



## **Occluding Effect of 8% Arginine Containing Toothpaste under Scanning Electron Microscopy: An In-Vitro Study.**

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**ABSTRACT**

**Background:** Dentine hypersensitivity is a common condition characterized by sharp pain arising from exposed dentin due to stimuli such as thermal, mechanical, or chemical factors. This condition is often a result of the exposure of dentinal tubules which are microtubules leading to the nerve endings within the dental pulp. Various strategies have been proposed to manage this sensitivity, including the utilization of dentifrices containing occlusive agents. One such promising agent is arginine, which has been shown to form a protective layer over the exposed dentin surfaces, effectively blocking the access of stimuli to the nerve endings.

**Aim:** The primary aim of this study was to evaluate the occlusal effect of an 8% arginine-containing toothpaste, specifically Colgate Pro Argin, on dentinal tubules using scanning electron microscopy (SEM).

**Materials and Methods:** A total of fifteen human premolars, extracted for orthodontic reasons, were collected for the experiment. These teeth were sectioned labiolingually, and dentine blocks measuring 2 mm x 1.5 mm x 1.5 mm were created from the cervical region. To prepare the samples, the dentine blocks were immersed in deionized water and subjected to ultrasonic sonication in distilled water for 30 seconds for cleaning and surface preparation. Each block was then mounted in acrylic resin. To simulate dental conditions, the blocks were etched using a 6% citric acid solution for two minutes, followed by rinsing and preservation in distilled water. For the intervention, all specimens were brushed twice daily with Colgate Pro Argin toothpaste for a duration of two minutes per session. After brushing, the blocks were cleaned with distilled water. This regimen was maintained for a continuous period of 40 days. Following treatment, the specimens underwent a drying process in a desiccator for two days, after which they were sputter-coated with a thin layer of gold to enhance conductivity for SEM analysis. The blocks were then assessed at baseline, as well as at 10, 20, 30, and 40 days, employing the tubule occlusion categorization scoring method as outlined by Pathan AB et al. (2016).

**Results:** Scoring of tubule occlusion revealed significant changes over the course of the study. At baseline (Day 0), the occlusion score was 5, indicating wide-open dentinal tubules. After 10 days of treatment, the score improved to 4, suggesting partial occlusion. By Day 20, the score decreased to 3, reflecting further blockage of tubules. On Day 30, the score was 2, indicating more substantial occlusion, and ultimately, by Day 40, the score reached 1, demonstrating significant occlusion of the tubules.

**Conclusion:** The findings from this study suggest that Colgate Pro Argin toothpaste is effective in providing relief for dentine hypersensitivity by occluding dentinal tubules as evidenced by SEM imaging. This occlusive property is likely attributed to the presence of 8% arginine, which enhances dentin sealing and reduces sensitivity by preventing stimuli from reaching the nerve endings. Therefore, the use of this dentifrice may be recommended for patients suffering from dentinal hypersensitivity as a part of their oral hygiene regimen.

**Keywords:** Dentinal tubule, 8% arginine toothpaste, Scanning electron microscope.

## Introduction

Short, sharp pain that comes from exposed dentin in reaction to stimuli that are usually thermal, evaporative, tactile, osmotic, or chemical is known as dentin hypersensitivity, and it cannot be linked to any other type of dental pathology or defect.<sup>1</sup> Dentin exposure to the oral environment is the hallmark of this excruciating clinical illness, which affects 8% to 57% of adults globally. It usually affects people between the ages of 20 and 49, with women being affected at a lower mean age.<sup>2</sup> Gysi's 1900 hydrodynamic theory and Brännstorm's 1966 scientific explanation provide an appropriate explanation of the process of the external stimulus that causes dentin hypersensitivity pain. Occluding open dentinal tubules and interruption of nerve transmission are the two primary methods of treating dentin hypersensitivity. The holy grail for treating dentin hypersensitivity has not yet been identified, despite the many clinical trials and investigations conducted by physicians around the world. The general public regularly uses over-the-counter desensitizing drugs that contain active chemicals such as potassium nitrate, oxalates, strontium-based compounds, citrate-based compounds, stannous fluoride, etc. in the form of mouth rinses and dentifrices.<sup>1</sup>

The need for frequent repetition to provide consistent and long-lasting pain alleviation is one of the main drawbacks of conventional therapy approaches. Usually, abrasion from a toothbrush, acid problems in the mouth, and/or coating material deterioration cause hypersensitivity to resurface. Recent additions of chemical

components in toothpaste include calcium sodium silicon phosphate and arginine, which work by mechanically blocking dentinal tubules.<sup>3</sup>

8% of formula for Colgate Pro Argin Toothpaste is arginine. Arginine balances the pH of your mouth, helps restore enamel twice, combats sensitivity in 60 seconds, and protects beneficial microorganisms from harmful ones. To strengthen enamel and give it long-lasting protection, arginine actively remineralizes it.<sup>4</sup> Thus, this invitro investigation was conducted to assess the blocking effects of dentinal tubules by 8% arginine desensitizing dentifrice utilizing SEM.

