

## Efficacy of Toothpaste Containing Nano Calcium in Dentin Remineralization

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### Abstract

Brushing teeth with toothpaste is the most effective method of removing plaque, preventing dental caries and repairing early caries. This research aims to conduct an efficacy test of toothpaste containing nano calcium as an active ingredient in repairing early caries over two weeks. A double-blind randomized parallel group clinical trial was conducted. Eighteen people were randomly assigned to use the test toothpaste, and eighteen others were assigned to use the control toothpaste. Assessments of early caries with DIAGNOdent Pen were performed before and after two weeks of toothpaste use. The results showed that the test toothpaste was more effective in repairing early caries over two weeks than the control toothpaste. Toothpaste containing nano-calcium has the potential to accelerate the healing of early dental caries.

### Abstrak

**Efikasi Pasta Gigi yang Mengandung Nano Kalsium dalam Memperbaiki Karies Dini.** Menyikat gigi dengan pasta gigi adalah cara mekanis yang paling efektif dalam menghilangkan plak, mencegah karies gigi dan memperbaiki karies dini. Penelitian ini bertujuan untuk menguji efektifitas pasta gigi yang mengandung nano kalsium sebagai komponen aktif untuk memperbaiki karies dini dalam dua minggu. Penelitian ini menggunakan desain *double-blind randomized parallel group clinical trial*. Delapan belas orang secara acak diberikan pasta gigi yang diuji, dan delapan belas lainnya diberikan pasta gigi kontrol. Pengukuran karies dini dilakukan dengan menggunakan *DIAGNOdent Pen* pada saat sebelum dan sesudah dua minggu pemakaian pasta giginya. Hasilnya menunjukkan bahwa pasta gigi uji secara signifikan efektif dalam memperbaiki karies dini dalam jangka waktu dua minggu, dibandingkan dengan pasta gigi kontrol. Pasta gigi yang mengandung nano-calcium memiliki potensi yang tinggi dalam mempercepat penyembuhan dari karies dini.

*Keywords: dentistry, DIAGNOdent pen, nano-calcium, preventive, promotive*

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### Introduction

The significant decline in caries in many countries in the world over the past 30 years has been attributed to the use of fluoride toothpaste. Numerous clinical studies have demonstrated that regular tooth brushing with well formulated fluoride toothpaste can reduce the incidence of dental caries.<sup>1,2</sup> Non-invasive techniques such as toothbrushing with effective toothpaste for treating early caries are low cost, feasible and sustainable.<sup>3-5</sup> Developing affordable toothpaste ingredients to increase toothpaste's effectiveness in repairing and maintaining dental health is therefore essential.<sup>6</sup> Although fluoride has received much attention, along with adding other minerals such as

calcium to toothpaste, to our knowledge, little research has been conducted on nano calcium.

Toothpaste containing nano-sized calcium has the potential to remineralize incipient enamel lesions due to its unique properties and the fact that it is retained on oral surfaces, thereafter releasing calcium ions into oral fluids.<sup>7,8</sup> Methods for detecting and evaluating early caries with high accuracy have been established and are being developed.<sup>9</sup> DIAGNOdent Pen is one of the most reliable and accurate methods in diagnosing dental caries in vivo.<sup>10-13</sup> The primary objective is to assess the efficacy of nano toothpaste in repairing early caries of dental enamel in vivo as assessed by DIAGNOdent Pen

over 2 weeks compared to that of a standard silica fluoride toothpaste.

