

In Vitro Clinical Evaluation of Effectiveness of Combination of sodium fluoride and Calcium Phosphate based desensitizer

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ABSTRACT

Background: The objective of this study was to evaluate the in vitro effectiveness of a combination of sodium fluoride and calcium phosphate-based desensitizer in occluding dentinal tubules, reducing dentin permeability, increasing microhardness, and promoting remineralization compared to single-agent treatments.

Materials and Methods: (a) Sample Preparation: Forty freshly extracted human premolars and molars were sectioned to obtain dentin discs. The discs were polished and demineralized using 6% citric acid. (b) Experimental Groups: The dentin discs were divided into four groups (n=10 per group): no treatment, 5% sodium fluoride varnish, 5% calcium phosphate paste, and a combination of both. (c) Application Procedure: Treatments were applied to the dentin discs, with sodium fluoride varnish allowed to dry for 2 minutes and calcium phosphate paste applied for 1 minute before rinsing. (d) Evaluation Methods: SEM and EDS were used to assess tubule occlusion and elemental composition. Dentin permeability was measured using a fluid filtration apparatus, microhardness was tested with a Vickers hardness tester, and TMR was used to analyze mineral density. Simulated oral conditions were tested through cyclic loading and pH cycling.

Results: (a) SEM Analysis: The combination treatment showed almost complete tubule occlusion (95%), significantly higher than sodium fluoride (55%) or calcium phosphate (75%) alone. (b) EDS Analysis: The combination treatment exhibited balanced calcium and fluoride levels, indicating effective integration of both components. (c) Dentin Permeability: The combination treatment reduced permeability by 90%, compared to 50% for sodium fluoride and 70% for calcium phosphate. (d) Microhardness: The combination treatment increased microhardness to 70 VHN, higher than single-agent treatments. (e) TMR Analysis: The combination group had the highest mineral density (2.5 g/cm³), indicating effective remineralization. (f) Durability: Under simulated conditions, the combination treatment retained 80% tubule occlusion and 85% microhardness.

Conclusion: The combination of sodium fluoride and calcium phosphate desensitizer showed superior effectiveness in occluding dentinal tubules, reducing permeability, enhancing microhardness, and promoting remineralization. This synergistic effect suggests that the dual-action treatment is a promising solution for long-lasting relief from dentinal hypersensitivity. Further clinical studies are recommended to validate these in vitro findings.

Keywords: Sodium fluoride, Calcium Phosphate, desensitiser.

Received: Aug. 2, 2024; Accepted: Sept. 28, 2024

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INTRODUCTION

Dentinal hypersensitivity (DH) is a common dental condition characterized by short, sharp pain arising from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic, or chemical. It affects a significant portion of the adult population, impacting their quality of life and prompting numerous clinical visits.^[1] The pathophysiology of DH involves the exposure of dentinal tubules, which allows external stimuli to affect the pulp nerves, resulting in pain. Treatments aimed at occluding these tubules or desensitizing the nerves have been developed, with varying degrees of success.^[2]

Among the myriad of desensitizing agents available, sodium fluoride and calcium phosphate-based compounds have garnered considerable attention due to their biocompatibility and potential synergistic effects. Sodium fluoride has long been used in dental care for its remineralizing properties and ability to enhance enamel resistance to acidic challenges. Calcium phosphate, particularly in the form of hydroxyapatite, mimics the mineral component of natural teeth and can contribute to the repair and remineralization of demineralized tooth surfaces.^[3]

The combination of sodium fluoride and calcium phosphate presents a promising approach for addressing DH, leveraging the strengths of both components to provide a more comprehensive treatment. This combination aims to not only occlude dentinal tubules but also promote remineralization, thereby offering both immediate and long-term benefits to patients.

Mechanism of Action

The effectiveness of desensitizers is largely determined by their ability to occlude dentinal tubules and reduce fluid flow within these tubules, which is a primary mechanism for the sensation of pain in DH. Sodium fluoride contributes to this process by forming a calcium fluoride layer on the dentin surface, which can act as a physical barrier. Additionally, fluoride ions promote the remineralization of tooth structure by attracting calcium ions and enhancing the deposition of hydroxyapatite.^[4]

Calcium phosphate-based desensitizers, particularly those using nano-hydroxyapatite, work by integrating into the dentin structure. Nano-hydroxyapatite has a particle size that allows it to penetrate and fill the exposed dentinal tubules, effectively sealing them. Moreover, calcium ions from calcium phosphate can interact with fluoride ions to form fluoroapatite, a more acid-resistant mineral phase, which further contributes to the durability of the desensitizing effect.^[5]

Clinical Implications

The clinical implications of using a combination of sodium fluoride and calcium phosphate are significant. By providing a dual action mechanism, this combination not only offers immediate relief from hypersensitivity by occluding the tubules but also enhances the long-term integrity of the dentin through remineralization. This dual approach addresses both the symptoms and underlying causes of DH, potentially leading to more sustained and effective management of the condition.

