Clin Oral Invest (2013) 17:775–783
DOI 10.1007/s00784-012-0772-7

ORIGINAL ARTICLE

Dentin tubule occlusion and erosion protection effects of dentifrice containing bioadhesive PVM/MA copolymers

Qin Wang · Yang Kang · Virginia Barnes
William DeVizio · Ajay Kashi · Yan-Fang Ren

Received: 23 June 2011 / Accepted: 11 June 2012 / Published online: 30 June 2012
© Springer-Verlag 2012

Abstract
Objectives To study the effectiveness of a dentifrice containing polyvinylmethyl ether-maleic acid (PVM/MA) copolymer in occluding dentin tubules and investigate the interaction between PVM/MA and type I collagen using surface plasmon resonance (SPR).
Materials and methods Fifteen volunteers brushed dentin discs in situ using dentifrices with and without PVM/MA copolymer in a cross-over design. Dentin tubule occlusion was evaluated after brushing, after overnight saliva challenge in vivo for 12 h and after drinking 250 ml of orange juice. Dentin tubule occlusion and tubule size were compared between the two groups using repeated ANOVA and before and after erosive challenges using paired *t* tests. SPR using type I collagen as ligand and PVM/MA as analyte was performed to evaluate the binding of the two macromolecules.
Results A median of 91 % of dentin tubules were occluded after a single brushing in the PVM/MA group, as compared to 9 % in the controls. After overnight saliva challenge and 10 min of erosion by orange juice, a median of 73 % of the dentin tubules remained fully occluded in the PVM/MA group as compared to zero in the controls. Dentin tubule size increased after orange juice erosion in the controls but not in the PVM/MA group. SPR study showed that PVM/MA bound readily to collagen molecules in a 4 to 1 ratio.
Conclusions Dentifrice containing PVM/MA could effectively occlude dentin tubules and prevent dentin erosion. PVM/MA may improve adhesive retention of intratubular dentifrice plugs through binding to dentin surface collagen.
Clinical relevance Brushing with dentifrice containing adhesive polymers has preventive effect against dentin erosion and dentin sensitivity.

Keywords Dentin sensitivity · Dental erosion · Dentifrice · Adhesive polymer

Q. Wang · Y. Kang · A. Kashi · Y.-F. Ren (✉)
University of Rochester Eastman Institute for Oral Health,
625 Elmwood Avenue,
Rochester, NY 14620, USA
e-mail: yanfang_ren@urmc.rochester.edu

V. Barnes · W. DeVizio
Colgate Palmolive Technology Center,
Piscataway, NJ, USA

The prevalence of dental erosion is trending higher due to increasing consumptions of soft drinks, including fruit juices, carbonated beverages and sport and energy drinks [1, 2]. Dental erosion contributes to erosive and abrasive tooth wear, which often leads to exposure of dentin tubules and results in dentin sensitivity [3, 4]. Management of dentin sensitivity in patients with dental erosion is challenging due to the perpetual exposures of dentin surfaces to challenges from dietary acids. Dentin tubule occlusion is a common approach for effective management of dental sensitivity for it can isolate dentinal nerves from external stimuli that elicit the painful sensitive responses [5–8]. Though many products have been developed to occlude dentin tubules, the effects are often temporary and dentin hypersensitivity recurs when the substances occluding the tubules are removed due to the erosive challenges from daily dietary activities. Repeated applications of dentin occlusion agents are necessary to maintain the desensitizing effects. Daily application through brushing with toothpastes is preferable for its ease of use and low costs as compared to frequent visits to dental offices.

For toothpaste to be effective in occluding dentin tubules, it needs to possess a unique physicochemical property, that is, adequate adhesion to dentin surfaces to resist erosive challenges from acidic beverages and food substances. As the usual brushing duration is only 30 to 80 s [9, 10], the toothpaste substances that block the dentin tubules must remain inside tubules beyond the duration of brushing to achieve the intended effects. As toothpaste formulations differ depending on their claimed benefits, they may have different physical and chemical properties that affect their adhesion to tooth surfaces after application. Those toothpastes that can 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 dentin hypersensitivity.

Dentifrice formulations usually include thickening and binding agents composed of natural and/or synthetic macromolecules that may potentially adhere to dentin surfaces. Dentifrice containing a polyvinylmethyl ether-maleic acid [PVM/MA) copolymer has been shown to adhere to dentin surfaces and resists oral fluid and dietary acid challenges in vitro [11]. The PVM/MA copolymer appeared to be responsible for the formation of a protective layer that largely remained on the dentin surfaces after brushing and following immersion in human saliva and erosive challenges by orange juices. Numerous studies indicate that dentifrices containing PVM/MA and the antimicrobial agent triclosan have a sustained action against oral bacteria after a single application [12, 13], presumably due to adhesive retention of the active ingredients on oral tissue surfaces. In fact, various PVM/MA formulations have been used as a controlled release drug delivery system through adhesions to mucosal tissues [14–16]. PVM/MA molecules may adhere to tissue surface through van der Waals force and formation of predominantly hydrogen bonds with surface proteins such as mucin, a large glycoprotein with a high content of serine, threonine, and proline residues [17]. Adhesion of PVM/MA to dentin may also occur because of the presence of abundant proteins on dentinal surfaces. Human dentin is comprised of approximately 70 % hydroxyapatite, 20 % protein and 10 % water [18]. Type I collagen, the main type of protein on dentin surfaces, is composed of three long chains of over 14,000 amino acids twisted into a triple helix 300 nm in length and 1.5 nm in width [19, 20]. Exposed collagen on acid eroded dentin surfaces could potentially interact with bioadhesive polymers through the formation of chemical bonds.