## Materials and Methods

After being extracted for orthodontic or periodontal reasons, fifteen mandibular premolars were collected and kept in saline. The study did not include teeth that were carious, repaired, hypoplastic, or shattered.

Following labiolingual sectioning and ultrasonic scaling, all samples were submerged in deionized water till preparation of dentine blocks. Cervical region was used for preparation of blocks measuring 2x1.5x1.5mm by wet ground to remove enamel from all surface and reveal a flat underlying dentine surface. One block was made from each sample and ultrasonically sonicated for 30seconds in distilled water to eliminate any particle matter formed during grounding, and mounted in cold cure acrylic resin to provide stability during brushing. 6% citric acid for 2minutes was utilized for etching and rinsing with distilled water to eliminate smear layer. Throughout the experiment, the prepared specimens were kept in distilled water. Twice a day, for two minutes, the blocks were brushed with a power brush utilizing 8% arginine toothpaste.

The specimens underwent a two-day desiccator drying process, a distilled water wash, a thin layer of gold sputter coating, and SEM analysis before being assessed using blocking of tubule categorization scoring system by Pathan AB et. al. (2016).<sup>5</sup>

1. Occluded (100% of tubules occluded);
2. Mostly occluded (50% to <100% of tubules occluded);
3. Partially occluded (25% to <50% of tubules occluded);
4. Mostly unoccluded (<25% of tubules occluded);
5. Unoccluded (0%, no tubule occlusion).

Colgate Pro Argin Toothpaste was used twice a day for 40 days, brushing each specimen for two minutes. SEM imaging was used to evaluate tooth sections at baseline, 10 days, 20 days, 30 days, and 40 days for dentinal tubule occlusion caused by Colgate Pro Argin toothpaste.

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## Results

Over the course of a 40-day observation period, the progression of blockage within dentinal tubules was meticulously assessed and recorded.

**Day 0:** Initial examinations revealed a score of 5, indicating a high level of patency in the dentinal tubules. The structural integrity of the tubules was optimal, with little to no obstruction present.

**Day 10:** A noticeable decline in the score was observed, with the score dropping to 4. This reduction suggests an early stage of obstruction, where some blockage began to manifest within the tubules, likely due to accumulating debris or changes in the surrounding environment.

**Day 20:** By this point, the score further decreased to 3. The blockage appeared to be more prominent, with approximately 40% obstruction observed. This indicates a progressive deterioration in the condition of the dentinal tubules, enhancing the likelihood of impaired functionality.

**Day 30:** Continuing this trend, the score fell to 2, demonstrating a significant increase in blockage. Most dentinal tubules exhibited signs of obstruction, approaching an estimated 60% to 80% blockage. The structural ramifications of this degree of blockage could potentially impact the overall health of the dental structure.

**Day 40:** Finally, the assessment recorded a score of 1, indicating severe blockage within the dentinal tubules. At this stage, it can be inferred that approximately 80% or more of the tubules were obstructed, highlighting a critical state of impairment.

The observation period suggests that the process of blockage in dentinal tubules takes between 20 to 30 days to escalate to a severity of 80% blockage. This timeline emphasizes the need for ongoing monitoring and potential intervention strategies to mitigate the progressive obstruction in dentinal tubules and preserve dental health.

## Discussion

The current in-vitro study was designed to evaluate the effectiveness of Colgate Pro Argin toothpaste in occluding dentinal tubules, using scanning electron microscopy (SEM) as the primary analytical method to assess alterations in dentinal structure over a specified duration of 40 days.

The research focused on the role of an arginine-based formulation, as arginine is known for its potential desensitizing properties. Throughout the 40-day observational period, the study aimed to provide a systematic analysis of how effectively the toothpaste could occlude dentinal tubules, which are microscopic channels within the dentin that can contribute to dentine hypersensitivity when exposed and unprotected.

Findings from the study indicated a progressive occlusion of dentinal tubules over the designated time frame. Initially, the tubules exhibited a notable degree of patency, but as the study advanced, the application of

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Colgate Pro Argin resulted in increasing levels of occlusion. This effect was documented through detailed SEM imaging, which provided high-resolution visual evidence of the structural changes occurring within the dentinal tubules.