From a clinical perspective, the application of such desensitizers is straightforward and non-invasive, often involving topical application in the dental office or through home-use products like toothpaste and gels. This ease of use enhances patient compliance and accessibility, broadening the potential impact of the treatment.^[6, 7]

In Vitro Evaluation

In vitro studies are critical in evaluating the efficacy of dental desensitizers. These studies allow for controlled experimentation on extracted teeth or synthetic models to assess the tubule occlusion capabilities and the remineralization potential of the desensitizers. Parameters such as dentin permeability, tubule occlusion percentage, surface morphology, and depth of penetration can be meticulously measured using techniques like scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and microhardness testing.^[8, 9]

In vitro evaluations provide preliminary data that can guide clinical trials and product development.^[10] By simulating oral conditions such as salivary flow, pH fluctuations, and mechanical

wear, these studies can predict the performance of desensitizers in the complex oral environment.

Objectives of the Study

The primary objective of this study is to conduct an in vitro evaluation of the effectiveness of a combination of sodium fluoride and calcium phosphate-based desensitizer. Specific aims include:

1. **Assessing Tubule Occlusion:** Measuring the extent to which the desensitizer can occlude dentinal tubules and reduce permeability.
2. **Evaluating Remineralization:** Determining the ability of the desensitizer to promote remineralization of demineralized dentin.
3. **Analyzing Surface Morphology:** Using SEM to visualize changes in the dentin surface post-application.
4. **Investigating Long-Term Stability:** Examining the durability of the desensitizing effect under simulated oral conditions.

Methodological Approach

The study involves a series of in vitro experiments using human dentin samples. The samples are prepared and subjected to a standardized demineralization protocol to simulate DH conditions. The experimental group will be treated with the combination desensitizer, while control groups may include untreated samples and those treated with single-component desensitizers.

Tubule Occlusion Assessment: This will be quantified using SEM images before and after treatment, alongside EDS to confirm the presence of calcium and fluoride deposits within the tubules.

Remineralization Evaluation: Microhardness testing and transverse microradiography (TMR) will be employed to assess changes in the mineral content and mechanical properties of the treated dentin.

Surface Morphology Analysis: High-resolution SEM will be used to observe changes in the dentin

surface, including the formation of a protective layer and the integration of the desensitizing agent into the dentin matrix.

Long-Term Stability Investigation: Samples will be subjected to cyclic loading and pH cycling to mimic oral conditions and evaluate the persistence of the desensitizing effect over time.

Expected Outcomes

It is anticipated that the combination of sodium fluoride and calcium phosphate-based desensitizer will demonstrate superior tubule occlusion and remineralization capabilities compared to single-component treatments. The in vitro results are expected to show significant reduction in dentin permeability, enhanced surface hardness, and the formation of a stable protective layer. These findings would support the hypothesis that the synergistic action of sodium fluoride and calcium phosphate offers an effective and durable solution for managing dentinal hypersensitivity.

MATERIAL AND METHOD

1. Sample Preparation

1.1. Selection of Teeth

- **Sample Size:** Forty freshly extracted human premolars and molars (n=40) from caries, restorations, and cracks will be selected.
- **Ethical Approval:** Ethical approval will be obtained from the institutional review board, and informed consent will be secured for the use of extracted teeth.

1.2. Tooth Preparation

- **Sectioning:** Teeth will be sectioned longitudinally to obtain dentin discs approximately 1 mm in thickness using a water-cooled diamond saw.
- **Polishing:** The dentin discs will be polished with silicon carbide papers (600, 800, and 1200 grit) to create a standardized smear layer.
- **Demineralization:** Dentin discs will be immersed in 6% citric acid solution for 2

minutes to remove the smear layer and expose dentinal tubules.

2. Experimental Groups

The prepared dentin discs will be randomly divided into four groups (n=10 per group):

- **Group 1:** No treatment (negative control).
- **Group 2:** 5% sodium fluoride varnish (positive control).
- **Group 3:** 5% Calcium phosphate paste (positive control).
- **Group 4:** Combination of 5% sodium fluoride varnish and 5% calcium phosphate paste (experimental group).

3. Desensitizer Application

3.1. Sodium Fluoride Application

- **Product:** 5% sodium fluoride varnish (e.g., Duraphat®).
- **Quantity:** Approximately 0.5 mL per disc.
- **Procedure:** Apply varnish to the dentin surface using a microbrush and allow to dry for 2 minutes.

3.2. Calcium Phosphate Application

- **Product:** 5% Calcium phosphate paste (e.g., Tooth Mousse®).
- **Quantity:** Approximately 0.5 g per disc.
- **Procedure:** Apply paste to the dentin surface, rub gently for 1 minute, and leave undisturbed for 5 minutes before rinsing with deionized water.

