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Fluoride release and anti-erosive effects of dentifrices containing PVM/MA copolymers

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ABSTRACT

Objective: To evaluate fluoride release from dentine discs and study the effects of dentine tubule occlusion and erosion prevention of dentifrices containing fluoride and PVM/MA copolymers in a cycling erosive challenge model.
Methods: Human dentine discs, 15 in each group, were eroded by 1.0% citric acid and treated by ProNamel® (PRN, 1450 ppm fluoride), Colgate® Total Sensitive (CTS, 1100 ppm fluoride), a prototype dentifrice containing 5000 ppm fluoride and 2% PVM/MA copolymers (PVD) and distilled water (control). Fluoride release from each dentine disc was evaluated every 2 h in a 12-h period. For cycling erosive challenges, dentine discs were treated by dentifrice slurries twice daily, followed by immersion in saliva and erosive challenges by orange juice. Dentine discs were stored in saliva between treatment cycles and the cycling erosive challenges were repeated for 15 days. On days 5, 10, and 15, size of dentine tubule openings and level of dentine tubule occlusion were evaluated with a 3D scanning microscope.
Results: PVD released more fluorides than other dentifrices in a 12-h period ($p < 0.05$). CTS released more fluorides than PRN at 2, 4, 6, and 8 h following a single application ($p < 0.05$). The size of the dentine tubules was smaller and the number of occluded dentine tubules was greater in the CTS and PVD groups than those in the control and PRN groups on day 15 of the erosive challenges.
Conclusion: Bioadhesive PVM/MA copolymers facilitate fluoride retention and release from dentine discs, and promote dentine tubule occlusion and erosion prevention when combined with hydrated silica particles.

1. Introduction

Dental erosion is the softening and loss of dental hard tissues by a chemical process involving dietary or gastric acids. The prevalence of dental erosion has been steadily increasing in the past decades with widespread use of acidic beverages including carbonated drinks, fruit juices, energy and sports drinks.[1] A common sequela of dental erosion is the exposure of dentine tubules following the wear of cervical enamel and cementum,[2,3] which may lead to dentine sensitivity. Dentine sensitivity associated with exposed dentine tubules may persist until the open tubules are sealed or isolated from external stimuli.[4]

As dental erosion involves dissolution of hydroxyapatite akin to the chemical process of dental caries, its prevention and treatment often includes fluoride therapy using products such as dentifrices, mouthwashes and varnishes.[5–8] Remineralization effects of these products were associated with the concentration and retention of fluoride in oral cavities.[9]

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Enamel treated by dentifrices with higher fluoride concentrations was significantly more resistant to erosive challenges than those with lower fluoride concentrations,[10–12] presumably owing to the formation of a thicker and more stable layer of calcium fluoride precipitate at high fluoride concentrations.[13] Recent studies showed that 5000 ppm fluoride were more effective than 1450 ppm fluoride against erosive wear.[7,8] Therefore, products with high concentrations of fluoride should be an important part of the armamentarium against dental erosion in clinical practices.

In case of dentine exposure as a consequence of dental erosion, products that can occlude the exposed dentine tubules are often used to prevent and manage dentine sensitivity through the formation of a physical barrier that isolates dentinal nerves from external stimuli.[14,15] Though many products have been developed for dentine tubule occlusions, their effects are often temporary and dentine sensitivity may recur when the substances occluding the tubules are removed due to erosive and abrasive forces. Repeated applications of dentine occlusion agents are often necessary to sustain the treatment effects. Daily application through brushing with dentifrices is preferable for its ease of use and low costs as compared to frequent visits to dental offices.[16]

For a dentifrice to be effective in delivering fluoride to eroded dentine surfaces and in occluding dentine tubules, it needs to possess a property that allows adequate retention of the toothpaste substances on dentine surfaces. As the usual brushing duration lasts only 30–80 s,[17,18] the effects of fluoride on dental hard tissues are limited if the toothpaste substances cannot be adequately retained on the tooth surfaces after brushing. Fluoride needs to remain on dentine surfaces or inside the dentine tubules for an extended period of time to maximize the intended effects. Though there is evidence that fluoride can be retained in oral mucosa, human saliva and dental plaques,[19,20] the retention potential of fluoride in dentifrices on eroded dentine surfaces has not been studied. Depending on their intended effects, dentifrices may have different physical properties that affect their adhesion to tooth surfaces after application. Dentifrices that adhere to tooth surfaces and resist erosive challenges from dietary acids may have advantages over those without such properties in terms of their ability to prevent dental erosion and dentine sensitivity. Polyvinylmethyl esther-malic acid copolymers (PVM/MA) is a common bioadhesive polymer and have been widely used to improve tissue adhesion and bioavailability of therapeutic agents.[21] Recent studies have shown that dentifrices containing PVM/MA are effective in occluding dentine tubules through the formation of stable intra-tubular plugs.[22] Dentifrice substances appear to be retained on the dentine surfaces for several hours following a single brushing in situ owing to the adhesive properties of PVM/MA.[23]