Though experimental study in vitro indicates that dentifrice containing the PVM/MA co-polymer could adhere to dentin surfaces and seemingly form a stable protective layer [11], it is not known if the laboratory findings can be translated into outcomes of oral applications in human subjects. The effects of salivary flow and acidic beverage consumption may not be replicated accurately in laboratory settings. Therefore, the purpose of the present study was to examine the dentin tubule occlusion and anti-erosion effects of dentifrice containing the bioadhesive polymer PVM/MA in a randomized and controlled clinical study in situ. We also studied the interactions between type I collagen and PVM/MA using surface plasmon resonance to elucidate the adhesive potential of these two macromolecules.

Methods

Study subject selection and enrollment

This study was a controlled, double-blind, and cross-over interventional study. Fifteen male or female subjects who were in good health and willing to sign an informed consent were recruited to participate in this study. Subjects with the following conditions were excluded from the present study: periodontal diseases, two or more untreated carious lesions, impaired salivary flow, immune deficiencies, smoking, and taking oral liquid or chewable medications. The study protocol and the informed consent form were reviewed and approved by the institutional review board of the authors' institution. In the first phase of the study, the subjects were randomly assigned one of the two study dentifrices that were masked and coded. After a 1-week washout period, the subjects were given the second test dentifrice and began the second phase of the study.

Dentin sample preparation and base line evaluation

Dentin sample preparation followed the same procedures as reported elsewhere [11]. Briefly, freshly extracted human third molars were sterilized with ethylene oxide for 12 h. A total of 60 dentin discs, approximately 1.0 mm thick, were prepared from coronal sections of the teeth as follows: the occlusal part of the enamel was first cut with a slow speed diamond saw (SYJ150, MTI Corp., Richmond, CA, USA) to expose the dentin. A parallel cut was then made above the cementoenamel junction to produce a dentin disc that was approximately 2 mm in thickness. The dentin discs were ground on a 320 grits carbide plate to remove any remnants of the enamel on the occlusal side and the pulp horn on the pulp side of the disc, and polished with 320, 600, and 1,200 grits carbide polishing papers (Extec Corp, Enfield, CT, USA). The prepared dentin discs were cleaned for 3 min in an ultrasonic cleaner using 1 % Micro-90® cleaner (International Product Corp., Burlington, NJ, USA).

Images of the dentin surfaces were obtained in an area of 1,000 x 1,000 μm in the center of specimens using focus-variation 3D scanning microscopy (IFM, InfiniteFocus® G4, Alicona Imaging, Graz, Austria) at a magnification of x2,000. 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 dentin tubules. The Cell Counter function of the ImageJ® software was utilized to mark the open and occluded tubules, and the rate of dentin tubule occlusion were 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 dentin tubule opening. Observations and measurements of the dentin specimens were completed by a single examiner (QW).

Intraoral appliance for dentifrice treatment in situ

Dentin discs were mounted on the buccal flanges of an intraoral mandibular appliance and fixed in place with adhesive tapes and a silicone-based denture reline material (Sofreliner™ Medium Soft, Tokuyama Dental Corp., Tokyo, Japan) with the dentin surface exposed and facing the buccal mucosa. The location of the dentin discs was at the second premolar and the first molar areas on each side of the appliance. Enrolled subjects would wear the dentin disc embedded appliance during the study period. Each intraoral mandibular appliance carried two dentin discs, one on each side, on the buccal flanges. The subjects wore the appliances for a 24-h period during each treatment phase. After the initial sterilization with ethylene oxide, dentin discs were disinfected in 0.5 % chlorhexidine plus 70 % ethanol for 30 min before insertion into the mouth. The appliance was worn from 9:00 am in the morning and overnight till 9:00 am next day. During the treatment period, only water drinking was allowed while the appliance was being worn. The appliances were removed during eating lunch and dinner and stored in tap water. The subjects were allowed to brush their teeth with their regular dentifrice before the insertion of the appliance and at the conclusion of the study at the following morning.

Dentifrice treatments, erosive challenges, and dentin tubule evaluations

Study subjects were randomly assigned to one of the two treatment dentifrices, that is, the study dentifrice containing PVM/MA copolymer (Colgate Total Sensitive®, Colgate Palmolive Co., New York, NY, USA) and a control dentifrice without the copolymer from the same company (Colgate Cavity Protection®), respectively. Other than the copolymer, the chemical compositions of the two dentifrices are similar, with equal concentrations of fluoride in the form of 0.24 % sodium fluoride in a paste form. Starting at 9:00 am in the morning, subject was instructed to wear the appliance for 15 min and brush the exposed dentin surfaces with the assigned dentifrice for 30 s and rinse with tap water for 5 s. Before brushing, all subjects went through a practice session to calibrate the brushing force with the aid of a laboratory scale. A soft-bristle toothbrush (Colgate Wave®) was used for the brushing at a pressure of approximately 200 mg. Immediately after brushing, the dentin discs were removed and imaged with the 3D focus-variation scanning microscope as described earlier to assess the open and occluded dentin tubules. The dentin discs were then mounted on the mandibular appliance again. Subjects were instructed to wear the appliance till 9:00 pm in the night, when the dentin surfaces were brushed again for 30 s followed by rinsing with tap water for 5 s. The appliances were worn overnight to allow the dentin surfaces be subjected to a minimum of 12 h of salivary flow in vivo and were only removed at 9:00 am the following morning when the subjects reported to the study clinic. Study subjects were then allowed to brush their teeth as usual and eat breakfasts without the appliances. After imaging evaluation with the 3D focus-variation scanning microscope, the dentin discs were again mounted on the mandibular appliance. Subjects were instructed to wear the appliance for 15 min and then drink 250 ml of orange juice (Minute Maid® Plus, pH 3.8) in a 10-min period. Following the orange juice challenge, the dentin discs were removed and evaluated for the final time with the 3D microscope.

