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J Dent Res 93(12):1264-1269, 2014

ABSTRACT

Dental professionals are seeing a growing population of patients with visible signs of dental erosion. The approach currently being used to address the problem typically leverages the enamel protection benefits of fluoride. In this report, an alternative new block copolymer with a hydrophilic polyacrylic acid (PAA) block and a hydrophobic poly(methyl methacrylate) (PMMA) block was developed to similarly reduce the mineral loss from enamel under acidic conditions. This series of PMMA-*b*-PAA block copolymers was synthesized by reversible addition fragmentation transfer (RAFT) polymerization. Their structures were characterized by gel permeation chromatography (GPC) and ¹H nuclear magnetic resonance (NMR) spectra. The molar fractions of acrylic acid (AA) in the final block copolymer were finely controlled from 0.25 to 0.94, and the molecular weight (Mn) of PMMA-*b*-PAA was controlled from 10 kDa to 90 kDa. The binding capability of the block copolymer with hydroxyapatite (HAP) was investigated by ultraviolet-visible spectroscopy (UV-Vis) and Fourier transform infrared (FTIR) spectroscopy. FTIR spectra confirmed that the PMMA-*b*-PAA block copolymer could bind to HAP *via* bridging bidentate bonds. Both UV-Vis and FTIR spectra additionally indicated that a high polymer concentration and low solution pH favored the polymer binding to HAP. The erosion-preventing efficacy of the PMMA-*b*-PAA block copolymer in inhibiting HAP mineral loss was quantitatively evaluated by atomic absorption spectroscopy (AAS). Based on the results, polymer treatment reduced the amount of calcium released by 27% to 30% in comparison with the unprotected samples. Scanning electron microscope (SEM) observations indicated that PMMA-*b*-PAA polymer treatment protected enamel from acid erosion. This new amphiphilic block copolymer has significant potential to be integrated into dentifrices or mouthrinses as an alternative non-fluoride ingredient to reduce tooth erosion.

KEY WORDS: surface, binding, enamel, hydroxyapatite, RAFT polymerization, dental erosion.

DOI: 10.1177/0022034514551609

Received April 30, 2014; Last revision August 18, 2014; Accepted August 22, 2014

A supplemental appendix to this article is published electronically only at <http://jdr.sagepub.com/supplemental>.

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Synthesis of Carboxylic Block Copolymers *via* Reversible Addition Fragmentation Transfer Polymerization for Tooth Erosion Prevention

INTRODUCTION

Dental erosion, the mineral loss due to non-bacteria-associated acids, is a growing concern for dental professionals (Touger Decker and van Loveren, 2003; Huysmans *et al.*, 2011; Lussi *et al.*, 2011). Particularly, because of the daily consumption of large volumes of soft drinks, tooth erosion has become an escalating problem in oral health (West *et al.*, 2011) and has only recently received the same level of research focus as in the well-documented studies demonstrating the benefits of fluoride-containing products for caries prevention and treatment. Currently, incorporation of fluoride ions, the representative caries-preventive agent, into dentifrices or mouthrinses is regarded as one of the most common strategies to prevent tooth erosion. Although fluoride can effectively reduce the effects of erosion by inhibiting demineralization and enhancing remineralization (Featherstone, 1999, 2000, 2008), there is a need to develop alternative technologies to complement fluoride's benefits to provide more effective treatment options.

Recent findings disclosed that tooth-enamel-associated proteins such as amelogenins, enamelin, and mucins play key roles in initiating nucleation and controlling crystal growth and three-dimensional organizations, as well as maintaining the healthy condition of teeth (Simmer and Fincham, 1995; Fincham, 1999; Van Nieuw Amerongen *et al.*, 2004). The binding mechanisms between the protein and hydroxyapatite (HAP) or enamel have been extensively studied (Moreno *et al.*, 1984; Tanaka *et al.*, 1989; Wassell *et al.*, 1995; Fu *et al.*, 2005; Shen *et al.*, 2008). Although the exact mechanism remains unclear, the phosphate functional groups as well as the secondary structure of proteins are believed to be important to the mineralization and protection process (George and Ravindran, 2010; Chen *et al.*, 2011a).