Based on the findings of these studies, we hypothesize that dentifrices containing PVM/MA copolymer facilitate the retention of fluoride on eroded dentine surfaces, thus enhancing the action of fluoride against dentine erosion and demineralization. To test this hypothesis, we investigated the potential for fluoride release from dentine discs following a single application of dentifrices containing PVM/MA copolymer and fluoride in different concentrations, and assessed the level of dentine tubule occlusion and the effect of erosion prevention by this dentifrice following erosive challenges by orange juice in vitro.

2. Materials and methods

A total of 60 freshly extracted human third molars were collected from oral surgery clinics following ethic guidelines from the authors' institution. The teeth were cleaned of soft tissues and sterilized with ethylene oxide for 12 h (Andersen Products Inc., Haw River, NC, USA) before use.

2.1. *Dentine discs preparation*

Dentine discs were prepared from coronal sections of third molars as described elsewhere.[22] Briefly, occlusal enamel was removed in the coronal plane using a low speed diamond saw (MTI Corporation, Richmond, CA, USA) to expose the dentine, a parallel cut was then made above the cementoenamel junction to produce a dentine disc that is approximately 2 mm in thickness. The dentine specimens were then ground under adequate water coolants to remove any remnant enamel and progressively polished with 360–1200 grit silicon carbide papers (Extec®, Enfield, CT, USA) using a polishing machine (MTI Corporation). The dentine discs were ground and polished to 1.00 mm in thickness (±0.02 mm) as measured by an electronic digital caliper with 0.01 mm resolution (Mitutoyo America, Aurora, IL, USA). After polishing, the dentine discs were cleaned in an ultrasonic device with 2% Micro-90® cleaner (International Product Corp., Burlington, NJ) for 3 min. A total of 60 dentine discs were prepared. The dentine discs were then subjected to erosive challenge by 1.0% (0.052 mol/l) citric acid (PH 3.5) for 5 min and rinsed thoroughly for 2 min in running water. Dentine disc surfaces were inspected under a scanning microscope to confirm patency of dentine tubules, and randomly divided into 4 groups of 15 each. The size and thickness of the dentine discs were measured again with the digital caliper to ensure bulk equality among the study groups. Prepared dentine discs were placed in fresh clarified human saliva before next step of the experiment.

2.2. *Preparation of dentifrice slurries*

Three dentifrices were selected to test the hypothesis of the present study: Synsodyne ProNamel® (PRN) (GlaxoSmithKline, Middlesex, UK), which contains 1450 ppm fluoride in the form of 0.315% NaF and is marketed as protective against dental erosion; Colgate Total Sensitive (CTS) (Colgate-Palmolive Co., New York, USA), which contains 2.0% PVM/MA copolymers and 1100 ppm fluoride in the form of 0.243% NaF, and is marketed as protective against dentine sensitivity; and a prototype dentifrice (PVD) which contains 2.0% PVM/MA copolymers and 5000 ppm fluoride in the form of 1.1% NaF. Compositions of the study dentifrices are shown in Table 1.

Slurries of the three dentifrices, PRN, CTS and PVD, were prepared as follows: 3 g of dentifrice were mixed with 10 ml distilled water and shaken thoroughly to produce uniform toothpaste slurries. The slurries were prepared immediately before use and kept at room temperature.

Table 1 – Compositions of study dentifrices.

