

# Effectiveness of Pro-Arginine containing eggshell toothpaste in occluding dentinal tubules: a comparative study

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## Abstract

**Background:** Dentin hypersensitivity, a prevalent condition characterized by acute pain from exposed dentin, can significantly impact quality of life. Treatments often involve occluding dentinal tubules or modulating pain responses. Recent interest in natural products has led to investigating the efficacy of eggshell powder combined with arginine for managing this condition.

**Aim:** To evaluate and compare the effectiveness of three different toothpaste formulations—Pro-Arginine Containing Eggshell Toothpaste (PA-ESTP), Eggshell Toothpaste (ESTP), and Pro-Argin™-Formulated Toothpaste—in occluding dentinal tubules and alleviating dentin hypersensitivity.

**Materials and methods:** Thirty human maxillary premolar teeth were prepared into dentin discs, etched, and treated with one of the three toothpaste formulations. A standardized brushing protocol was simulated using a powered toothbrush. Dentin tubule occlusion was analysed using Scanning Electron Microscopy (SEM) to assess the number of open, partially closed, and completely closed tubules. ANOVA was used to examine differences in tubule occlusion among the three groups.

**Results:** PA-ESTP demonstrated superior performance with  $85.51 \pm 1.33$  completely closed tubules and significantly fewer open tubules than ESTP and Pro-Argin™-Formulated Toothpaste. Statistical analysis confirmed these differences were significant ( $p < 0.001$ ).

**Conclusion:** Pro-Arginine Containing Eggshell Toothpaste (PA-ESTP) is more effective in managing dentin hypersensitivity than the other tested formulations. The combination of arginine and eggshell powder enhances tubule occlusion, offering a promising treatment option. Further, clinical trials are needed to confirm these findings and evaluate long-term efficacy.

**Keywords:** Dentin hypersensitivity, dentinal tubule occlusion, Pro-Arginine containing Eggshell Toothpaste, Eggshell Toothpaste, Pro-Argin™-Formulated Toothpaste, Scanning Electron Microscopy.

## 1. Introduction

Dentin hypersensitivity is a condition characterized by a sharp, transient response resulting from exposed dentin. This hypersensitivity can be triggered by various stimuli including evaporative, chemical, thermal, tactile, or osmotic, and its intensity is influenced by the duration of the stimulus [1]. The prevalence of dentin hypersensitivity ranges between 8% and 57% among adults [2], with even higher rates, from 72.5% to 98%, observed in patients with periodontal disease [3]. Factors such as gingival recession, cervical lesions, periodontal disease, and enamel erosion can exacerbate the severity of hypersensitivity [4]. Despite the availability of three proposed mechanisms for dentin hypersensitivity, no single theory has yet comprehensively explained the condition [1].

Dentin hypersensitivity arises when dentin is exposed, making the dentinal tubules accessible from the pulp to the oral cavity. The severity of hypersensitivity intensifies as the number and diameter of open tubules increase [5]. Current management strategies for dentin hypersensitivity focus on diminishing the neural response to pain stimuli or occluding the exposed tubules. These desensitizing approaches include both home-based and professional treatments, such as the application of desensitizing toothpaste.

Dentifrices are commonly used for treating dentin hypersensitivity due to their affordability, convenience, and suitability for home use. They contain various active

ingredients, including strontium chloride, potassium nitrate, dibasic sodium citrate, formaldehyde, sodium fluoride, sodium monofluorophosphate, and stannous fluoride [6-9]. Fluorides, in particular, help form a protective barrier by precipitating calcium fluoride crystals, which typically form at the openings of the dentinal tubules. The calcium fluoride precipitate created by fluorides is slowly soluble in saliva, which may account for the temporary nature of this barrier [10]. Additionally, abrasive components in dentifrices, such as calcium phosphate, calcium carbonate, silicate, or aluminium, can help close dentinal tubules through precipitation or smear layer formation during brushing [7,8]. The variety of dentin hypersensitivity products available highlights that an ideal treatment has yet to be developed. The selection of a treatment method and product is often guided by the clinician's experience, preferences, and expertise.