Inspired by the strong tooth binding capability of the phosphate and carboxylic groups, this report describes the synthesis of a block copolymer containing one hydrophilic block (*e.g.*, polyacrylic acid, PAA) and another hydrophobic block [*e.g.*, polymethyl methacrylate (PMMA)] for the prevention of tooth erosion. The carboxylic block binds strongly to enamel, while the hydrophobic block has been designed to physically obstruct the acid attack, thus effectively reducing dental erosion. The block copolymers with varied molecular weights and acrylic acid (AA)/methyl methacrylate (MMA) ratios were synthesized by reversible addition fragmentation transfer (RAFT) polymerization (see online Appendix). HAP, the major mineral component of enamel, was used to assess the binding and erosion protection potential of the designed block copolymers. The HAP/polymer interactions were qualitatively and quantitatively evaluated by Fourier transform infrared (FTIR) and ultraviolet-visible (UV-Vis) spectroscopy. The efficiency of the carboxylic acid

block copolymers to reduce acid erosion on enamel was also evaluated by scanning electron microscopy (SEM).

MATERIALS & METHODS

Quantitative Evaluation of Erosion Prevention by Calcium Release from HAP Discs

Sintered HAP discs (Himed Inc., Old Bethpage, NY, USA) were pre-conditioned by immersion in 1% citric acid (pH = 2.5) for 15 min, soaked in water for 2 min, then sonicated in water for 30 min, and finally were washed with distilled water. After the HAP discs were mounted in 6-well plates by means of a Kerr impression compound (Kerr Inc., Orange, CA, USA), they were challenged with 5 mL of 1% citric acid (pH = 3.8) for 15 min in a water bath at 37°C with a shaking speed of 50 rpm. Aliquots of solution were then removed for determination of the initial calcium concentration, $[Ca]_{ref}$, by atomic absorption spectroscopy (AAS). The HAP discs were then washed in a 5 mL phosphate-buffered solution (PBS, pH = 7.0) and treated with the polymer solution for 2 min. After being washed with PBS, the HAP discs were again challenged with citric acid for another 15 min. Aliquots of solution were again removed for the measurement of calcium, $[Ca]_{treat}$. The relative calcium level (Ca level), $[Ca]_{treat}/[Ca]_{ref} \times 100\%$, was calculated to assess the protective efficiency against acid erosion. At least 4 HAP samples *per* treatment were evaluated. The control HAP discs were treated with Gantrez™ S95 (Ashland Inc., Covington, KY, USA), a copolymer with alternating/random maleic acid and methyl vinyl ether monomer units.

Morphological Evaluation of Erosion Prevention by SEM Observation on Enamel Blocks

Bovine teeth (Southeastern Dental Research Corp., Baton Rouge, LA, USA) were cut into 6 x 6-mm sections (also referred to as blocks). The surfaces of the enamel blocks were flattened by means of a 15- μ diamond polishing disk, then polished with Metadi 6- μ diamond suspension and Masterprep 0.05- μ polishing suspension (Buehler, Lake Bluff, IL, USA), respectively. The surface of each enamel block was pre-conditioned by immersion in citric acid solution (1%, pH = 3.8) for 5 min. Polymer solution (1.0 g/L at pH = 4.2) was used to treat the enamel surface for 2 min at room temperature. After the enamel was washed with PBS solution for 5 min, the polymer-treated enamel block was then subjected to an acid challenge with citric acid (1%, pH = 3.8) for 15 min at room temperature, without being stirred. Both the pre-conditioned and polymer-treated enamel samples were air-dried in preparation for SEM imaging.

Binding of the Block Copolymer to HAP Powder

The binding capacity of block copolymers onto HAP powder (< 200 nm, Sigma-Aldrich, St. Louis, MO, USA) was evaluated by UV-Vis spectroscopy based on the characteristic absorption from the C=S functional group associated with the chain transfer agent within the block copolymer (Skrabania *et al.*, 2011). Briefly, 5 mL of the block copolymer solution with different

Figure 1. Synthesis of PMMA-*b*-PAA via reversible addition fragmentation transfer (RAFT) polymerization and hydrolysis.

concentrations (0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 g/L) and pH (4.0 and 7.0) were mixed with 50 mg of HAP powder for 2 hr at room temperature. After centrifugation at 10,000 rpm for 10 min, the UV-Vis absorbance of polymer solution (*i.e.*, supernatant) before and after exposure to the HAP powder was recorded. The calibration curve was created with block copolymer solutions of known concentrations. HAP powder was separated by centrifugation. FTIR measurements of the air-dried powder were taken.