	ProNamel	Colgate Total Sensitive	Prototype
Active ingredients	Sodium fluoride 0.315% Potassium nitrate 5%	Sodium fluoride 0.243% Triclosan 0.3%	Sodium fluoride 1.1%
Abrasives	Hydrated silica	Hydrated silica	–
Bioadhesive copolymers	–	PVM/MA copolymer	PVM/MA copolymer
Other ingredients	Glycerin PEG-8 Cocamidopropyl betaine Sodium hydroxide Sodium saccharin Sorbitol Xanthan gum Titanium dioxide	Glycerin Propylene glycol Carrageenan Sodium lauryl sulphate Sodium hydroxide Sodium saccharin Sorbitol Carboxymethylcellulose Titanium dioxide	Hydroxyethylcellulose Pluronic F-127 Sorbitol Sodium saccharin Methyl paraben Propylparaben Titanium dioxide

2.3. *Determination of fluoride release after dentifrice treatment*

To determine the rate of fluoride release from the study dentifrices, dentine discs were divided into 4 treatment groups: PRN, CTS, PVD and distilled water (Control). The dentine discs, 15 in each group, were treated by dentifrice slurries or water for 2 min in a shaking incubator at 35 °C, then gently rinsed for 5 s and each disc immersed in 1.0 ml distilled water. Fluoride release from each dentine disc was measured every 2 h for a 12-h period (8:00 am to 8:00 pm). The dentine discs were transferred to fresh distilled water every 2-h during the 12-h period and fluoride release from each dentine discs into 1.0 ml distilled water was measured using a fluoride selective electrode (Thermo Electron, Bedford, OH, USA).

Fluoride concentration in distilled water was determined as described elsewhere.[24] Briefly, the collected fluid was transferred into a 15 ml tube, and buffered with 1.0 ml TISAB (Thermo Fisher, Waltham, MA, USA). The fluorometer was first calibrated using standard solutions (0.001, 0.01, 0.1, 1, and 10 ppm). A liner equation correlating the logarithm of F concentration (ppm) and mV was constructed with $r^2 > 0.95$. The mV and the ppm values were consistently in the liner region of the standard curve.

2.4. *Dentifrice treatment and erosive challenge cycling of dentine discs*

Following fluoride release test, the specimens were stored in fresh clarified human saliva overnight. Dentifrice slurries and distilled water were applied, respectively, to the dentine discs in the 4 treatment groups for 2 min each time in a shaking incubator at 35 °C. The dentine discs were then washed with distilled water for 10 s, stored in fresh human saliva for 30 min, and subjected to erosive challenge by orange juice. Each dentine disc was immersed in 2.0 ml orange juice (pH 3.8, Minute Maid™ Premium, Sugar Land, TX, USA) for 10 min in 24-well cell culture plates in an incubator gently rocking at 60 rpm and 35 °C to keep the solution agitated and simulate the fluid movement during the sipping of juice. Dentifrice treatments and subsequent erosive challenges were performed twice daily, at 9:00 am and 5:00 pm, respectively. Dentine discs were stored in fresh clarified human saliva when not subjected to dentifrice treatments and erosive challenges. Dentifrice treatment and erosive challenge cycles were repeated for a total of 15 days.

2.5. *Evaluation of dentine tubule occlusion and size*

At the end of 5, 10, 15 days, the size of dentine tubule opening and the level of dentine tubule occlusion were evaluated on images of the dentine surfaces obtained in the centre of specimens using focus-variation 3D scanning microscopy (IFM, InfiniteFocus® G4, Alicona Imaging, Graz, Austria) at a magnification of 2000×. The images were imported into a public domain imaging program (ImageJ®, developed at the U.S. National Institutes of Health) for analysis of open and occluded tubules and for measurement of the size of the dentine tubules. The Cell Counter function of the ImageJ® software was utilized to mark the open and the partially or fully occluded dentine tubules, and the rate of dentine tubule occlusion was calculated as percentage of tubules that were occluded in the examination fields. The size of the tubule was measured as the longest dimension of the dentine tubule opening.

2.6. *Statistics*

Fluoride release in ppm was compared among the 4 groups at 2, 4, 6, 8, 10, and 12 h after a single application. Dentine tubule size and percent of occluded dentine tubules were compared among the 4 groups on days 5, 10, and 15 of the dentifrice treatment and erosive challenge cycles. All data were expressed as median and interquartile range and nonparametric Kruskal–Wallis tests were used to compare fluoride release, dentine tubule size, and percentage tubule occlusion among the 4 study groups. A post hoc Mann–Whitney test with Bonferroni correction was used for pair-wise comparison between the study groups. Sample size estimation was based on the findings of published studies using dentine tubule occlusion as the primary outcome measure. A sample size of 14 in each group would have 80% power to detect a 20% difference in the mean percentage of occluded tubules between the two groups. We prepared 15 discs for each group.

Table 2 – Median fluoride release (ppm) from all dentine discs in each group at 2–12 h after a single application.

