Efficacy of a mouthwash containing 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride compared to a negative control mouthwash on dentin hypersensitivity reduction. A randomized clinical trial

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ABSTRACT

Objective: The objective of this eight week, single-center, two-cell, double-blind, and randomized clinical study was to evaluate the dentin hypersensitivity reduction efficacy of a mouthwash using Pro-Argin™ Mouthwash Technology containing 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride in an alcohol-free base (“Arginine Mouthwash”) compared to an ordinary mouthwash without any active ingredients (“Negative Control”).

Methods: Qualifying subjects who presented two hypersensitive teeth with a tactile hypersensitivity score between 10 and 50 g of force, and an air blast hypersensitivity score of 2 or 3 participated in this study and were randomized into one of two treatment groups. Subjects brushed with the toothbrush and fluoride toothpaste provided and then rinsed with 20 mL of their assigned mouthwash for 30 s twice daily. Subjects refrained from eating or drinking for 30 min after rinsing. Dentin hypersensitivity assessments, as well as examinations of oral hard and soft tissues, were conducted at the baseline visit and again after two weeks, four weeks and eight weeks of product use.

Results: Ninety (90) subjects entered and completed the eight week study. After two weeks, four weeks and eight weeks of product use, subjects in the Arginine Mouthwash group exhibited statistically significant (p < 0.05) improvements in mean tactile and air blast hypersensitivity scores as compared to the Negative Control mouthwash.

Conclusion: The results of this study support the conclusion that the Arginine Mouthwash provides a significant reduction in dentin hypersensitivity after eight weeks of product use as compared to a Negative Control mouthwash.

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1. Introduction

Dentin hypersensitivity has been the subject of many scientific publications over the past several years. The articles have ranged from discussions of its causes and management to current and new agents that have been incorporated into oral health product formulations which have been clinically proven to treat dentin hypersensitivity.1–8
The term dentin hypersensitivity is almost always described as a short episode of sharp pain that arises from exposed dentin, typically in response to chemical, thermal, tactile and osmotic stimulation.\(^9\) Up to 57% of patients have been reported to be affected by this condition.\(^{10-13}\) Most hypersensitive teeth are accompanied by gingival recession, the result of periodontal disease, periodontal therapy or improper brushing.\(^{14,15}\) In a healthy tooth, the dental pulp is covered by dentin which is protected by enamel above the gingiva and cementum and the gingiva themselves below the gingiva.\(^{16}\) Just as the enamel covers and protects the underlying dentin from external stimuli, the gingiva protects the underlying cementum and root dentin. When the gingiva recedes, the protective cementum can be easily removed so that the dentin tubules are exposed and open, thereby transmitting the pain producing stimuli.

The hydrodynamic theory of dentin hypersensitivity proposed by Brannstrom in 1963 remains the widely accepted theory of how dentin hypersensitivity occurs.\(^{17,18}\) It attributes fluid movement within exposed dentin tubules to the transmission of painful sensation. Specifically, non-noxious stimuli at the tooth surface can trigger fluid movement within the dentin tubules affecting the pulpal mechanoreceptors and resulting in the sensation of pain. Under a microscope, a sensitive tooth shows widened dentin tubules, as much as two times larger than tubules of normal dentin, and in greater number per area compared to a tooth without dentin hypersensitivity.\(^{19}\) Treatment and prevention of dentin hypersensitivity focuses on eliminating the ability of external stimuli to trigger pain. Currently one of two approaches is typically used. The first is to block the triggers of nerve activity by treating the tooth with a physical or chemical agent that forms a layer which mechanically occludes the dentin tubules and prevents pulpal fluid flow. The second is by interrupting the neural response to pain stimuli by delivering potassium salts to the tubule area where they have a depolarizing effect on electrical nerve conduction, causing nerve fibers to be less excitable to the stimuli, thereby reducing a patient’s sensation of pain. The occluding agent or potassium salt is generally delivered by incorporating it into an oral health product so that patients can treat the condition at home during normal oral hygiene procedures.\(^{20-23}\)