RESULTS

Synthesis of Block Copolymers by RAFT Polymerization

Fig. 1 illustrates the synthetic route for making the block copolymer *via* RAFT polymerization. Rather than direct polymerization, indirect polymerization (*i.e.*, polymerization of the hydrophobic monomer as the precursor, followed by hydrolysis to generate the hydrophilic carboxylic acid group) was utilized for the synthesis of the acidic blocks according to established methods (Ma and Wooley, 2000; Chen *et al.*, 2008). Specifically, because *tert*-butyl acrylate (tBA) can be hydrolyzed by trifluoroacetic acid (TFA) under mild conditions, the hydrophobic monomer of tBA was used as the precursor to generate the hydrophilic PAA block. Another hydrophobic monomer, methyl methacrylate (MMA), which was least affected by TFA hydrolysis, was used for constructing the hydrophobic block. Typical ¹H nuclear magnetic resonance (NMR) spectra of one block copolymer before and after hydrolysis and gel permeation chromatography (GPC) traces of all block copolymers (named as tBE1 – tBE5; see their compositions from online Appendix Table 2) are shown in Fig. 2. The detailed methods and characterization results are also described in the online Appendix.

Binding of Block Copolymer to HAP Powder

Polymer binding to HAP was evaluated by FTIR and UV-Vis spectroscopy. Based on the absorption of the C=S functional group (308 nm), UV-Vis spectroscopy was used for quantitative evaluation of the binding of the block copolymers to HAP (Skrabania *et al.*, 2011). Fig. 3A shows the absorbance of one of the block copolymers, tBE5, under different pH conditions. The

Figure 2. ¹H nuclear magnetic resonance (NMR) spectra of 1 block copolymer before and after hydrolysis. (A) PMMA-*b*-PtBA before hydrolysis in CDCl₃ and (B) after hydrolysis (converted into PMMA-*b*-PAA) in dimethyl sulfoxide (DMSO)-*d*₆, and (C) gel permeation chromatography (GPC) traces of PMMA-*b*-PtBA. The asterisks (*) indicate the peaks from solvents and impurities.

Figure 3. Evidence of polymer binding to HAP. (A) The ultraviolet–visible spectroscopy (UV-Vis) absorbance (308 nm) of the PMMA-*b*-PAA polymer solution before and after binding to hydroxyapatite (HAP). (B) The polymer quantity adsorbed onto HAP calculated based on the difference from Fig. 3A. (C, D) The Fourier transform infrared (FTIR) spectra of HAP (curve a), block copolymer (curve h), and HAP treated with the block copolymer at varied concentrations (curve b, 0.05 g/L; curve c, 0.1 g/L; curve d, 0.2 g/L; curve e, 0.5 g/L; curve f, 1.0 g/L; curve g, 2.0 g/L) and different pH (C, pH = 4.0; D, pH = 7.0).

characteristic absorbance of C=S was independent of pH and linearly increased with increasing polymer concentration. In the presence of HAP powder, the absorbance of the polymer aqueous solution decreased. The reduced absorbance reflects the loss of tBE5 from the solution phase, providing indirect evidence for

its adsorption to the HAP powder (Fig. 3A). The amount of polymer adsorbed onto HAP powder was calculated based on the absorbance-concentration calibration curve. Fig. 3B shows the amount of polymer adsorbed onto the HAP surface at varied polymer concentrations and pHs. At both pH values tested, the amount of the polymer adsorbed increased with increasing polymer concentration. The absorbance was independent of pH at polymer concentrations below 0.2 g/L. However, when the polymer concentration was higher than 0.2 g/L, more polymers were adsorbed at the lower pH (pH = 4.0) than at the higher pH (pH = 7.0). For example, when the original polymer concentration was 1.0 g/L, the polymer adsorbed onto HAP was 100 mg/g HAP at pH = 4.0, but only 48 mg/g HAP at pH = 7.0, respectively.