Surface plasmon resonance spectroscopy

Surface plasmon resonance (SPR) is a biosensing technology widely used to detect interactions among large molecules including DNA, proteins, and polymers [21, 22]. SPR-based optical sensors use special electromagnetic waves to probe interactions between an analyte molecule dissolved in solution and a ligand molecule immobilized on the SPR sensor surface. As the two molecules bind, the analyte molecule accumulates on the sensor surface and results in an increase in refractive index. This change in refractive index is measured in real time, and the result plotted as response or resonance units (RUs) versus time [23]. In this study, a Biacore-T100 SPR system (Biacore AB, Uppsala, Sweden) was used to explore the binding potential between PVM/MA molecules (Gantrez® AN119, ISP Inc., Wayne, NJ, USA) and bovine type I collagen (Kensey Nash Corporation, Exton, PA, USA). Briefly, 100 μg/ml type I collagen in 10 mM acetate coupling buffer was immobilized on a gold CM5 sensor chip™ (GE Healthcare, Piscataway, NJ, USA). Control flow cells were prepared using the coupling buffer only. After activating and blocking chip surfaces as described elsewhere [24], PVM/MA solution was series diluted to 50, 25, 12.5, 6.25, and 3.125 μM in the SPR flow buffer [0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005 % *v*/*v* Tween 20]. Samples were injected consecutively at 10 μl/min flow rate at 25 °C. Between runs, sensor surfaces were regenerated with buffer B [0.01 M HEPES pH 7.4, 4 M NaCl, 3 mM EDTA, 0.005 % *v*/*v* Tween 20] for 1 min. Results were analyzed using the Biocore T100 Evaluation Software (version 2.0.2). For calculation of the stoichiometry of analyte:ligand ($S_{A:L}$), the following equation was used:

$$S_{A:L} = \frac{R}{R_L * (MW_A / MW_L)} \quad (1)$$

[23]

Where R is the response units (RU) change when ligand is bound with analyte; R_L is the RU of ligand coupled to obtain R; MW_A and MW_L are molecular weights of analyte (PVM/MA, 200 kDa) and ligand (type I collagen, 300 kDa), respectively.

Statistical analyses

Dentin tubule occlusion was evaluated as fully occluded, partially occluded and fully open depending on the degree of obturation. Median and interquartile range (IQR) of fully and partially occluded and fully open dentin tubules were compared between the two study groups at different stages of the study using the Mann–Whitney test. Dentin tubule sizes were compared between the baseline and the final evaluation after orange juice erosion in the two groups using paired t test. Sample size estimation was based on the findings of laboratory study using dentifrice for dentin tubule occlusion. We estimated that dentifrice containing the PVM/MA copolymer could effectively occlude a mean of 80 % of dentin tubules (SD 15 %). 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 decided to enroll 15 subjects in each group.

Results

Dentin tubule occlusion

Brushing with dentifrice containing PVM/MA copolymer resulted in occlusion of a majority of the dentin tubules while brushing with the control dentifrice did not have such effect. As shown in Table 1, a median of 91.0 % of the dentin tubules were fully or partially occluded after a single brushing in the PVM/MA group, as compared to only 9.0 % in the control group. After wearing the appliance overnight, the proportion of fully occluded dentin tubules were 78.5 % in the PVM/MA group and 7.0 % in the control group. Acidic erosion challenge for 10 min with orange juice caused opening of almost all dentin tubules in the control group. After overnight salivary flow challenge and 10 min of erosion by orange juice, 73.0 % of the dentin tubules remained fully occluded in the PVM/MA group as compared to zero in the control group. Mann–Whitney tests showed that there were statistically significant differences between the groups in fully occluded and fully open dentin tubules at different measurement times (after brushing, after overnight and after orange juice erosion) (p<0.0001) (Table 2). Overnight salivary flow challenge and orange juice erosion caused a reduction in fully occluded dentin tubules and an increase in fully open dentin tubules in the control group but not in the PVM/MA group. The median proportion of fully open dentin tubules remained to be zero in the PVM/MA group in all stages of the experiments. The change in proportions of partially occluded dentin tubules showed a diverging trend between the two groups, with the proportion increasing from 9.0 % to 23.0 % in the PVM/MA group but decreasing from 17.0 % to 8.0 % in the control group (Table 2). As illustrated in Table 1, some of the fully occluded tubules became partially occluded but did not became fully open after overnight salivary flow and orange juice erosion challenges in the PVM/MA group, while the few fully and partially occluded dentine tubules became fully open after the saliva and orange juice challenges in the control group. At the conclusion of the study, a median of zero dentin tubules were fully open in the PVM/MA group as compared to 88.0 % in the control group.

