconstruction.[22] In this study there was no visible pore size which was included in the classification of microporous and mesoporous. This is caused by the use of SEM cannot identify very small structures. Microstructural observations using SEM show porous materials, but to see very small pore sizes with a clearer picture, it is recommended to use equipment that has higher magnification capabilities such as Field Emission Scanning Electron Microscopy (FE-SEM) with a magnification of up to 300,000x or Transmission Electron Microscopy (TEM) with a magnification of up to 600,000x.[23]

Acid-base reactions are chemical reactions that involve acidic compounds and basic compounds and produce products in the form of salts and water.[24] The combination of EGCG containing phenolic acid in the form of gallic acid with basic compounds will produce an acid-base reaction.[25] Meanwhile, calcium hydroxide is widely used because it is a strong base resulting from the breakdown and release of hydroxyl ions with a pH of 12.5-12.8.[11] The more water content, the porosity of the material will increase, directly proportional to the pore size. In the freeze-drying process, a drying process occurs which results in a reduction of water up to 95%. The result of this process will also form pores caused by sublimation by water that has been frozen in the previous process.[26,27]

Porosity and pore size are also very dependent on the viscosity of a mixture of materials. The reaction of the combination of $Ca(OH)_2$ with EGCG will decrease the viscosity because it contains more water. Viscosity is the frictional force created by a moving fluid. In liquids, viscosity occurs due to the attractive force between molecules of the same type and is influenced by the concentration of the solution, the weight and size of the dissolved molecules, the intermolecular forces, temperature, and pressure.[28,29]

There is an effect of adding EGCG to $Ca(OH)_2$ on the surface porosity of the material. In conclusion, the surface porosity percentage of the addition of EGCG to $Ca(OH)_2$ was higher compared to $Ca(OH)_2$ with aquadest.

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