	2 h		4 h		6 h		8 h		10 h		12 h	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Control	0.004a	0.004	0.004a	0.001	0.004a	0.001	0.004a	0.001	0.004a	0.002	0.004a	0.002
PRN	1.137b	0.875	0.126b	0.086	0.062b	0.044	0.036b	0.020	0.028b	0.026	0.014b	0.015
CTS	2.214c	1.037	0.355c	0.266	0.113c	0.080	0.055c	0.032	0.031b	0.023	0.021b	0.019
PVD	4.540d	5.960	0.404c	0.244	0.132d	0.135	0.096d	0.111	0.060c	0.034	0.056c	0.027

Kruskal–Wallis and Mann–Whitney tests: different letters in the same column denote statistically significant difference in pair-wise comparisons ($p < 0.05$). PRN, ProNamel; CTS, Colgate Total Sensitive; PVD, 5000 ppm F with PVM/MA.

3. Results

3.1. *Fluoride release from dentine discs*

Median (IQR) fluoride release from dentine discs is listed in Table 2. There were statistically significant differences in fluoride release among the study groups. All 3 dentifrices exhibited more fluoride release than the control group ($p < 0.05$) (Fig. 1). Dentine discs in PVD group released more fluorides than other dentifrices at 2, 6, 8, 10 and 12 h following the treatment ($p < 0.05$). But there was no statistically significant difference in fluoride release between the PVD and CTS groups at 4 h after treatment ($p > 0.05$). CTS released more fluorides than PRN at 2, 4, 6, and 8 h ($p < 0.05$) but not at 10 and 12 h after the treatment.

3.2. *Size of dentine tubule openings*

Diameters of dentine tubule openings at different stages of the experiments are listed in Table 3. There were statistically significant differences in size of dentine tubule openings on days 5, 10 and 15 of the erosive challenges but not at baseline. On days 5 and 10, dentine tubule sizes were significantly greater in the Control, the PRN and the PVD groups than in the CTS group ($p < 0.05$). On day 15, dentine tubule sizes were significantly greater in the Control and the PRN groups than in the CTS and the PVD groups ($p < 0.05$). There were no statistically significant differences in dentine tubule size between the CTS and the PVD groups on day 15 of cycling erosive challenges ($p > 0.05$).

3.3. *Dentine tubule occlusion*

Proportions of fully and partially occluded dentine tubules at different stages of dentifrice treatment and erosive challenge cycles are listed in Table 4. CTS group had significantly higher percentage of fully occluded and lower percentage of open dentine tubules than those in the PRN and the PVD groups on day 5 and day 10 ($p < 0.05$). On day 15, both the CTS and the PVD groups have higher percentage of fully occluded and lower percentage of open dentine tubules than those in the PRN group ($p < 0.05$) (Table 4).