As shown in Fig. 1, dentin tubules were almost uniformly occluded by intra-tubular plugs after brushing and rinsing in the PVM/MA group. The dentifrice substances adhered on the dentin surfaces and intra-tubular plugs were mostly retained after overnight salivary flow challenges in vivo (Fig. 1C). The intra-tubular plugs appeared to be stable and resistant to acidic erosion by

Table 1 Proportions of fully, partially occluded, and open dentin tubules after brushing with the study dentifrices, after overnight for a minimum of 12 h and after erosion by orange juice for 10 min

	PVM/MA						Control					
	Full		Partial		Open		Full		Partial		Open	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
After brushing	91.0	22.0	9.0	26.0	0.0	3.0	9.0	22.0	17.0	20.0	68.0	30.0
After overnight	78.5	49.5	18.0	26.0	0.0	4.8	7.0	15.0	16.0	26.0	74.0	40.8
After OJ erosion	73.0	39.0	23.0	31.5	0.0	4.0	0.0	8.8	8.0	12.0	88.0	29.0

Table 2 Results of Mann–Whitney *U* test in fully, partially occluded and open dentin tubules between the PVM/MA and control groups at different measurement times

	Full		Partial		Open	
	Z	*p*	*Z*	*p*	*Z*	*p*
After brushing	6.402	<0.0001	2.166	0.0303	6.224	<0.0001
After overnight	5.586	<0.0001	0.373	0.7083	5.378	<0.0001
After OJ erosion	6.116	<0.0001	2.812	0.0049	6.198	<0.0001

orange juice. Erosive challenge by drinking 250 ml of orange juice in 10 min did not significantly affect the appearance of the occluded dentin tubules in a majority of the cases (Fig. 1D). Though some fully occluded dentin tubules became partially open after the erosive challenges, a thin layer of the toothpaste substances appeared to remain on the tubule walls (Fig. 1).

In contrast, most dentin tubules remained to be patent after brushing in the control group (Fig. 1A′–D′). Overnight salivary flow challenge in vivo and acidic erosion by orange juice for

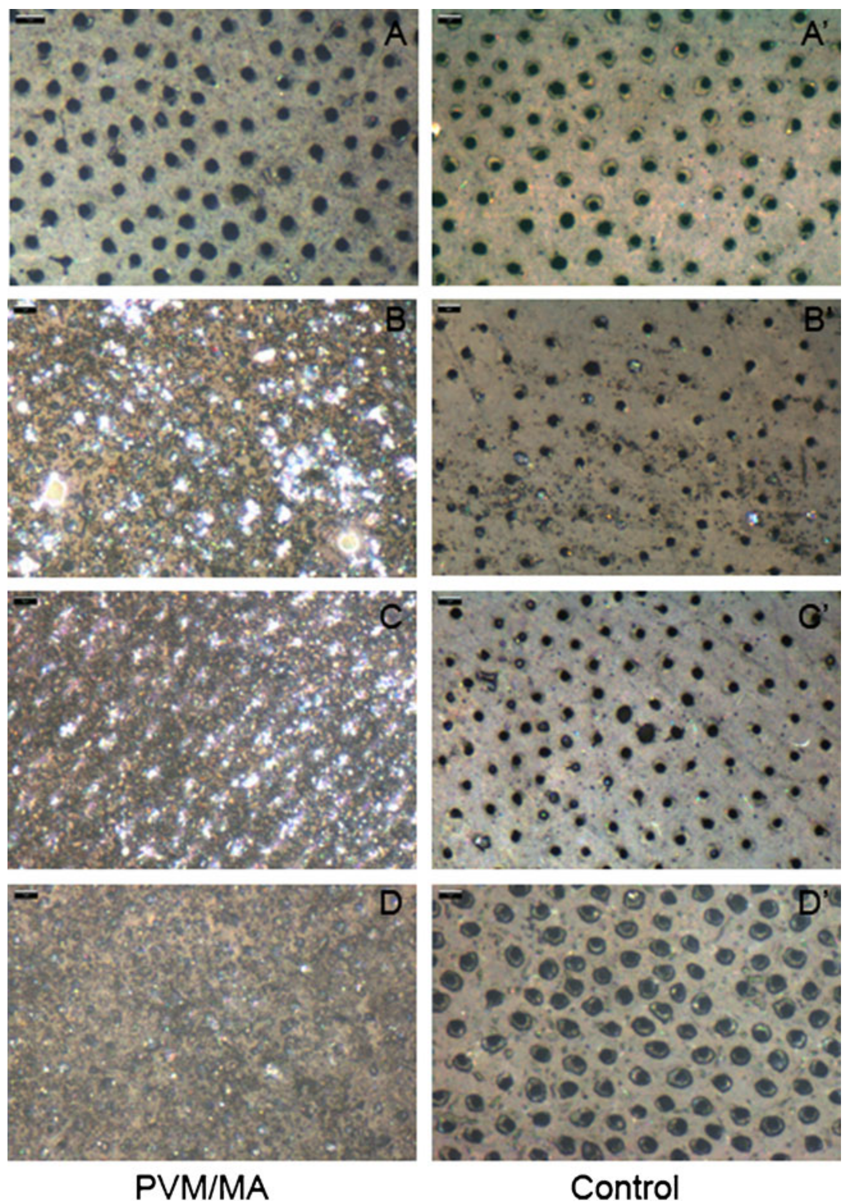

Fig. 1 Dentin discs at baseline, after brushing with dentifrices, after overnight, and after erosive challenges by drinking 250 ml of orange juice in 10 min in the PVM/MA (**A**–**D**) and the control group (**A′**–**D′**) in the same individual. Bar length=5 μm

10 min rendered fully open of most dentin tubules in the dentin discs (Fig. 1D′).