The most common products used by patients seeking pain relief from dentin hypersensitivity are desensitizing dentifrices. In the past, these dentifrices usually contained potassium salts – potassium nitrate, potassium citrate, potassium chloride – which are believed to have a depolarizing effect on electrical nerve conduction, causing nerve fibers to be less excitable to the stimuli.\(^{24-39}\) Recently, a new dentifrice product was introduced which has been clinically proven to provide superior dentin hypersensitivity relief. The dentifrice contains 8% arginine, calcium carbonate and 1450 ppm fluoride as sodium monofluorophosphate. Arginine, an amino acid, historically has been studied for its potential oral health benefits. It was shown that a combination of arginine and calcium carbonate when deposited on exposed dentin surfaces is able to physically block and seal open dentin tubules. The novel dentifrice has been reported in numerous clinical studies to provide superior relief of dentin hypersensitivity when compared to a leading sensitivity dentifrice containing potassium ion.\(^{40-42}\) This technology has also been shown to provide instant relief after a single direct topical application of the dentifrice.\(^{43-45}\)

There are a variety of dental products known to successfully address dentine hypersensitivity. A new mouthwash using the Pro-Argin™ Mouthwash Technology was designed to effectively reduce dentin hypersensitivity. The mouthwash efficacy is based on occlusion of the dentin tubules,\(^{46,47}\) provided by a proprietary formulation of arginine, PVM/MA copolymer and pyrophosphates.

Therefore, the aim of this eight week, single-center, two-cell, double-blind, and randomized clinical study conducted in the Chengdu, China area, was to evaluate the dentin hypersensitivity reduction efficacy of two mouthwashes, one containing 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride (“Arginine Mouthwash”) and the other containing 0.05% sodium fluoride (“Negative Control”).

2. Materials and methods

This clinical study employed a randomized assignment, double-blind, two-treatment, parallel-group design. Adult subjects from the Chengdu, China area were enrolled in the study.

2.1. Sample size determination

The sample size of 90 (45 per group) was determined based on a standard deviation (SD), for the response measure tactile sensitivity (or air blast) of 3.34 (or 0.31), a significance level of \(\alpha = 0.05\), a 10% attrition rate and an 80% level of power. The study was powered to detect a minimal statistically significant difference between the study means of 20%. The sample size calculation utilized a historical data from previous studies.

2.2. Inclusion criteria

(i) had to be between the ages of 18 and 70 (inclusive) and in general good health,
(ii) required to possess a minimum of two hypersensitive teeth which were anterior to the molars and demonstrated cervical erosion/abrasion or gingival recession; and for which a tactile sensitivity stimuli score of 10–50 g of force (Yeaple Probe) and an air blast stimuli score of 2 or 3 (Schiff Cold Air Sensitivity Scale) were presented at the baseline examination,
(iii) required to be available for the eight week duration of the study and to sign an informed consent form.

Subjects were excluded from the study if they:

(i) had gross oral pathology, chronic disease, advanced periodontal disease, had undergone treatment for periodontal disease (within the last 12 months), or if they had hypersensitive teeth with a mobility greater than one,
(ii) had teeth with extensive/defective restorations (including prosthetic crowns), suspected pulps, caries, cracked enamel or that were used as abutments for removable
partial dentures. Began taking anticonvulsants, antihistamines, antidepressants, sedatives, tranquilizers, anti-inflammatory drugs or daily analgesics within one month prior to the start of the study or if they started taking them during the course of the study,

(iii) were pregnant or lactating women, individuals who were participating in any other clinical study or who had participated in a desensitizing dentifrice study or who used a desensitizing dentifrice within the last three months,

(iv) had a history of allergy to the test products, or allergies to oral care/personal care consumer products or their ingredients, or had existing medical conditions, which precluded them from eating and drinking for periods up to 4 h.

Prospective study subjects reported to the clinical facility having refrained from all oral hygiene procedures, from chewing gum for 8 h, and from eating and drinking for 4 h prior to their examination. All prospective subjects who met the inclusion/exclusion criteria and signed an informed consent form received a baseline tactile hypersensitivity and an air blast hypersensitivity evaluation, along with an oral soft and hard tissue assessment. Qualifying subjects were sequentially randomized using a list of random numbers provided by study sponsors.