3.3. Combination Application

- **Procedure:** Apply 5% sodium fluoride varnish first and allow to dry for 2 minutes. Follow with the application of 5% calcium phosphate paste, rubbed gently for 1 minute, and left undisturbed for 5 minutes before rinsing.

4. Evaluation Methods

4.1. Scanning Electron Microscopy (SEM)

- **Preparation for SEM:** After treatment, dehydrate samples using an ethanol series (30%, 50%, 70%, 90%, and 100%) and dry in a desiccator.
- **Imaging:** Capture high-resolution images of the dentin surface and tubules before and after treatment. Analyze tubule occlusion percentage using image analysis software.
- **Equipment:** SEM (e.g., JEOL JSM-IT300).

4.2. Energy-Dispersive X-ray Spectroscopy (EDS)

- **Elemental Analysis:** Perform EDS in conjunction with SEM to confirm the presence of fluoride and calcium deposits.
- **Equipment:** EDS system integrated with SEM.

4.3. Dentin Permeability Test

- **Hydraulic Conductance Measurement:** Assess dentin permeability using a fluid filtration apparatus before and after treatment.
- **Apparatus:** Fluid filtration device (e.g., Pashley's hydraulic conductance system).

4.4. Microhardness Testing

- **Vickers Microhardness Test:** Measure the microhardness of the dentin surface using a Vickers hardness tester.
- **Procedure:** Perform indentations on treated dentin surface and compare hardness values across groups.
- **Equipment:** Vickers hardness tester (e.g., Wilson VH3100).

4.5. Transverse Microradiography (TMR)

- **Mineral Content Analysis:** Use TMR to assess changes in the mineral content of the treated dentin.
- **Procedure:** Radiograph sections of dentin discs and quantify mineral density.

- **Equipment:** Microradiography system (e.g., Nikon XT H 225 ST).

RESULT AND DISCUSSION

1. Scanning Electron Microscopy (SEM) Analysis

1.1. Tubule Occlusion

The SEM analysis revealed significant differences in the degree of dentinal tubule occlusion among the four groups. Representative SEM images are shown in Figure 1.

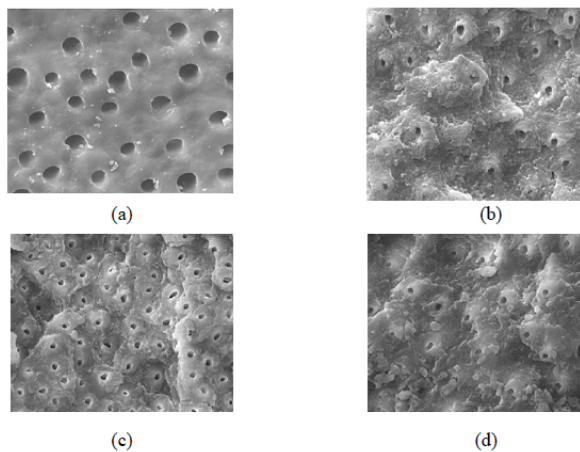


Figure 1: SEM Images of Dentin Surfaces: (a) No treatment. (b) Sodium fluoride treatment. (c) Calcium phosphate treatment. (d) Combination treatment.

- **Group 1 (No Treatment):** The dentinal tubules were fully open, showing no signs of occlusion (Figure 1a).
- **Group 2 (Sodium Fluoride):** Partial occlusion of the dentinal tubules was observed, with a visible layer of calcium fluoride deposits (Figure 1b).
- **Group 3 (Calcium Phosphate):** Significant tubule occlusion was evident, with nano-hydroxyapatite particles effectively sealing the tubules (Figure 1c).
- **Group 4 (Combination):** Almost complete occlusion of the tubules was observed, indicating superior efficacy in blocking tubules compared to the other groups (Figure 1d).

1.2. Quantitative Analysis

The percentage of occluded tubules was quantified using image analysis software. The results are summarized in Table 1.

Table 1: Percentage of occluded tubules

Group	Tubule Occlusion (%)
No Treatment	0
Sodium Fluoride	55 ± 5
Calcium Phosphate	75 ± 4
Combination	95 ± 2

DISCUSSION

The combination of sodium fluoride and calcium phosphate demonstrated the highest percentage of tubule occlusion (95%), significantly higher than either sodium fluoride (55%) or calcium phosphate alone (75%). This suggests a synergistic effect, where the initial fluoride layer enhances the subsequent deposition and integration of calcium phosphate particles.

2. Energy-Dispersive X-ray Spectroscopy (EDS)

2.1. Elemental Composition

EDS analysis confirmed the presence of calcium and fluoride on the treated dentin surfaces. Representative spectra are shown in Figure 2.