Arginine, an essential amino acid with an alkaline pH, has demonstrated dentin-desensitizing effects when combined with calcium carbonate, which provides a rich source of calcium ions. This interaction has been validated through both *in vitro* and clinical studies [11-18]. At physiological pH, where arginine and calcium ions are positively charged due to the bicarbonate buffer, they adhere to the negatively charged surface of dentin. This process results in a calcium-rich layer formation that aids in desensitizing dentin [11].

Recently, the use of natural products as affordable treatment options has gained significant interest. Eggshell powder (ESP) has emerged as a notable choice, particularly for its vitamin and mineral enrichment, which is beneficial for postmenopausal women in osteoporosis prevention and treatment [19,20]. Rich in calcium, phosphorus, strontium, zinc, fluoride, and copper, ESP supports remineralization [21,22]. It has been shown to enhance bone density [23] and reduce resorption and pain. Additionally, ESP has demonstrated an antirachitic effect in various animal and human studies. In dentistry, ESP is being explored for its potential as a pulp-capping material [24] and in managing enamel erosion [25]. So, this study aimed to evaluate and compare the effectiveness of three different toothpaste formulations—Pro-Arginine Containing Eggshell Toothpaste (PA-ESTP), Eggshell Toothpaste (ESTP), and Pro-Arginine-Formulated Toothpaste—in occluding dentinal tubules and alleviating dentin hypersensitivity.

## 2. Materials and methods

### 2.1 Sample size

Ethical clearance was obtained from the institutional ethical committee (GSLDC/IEC/2024/023). In the current study, 30 human maxillary premolar teeth extracted for orthodontic purposes other than dental caries and with no history of oral prophylaxis in the past 6 months were included in the study. Teeth with dental caries and with restorations were excluded.

### 2.2 Preparation of Eggshell Toothpaste [26]

To prepare the eggshell toothpaste, twenty eggshells were collected and dried under sunlight until all moisture was removed. The dried eggshells were then ground into a fine, smooth powder. This powder was combined with Clove Oil

(Dabur India Ltd), Coconut Oil (Parachute, Marico Ltd), Peppermint Oil (PT invent India Pvt Ltd), and Baking Soda (Desire Foods India). The mixture was blended thoroughly in a bowl to form a paste. The resulting toothpaste was stored in an airtight jar to preserve its freshness and effectiveness.

### 2.3 Preparation of dentin discs

Mid-coronal dentin discs (Figure 1), approximately 1 mm thick, were created using a diamond disc and mounted in self-cure acrylic resin. The discs were then etched with 37% phosphoric acid (Dentsply India Pvt. Ltd) for 60 seconds [26], followed by washing with distilled water and air drying for a few minutes. After etching, the dentin discs were randomly assigned to one of three groups (Figure 2).

**Group 1:** Egg Shell prepared Toothpaste (ESTP).

**Group 2:** Pro-Arginine™-formulated toothpaste (Colgate Sensitive ProRrelief™, India).

**Group 3:** Pro Arginine containing Egg Shell toothpaste (PA-ESTP) (Shanghai Wentai Bio Technology Co., Ltd, China).



Figure 1. Dentin Discs Obtained from Sectioning Maxillary Premolar Teeth



Figure 2. Syringes containing toothpaste formulations used in the study. Group 1, Eggshell toothpaste, Group 2, Pro-Arginine toothpaste, and Group 3. Eggshell-based Pro-Arginine toothpaste.

