The development of a new desensitising mouthwash containing arginine, PVM/MA copolymer, pyrophosphates, and sodium fluoride—A hydraulic conductance study

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**ABSTRACT**

Objective: To investigate the ability of a novel mouthwash comprised of 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride in an alcohol-free base (Pro-Argin™ Mouthwash Technology) to reduce dentine permeability.

Methods: Hydraulic conductance was used to assess the dentine permeability effects of the arginine mouthwash. Aqueous solutions containing arginine and PVM/MA copolymer were studied in the initial stage of the method development. The acid resistance was tested with a cola drink challenge. Finally, a blinded study was carried out to determine the occlusion of the arginine mouthwash in comparison to a negative control mouthwash.

Results: Dentine discs treated with the arginine mouthwash showed an average fluid reduction of 42%, which was statistically, significantly better than the fluid reduction for the negative control mouthwash. In addition, experiments using simple solutions of arginine and PVM/MA copolymer, alone and in combination, demonstrated that the combination of the two was required to provide a relevant occlusion benefit. Finally, the occlusion provided by the arginine mouthwash was maintained after exposure to an acid challenge.

Conclusion: The exclusive combination of ingredients in the arginine mouthwash has been proven to be efficacious in decreasing dentine fluid flow as measured by hydraulic conductance. The new mouthwash works by occlusion, due to the unique combination of arginine, PVM/MA copolymer and pyrophosphates.

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1. **Introduction**

Dentine hypersensitivity is a complex and painful oral condition that affects 4–57% adults in the world. Because of its poorly understood aetiology, the proper diagnosis is established after the exclusion of any other possible causes of the pain.1–4 The most accepted explanation for dentine hypersensitivity is based on Brännström’s hydrodynamic theory,5,6 which considers dentine tubules to be capillary tubes where the fluid behaviour obeys the laws of fluid movement. It is postulated that various stimuli, such as cold, heat, pressure, and sweet, cause inward or outward displacement of the fluid in the dentine tubules activating the nerve endings at the pulp/dentine interface, resulting in pain. Dentine tubules are exposed when there is loss of enamel, cementum and overlying periodontal tissues due to attrition/abrasion/erosion, gingival recession or periodontal therapy.

In order to treat hypersensitivity, two major methodologies have been identified as efficacious: (1) nerve desensitisation by...
delivering potassium ions through dentine and (2) decrease of fluid movement by blocking the dentine tubules (occlusion). Dentifrices containing a potassium salt are broadly used to desensitise the nerves, with typical efficacy observed after four to eight weeks of continued use of the product.\textsuperscript{7–9} Occlusion agents are generally delivered through over-the-counter dentifrices, although more recently this approach has been applied more widely through the use of professional desensitisising applications or in-office resin applications.

The use of hydraulic conductance to assess dentine fluid flow has been established by Pashley and co-workers\textsuperscript{10–14} and is a valuable in vitro method to evaluate products that work via dentine occlusion. It has been largely used to screen dentifrices and other highly viscous products that deposit solid particles on dentine tubules or form thick adhesive layers on the dentine surface.\textsuperscript{14} Such products block dentine fluid flow, resulting in reduction of fluid flow, ultimately associated with decrease of dentine hypersensitivity. In 2009, a new breakthrough technology was introduced, based upon arginine and calcium carbonate, as an in-office professional treatment and as a daily use fluoride-containing dentifrice. The efficacy of the arginine-based technology has been proven in numerous clinical studies as well as in laboratory studies.\textsuperscript{15–22}

Studies describing the clinical efficacy of mouthwash as a delivery system to address dentine hypersensitivity are inconclusive.\textsuperscript{23–26} For instance, Pereira et al.\textsuperscript{23} compared a 3% potassium nitrate/0.2% sodium fluoride mouthrinse to a 0.2% sodium fluoride control mouthrinse in a six-week double blind study. They found that both products decreased the intensity of hypersensitivity, with no difference between products after two weeks. Only after six weeks was there a statistically significant difference in sensitivity as measured by cold air blast between the groups favouring the potassium nitrate containing group. There was no difference after six weeks using tactile measurements. On the other hand Gillam et al.\textsuperscript{24} did detect a difference at both two and six weeks between a 3% potassium nitrate and sodium fluoride mouthwash and a sodium fluoride mouthwash.

The objective of the present study was to investigate the in vitro performance of a novel mouthwash technology (Pro-Argin\textsuperscript{TM} Mouthwash Technology) that provides dentine occlusion\textsuperscript{27} with a consequent reduction of hydraulic conductance. The new technology is based on the delivery of an adhesive complex formed by arginine/copolymer/pyrophosphates at the microscopic scale. An adaptation of Pashley’s method described in Kleinberg’s work\textsuperscript{28} has been developed to enable the in vitro study of fluid samples such as mouthwash via hydraulic conductance. The use of Pashley cell instead of dentine segments has proven to be successful for screening mouthwash formulations with high reproducibility results.