Figs. 3C and 3D show the FTIR spectra of pristine HAP, polymer-treated HAP, and the pure block copolymer. The polymer-treated HAP exhibited a new peak at 1,550 cm⁻¹, whose intensity increased with increasing polymer concentration. This concentration-dependent increase suggests that the new peak is associated with polymer-bound HAP. When the polymer concentration was 2.0 g/L, another peak at 1,700 cm⁻¹ was detected for the polymer-treated HAP. This peak could be associated with the symmetric stretching vibration of the free carboxylate group. The observation of this band could suggest that there is another type of bond that forms between –COO⁻ and the

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Ca mineral at higher (e.g., 2.0 g/L) polymer concentrations.

Reduction of Acid Erosion

SEM images (Fig. 4) of the acid-challenged enamel blocks with or without polymer treatment were examined for comparison of the surface morphology of enamel and evaluation of the enamel-protective efficacy of the prepared polymers. The polymer-protected enamel after erosion (Fig. 4D) shows surface characteristics similar to those of sound enamel (Fig. 4B). In contrast, the unprotected enamel after erosion (Fig. 4C) shows a rougher surface structure and wider enamel inter-prism gaps than the polymer-treated enamel in Fig. 4D.

In addition to the change in enamel surface morphology, the mineral loss of HAP under acidic conditions was measured by AAS for quantitative evaluation of the degree of erosion prevention of the PMMA-*b*-PAA polymer. Fig. 4E shows that the block copolymers could reduce the Ca level from 90.4% measured for untreated control to 65.3% and 58.8% for the HAP discs treated with tBE4 and tBE5, respectively. No significant difference was observed between the HAP discs treated with tBE3, tBE4, and tBE5. More importantly, the PAA homopolymer ($M_w = 50$ kDa, Polysciences Inc., Warrington, PA, USA) and the alternating copolymer containing carboxylic acid (Gantrez™) both exhibited no significant difference in reducing the release of Ca ion in comparison with the untreated control.

DISCUSSION

The RAFT polymerization technique has previously been used to synthesize block copolymers, given the relatively mild requirements, good controllability of the polymer structure, and biocompatibility of reagents used (McCormick and Lowe, 2004; Boyer *et al.*, 2011). An indirect polymerization method that involved preparation of the PtBA hydrophobic block followed by hydrolysis to form the PAA hydrophilic block (Ma and Wooley, 2000; Strandman *et al.*, 2006; Chen *et al.*, 2008) was chosen to synthesize the amphiphilic block copolymers, PMMA-*b*-PAA. The indirect method avoided some of the disadvantages of the direct polymerization method, e.g., difficulty in choosing solvent because of the presence of both polar and non-polar monomers, poor controllability of the polymer structure, high viscosity when the molecular weight (M_n) of the amphiphilic (co)polymers reached high levels, inaccuracy of characterization because of micelle formation, and possible cleavage of S-C=S bonds from the chain transfer agent under acidic conditions.

Analysis of both GPC and ^1H NMR data confirmed the success of RAFT synthesis of the block copolymers. The variation in the elution time in GPC (Fig. 2C) confirmed the ability of the RAFT method to control the molecular weight. The

Figure 4. SEM images of enamel blocks under various treatment conditions. (A) Sound enamel without polymer treatment or acid challenge. (B) Enamel treated by block copolymer. (C) Enamel without polymer treatment after acid erosion of 15 min. (D) Polymer-treated enamel with acid erosion of 15 min. (E) The level calcium released from polymer-treated hydroxyapatite (HAP) due to acid erosion. ‘—’ indicates no statistically significant difference, p value $> .05$. The HAP was treated with different polymers, e.g., homopolymer polyacrylic acid (PAA), an alternating copolymer Gantrez™, and carboxylic block copolymers with various molecular weights (tBE3, tBE4, tBE5).

disappearance of the peak of the *tert*-butyl groups ($\delta = 1.3$ – 1.5) in the ^1H NMR spectra of PMMA-*b*-PAA in comparison with PMMA-*b*-PtBA (Figs. 2A, 2B) indicated that tBA was completely converted to acrylic acid by hydrolysis. The compositions of the block copolymers, including the M_n and mole fraction of each monomer, are shown in Appendix Table 2. Based on the one-pot RAFT subsequent polymerization described in this study, the M_n of PMMA block within each PMMA-*b*-PAA block copolymer was well-controlled, as evidenced by the similar PMMA lengths. The PAA block mole fractions varied from 0.23 to 0.94 based on the GPC and NMR calculations. In summary, PMMA-*b*-PAA with controlled molecular weight and mole fraction of the hydrophilic block could be successfully prepared by the indirect RAFT polymerization method, followed by selective hydrolysis.