### 2.4 Tooth Brushing Simulation

Slurry of each toothpaste was prepared at a ratio of 1:3 with distilled water. To mimic three weeks of regular use, brushing was carried out using a powered toothbrush in a circular motion twice daily for three minutes per session. For the calculation, assuming brushing three times per day for 28 teeth, the brushing time per tooth was determined using the formula [26];

$$\text{Brushing time (Minutes)} = \frac{(\text{Number of brushings per day} \times \text{Time of brushing (Minutes)}) \times \text{Number of teeth in a mouth} \times \text{Number of days}}$$

The brushing time was then converted to cycles, with each cycle lasting one second (60 cycles/minute), using the formula [26];

**Number of Brushing cycles = Brushing time (Minutes) X Number of cycles per minute**

This approach ensured a consistent and quantifiable brushing regimen throughout the experiment. At each time point, dentin discs were removed, washed with distilled water, and allowed to air dry.

### 2.5 Scanning Electron Microscopic (SEM) evaluation

Dentin discs were subjected to a drying process involving serial dilution with alcohol, followed by critical drying for 30 minutes. The discs were then mounted on metal stubs, coated with carbon and analysed using scanning electron microscopy (SEM, JEOL JSM 6480LV, United States). For each group, the mean  $\pm$  standard deviation (SD) of the diameter and the number of open, partially closed, and completely closed dentinal tubules within an area of  $125 \times 90 \mu\text{m}^2$  were calculated after two weeks.

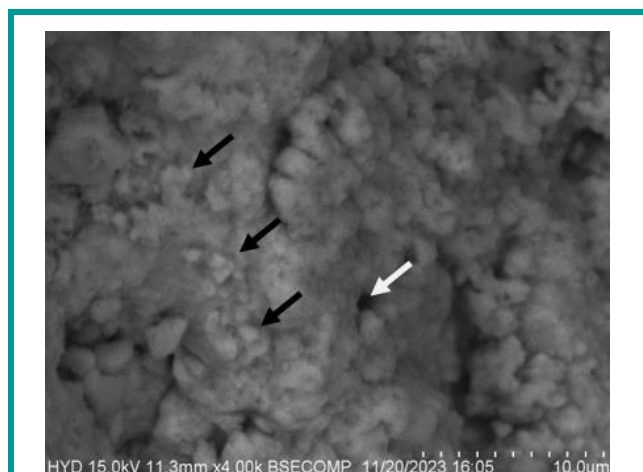
### 2.5 Statistical analysis

Statistical analysis was performed using IBM SPSS version 26.0, IBM Corporation, USA. For the analysis of the number of open, partially closed, and closed dentinal tubules, a Two-Way ANOVA (Analysis of Variance) test was performed. Continuous variables were expressed as means  $\pm$  SD and  $p$  less than 0.001 was considered statistically significant.

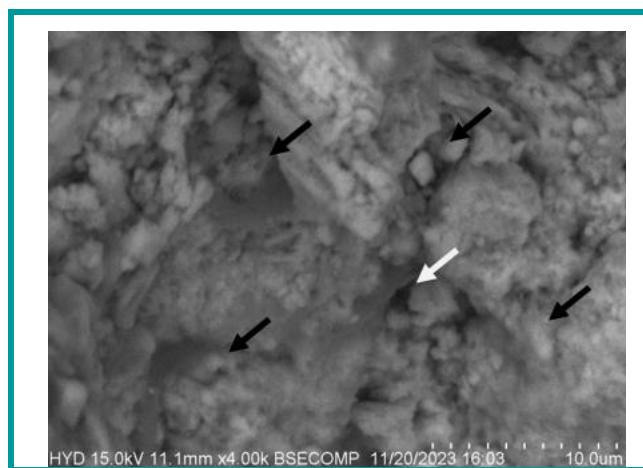
## 3. Results

In the SEM analysis, the dentinal surface treated with Egg Shell prepared Toothpaste (ESTP) demonstrated with more occluded surface, with a denser and more compact structure, suggesting that the dentinal tubules are mostly covered (Figure 3). However, some areas seem rough or uneven, indicating partial occlusion, with a few open tubules visible. The dentinal surface treated with Pro-Argin™-formulated toothpaste (Colgate Sensitive ProRrelief™) showed partial occlusion of dentinal tubules, with a mix of both covered and exposed tubules (Figure 4). The dentinal surface treated with Pro Argine containing Egg Shell tooth paste (PA-ESTP) exhibited with extensive occlusion of dentinal tubules (Figure 5). The compact and dense surface structure indicates effective coverage, minimizing the potential for dentinal sensitivity.