For each subject who qualified for participation in the study, two hypersensitive teeth that satisfied the tactile and air blast hypersensitivity enrollment criteria were identified for evaluation throughout the study. Qualifying subjects were stratified based on mean tactile and air blast hypersensitivity baseline scores and randomly assigned within strata to one of two study treatments: (1) a test mouthwash containing 0.8% arginine, polyvinylmethyl ether/maleic acid (PVM/MA) copolymer, pyrophosphates, and 0.05% sodium fluoride in an alcohol-free base (“Arginine Mouthwash”) (Colgate-Palmolive Company, New York, NY), or (2) a mouthwash (“Negative Control”) in an alcohol-free base without any active ingredients (Colgate-Palmolive Company, New York, NY).

 Mouthwashes were over-wrapped to maintain the binding of the study participants, examiners and all study personnel. Site personnel not involved in the clinical evaluations distributed all test products in sealed opaque bags in an area separate from the examination room. The three products were letter coded.

Following treatment assignment, subjects were provided with toothpaste (Colgate® Cavity Protection containing 1450 ppm fluoride) and a soft toothbrush (Colgate® Adult Extra Clean) for home use. All mouthwashes were over-wrapped in their original package to maintain the double-blind study design. Subjects were instructed to brush their teeth and then to immediately rinse their mouth with 20 mL of their assigned mouthwash for 30 s twice daily (morning and evening) and to use only the toothpaste, toothbrush and mouthwash provided. Subjects were not allowed to floss or use inter-dental stimulators or to eat or drink for 30 min after rinsing. There were no restrictions regarding diet or smoking habits during the course of the study.

Oral soft and hard tissue assessments, as well as tactile and air blast hypersensitivity follow-up evaluations of baseline-designated study teeth, were conducted after two, four and eight weeks of product use. Subjects were requested to return to the clinical facility having refrained from all oral hygiene procedures and from chewing gum for 8 h, and from eating and drinking for 4 h prior to their scheduled visit. All examinations were performed by the same dental examiner, using the same procedures as employed at baseline. Subjects were also interviewed with respect to the presence of adverse events and the use of concomitant medications.

3. Clinical scoring procedures

3.1. Tactile dentin hypersensitivity assessment

Tactile dentin hypersensitivity was assessed by use of the Model 200A Electronic Force Sensing Probe developed by Yeaple Research of Pittsford, NY. The application of this probe for dental hypersensitivity testing utilizing a #19 explorer tip at a pre-set force measured in grams was employed.

 Teeth were evaluated for tactile hypersensitivity in the following manner:

(1) The subject was instructed to respond at the point where he/she first experienced discomfort.
(2) The explorer tip of the probe was applied to the buccal surface of each hypersensitive tooth at the CEJ.
(3) The explorer tip was stroked perpendicular to the tooth beginning at a pre-set force of 10 g (0.01 kg) and increased by 10 g (0.05 kg) increments until the subject experienced discomfort, or until 50 g (0.05 kg) of force was applied.

 Subjectwise scores were calculated by averaging the values measured on the two baseline-designated study teeth.

3.2. Air blast dentin hypersensitivity assessment

Teeth were evaluated for air blast dentin hypersensitivity in the following manner:

(1) The sensitive tooth was isolated from the adjacent teeth (mesial and distal) by placement of the examiner’s fingers over the adjacent teeth.
(2) Air was delivered from a standard dental unit air syringe at 60 psi (4.22 kgf/cm²) ± 5 psi (0.35 kgf/cm²) and 70°F (21) ± 3°F (−16). The air was directed at the exposed buccal surface of the hypersensitive tooth for 1 s from a distance of approximately 1 cm.
(3) The Schiff Cold Air Sensitivity Scale was used to assess subject response to this stimulus. This scale was scored as follows:

0 – Subject did not respond to air stimulus.
1 – Subject responded to air stimulus but did not request discontinuation of stimulus.
2 – Subject responded to air stimulus and requested discontinuation or moved from stimulus.
3 – Subject responded to air stimulus, considered stimulus to be painful and requested discontinuation of the stimulus.
3.3. **Oral soft and hard tissue assessment**

The dental examiner visually examined the oral cavity and peri-oral area using a dental light and dental mirror at all hypersensitivity evaluations. This examination included an evaluation of the soft and hard palate, gingival mucosa, buccal, mucosa, mucogingival fold areas, tongue, sublingual and submandibular areas, salivary glands, and the tonsil and pharyngeal areas.

3.4. **Adverse events**

Adverse events were obtained from an interview with the subjects and from a dental examination by the investigator.