The FTIR and UV-Vis measurements (Fig. 3) provided qualitative and quantitative evidence of polymer-HAP binding, which is an essential requirement for polymer-associated protection against erosion. Although the UV-Vis absorbance of the pure polymer solution was independent of pH, polymer adsorption onto HAP was pH-dependent, particularly when the polymer concentration exceeded 0.2 g/L. When the polymer concentration was higher than 0.2 g/L, a greater amount of polymer was adsorbed to HAP at pH 4 in comparison with that in neutral conditions (pH = 7). The quantity of the adsorbed polymer in Fig. 3B confirmed that more polymers were adsorbed onto HAP at higher polymer concentrations. However, polymer binding to HAP shows different behavior at low polymer concentrations in comparison with that at high polymer concentrations. At polymer concentrations less than 0.2 g/L, polymer binding was less affected by pH. This implied that most of the polymers could be adsorbed onto HAP surfaces when the polymer/HAP ratio was relatively low. Under acidic conditions, HAP powder was capable of binding more than two-fold the amount of the block copolymer in comparison with what occurred under neutral conditions. This result could be attributed to the increased HAP surface area resulting from acid

etching. This phenomenon is consistent with other reports describing the adsorption of bovine serum albumin (Wassell *et al.*, 1995), dentin proteins (Boonstra *et al.*, 1992), and amino acids (Tanaka *et al.*, 1989) onto HAP.

Analysis of the FTIR data further confirmed the polymer binding to HAP. The vibration frequencies for $V_{\text{asym}}(\text{COO}^-)$ and $V_{\text{sym}}(\text{COO}^-)$ at 1,550 and 1,410 cm^{-1} , respectively, suggested the formation of the bridging bi-dentate structure between the COO^- and Ca^{2+} (McCluskey *et al.*, 1989; Jones *et al.*, 1998; W Chen *et al.*, 2011). The new peak at 1,550 cm^{-1} from the polymer-treated HAP confirmed that the polymer was adsorbed onto HAP. Unlike the characteristic peak of carbonyl at 1,700 cm^{-1} of the pure PMMA-*b*-PAA, this peak has a significant red-shift. The red-shift of carbonyl from 1,700 cm^{-1} to 1,550 cm^{-1} indicated that the carboxylic block copolymer could strongly bind with calcium *via* a 'bi-dentate bond' (Jones *et al.*, 1998). At 2.0 g/L of polymer, the peak at 1,700 cm^{-1} , which was similarly observed for the free, unbound polymer, was detected again. The appearance of the 1,700- cm^{-1} band suggested that the polymer coordination to HAP at the higher concentrations may also include the physically adsorbed polymer without strong interaction.

The SEM and particularly the AAS results provided evidence that the PMMA-*b*-PAA block copolymer protected the enamel surface from acid erosion. Based on the SEM images, the surface morphology of the polymer-treated enamel was less affected by the acid challenge in comparison with the untreated surface (Figs. 4B, 4D). Conversely, the untreated enamel surface was rougher, with wider inter-prism gaps after the acid challenge (Figs. 4A, 4C). This confirmed that the carboxylic block copolymer could effectively protect the integrity of the enamel surface.

AAS (Fig. 4E) provided quantitative information on erosion prevention introduced by PMMA-*b*-PAA. The decreased calcium level measured *in vitro* for the block copolymer-treated HAP discs (tBE3, tBE4, and tBE5) compared with the untreated HAP indicated an approximate 27% to 30% protective benefit from the polymer treatment. Moreover, the Ca release from the polymer-treated HAP was less than from the PAA homopolymer as well as the GantrezTM-treated HAP discs. GantrezTM, an ingredient found in toothpaste (*e.g.*, Colgate TotalTM), is an alternating copolymer containing hydrophobic methyl vinyl ether and hydrophilic carboxylic acid and is somewhat compositionally similar to, but structurally quite different from, the block copolymers described in this article. Although it is true that the Mn and mole fraction of the hydrophilic group/hydrophobic group of GantrezTM are not the same as those of the prepared block copolymers, GantrezTM does possess the critical carboxylic acid groups to bind to HAP and possesses the hydrophobic functionality that could repel acids. Clearly, its reduced ability to protect against acid challenges suggests that a block structure may provide more protective benefits than an alternating/random polymer design. The importance of the hydrophobic block is further demonstrated in light of the results obtained for the PAA homopolymer, which offered less protection than every PMMA-*b*-PAA block copolymer described in this publication.