## Methods

Forty volunteers aged 19-30 years were selected for the study. To be included in the study, subjects were required to be willing to participate, to sign the informed consent, comply with the study procedures, have no medical conditions that prevented them from brushing their teeth, practice good oral hygiene, and have early dentinal caries. The participants were required to comply with the clinical research protocol and to abstain from the use of oral hygiene products other than those provided for this study. The participants were in good general and oral health and presented a normal salivary flow rate.<sup>14,15</sup> The volunteers were clinically evaluated after professional dental prophylaxis to detect active caries lesions and periodontal disease. The exclusion criteria were current or recent use of any form of medication that affects salivary flow, use of fixed or removable orthodontic appliances, dental treatment, presence of active caries lesions or periodontal disease, being pregnant or breast-feeding, being a smoker, or having systemic illness.<sup>16</sup> A meeting was organized with the volunteers to present the research project, its objectives and the experimental design. The volunteers received oral and written information regarding the procedures to be performed during the experiment and were asked to refrain from using any antibacterial or fluoridated product. Moreover, the exclusion criteria included the occurrence of any adverse event, withdrawal, or sickness that could bias the results and not complying with the study procedures in a way that could bias the research results, such as using xilytol gum, casein phosphopeptide-amorphous calcium phosphate, or gargling solution.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics in Research Committee of Dental Faculty University of Indonesia (approval number 54) in 2014. The study aim, procedures, possible discomforts, risks, safety, and

benefits were fully explained to the subjects. Informed consent was obtained from all volunteers prior to the investigation.

This parallel randomized double blind clinical trial study included male subjects aged 18 years and over. Oral soft tissues were assessed at each examination for any adverse event, such as allergic reaction. The subjects were given a prophylaxis and a silica fluoride toothpaste to use for 1 week as the run-in phase. Baseline oral hygiene scores were taken. The respondent were randomly allocated to one of the two groups for 2 weeks, consistent with previous studies.<sup>15,17,18</sup> The subjects used the study products as part of their normal oral hygiene regimen. They were instructed to brush their teeth twice daily, after breakfast and before going to bed, with a pea size amount of toothpaste. New toothpaste was given to the subjects. The ingredients of the test toothpaste and the control toothpaste are described in Table 1.

Clinical assessment of inner half and outer half enamel caries on posterior occlusal tooth fissures were assessed before and after 2 weeks of use of the test toothpaste by DIAGNOdent Pen. Levels of early caries were measured at screening, baseline and after 1 and 2 weeks of product use. DIAGNOdent Pen from Kavo was used to measure the progression of the caries. DIAGNOdent Pen uses laser technology to detect and quantify hidden or sub-surface caries by measuring laser fluorescence within the tooth structure. The device operates at a wavelength of 655 nm. At this specific wavelength, clean, healthy tooth structure exhibits little or no fluorescence, resulting in very low-scale readings on the display. Altered tooth substances and bacteria, including caries, will fluoresce and the DIAGNOdent Pen will react with elevated scale readings on the display. The numbers on the DIAGNOdent Pen measurement are described in Table 2.

The early detection of caries lesions is important to provide proper and noninvasive management; lesions at this stage have the potential to be remineralized and can be monitored over time.<sup>19</sup>

**Table 1. Toothpaste Ingredients**

Toothpaste	Ingredients
Tested toothpaste	Calcium Carbonate, Nano sized Calcium Carbonate, Glycerin, Water, PEG-8, Sodium Lauryl Sulfate, Silica, Flavor, Sodium Monofluorophosphate 100 ppm, Carrageenan, Hydroxyethylcellulose, Sodium Saccharin, PEG-20 Hydrogenated Castor Oil, Caprylic/Capric Triglyceride, Methylparaben, Butylparaben, PEG 5M, Sodium Silicate
Control toothpaste	Calcium Carbonate, Erythritol, Glycerin, Water, PEG-8, Sodium Lauryl Sulfate, Silica, Flavor, Sodium Monofluorophosphate 1000 ppm, Carrageenan, Hydroxyethylcellulose, Sodium Saccharin, Dipotassiumglycyrrhizinate, PEG-20 Hydrogenated Castor Oil, Caprylic/ Capric Triglyceride, O-Cymen-5 Ol (IPMP), Methylparaben, Butylparaben, PEG 5M, Sodium Silicate

**Table 2. Category of Diagnosis of DIAGNOdent Pen Measurement**

Range	Category
0-10	Healthy Tooth Structure
11-20	Outer Half Enamel Caries
21-30	Inner Half Enamel Caries
30+	Dentin Caries

**Table 3. DIAGNOdent Pen Results (Mean±SD) Before and After Two Weeks of Toothpaste Usage**

	N teeth	Baseline	After 2 weeks	p-value
Control toothpaste	142	18 (5)	21 (7)	0.001
Tested toothpaste	157	19 (6)	14 (5)	0.001
p-value		0.208	0.001	