4. **Statistical methods**

Statistical analyses were performed separately for the tactile hypersensitivity assessments and air blast hypersensitivity assessments. Comparisons of the treatment groups with respect to gender were performed using a chi-square analysis and for age an analysis of variance (ANOVA). Comparisons of the treatment groups with respect to baseline-adjusted tactile hypersensitivity and air blast hypersensitivity scores at the follow-up examinations were performed using an analysis of covariance (ANCOVA). All statistical tests of hypotheses were two sided, and employed a level of significance of $\alpha = 0.05$.

5. **Results**

Ninety (90) subjects entered the study, from December 29, 2010 to February 28, 2011 complied with the protocol, and completed the eight week clinical study (Fig. 1). A summary of the gender and age of the study population is presented in Table 1. The treatment groups did not differ significantly with respect to either of these characteristics. Throughout the study, there were no adverse events on the oral soft or hard tissues of the oral cavity observed by the examiner or reported by the subjects when questioned.

Table 2 presents a summary of the mean tactile hypersensitivity scores for each of the treatment groups at each of the measurement time points. Table 3 presents a summary of the mean air blast scores for each of the treatment groups at each of the measurement time points. Tables 2 and 3 also contain a comparison of the percent difference in the two treatments at each of the time points. At baseline there was not a statistically significant difference between the two treatment groups with respect to either mean tactile or mean air blast hypersensitivity scores.

After two weeks of product use, the Arginine Mouthwash group exhibited a statistically significant improvement in
6. Discussion

Dentin is structurally composed of hydroxyapatite mineral and organic components and is formed by the odontoblasts during tooth development. It contains thousands of tubules which run perpendicular to the pulp chamber and which are formed as the odontoblasts migrate away from the dentin-enamel junction during dentin formation.\(^{51,52}\) Dentin is normally covered by enamel or cementum. When teeth erupt, the gingival margin seals the teeth leaving the coronal portion exposed in the oral cavity and the root portion of the tooth protected from the external environment. Exposed dentin and open tubules patent to the pulp cause hypersensitive teeth.\(^{10}\)

The mechanism by which dentin hypersensitivity occurs is that external stimuli triggers a pressure change in the dentin fluid, the fluid movement transmits a signal to the odontoblast process, and the process carries the stimulus from the tooth surface toward the nerve ending in the dentin tubule, resulting in pain. Once the stimulus is removed, the pressure within the tubule returns to normal and the pain is alleviated.

There are two scientifically accepted approaches to treat dentin hypersensitivity. One is to occlude exposed tubules and the other is to suppress nerve activity. With tubular occlusion, the tooth is treated with a physical or chemical agent that forms a layer that mechanically occludes the dentin tubules and prevents pulpal fluid flow, thereby, decreasing dentin hypersensitivity.\(^{53,54}\) In suppressing nerve activity, the tooth is treated with an agent that increases the nerve depolarization threshold, and thus modulates or suppresses the sensation of pain.\(^{55,56}\)

Research has indicated that the ideal dentin hypersensitivity treatment should mimic natural desensitizing processes leading to spontaneous occlusion of open dentin tubules.\(^9\) Kleinberg and coworkers developed a dentin hypersensitivity treatment consisting of 8% arginine (an amino acid found in saliva), and calcium carbonate. This technology mimics saliva’s natural process of plugging and sealing open dentin tubules.\(^{57}\) When applied to exposed dentin, open dentin tubules are sealed with a plug that reduces dentin hypersensitivity.\(^7\) This technology was first introduced as a desensitizing prophylaxis paste with 8% arginine and calcium carbonate for professional use.

Research continued with the arginine/calcium carbonate combination and led to the development of a dentifrice containing 8.0% arginine, calcium carbonate, and sodium monofluorophosphate. The dentifrice physically seals dentin tubules with a plug that contains arginine, calcium carbonate and phosphate. The plug is resistant to normal pulpal pressures and to acid challenge and thereby reduces dentin fluid flow and reduces dentin hypersensitivity. Clinical studies have shown that this dentifrice containing 8% arginine, calcium carbonate and 1450 ppm sodium monofluorophosphate provides superior efficacy in reducing dentin hypersensitivity as compared to a leading desensitizing dentifrice containing 2% potassium ion.\(^{40-42}\) In addition, direct topical self-application of the product to the sensitive site has been shown to provide instant relief of dentin sensitivity.\(^{43-45}\)