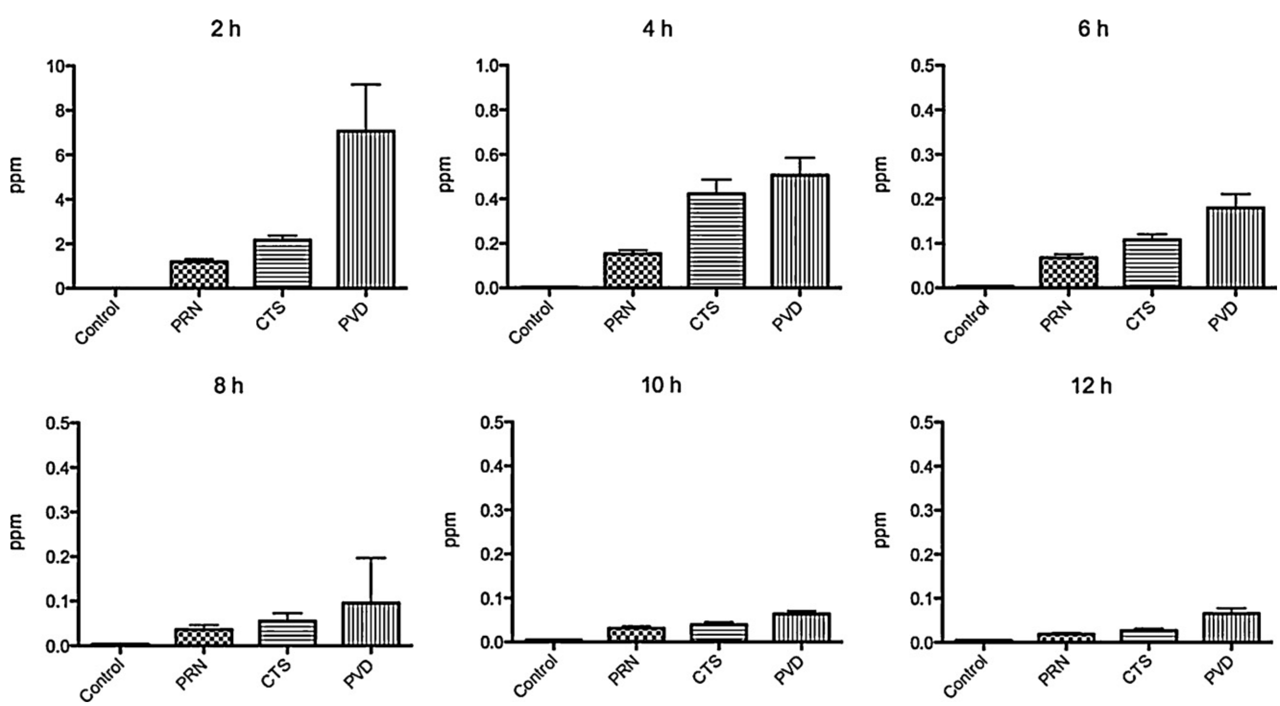

Fig. 1 – Fluoride release (ppm) from eroded dentine discs after a single application for 2 min.

Table 3 – Size of dentine tubule openings (μm) at different stages of the experiments.

	Baseline		Day 5		Day 10		Day 15	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Control	2.426a	0.502	2.794a	0.463	3.063a	0.422	3.619a	0.578
PRN	2.464a	0.384	2.698a	0.296	3.269a	0.667	3.524a	0.572
CTS	2.512a	0.419	2.500b	0.434	2.679b	0.221	2.764b	0.754
PVD	2.472a	0.679	2.716a	0.484	2.900a	0.519	2.846b	0.476

Kruskal–Wallis and Mann–Whitney tests: different letters in the same row denote statistically significant difference in pair-wise comparisons ($p < 0.05$). PRN, ProNamel; CTS, Colgate Total Sensitive; PVD, 5000 ppm F with PVM/MA.

4. Discussion

The findings of the present study indicate that dentifrice containing PVM/MA copolymers could improve the retention of fluoride on eroded dentine surfaces and sustain the release of fluoride for up to 8 h after a single brushing application when compared to dentifrice without the bioadhesive copolymers. The dentifrice with 5000 ppm fluoride and the copolymers released significantly higher amount of fluoride than those with lower concentrations of fluoride (1100–1450 ppm) in a 12-h period after one application. Dentine tubule occlusion appeared to be associated with the presence of the inorganic fillers and the PVM/MA copolymers, as the dentifrice that contains both (CTS) performed significantly better in occluding dentine tubules than those that contains either the filler (PRN) or the copolymers (PVD) alone. Dentine erosion prevention, as measured by the size of dentine tubule openings, is largely dependent on the ability of the dentifrice in occluding the dentine tubules, especially in the early stage of the 15-day erosive challenge cycles.

Though it is expected that the dentifrice with the higher concentration of NaF release more fluoride than those with lower concentrations, it is interesting to observe that CTS, which contains 0.243% NaF (or 1100 ppm F), had a consistently higher fluoride release than PRN, which contains 0.315% NaF (or 1450 ppm F) (Table 1). At 2, 4, 6 and 8 h after a single application of the dentifrices for 2 min, the amount of fluoride ions released from the dentine discs were 49%, 65%, 45% and 35% higher in CTS than in PRN, respectively. Improved fluoride release of CTS is likely associated with the ability of this dentifrice in adhering on the surfaces and tubule walls of the dentine. Previous studies have shown that brushing with CTS could result in the formation of intra-tubular plugs that were resistant to erosive challenges by orange juice in vitro[22] and in situ.[23] The PVM/MA copolymers are commonly used bioadhesives for controlled-release drug delivery as they could form weak chemical bonds with tissue surface proteins that aid the retention and gradual release of the therapeutic agents.[21,25,26] It has been shown that PVM/MA copolymers at 1.0% concentration can bind with Type I collagen, the most common protein on eroded dentine surfaces, in a 4:1 ratio.[23] Fluoride in dentifrices can be retained on dentine surfaces and inside dentine tubules and released with the hydrolysis of PVM/MA copolymers. Sustained release of low concentrations of fluoride after tooth brushing should improve acid resistance of dentine surfaces.