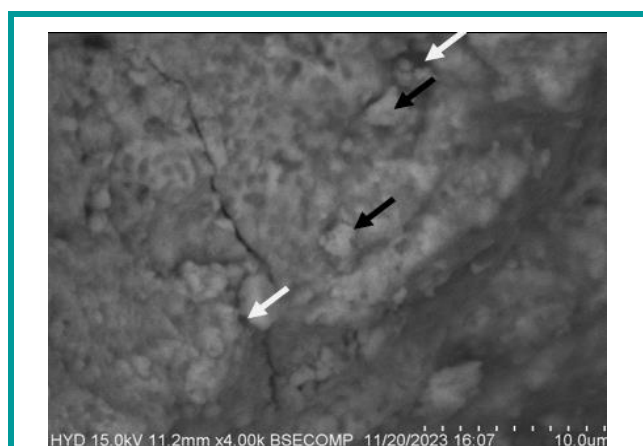
Table 1 presents a comparative analysis of dentinal tubule occlusion among three different toothpaste treatments over two weeks. The data reveal that Group 3 (Pro-Arginine Containing Eggshell Toothpaste - PA-ESTP) was significantly more effective at closing dentinal tubules, with a mean of  $85.51 \pm 1.33$  completely closed tubules, compared to Group 1 (Eggshell Toothpaste - ESTP) and Group 2 (Pro-Argin™-Formulated Toothpaste). In Group 3, the number of open tubules was markedly lower ( $16.61 \pm 2.04$ ) and the number of closed tubules was higher than in the other groups. Group 1 and Group 2 showed similar results in terms of the number of open and partially closed tubules, but Group 1 had slightly higher counts for these categories compared to Group 2. The statistical significance of these findings, with a  $p$ -value of less than 0.001, underscores the superior efficacy of PA-ESTP in managing dentin hypersensitivity through more effective dentinal tubule occlusion.



**Figure 3.** Dentinal surface treated with Egg Shell prepared Toothpaste (Black arrows- closed dentinal tubules; white arrows- open dentinal tubules).



**Figure 4.** The dentinal surface treated with Pro-Argin™-formulated toothpaste (Black arrows- closed dentinal tubules; white arrows- open dentinal tubules).



**Figure 5.** The dentinal surface treated with Pro Argine containing Egg Shell tooth paste (Black arrows- closed dentinal tubules; white arrows- open dentinal tubules).

Table 2 presents the comparison of dentinal tubule occlusion across the three different toothpaste treatments using two-way ANOVA results. The data indicates that there were statistically significant differences ( $p$ -value

<0.001) between the groups for all three categories of tubule occlusion: open, partial, and closed tubules. The sum of squares and mean square values highlight substantial variation in the extent of dentinal tubule occlusion among the groups. Specifically, the F-values for open, partial, and closed tubules are 455.636, 463.091, and 1335.025, respectively, with p-values all less than 0.001. This significance suggests that the differences in dentinal tubule occlusion observed among the toothpaste treatments were not due to chance, but rather reflect the varying efficacy of each treatment in managing dentin hypersensitivity. Two-way ANOVA results underscore the effectiveness of the Pro-arginine-containing Eggshell Toothpaste (PA-ESTP) in achieving superior dentinal tubule occlusion compared to the other treatments.