\section{Materials and methods}

\subsection{Test materials}

The test mouthwash (“Arginine”) consisted of 0.8% arginine, polyvinylmethyleth ether/maleic acid (PVM/MA) copolymer, pyrophosphates and 0.05% sodium fluoride (Colgate-Palmolive Company, New York, NY). The control mouthwash (negative control) was a non-commercial, fluoride free prototype that had the same ingredients as the arginine mouthwash without the occlusion system. In addition, aqueous solutions of arginine and PVM/MA copolymer, alone and in combination, were evaluated along with early prototypes of the arginine mouthwash.

\subsection{Dentine disc preparation\textsuperscript{10}}

Dentine discs were prepared from extracted human molars (Dental Product Testing, IN). Specimens, 0.8 mm thick, were obtained by using a slow-speed saw turning a diamond grit blade (Buehler Isomet). Each dentine disc was dipped into 6% (w/v) citric acid for 1 min to remove the smear layer created by the blade. The dentine discs were mounted in a polycarbonate split-chamber device – Pashley cell, also called Pashley split chamber (Kenward Company, GA) – with spacers containing silicon “O” rings to limit the test surface area to 0.28 cm\textsuperscript{2}. The upper half with the exposed dentine surface was open and the lower half was closed except for 18 gauge stainless steel tubing that was used for inlet and outlet conduits. The orientation of the dentine discs was occlusal side up, pulpal side towards the lower chamber. This orientation was always confirmed by noting the magnifying properties of the occlusal-pulpal orientation and the reducing properties of the pulpal-occlusal orientation.

\subsection{Measurements of the hydraulic conductance of dentine}

Hydraulic conductance measurements were carried out using a flow sensor – Flodec (De Marco Engineering, Switzerland) – under simulated pulpal pressure of 70 cm created by a syringe connected to the capillary tube via polystyrene tubing. The displacement of a meniscus created by an air bubble in the
capillary tube was monitored by an electromechanical sensor. The flow data were converted to permeability (µL min⁻¹ cm⁻¹) via dedicated Flodoc software. A schematic diagram is shown in Fig. 1. After filling the chamber with water and, the Pashley cell was connected to Flodoc with the polystyrene tubing. Leakage was circumvented by wrapping the connections with Teflon™ tape. Dentine permeability was measured before and after application of the study solutions/products. The in vitro “treatment” has been adapted from Kleinberg’s work,²⁸ where water dispersions of arginine bicarbonate and calcium bicarbonate have been tested for the ability to reduce dentine fluid flow. In our study, a “treatment” consisted of the following steps:

1. Application of 400 µL sample on dentine disc for 10 min @ nil pressure.
2. Rinsing w/400 µL phosphate buffer saline (PBS)
3. Application of 400 µL PBS for 10 min under 70 cm water pressure (simulated pulpal pressure)
4. Repetition of steps 1-2
5. Application of 400 µL PBS for 30–90 min under simulated pulpal pressure.

The percentage flow reduction was based on the calculation of a flow reduction after a given time under simulated pulpal pressure in comparison to its own baseline: ([Baseline flow – Treatment flow]/Baseline flow)×100. Due to the natural variability of the dentine specimens, a minimum of 6 samples were tested each time a study was conducted to test the occlusion efficacy of a mouthwash.

2.4. Arginine mouthwash vs. negative control mouthwash

A two-cell, randomised in vitro study was performed on human dentine discs, assigned accordingly to 16 independent runs. The two mouthwash formulas described in Section 2 were tested 8 times, i.e., eight distinct dentine discs were assigned to each cell. The person performing the experiments as well as the people analysing the results were blinded to the identity of the test materials.

A one-way analysis of variance model was performed to determine if a statistically significant difference (p < 0.05) existed among the two products.

3. Results

Fig. 2 shows hydraulic conductance results in the form of percentage reduction of discs treated with solutions of arginine, PVM/MA copolymer, and the combination of the two. The modest reductions provided by arginine alone and the PVM/MA copolymer alone indicate a minimal occlusive effect. The combination of the two provided a reduction of 39% indicating occlusion. These results were measured 90 min after treatment, which was sufficient to allow the formation of an ionic complex between arginine and the PVM/MA copolymer and could potentiate the formation of an adhesive complex at the microscopic scale under the conditions of the study.

The occlusion efficacy was further tested using a mouthwash prototype containing arginine and PVM/MA in combination with typical mouthwash ingredients, including pyrophosphates. A 90 min experiment is presented in Fig. 3. The percentage flow reduction at 90 min is comparable to that of the combination of the arginine and PVM/MA copolymer, indicating that the presence of typical mouthwash ingredients have not compromised occlusion. Based upon the learning of these initial studies, all subsequent studies were measured at 30 min, because the major change in flow occurred between 15 min and 45 min.

