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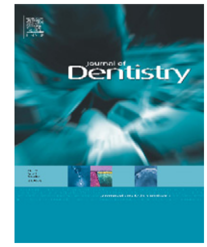

Mode of action studies of a new desensitizing mouthwash containing 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride

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ABSTRACT

Objective: The mode of action of an arginine mouthwash using the Pro-Argin™ Mouthwash Technology, containing 0.8% arginine, PVM/MA copolymer, pyrophosphates and 0.05% sodium fluoride, has been proposed and confirmed as occlusion using a variety of in vitro techniques.

Methods: Quantitative and qualitative laboratory techniques were employed to investigate the mode of action of the new arginine mouthwash. Confocal laser scanning microscopy (CSLM) and atomic force microscopy (AFM) investigated a hydrated layer on dentine surface. Electron spectroscopy for chemical analysis (ESCA), secondary ion mass spectroscopy (SIMS) and near-infrared spectroscopy (NIR) provided information about its chemical nature.

Results: CLSM was used to observe the formation of a hydrated layer on exposed dentine tubules upon application of the arginine mouthwash. Fluorescence studies confirmed penetration of the hydrated layer in the inner walls of the dentinal tubules. The AFM investigation confirmed the affinity of the arginine mouthwash for the dentine surface, supporting its adhesive nature. NIR showed the deposition of arginine after several mouthwash applications, and ESCA/SIMS detected the presence of phosphate groups and organic acid groups, indicating the deposition of copolymer and pyrophosphates along with arginine.

Conclusion: The studies presented in this paper support occlusion of the dentine surface upon the deposition of an arginine-rich layer together with copolymer and phosphate ions from an alcohol-free mouthwash containing 0.8% arginine, PVM/MA copolymer, pyrophosphates and 0.05% sodium fluoride.

1. Introduction

Tooth sensitivity has been recognized as one of the earliest maladies of the mouth reported in history. Tooth sensitivity is a common name for dentine hypersensitivity or root sensitivity. It is estimated that up to 57% of the population in the United States suffers from dentine hypersensitivity.[1] Dentine hypersensitivity is characterized by short, sharp pain arising from exposed dentine in response to stimuli, typically

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thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other dental defect or pathology.[2] Diverse in-office treatments have been reported to address dentine hypersensitivity, such as desensitizing pastes, fluoride varnishes, and restorative resins.[3–13] Over-the-counter products that treat dentine hypersensitivity are normally toothpastes formulated with potassium ions or dentine occlusion agents.[14–21] The mode of action of desensitizing products can be divided in two main groups: (1) nerve desensitization by potassium ions and (2) decrease in dentine permeability by occlusion agents. Historically, marketed anti-hypersensitivity toothpastes were recommended for a minimum of two weeks use, in order to allow the accumulation of an effective concentration of potassium ions to desensitize the nerve endings. The over-the-counter occlusion treatment approach has been traditionally reported as the deposition of solid particles or mineralized crystals, such as stannous, strontium and oxalates.[4,5] More recently, a breakthrough occlusion technology containing arginine and calcium carbonate has been formulated into toothpaste and in-office products.[7,20–24]

In vitro investigations of dentine occlusion systems are commonly carried out via Pashley's hydraulic conductance method[25–29] and surface analysis techniques.[3,23] Also, Kolker et al.[30] studied the in vitro performance of different synthetic resins, showing effective reduction of hydraulic conductance in the range of 40–60%, for different coatings with diverse chemical compositions. In contrast to mineral solids occlusion, the mechanism of action of adhesive coatings depends on the physical adsorption of organic materials, their diffusion through porous dentine, the chemical bonding or curing and electrostatic interactions. The viscosity and contact time of the polymeric layer are also important to allow the coating effect (film-forming) and ensure enough cohesive forces to increase the life-time of the coating. Most recently, hydraulic conductance studies have demonstrated a significant reduction in fluid flow for a toothpaste[23] and for a mouthwash.[31]