As tooth surfaces are often exposed to erosive challenges by acidic soft drinks, frequent treatments with high concentrations of fluoride have been shown to be more effective than other treatment regimes.[27] The prototype dentifrice containing 5000 ppm fluoride (PVD) was developed as a prescription only brush-on gel for dental erosion prevention. PVM/MA copolymers were added to PVD to improve the retention of fluoride on dentine surfaces based on the bioadhesive properties of these copolymers as described above. To minimize the potential abrasive effects on eroded tooth surfaces, no abrasive particles such as hydrated silica were added to this gel-like dentifrice. The PVD group did exhibit a superior fluoride release profile as compared to dentifrices with lower fluoride concentrations during a 12-h period following a single application (Fig. 1). However, the erosion prevention effects of PVD were less than ideal, especially at the early stages of the cycling erosive challenges. As the prescription strength fluoride gel in the PVD groups contains no abrasive fillers such as hydrated silica as in CTS and PRN, it did not adequately occlude the open dentine tubules when applied as slurry to the dentine surfaces. On days 5 and 10 of the 15-day erosive challenge cycling, most dentine tubules remained open, and the size of the dentine tubule openings increased as compared to the baseline, indicating dentine tissue loss. The erosive tissue loss appeared to be halted only after 10 days of use of the 5000 ppm fluoride gel in the PVD group as no significant changes in dentine tubule size was

Table 4 – Proportions of fully, partially occluded and open dentine tubules in the study groups at different stages of experiment.

	Full		Partial		Open	
	Median	IQR	Median	IQR	Median	IQR
Day 5						
PRN	10.3a	12.9	30.8	13.6	54.5a	16.3
CTS	42.3b	24.8	26.3	17.9	24.0b	21.6
PVD	10.0a	33.8	12.5	9.1	76.6a	46.2
Day 10						
PRN	34.0a	32.5	32.1	19.2	26.4a	26.3
CTS	50.7b	15.0	38.8	11.7	11.1b	7.6
PVD	26.1a	23.9	19.4	11.0	55.3c	32.5
Day 15						
PRN	34.0a	13.7	37.9	30.0	28.0a	27.0
CTS	61.8b	56.8	29.4	37.5	7.9b	18.3
PVD	68.4b	34.3	20.5	14.0	8.3b	26.2

Kruskal–Wallis and Mann–Whitney tests: different letters in the same row denote statistically significant difference in pair-wise comparisons ($p < 0.05$). PRN, ProNamel; CTS, Colgate Total Sensitive; PVD, 5000 ppm F with PVM/MA.

found from day 10 to day 15, and more dentine tubules were occluded on day 15 of the erosive challenge cycling (Tables 3 and 4). These findings suggest that the silica particles in dentifrices may have played an important role in dentine tubule occlusion and erosion prevention at an early stage of the dentifrice application. Addition of silica particles into PVD should improve its potential in dentine tubule occlusion immediately following the application, thereby improving its overall effectiveness in erosion prevention. This hypothesis awaits further experimental testing.

The findings of the present study provide important evidence for further refinements of multifunction dentifrices for oral health improvement.[28] With wide spread use of acidic beverages and ageing of the population, dental erosion and root dentine exposure are increasingly becoming a dental public health concern. Dentifrices that facilitate the retention and gradual release of therapeutic agents such as fluoride ions on eroded dentine surfaces and in dentine tubules will be beneficial for these patients. Dentifrices formulated with bioadhesive polymers and proper abrasive fillers appeared to be effective in occluding dentine tubules and prolong the retention and release of fluoride. However, these dentifrices as consumer products typically contain fluoride in concentrations below 1500 ppm. As the acidic challenges associated with dental erosion are particularly strong, oral applications of fluoride in higher concentrations ($\geq$5000 ppm) were more effective in erosion prevention than those in lower concentrations.[7,8,12,29] Addition of bioadhesive polymers and inorganic fillers such as hydrated silica particles may substantially improve the therapeutic efficacy of prescription products with high concentrations of fluoride.

In summary, the results of the present study support the hypothesis that bioadhesive PVM/MA copolymers facilitate fluoride release from dentine discs and enhance anti-erosive effects of dentifrices. Dentifrice containing both the PVM/MA copolymers and hydrated silica particles are more effective in dentine tubule occlusion and erosion prevention than those without the copolymers or the silica particles. These findings provide evidence for further improvement of oral hygiene products for prevention and management of dental erosion and dentine sensitivity. Dentifrice containing high concentration of fluoride, bioadhesive polymers and proper abrasive fillers may benefit patients with dental erosion and dentine sensitivity.

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