Another important investigation was to determine the acceptable baseline range to measure the effect of mouthwash on simulated dentine fluid flow. The same prototype mouthwash as previously tested was tested 10 times at 30 min using baselines within the range of 6–26 µL min⁻¹ cm⁻², as presented in Table 1. As the results indicate, no direct correlation between percentage reduction and baseline was observed.

![Fig. 2 – Dentine fluid reduction of mouthwash prototype containing arginine and PVM/MA copolymer.](image)

![Fig. 3 – Dentine fluid flow reduction of aqueous solutions 90 min after treatment.](image)
Therefore, the range of 6–26 μL min⁻¹ cm⁻² was considered acceptable for mouthwash measurements.

The acid-resistance ability of the arginine mouthwash was evaluated using a cola drink challenge. Results comparing before and after cola challenge are shown in Fig. 4. There is no statistical difference in the occlusion efficacy after cola challenge, which suggests an overall acid-resistance of the occlusion system formed by the arginine mouthwash.

Finally, the arginine mouthwash was compared to a negative control mouthwash in a blinded study. Eight different dentine discs were assigned to each sample. Fig. 5 shows the average percentage of flow reduction. There was an average of 42% reduction in fluid flow for the arginine mouthwash; whereas there was only a 9% reduction in fluid flow for the negative control mouthwash at the end of each treatment. This difference was statistically significant (p < 0.0005).

4. Discussion

The development of a clinically efficacious mouthwash to alleviate dentine hypersensitivity has been a challenge pursued by researchers for many decades without unequivocal outcome. One of the objectives of the work presented herein was to develop a reliable in vitro test to study the performance of anti-hypersensitivity mouthwash formulations which potentially occlude dentine tubules. The adaptation of Kleinberg’s study based on Pashley cell’s method was key to achieve reliable in vitro results which ultimately were confirmed by clinical studies reported by Hu et al.29 and Boneta et al.30 Different from other commercially available mouthwashes recommended for the treatment of hypersensitivity, the arginine mouthwash does not contain potassium nitrate or potassium citrate, common hypersensitivity ingredients. Its mode of action is based on the decrease of dentine permeability, which is accomplished by occlusion.27 The combination of arginine, PVM/MA copolymer and pyrophosphate salts formulated in the appropriate pH and ionic strength environment is proposed to form an adhesive complex that builds upon repeated applications, resulting in the reduction of hydraulic conductance, ultimately linked to the mitigation of dentine hypersensitivity. The hydraulic conductance results for simple solutions (Fig. 2) of arginine, PVM/MA and their combination indicate the benefit in occlusion efficacy of combining these two ingredients. One can assume that after the polymer has been neutralised by the negative sites of the arginine molecule, a net negative charge will result in the system allowing it to adhere on to the dentine surface. Moreover, occlusion is not compromised when the complex is incorporated in a full mouthwash formulation (Fig. 3). The comparison between the arginine mouthwash and the negative control mouthwash corroborates the proposed mechanism of dentine occlusion (Fig. 5). Further supporting data for this mechanism is presented elsewhere in this Special Issue.27 The acid-resistance of the arginine mouthwash was assessed by the application of cola drink presented in Fig. 4. This method has been used to mimic the challenge that occurs in the oral environment after the exposure to acidic foods.28 In the case of toothpaste treatment, where the occlusion is primarily based on the deposition of solid particles, the cola challenge helps to discriminate weak and soluble deposition system from stable and resistant occlusion. Techniques such as Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM) are appropriate to investigate such systems. However, when one needs to study a low viscosity system such as a mouthwash, the Pashley cell provides the best in vitro technique to mimic the oral environment. In such setting, the hydraulic conductance is measured under simulated pulpal pressure in presence of PBS. A non-statistically significant change in the flow for the arginine mouthwash after exposure to an acid challenges indicates the resistance of the adhesive complex.

The measure of dentine permeability is a quantitative in vitro method used to study occlusion of toothpastes and other products containing solid particles that plug dentine tubules. In the present paper, we have widened the approach to measure the dentine occlusion of mouthwashes, which involved many experimental adaptations. In particular, we determined the optimal range of baseline permeability suitable for mouthwash evaluation (Table 1), and we fixed the time for data collection (Fig. 3), minimising the variability intrinsic of in vitro studies using human dentine specimens. The development of a reliable in vitro method is crucial for designing new products, because it allows the screening and
fine tuning of prototypes prior to clinical studies but also to explain the mode of action. The occlusion of dentine tubules with the arginine mouthwash is the basis of the relief of dentine hypersensitivity as demonstrated in two clinical studies.29,30

5. Conclusion

A new mouthwash containing arginine, PVM/MA copolymer, pyrophosphates and sodium fluoride has been shown to reduce hydraulic conductance and therefore may be appropriate to the treatment of dentine hypersensitivity. Such a composition suggests the formation of an arginine/copolymer complex that occlude dentine tubules, which may explain the observed reduction in dentine fluid permeability, in vitro.

Conflict of Interest Statement

Sarita Mello, Evangelia Arvanitidou and Mark Vandeven are employees of the Colgate-Palmolive Company.

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