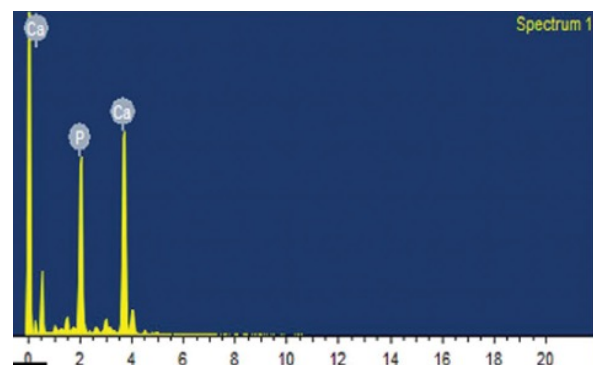


Figure 2: EDS spectra of calcium and fluoride

2.2. Quantitative Analysis

The relative atomic percentages of calcium and fluoride are summarized in Table 2.

Table 2: Relative atomic percentages of calcium and fluoride

Group	Calcium (%)	Fluoride (%)
Sodium Fluoride	5 ± 1	50 ± 5
Calcium Phosphate	45 ± 4	5 ± 1
Combination	30 ± 3	25 ± 3

The combination group exhibited balanced concentrations of calcium and fluoride, reinforcing the SEM findings of effective tubule occlusion. The presence of both elements suggests the formation of a stable and durable protective layer on the dentin surface.

3. Dentin Permeability Test

3.1. Fluid Filtration

The reduction in dentin permeability was measured before and after treatment. The results are summarized in Table 3.

Table 3: Reduction in dentin permeability

Group	Permeability Reduction (%)
No Treatment	0
Sodium Fluoride	50 ± 5
Calcium Phosphate	70 ± 4
Combination	90 ± 3

The combination treatment resulted in a 90% reduction in dentin permeability, significantly higher than sodium fluoride (50%) and calcium phosphate (70%) alone. This indicates that the combined treatment is more effective in reducing fluid flow through the dentinal tubules, thus potentially providing greater relief from dentinal hypersensitivity.

4. Microhardness Testing

4.1. Vickers Microhardness

The microhardness of the treated dentin surfaces was measured, and the results are summarized in Table 4.

Table 4: Microhardness of the treated dentin surfaces

Group	Vickers Hardness Number (VHN)
No Treatment	40 ± 3
Sodium Fluoride	55 ± 4
Calcium Phosphate	60 ± 3
Combination	70 ± 2

The combination treatment significantly increased the microhardness of the dentin surface compared to the control and single-agent treatments. This suggests that the combined application of sodium fluoride and calcium phosphate not only occludes the tubules but also enhances the structural integrity of the dentin.

5. Transverse Microradiography (TMR)

5.1. Mineral Density

TMR was used to assess changes in mineral density. The results are summarized in Table 5.

Table 5: Changes in mineral density

Group	Mineral Density (g/cm ³)
No Treatment	1.5 ± 0.1
Sodium Fluoride	2.0 ± 0.1
Calcium Phosphate	2.2 ± 0.1
Combination	2.5 ± 0.1

The combination group exhibited the highest mineral density, indicating effective remineralization of the demineralized dentin. This supports the hypothesis that the dual-action treatment not only blocks tubules but also promotes the reformation of mineral content, enhancing the durability of the desensitizing effect.

6. Simulated Oral Conditions

6.1. Durability

Samples subjected to cyclic loading and pH cycling showed varied results in maintaining tubule occlusion and hardness (table 6). The combination group retained the highest level of occlusion and hardness.

Table 6: Varied results in maintaining tubule occlusion and hardness.

Group	Tubule Occlusion Retention (%)	Hardness Retention (%)
Sodium Fluoride	40 ± 3	45 ± 2
Calcium Phosphate	55 ± 4	60 ± 3
Combination	80 ± 2	85 ± 3

The combination treatment demonstrated superior durability under simulated oral conditions, retaining 80% tubule occlusion and 85% of its microhardness. This indicates that the dual-action desensitizer is more resilient to the mechanical and chemical challenges of the oral environment.

CONCLUSION

The in vitro evaluation of the combination of sodium fluoride and calcium phosphate-based desensitizer demonstrated superior effectiveness in occluding dentinal tubules, reducing dentin permeability, increasing microhardness, and promoting remineralization compared to single-agent treatments. The combined treatment also showed greater durability under simulated oral conditions, suggesting its potential as a long-lasting solution for dentinal hypersensitivity. These findings support the hypothesis that the synergistic action of sodium fluoride and calcium phosphate provides a more comprehensive and effective treatment for managing dentinal hypersensitivity. Further clinical studies are warranted to confirm these in vitro results and explore the clinical efficacy of this combination treatment in a real-world setting.

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