Although a variety of dental products have been employed successfully to address dentine hypersensitivity, there is still an absence of effective mouthwash products and only a few scientific papers have been published.[32–35] Herein we present the in vitro evaluation of a new mouthwash designed to effectively reduce dentine hypersensitivity through the occlusion of the dentine surface via a clinically proven formulation of arginine, PVM/MA copolymer and pyrophosphates.[36,37] Polymethylvinyl ether/maleic acid (PVM/MA) copolymer and pyrophosphates have been associated with dentine occlusion in toothpaste formulations by Miller et al.[38] and Mason et al.[39] based on in vitro measurements of hydraulic conductance and surface analysis techniques. The mechanism of occlusion presented here differs from Miller et al.[38] due to several factors, especially the presence of arginine, other formula ingredients, pH, and delivery in the absence of brushing (toothpaste vs mouthwash). The mode of action of the new mouthwash containing the Pro-Argin™ Mouthwash Technology is based on occlusion of the dentine tubules from the deposition of arginine, PVM/MA copolymer and polyphosphates onto the dentine, which after repeated application, decreases dentine permeability and reduces pain-causing stimuli. The mechanism of action was investigated via several laboratory techniques, namely confocal laser scanning microscopy (CSLM), atomic force microscopy (AFM), electron spectroscopy for chemical analysis (ESCA), secondary ion mass spectroscopy (SIMS) and near-infrared spectroscopy (NIR).

2. Materials and methods

2.1. Test products

The test mouthwash (''arginine'') consisted of 0.8% arginine, polyvinylmethyl ether/maleic acid (PVM/MA) copolymer, pyrophosphates and 0.05% sodium fluoride (Colgate-Palmolive Company, New York, NY) in an alcohol-free base. The control mouthwash (negative control) (Colgate-Palmolive Company, New York, NY) did not contain any of the ingredients mentioned above. In addition, a commercial fluoride toothpaste (Colgate-Palmolive Company, New York, NY) was employed during one of the evaluations.

2.2. Dentine disc preparation

Dentine discs used in all tests were prepared from extracted human molars. An average of 3 discs per molar were cut and prepared accordingly to each to be used in different experiments. At least 6 discs were randomly assigned per sample in all tests. Specimens, 0.8 mm thick, were obtained by using a slow-speed saw turning a diamond wafering blade (Isomet, Lake Bluf, USA). Afterwards, discs were sanded 600 grit paper discs (Carbimet, Buehler, Lake Bluf, USA) and polished with soft plane cloth (P4000, Silicon Carbide using a Variable Speed Grinder-Polisher EcoMet® 3000, Buehler, Lake Bluf, USA). Each dentine disc was sonicated in deionized water and dipped into 6% (w/v) citric acid for 1 min to remove the smear layer.[28]

2.3. Surface and chemical characterization

2.3.1. Confocal laser scanning microscopy (CLSM)

A Leica TCS SP confocal laser scanning microscope equipped with a spectral detection system was used to visualize the dentine surfaces. The 488 nm line from an argon laser along with a PLO APO 50× objective was used in all experiments. Dentine discs were prepared according to the procedure described above. Images were taken with a 50× objective and optical zoom of 2×. Distinct treatment procedures were developed for each detection mode in order to assess the relevant aspect of the coating mechanism, i.e. a thicker coating to allow the tubule blocking visualization (10 applications) and a thinner coating (2 applications) for the fluorophore detection inside the tubules.

Reflectance mode: on dentine discs pre-conditioned in phosphate buffer saline (PBS), 15 μL of mouthwash was applied 10 times for 1 min, with intervals of 10 min between each application.

Fluorescent mode: fluorescein–cadaverine (molecular probes) was added to both mouthwash samples prior to treatment, to a final concentration of 0.4 μM. A mouthwash aliquot of