Size of dentin tubule opening before and after erosion

The size of dentin tubules, as measured by the longest dimension of tubule openings at baseline and after orange juice erosion, is listed in Table 3. There was a statistically significant increase in the size of dentin tubules after orange juice erosion in the control group ($p<0.0001$) but not in the PVM/MA group ($p>0.05$). As shown in Fig. 2, the diameters of the dentin tubule openings in the control group increased from a mean of 2.414 μm (SD 0.38) at baseline to a mean of 2.999 μm (SD 0.61), signifying a 24 % increase in diameter after orange juice erosion ($t=5.293$, $p<0.0001$), while those in the PVM/MA group remained unchanged after the erosive challenges (2.590±0.38 vs. 2.624±0.57 μm, $p>0.05$).

Surface plasmon resonance spectroscopy

The SPR kinetic sensogram and steady state affinity curve are shown in Fig. 3. PVM/MA molecules could readily bind to type I collagen as indicated by the rapid rise of the association phase (Fig. 3A). The binding between the two molecules were likely through weak, non-covalent chemical bonds as indicated by the rapid fall of the dissociation phase. In this study, 800 RU (R_L) of collagen was amine-coupled to flow cells using the immobilization wizard. The acquired RU (R) was 2,200 when PVM/MA concentration reached 50 μM (Fig. 3A and B). Using Eq. 1, the stoichiometry of PVM/MA:collagen ($S_{A:L}$) was calculated as 4, signifying that four PVM/MA molecules were bound to each collagen molecule on the sensor surface. As shown in Fig. 3B, PVM/MA collagen binding did not saturate (peak) at the PVM/MA concentration of 50 μM (1 %*w*/*v*), and increasing the concentration of PVM/MA is expected to result in higher number of PVM/MA molecules bound to collagen.

Discussion

The findings of this clinical study indicate that dentifrice containing PVM/MA copolymer was more effective in occluding dentin tubules than dentifrice without such a bioadhesive polymer in situ. Most of the intra-tubular plugs withstood challenges from overnight salivary flow and erosion by orange juice drinking in vivo. These findings largely confirmed the previous laboratory study in vitro and demonstrated that brushing with dentifrice containing PVM/MA copolymer should be helpful in reducing dentin sensitivity by occluding the dentin tubules. More importantly, the study dentifrice formed a protective layer on the dentin surfaces, which served as an effective barrier against further acidic challenges from orange juice and prevented erosive tissue loss.

Stability of the dentifrice plugs is essential for occluding the dentin tubules for the intervals that span the two brushing periods and maintains a sustained protective effect against erosion and dentin sensitivity. Addition of PVM/MA copolymer into the dentifrice formulation can account for the improved retention of dentifrice substances on the enamel surfaces through bioadhesion. Bioadhesion refers to the ability of synthetic and biological macromolecules and hydrocolloids to adhere to biological tissues [25]. Adhesion of polymers to dentin surfaces can potentially occur through four mechanisms: physical or mechanical bonds, electrostatic forces, secondary chemical bonds (such as hydrogen bonds), and primary or covalent chemical bonds. The best example of bioadhesion is resin adhesives used for composite resin restoration in dentistry, where all four bonding mechanisms may contribute to the permanent adhesion of resin composites to enamel and dentin surfaces [26]. Bioadhesives used in consumer hygiene products such as dentifrice must not interfere with the primary purpose of tooth surface cleaning, nor adversely affect the consistency and flavor of the dentifrice, which precludes the use of many polymers that may form strong bonds with dental hard tissues. The findings of the present clinical study showed that dentifrice containing PVM/MA adhered strongly to dentin surfaces when compared to that without such copolymer. Surface plasmon resonance showed that PVM/MA molecules bound readily with type I collagen, the main protein in human dentin. At the concentration of 50 μM (1 %*w*/*v*), one collagen molecule could bind at least four PVM/MA molecules on the gold sensor surfaces. In clinical applications on tooth surfaces, mechanical interlocking and electrostatic forces also play important roles, which will further enhance the adhesion of PVM/MA on dentin [26, 27]. The bioadhesive polymer bound on dentin surfaces constitutes a protective barrier that prevented the dietary acids from reaching the mineral substrates of dentin, thus reducing the risk of erosive tissue loss. As shown in Fig. 2, widening of dentin tubules after orange juice challenge was evident in the control group, but not in the PVM/MA group. Though some dentin tubules in the PVM/MA group were only partially occluded, dentifrice remnants on tubule wall surfaces

Table 3 Dimensions of dentin tubule openings at baseline and at final evaluation after orange juice erosion in the PVM/MA and control groups

	Baseline		After OJ erosion		*t* test	
	Mean	SD	Mean	SD	*t*	*p*
PVM/MA	2.590	0.383	2.624	0.567	0.247	0.8067
Control	2.414	0.378	2.999	0.610	5.293	<0.0001