As the days progressed, the results underscored the potential efficacy of arginine-based desensitizing agents, demonstrating their capacity to effectively reduce the diameter of the tubules and potentially block pathways associated with pain transmission in the dental pulp. The progressive occlusion observed suggests that regular use of the Colgate Pro Argin toothpaste could be beneficial in the management of dentine hypersensitivity, providing a mechanism for relief by reducing the exposure of sensitive dentinal tissues to external stimuli.

Overall, this study contributes valuable evidence to the understanding of how arginine-based formulations can be effectively utilized in dental care, particularly for individuals suffering from dentine hypersensitivity. The findings advocate for the integration of such desensitizing agents into dental hygiene regimens as a proactive approach to managing oral sensitivity issues.

### **Mechanism of Action**

Arginine, an amino acid, has been shown to play a critical role in managing dentinal hypersensitivity. The occlusion of dentinal tubules is believed to occur as arginine interacts with calcium phosphate in saliva, forming a protective layer that physically blocks the tubules. This biochemical interaction possibly reinforces the structure of the dentin and provides a barrier against external stimuli, such as temperature changes and osmotic pressure, which can lead to discomfort in hypersensitive teeth.<sup>6</sup>

### **SEM Analysis Findings**

The SEM analysis employed in this study allowed for a detailed evaluation of the occlusion process over specified intervals.<sup>7</sup> At the baseline, all dentinal tubules appeared open, confirming the initial state of the samples prior to treatment. It is notable that after just 10 days of twice-daily brushing with the toothpaste, substantial occlusion was observed, correlating to a score of 4 on the classification system. This implies that the arginine was effective early in the treatment process, suggesting that significant changes in tubule morphology can occur relatively quickly with consistent use of the product.

By day 20, the score decreased to 3, indicating a further reduction in open tubules, while scores of 2 and 1 observed at days 30 and 40 respectively indicate that the occlusion progressively improved, ultimately achieving complete occlusion by the final assessment. The finding that approximately 80% of occlusion was achieved between days 20 and 30 highlights the importance of adherence to the treatment regimen, emphasizing the need for patients to consistently use the toothpaste for optimal results.

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## Clinical Implications

Dentine hypersensitivity is a common condition affecting a significant portion of the population, often caused by exposed dentin due to factors such as gum recession, enamel wear, and abrasive brushing. The successful occlusion of dentinal tubules implies that 8% arginine toothpaste could be an effective tool for alleviating hypersensitivity symptoms in patients, serving as a non-invasive treatment alternative to more aggressive procedures such as restorative dentistry or surgical interventions.<sup>1</sup>

Moreover, the rapid onset of action observed in this study could enhance patient compliance, as individuals would likely appreciate the immediate effects of using an effective therapeutic product. This could lead to improved patient satisfaction and a reduction in the need for additional dental treatments resulting from untreated sensitivity.

## Limitations of the Study

Several factors may limit the generalizability of the findings from this in-vitro study. First and foremost, the small sample size may affect the statistical power of the results and their applicability to broader populations. In addition, the variability in salivary content among individuals can impact the toothpaste's effectiveness, as saliva plays a crucial role in oral health and can influence the clearance and distribution of the active ingredients within the mouth. Moreover, individual oral hygiene practices, which can vary significantly from person to person, might further affect how effectively the toothpaste works in a real-world context. Other individual patient variables, such as age, dietary habits, and the presence of other dental conditions, could also significantly influence the outcomes. These limitations suggest that further research is necessary to fully understand the clinical applicability of these findings.

## Recommendations for Future Research

To enhance the robustness of future studies, it would be beneficial to utilize larger sample sizes that can provide more reliable data and increase the statistical significance of the results. Additionally, future research could explore various concentrations of arginine beyond the 8% used in this study, as well as different formulations that may combine arginine with other desensitizing agents. Such combinations could potentially yield synergistic effects, enhancing the overall efficacy of the treatment. It would also be advantageous to include a more diverse population in future research to determine how different demographic factors might influence the effectiveness of the toothpaste.

Furthermore, investigating alternative application methods, such as varying brushing techniques, the duration of brushing, or the frequency of application, could offer valuable insights into how best to optimize treatment protocols for patients experiencing dentine hypersensitivity. Taking these factors into consideration may lead

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to more tailored and effective management strategies for individuals suffering from this condition.

## Conclusion

The study's findings demonstrate that the occlusive efficiency of the 8% arginine-containing toothpaste plays a significant role in managing dentine hypersensitivity, as evidenced by observable changes in the morphology of dentinal tubules when visualized using scanning electron microscopy (SEM). The progressive occlusion of the tubules over time, along with the rapid onset of its effectiveness, highlights the therapeutic potential of this desensitizing agent. These results suggest that arginine-based formulations could be a promising option for alleviating the discomfort associated with dentine hypersensitivity, providing a valuable addition to dental care paradigms aimed at enhancing patient comfort and oral health.

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