**Table 1. Intra group comparison of dentinal tubules occlusion**

GROU PS	Open tubules (Mean±SD#)	Partial tubules (Mean ± SD#)	Closed tubules (Mean ± SD#)	p-value
GROUP 1	43.00±2.70	89.47±2.02	43.60±2.36	<0.001
GROUP 2	41.50±1.71	87.80±2.45	42.71±2.46	
GROUP 3	16.61±2.04	64.24±1.61	85.51±1.33	

#Standard deviation.

**Table 2. Intra group comparison of dentinal tubule occlusion (ANOVA)**

		Sum of Squares	df*	Mean Square	F-value	p-Value
Open	Between Groups	4393.981	2	2196.990	455.636	0.000
	Within Groups	130.189	27	4.822		
Partial	Between Groups	3981.385	2	1990.692	463.091	0.000
	Within Groups	116.065	27	4.299		
Closed	Between Groups	11963.601	2	5981.800	1335.025	0.000
	Within Groups	120.978	27	4.481		

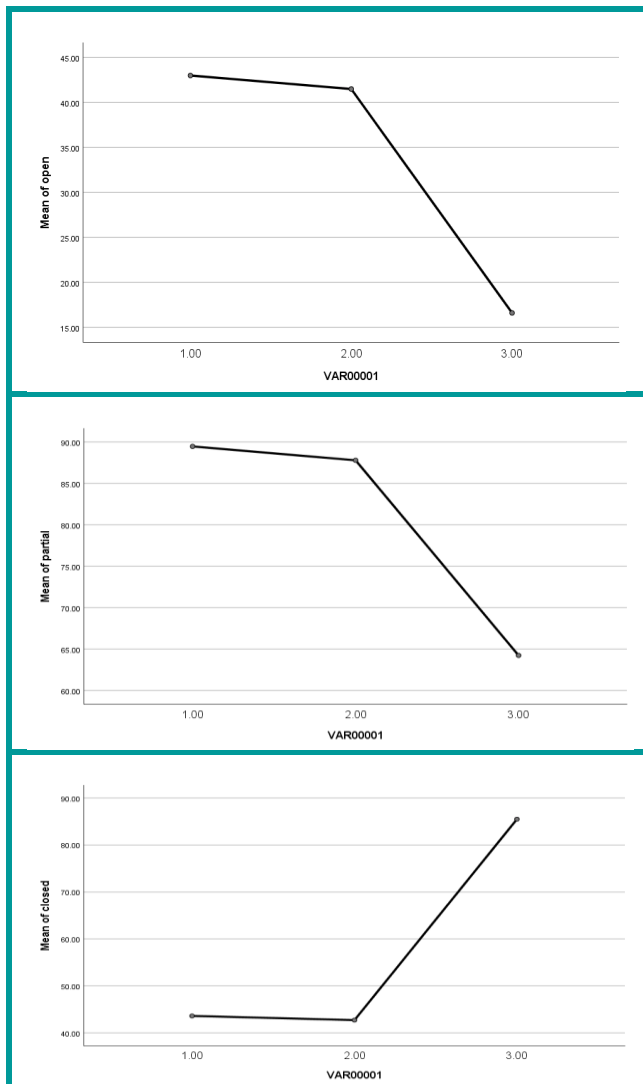
\*df- Degree of freedom.

Figures 6-8 collectively illustrate the efficacy of different toothpaste treatments in managing dentinal tubule occlusion. Figure 6 shows that Pro-Arginine Containing Eggshell Toothpaste (PA-ESTP) results in the fewest open tubules compared to Eggshell Toothpaste (ESTP) and Pro-Argin™-Formulated Toothpaste, highlighting its superior performance in reducing tubule exposure. Figure 7 indicates that PA-ESTP also leads to the lowest average number of partially closed tubules, suggesting enhanced progression towards full occlusion. Finally, Figure 8 demonstrates that PA-ESTP achieves the highest average number of completely closed tubules, confirming its effectiveness in fully occluding dentinal tubules. Together, figures 6 – 8 support the statistical findings from the two-way ANOVA analysis, underscoring PA-ESTP's effectiveness over the other treatments in alleviating dentin hypersensitivity.