Although the above conclusion may not be generalized to any other block copolymers, the better anti-erosion effect of the block copolymer than those of the PAA homopolymer and GantrezTM alternating copolymer confirmed the potential for the incorporation of these block copolymers into dentifrices or mouthrinses for erosion prevention. However, additional experiments such as contact angle and surface characterization are necessary in the future to provide further evidence to support our hypothesis that the hydrophilic PAA block binds to HAP, while the PMMA block forms a hydrophobic and resistant layer, which prevents acid attack.

Although we hypothesized that the variation of the hydrophobic/hydrophilic block ratio and Mn of the block copolymer might affect the anti-erosion efficacy of the prepared block copolymers, the significant difference between the molecular weight and block ratio for erosion prevention was not observed in this study. According to the hypothesis, controlling the hydrophobic/hydrophilic block ratios improves the binding to HAP (*via* the hydrophilic group) and allows for the formation of a hydrophobic layer on HAP to limit acid access to the mineral. However, there was no erosion protection advantage in increasing the molecular weight of the PMMA-*b*-PAA from 10 kDa to 90 kDa.

A possible reason might be due to the fact that the anti-erosion efficacy is a tradeoff between the amount of polymer adsorbed onto HAP and the hydrophobicity of the polymer. While the polymer adsorption depends on the hydrophilic PAA block length, the hydrophobicity depends on the PMMA block. A longer PAA segment may lead to stronger binding and greater quantities of polymers adsorbed to HAP. However, more acid groups from the longer PAA block could lead to increased hydrophilicity, thus decreasing the anti-erosion effect. Conversely, a longer hydrophobic block would presumably lead to greater anti-erosion efficacy, but its reduced water solubility from the longer hydrophobic PMMA block led to less polymer-HAP binding. Therefore, although tBE3 has relatively more hydrophobic MMA than tBE5, the relatively low water solubility of tBE3 in comparison with tBE5 may limit its adsorption onto HAP, thus no significant difference was observed between tBE3, tBE4, and tBE5 (see the online Appendix) (tBE1 and tBE2 could hardly dissolve in water, and thus no anti-erosion experiments were completed for these block copolymers). The tradeoff between hydrophobic PMMA block and the adsorption onto HAP determined by the PAA block may lead to an optimized fraction of MMA/AA and Mn for the best anti-erosion effect. To determine the above variables, more work has to be completed for precise control of the block copolymer structure and optimization of the hydrophobic/hydrophilic ratio.

In summary, carboxylic acid block copolymers with controlled hydrophobic/hydrophilic ratios were synthesized by indirect RAFT polymerization, followed by hydrolysis. Polymer compositions were characterized by gel permeation chromatography (GPC) and ¹H NMR spectroscopy. These carboxylic acid block copolymers could strongly bind to HAP, presumably through bridging bi-dentate bonds. Higher polymer concentrations and lower pH values facilitated polymer binding to HAP

and enamel. The polymer treatment could effectively reduce mineral loss from HAP by 27% to 30% in comparison with the unprotected HAP and was able to effectively protect the surface integrity of enamel from acid erosion. Therefore, this amphiphilic block copolymer has great potential to be used as an alternative non-fluoride ingredient in dentifrices or mouthrinses to reduce tooth erosion.

ACKNOWLEDGMENTS

This project is financially supported by Colgate-Palmolive Company and by the National Institutes of Health (NIH) of the USA (NIH/NIDCR grant R01DE021786). We also thank Dr. Earl M. Kudlick for initiating this exciting project, Dr. Charles Hosten for assistance with atomic absorption testing, and Dr. Laurence C. Chow (American Dental Association Foundation, Dr. Anthony Volpe Research Center, National Institute of Standards and Technology, Gaithersburg, MD, USA) for helpful discussions. Dr. Wang reports one research grant from Colgate-Palmolive Company, during the conduct of the study, but that had no influence or bias on the research results. In addition, Dr. Wang has a patent application pending. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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