As an alternative or a complement to using a desensitizing dentifrice, a new mouthwash formulation has been developed which contains 0.8% arginine, in combination with pyrophosphates and a PVM/MA copolymer to trigger tubule occlusion, and 0.05% sodium fluoride. This new mouthwash was compared to a Negative Control mouthwash containing 0.05% sodium fluoride to determine its relative effects in relieving dentin hypersensitivity over an eight week period.

| Table 1 – Summary of age & gender for subjects in the eight week clinical study. |
|---------------------------------|-----------|-----------|-----------|
| Treatment                        | Male      | Female    | Total     | Age       |
|                                 |           |           |           | Mean | Range |
| Arginine Mouthwash               | 20        | 25        | 45        | 46.2 | 21–63 |
| Negative Control                 | 20        | 25        | 45        | 46.6 | 25–67 |

Baseline-adjusted mean tactile hypersensitivity score to relative to the Negative Control group by 22.1%. With respect to air blast scores, the Arginine group exhibited a statistically significant reduction in baseline-adjusted mean hypersensitivity score relative to the Negative Control group by 14.2%.

After four weeks of product use, the Arginine Mouthwash group exhibited a statistically significant improvement in baseline-adjusted mean tactile hypersensitivity score relative to the Negative Control group by 37.1%. With respect to air blast scores, the Arginine group exhibited a statistically significant reduction in baseline-adjusted mean hypersensitivity score relative to the Negative Control group by 24.1%.

After eight weeks of product use, the Arginine Mouthwash group exhibited a statistically significant improvement in baseline-adjusted mean tactile hypersensitivity score relative to the Negative Control group by 44.6%. With respect to air blast scores, the Arginine group exhibited a statistically significant reduction in baseline-adjusted mean hypersensitivity score relative to the Negative Control group by 24.0%.

| Table 2 – Summary of the tactile hypersensitivity scores for subjects who completed the eight-week study. |
|---------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Treatment                        | No.       | Baseline\(^a\) (mean ± SD) | 2-Week\(^b\) (mean ± SD) | % Difference 2-Week\(^b\) | 4-Week (mean ± SD) | % Difference 4-Week\(^b\) | 8-Week (mean ± SD) | % Difference 8-Week\(^b\) |
| Arginine Mouthwash               | 45        | 17.22 ± 4.20 | 26.33 ± 11.79 | 22.1% | 32.44 ± 12.41 | 37.1% | 38.22 ± 10.23 | 44.6% |
| Negative Control                 | 45        | 17.00 ± 5.78 | 21.56 ± 8.84  |       | 23.67 ± 12.13 |       | 26.44 ± 12.60 |       |

\(^a\) Treatments are not statistically significantly different from each other at baseline.

\(^b\) At each time point, statistically significantly percentage differences (\(p < 0.05\)) as per the ANCOVA comparison of baseline-adjusted means are shown.
The results from the study indicate that:

- after two weeks, four weeks and eight weeks of product use, the Arginine Mouthwash group exhibited statistically significant ($p < 0.05$) improvements (22.1%, 37.1% and 44.6% respectively) in tactile hypersensitivity scores as compared to the Negative Control mouthwash group.

- after two weeks, four weeks and eight weeks of product use, the Arginine Mouthwash group exhibited statistically significant ($p < 0.05$) reductions (14.2%, 24.1% and 24.0% respectively) in air blast hypersensitivity scores as compared to the Negative Control Mouthwash group.

7. Conclusion

The results of this double-blind clinical study support the conclusion that the Arginine Mouthwash using the Pro-Argin™ Mouthwash Technology and containing 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride in an alcohol-free base provides a significant reduction in dentin hypersensitivity after eight weeks of product use as compared to a Negative Control mouthwash in an alcohol-free base.

The present study was approved and registered in "Ethics Committee of State Key Laboratory of Oral Diseases, China Clinical Research, Institutional Review Board" Registered under 2010011.

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Conflict of interest statement

Bernal Stewart, Foti Panagakos and Yun Po Zhang are employees of Colgate-Palmolive Company. Wei Yin is an employee of West China College of Stomatolgy. Deyu Hu is an employee of West China College of Stomatology, Sichuan University.

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