#### 4. Discussion

Dentin hypersensitivity, characterized by sharp pain due to exposed dentin, is commonly treated through strategies that either occlude dentinal tubules or alter the neural pain

response. This study evaluated three toothpaste formulations: Pro-Arginine Containing Eggshell Toothpaste (PA-ESTP), Eggshell Toothpaste (ESTP), and Pro-Argin™-Formulated Toothpaste, to determine their efficacy in alleviating dentin hypersensitivity.



**Figures 6-8 (Top to down). The line graph represents the mean percentage of dentin tubule occlusion across three different treatment groups. The graphs show mean values for "open," "partial," and "closed" conditions of dentinal tubules across three groups.**

PA-ESTP emerged as significantly more effective than ESTP and Pro-Argin™-Formulated Toothpaste in mitigating dentin hypersensitivity. As detailed in Table 1, PA-ESTP achieved a mean of 85.51 ± 1.33 completely closed tubules, which is notably higher than the values recorded for ESTP and Pro-Argin™-Formulated Toothpaste. Furthermore, PA-ESTP had the lowest number of open tubules at 16.61 ± 2.04, underscoring its superior performance in tubule occlusion. The statistical analysis of this study (Table 2) supports these findings, with p-values well below 0.001 confirming the significance of the observed differences.

The enhanced effectiveness of PA-ESTP is attributable to its dual-component formulation of arginine and eggshell powder. Arginine has been extensively studied for its role in managing dentin hypersensitivity. Previous research by Sharif MO *et al.* (2013) [27] and Mohammadipour HS *et al.*

(2024) [28] demonstrated that arginine facilitates the formation of a calcium-rich layer over dentinal tubules, leading to effective occlusion and reduced sensitivity. The addition of eggshell powder, rich in calcium and other minerals, likely amplifies this effect by further promoting tubule occlusion and remineralization. This is supported by Kunam D *et al.* (2020) [29] and Sanosh KP *et al.* (2022) [30], who highlighted that calcium-rich agents contribute significantly to reducing hypersensitivity and enhancing tubule occlusion.

ESTP, though beneficial due to its eggshell content, lacks arginine, which could explain its lower efficacy relative to PA-ESTP. This observation aligns with Onwubu SC *et al.* (2019) [31], which found that products combining arginine with mineral agents are more effective than those containing only one active component. Pro-Argin™-Formulated Toothpaste, while containing arginine, does not include additional minerals, which may limit its effectiveness in tubule occlusion. Arantes DC *et al.* (2019) [14] similarly noted that arginine alone might not achieve the same level of tubule occlusion as formulations with combined active ingredients.

The superior performance of PA-ESTP suggests it could be a valuable option for treating dentin hypersensitivity. The combination of arginine and eggshell powder provides a dual-action approach—desensitizing and enhancing mineralization—which could lead to improved clinical outcomes and patient satisfaction.

While the results are promising, they are based on an *in vitro* study. Validation through clinical trials is necessary to assess real-world applicability and effectiveness. Future research should focus on clinical studies to confirm the efficacy of PA-ESTP, evaluate long-term benefits, and explore patient-reported outcomes and satisfaction to fully understand its comparative effectiveness.

This comprehensive approach highlights the potential of combining natural and cost-effective ingredients in dental care, aiming for enhanced management of dentin hypersensitivity.

## 5. Conclusion

Pro-Arginine Containing Eggshell Toothpaste (PA-ESTP) demonstrated superior efficacy in managing dentin hypersensitivity, as evidenced by its effectiveness in occluding dentinal tubules. The combination of arginine and eggshell powder offered a promising approach for treating dentin hypersensitivity.

**Conflicts of interest:** Authors declared no conflicts of interest.

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