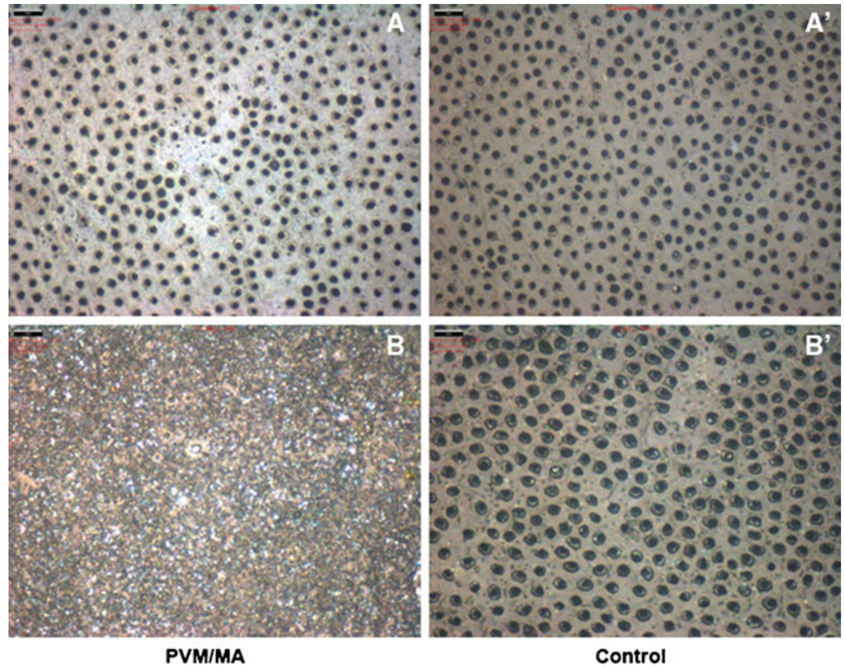

Fig. 2 Dentin discs at baseline (**A**, **A′**) and after erosive challenges by drinking 250 ml of orange juice in 10 min (**B**, **B′**). Erosion of dentin surface is evident in the control group as shown by enlarged dentin tubules after erosive challenges (**A′**) as compared to baseline (**A**). Dentin tubule enlargement is not seen in the PVM/MA group after erosive challenges (**B**) as compared to baseline (**A**). Bar length=10 μm

could still effectively fend off dietary acid challenges and keep the dentine tubule intact.

Numerous studies have shown the usefulness of macromolecule-based bioadhesives in clinical therapeutics because of their unique abilities in forming weak chemical bonds with human soft tissues, especially in controlled-release drug delivery for treatments of chronic diseases [28, 29]. The concept of controlled-release is also gaining increased popularity in dental applications in recent years [15, 30–32]. Macromolecules containing the hydroxyl (–OH) and/or carboxyl (–COOH) groups were considered to be the prime candidates for bioadhesive applications [25]. PVM/MA copolymer contains abundant carboxyl groups and has long been proven successful in dental applications [33–35]. It is believed that PVM/MA binds to the glycoprotein mucin on mucosal surfaces through mechanical interpenetration and weak chemical bonds [25, 36]. The findings of the present study showed evidence that PVM/MA molecules could readily bind to dentin surfaces, at least partly

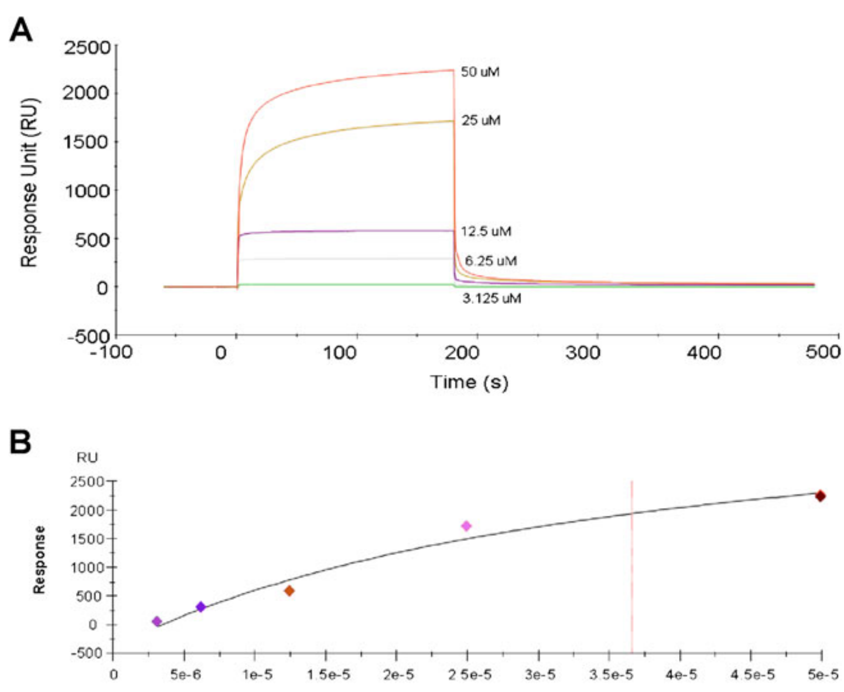

Fig. 3 Surface plasmon resonance of molecular binding between PVM/MA and type I collagen. **A** Kinetic sensogram of PVM/MA and collagen binding. Collagen (ligand) was immobilized on the sensor chip, and PVM/MA (analyte) was injected over the sensor surface at increasing concentrations as shown to the right of the sensogram. **B** Steady state affinity between PVM/MA and collagen, showing binding between the two macromolecules increases with increasing concentrations of the PVM/MA polymer