Conventional methods for caries detection are not capable of quantifying the mineral loss or gain occurring as a result of demineralization and remineralization processes, respectively.<sup>16</sup> In this context, quantitative methods have been developed for caries detection and for monitoring changes in mineral content.<sup>20</sup> Some of these methods are based on the fluorescence of bacterial porphyrins (fluorophores), molecules that are excited by a light source with a specific excitation wavelength.<sup>21</sup> The laser fluorescence DIAGNOdent Pen (LFpen; DIAGNOdent 2190, KaVo) is able to capture, analyze and quantify the fluorescence emitted from bacterial porphyrins and other chromophores.<sup>22</sup> Some studies have evaluated the performance of the Laser Fluorescence pen devices in detecting or monitoring caries development and in monitoring the remineralization process.<sup>16,23-27</sup>

A sample size of 27 subjects completing the study is sufficient to detect a statistically significant ( $p < 0.05$ ) difference between before and after intervention with a power of 80%, assuming a difference of 50%. Forty subjects meeting the inclusion/exclusion criteria were recruited to the study. The aim was to complete the study with at least 27 subjects. Subjects who withdrew from the study after visit 1 were not replaced.

Wilcoxon statistical tests were used to compare the proportion of caries before and after two weeks. Mann-Whitney statistical tests were conducted to compare the means between toothpastes at baseline. Moreover, values between toothpaste use after two weeks were statistically tested with independent T-test. SPSS 20.0 was used to analyse the data, with a p-value of 0.05 indicating significance.

## Results and Discussion

The measurements were initially taken for forty men. Over the course of the study, four subjects withdrew.

Data were analyzed from eighteen placebo group participants and eighteen test group participants. No side effects or adverse events occurred. Research results, presented in Table 3, show the mean DIAGNOdent Pen measurements results before and after two weeks of toothpaste usage. After two weeks of the intervention, the test toothpaste showed a significant increase in repairing teeth compared to the control toothpaste ( $p < 0.05$ ).

This study employed DIAGNOdent Pen to analyse in vivo mineralization changes.<sup>28</sup> The results showed that the tested toothpaste containing nano calcium had significantly higher efficacy in repairing early caries. It should be noted that nano calcium has an advantage over water-soluble calcium salts. As demonstrated in this study, nano calcium possesses a good retention property on the surface of the oral cavity due to its colloidal partial size, followed by calcium ion delivery. For calcium ions to exert a caries prevention effect, continuous calcium delivery with higher calcium concentration would be required. Water soluble calcium salts are thought to be readily washed away from the oral cavity. On the other hand, nano calcium absorbed to oral surfaces as solid particles would be capable of continuously supplying calcium ions to oral fluids due to the slow release of calcium ions. Moreover, nano calcium has the potential to increase the fluid pH surrounding the lesion enamel. Dentifrice containing nano calcium has premise for remineralization of incipient enamel lesions.<sup>10,29</sup>

The prevention of tooth decay and the treatment of lesions are ongoing challenges in dentistry, and nanotechnology has been viewed as one of the most revolutionary approaches in this field.<sup>30</sup> However, at the moment, the applied and marketable dental products have rarely been studied.<sup>31</sup> The advantage of the nano calcium toothpaste revealed in this study is that it has higher remineralizing effects than the control toothpaste. The disadvantage is that several individual factors could have potential impact on remineralization (e.g., behavioral changes, activity of the lesion, depth of the lesion, diet, and stimulation of salivary flow) and these factors may modify the natural process of lesion arrest (or repair).

In summary, this study was carried out to investigate the in vivo remineralization efficacy of a test dentifrice containing nano-sized calcium. It may be concluded that toothpaste with Nano Calcium is effective in repairing early caries over 2 weeks of use compared to the placebo. However, larger studies, including a direct comparison with calcium alone, should be carried out before clear public health implications can be drawn.

## Conclusions

Toothpaste containing nano-calcium has the potential to accelerate the healing of early dental caries. Therefore, the use of toothpaste containing nano-calcium would be

beneficial for the community as an early prevention and promotion of dental health.

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