through interactions with type I collagen. The most likely binding mechanism was that of adsorption through weak chemical bonds including hydrogen bonds and van der Waals force because the affinity between the two macromolecules were not particularly strong as indicated by surface plasmon resonance (Fig. 3). It has recently been reported that type I collagen macromolecule could form various types of hydrogen bonds with synthetic polymer macromolecules [37]. Hydrogen bonds can be formed between the carboxyl groups of PVM/MA copolymer and the carboxyl, amide, and hydroxyl groups of collagen. No permanent covalent chemical bonds were formed between PVM/MA copolymer and collagen as shown by the steep dissociation curve of the molecule binding sensogram (Fig. 3). The impermanent nature of polymer-collagen binding is particularly desirable for controlled-release purposes for it will allow repeated delivery of active therapeutic agents in situ. Addition of bioadhesive polymers into oral hygiene products may significantly improve therapeutic efficacies by prolonged retention and sustained release of active ingredients such as fluoride and antimicrobial agents. In addition to PVM/MA copolymer, other macromolecules including polyacrylic acid, and combinations of carboxymethylcellulose, xanthan gum and polyvinylpyrrolidone-vinylacetate (PVP/VP copolymer) have been tested in laboratory studies and showed promising results in retention of triclosan and fluoride on dental hard tissue surfaces [31, 38]. Whether the beneficial findings of these studies in vitro can be translated into therapeutic efficacies in clinical applications still awaits further testing in clinical studies in vivo.

As the two dentifrices used in the present study have minor differences in formulation other than the presence of PVM/MA, there may be uncertainty with regard to the potential effects of other dentifrice ingredients on dentin tubule occlusion. As the ingredients that differ between the two dentifrices are small molecules (triclosan in the PVM/MA dentifrice and tetrasodium pyrophosphate in the control dentifrice) with no known adhesive or binding properties, their effects on dentifrice consistency and on dentin tubule occlusion are presumably negligible. Nonetheless, the potential effects of dentifrice formulations on dentin tubule occlusion and on resistance of intratubular plug to acidic challenges deserve further investigation. It is worth mentioning that the purpose of the present study is limited to the effects of dentifrices containing PVM/MA copolymer on dentin tubule occlusion and dentin erosion. The potential effects of this dentifrice on enamel erosion is not known. As the mineral and collagen contents are significantly different between dentin and enamel, the PVM/MA copolymer may show different adhesive properties on enamel in contrast to dentin, resulting in different outcomes after application. Recent evidence indicates that bioadhesion polymers could indeed adhere on enamel surfaces and play an important role in prevention of enamel erosion [31, 38]. We are encouraged to conduct further studies to investigate the effects of dentifrices containing PVM/MA copolymer on enamel erosion.

In summary, brushing in situ dentin samples with dentifrice containing PVM/MA copolymer could occlude dentin tubules and form a protective barrier that could prevent dentin erosion by orange juice. The intra-tubular plugs were stable and resistant to salivary and dietary acid challenges in vivo. Besides mechanical interlocking and electrostatic forces, PVM/MA could bind to dentin surface collagen through weak chemical bonds, contributing to the adhesion of dentifrice substances to dentin surfaces. These findings show that dentifrice containing bioadhesive polymers may have relevance clinically in treating dentin erosion and dentin sensitivity.

Acknowledgment This study was supported in part by the Basil G. Bibby Research Fellowship stipend to Dr. Qin Wang and a grant from Colgate Palmolive Company.

Conflict of interests Drs. Virginia Barnes and William De Vizio are employees of Colgate Palmolive Company.

References

1. El Aidi H, Bronkhorst EM, Truin GJ (2008) A longitudinal study of tooth erosion in adolescents. J Dent Res 87:731–735
2. Jaeggi T, Lussi A (2006) Prevalence, incidence and distribution of erosion. Monogr Oral Sci 20:44–65
3. Addy M, Pearce N (1994) Aetiological, predisposing and environmental factors in dentine hypersensitivity. Arch Oral Biol 39 (Suppl):33S–38S
4. West NX (2006) Dentine hypersensitivity. Monogr Oral Sci 20:173–189
5. Lee SY, Kwon HK, Kim BI (2008) Effect of dentinal tubule occlusion by dentifrice containing nano-carbonate apatite. J Oral Rehabil 35:847–853
6. Gandolfi MG, Silvia F, H PD, Gasparotto G, Carlo P (2008) Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine. J Dent 36:565–578
7. Fu B, Shen Y, Wang H, Hannig M (2007) Sealing ability of dentin adhesives/desensitizer. Oper Dent 32:496–503
8. Kolker JL, Vargas MA, Armstrong SR, Dawson DV (2002) Effect of desensitizing agents on dentin permeability and dentin tubule occlusion. J Adhes Dent 4:211–221
9. Macgregor IDM, Rugg-Gunn AJ (1985) Toothbrushing duration in 60 uninstructed young adults. Comm Dent Oral Epidemiol 13:121–122
10. Saxer UP, Barbakow J, Yankell SL (1998) New studies on estimated and actual toothbrushing times and dentifrice use. J Clin Dent 9:49–51
11. Liu X, Barnes V, DeVizio W, Yang H, Malmstrom H, Ren Y (2011) Effects of dentin tubule occlusion by dentifrice containing a PVM/MA bioadhesive copolymer in a silica base. J Dent 39:293–301
12. Arweiler NB, Auschill TM, Reich E, Netuschil L (2002) Substantivity of toothpaste slurries and their effect on reestablishment of the dental biofilm. J Clin Periodontol 29:615–621
13. Barnes VM, Richter R, DeVizio W (2010) Comparison of the short-term antiplaque/antibacterial efficacy of two commercial dentifrices. J Clin Dent 21:101–104
14. Andrews GP, Donnelly L, Jones DS, Curran RM, Morrow RJ, Woolfson AD et al (2009) Characterization of the rheological,

mucoadhesive, and drug release properties of highly structured gel platforms for intravaginal drug delivery. Biomacromolecules 10:2427–2435
15. Kockisch S, Rees GD, Tsibouklis J, Smart JD (2005) Mucoadhesive, triclosan-loaded polymer microspheres for application to the oral cavity: preparation and controlled release characteristics. Eur J Pharmacol Biopharmacol 59:207–216
16. Kockisch S, Rees GD, Young SA, Tsibouklis J, Smart JD (2003) Polymeric microspheres for drug delivery to the oral cavity: an in vitro evaluation of mucoadhesive potential. J Pharmacol Sci 92:1614–1623
17. Hombach J, Bernkop-Schnürch A (2010) Mucoadhesive Drug Delivery Systems. Handb Exp Pharmacol 197:251–266
18. Driscoll CO, Dowker SEP, Anderson P, Wilson RM, Gulabivala K (2002) Effects of sodium hypochlorite solution on root dentine composition. J Mater Sci Mater Med 13:219–223
19. Brodsky B, Ramshaw JAM (1997) The collagen triple-helix structure. Matrix Biol 15:545–554
20. Woodcock SE, Johnson WC, Chen Z (2005) Collagen adsorption and structure on polymer surfaces observed by atomic force microscopy. J Colloid Interface Sci 292:99–107
21. Maynard JA, Lindquist NC, Sutherland JN, Lesuffleur A, Warrington AE, Rodriguez M et al (2009) Surface plasmon resonance for high-throughput ligand screening of membrane-bound proteins. Biotechnol J 4:1542–1558
22. Willander M, Al-Hilli S (2009) Analysis of biomolecules using surface plasmons. Method Mol Biol 544:201–229
23. van der Merwe PA (2001) Surface plasmon resonance. In: Harding SE, Chowdry BZ (eds) Protein–ligand interactions: hydrodynamics and calorimetry. Oxford University Press, Oxford, pp 137–170
24. Xu Y, Gurusiddappa S, Rich RL, Owens RT, Keene DR, Mayne R et al (2000) Multiple binding sites in collagen type I for the integrins Œ ±1Œ≤1 and Œ±2Œ≤1. J Biol Chem 275:38981–38989
25. Peppas NA, Buri PA (1985) Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. J Control Release 2:257–275
26. Marshall SJ, Bayne SC, Baier R, Tomsia AP, Marshall GW (2009) A review of adhesion science. Dent Mater 26:e11–e16
27. Stupp SI, Kusleika R (1985) Surface charge on polymeric implants and calcified tissues: interfacial implications. J Biomed Mater Res 19:321–334
28. Andrews GP, Laverty TP, Jones DS (2009) Mucoadhesive polymeric platforms for controlled drug delivery. Eur J Pharmacol Biopharmacol 71:505–518
29. Piao J, Lee J-E, Weon K-Y, Kim D-W, Lee JS, Park JDS et al (2009) Development of novel mucoadhesive pellets of metformin hydrochloride. Arch Pharmacol Res 32:391–397
30. Owens TS, Dansereau RJ, Sakr A (2005) Development and evaluation of extended release bioadhesive sodium fluoride tablets. Int J Pharm 288:109–122
31. Zaman MA, Martin GP, Rees GD (2010) Bioadhesion and retention of non-aqueous delivery systems in a dental hard tissue model. J Dent 38:757–764
32. Zaman MA, Martin GP, Rees GD (2008) Mucoadhesion, hydration and rheological properties of non-aqueous delivery systems (NADS) for the oral cavity. J Dent 36:351–359
33. Andrade Acevedo R, Machon L, Chavez N (2009) Effectiveness of a mouthwash containing Triclosan and Gantrez in the reduction of biofilm and gingivitis: a clinical pilot study. J Contemp Dent Pract 10:E033–E040
34. Bolden TE, Zambon JJ, Sowinski J, Ayad F, McCool JJ, Volpe AR et al (1992) The clinical effect of a dentifrice containing triclosan and a copolymer in a sodium fluoride/silica base on plaque formation and gingivitis: a six-month clinical study. J Clin Dent 3:125–131
35. Panagakos FS, Volpe AR, Petrone ME, DeVizio W, Davies RM, Proskin HM (2005) Advanced oral antibacterial/anti-inflammatory technology: a comprehensive review of the clinical benefits of a triclosan/copolymer/fluoride dentifrice. J Clin Dent 16(Suppl):S1–S19
36. Esposito P, Colombo I, Lovrecich M (1994) Investigation of surface properties of some polymers by a thermodynamic and mechanical approach: possibility of predicting mucoadhesion and biocompatibility. Biomaterials 15:177–182
37. Sionkowska A, Skopinska-Wisniewska J, Wisniewski M (2009) Collagen-synthetic polymer interactions in solution and in thin films. J Mol Liq 145:135–138
38. Gracia LH, Brown A, Rees GD, Fowler CE (2010) Studies on a novel combination polymer system: in vitro erosion prevention and promotion of fluoride uptake in human enamel. J Dent 38: S4–S11

Copyright of Clinical Oral Investigations is the property of Springer Science & Business Media B.V. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.