Published in final edited form as:

RSC Adv. 2014 January 1; 4(90): 49053–49060. doi:10.1039/C4RA08377F.

Bioinspired amphiphilic phosphate block copolymers as non-fluoride materials to prevent dental erosion

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Abstract

Inspired by the fact that certain natural proteins, e.g. casein phosphopeptide or amelogenin, are able to prevent tooth erosion (mineral loss) and to enhance tooth remineralization, a synthetic amphiphilic diblock copolymer, containing a hydrophilic methacryloyloxyethyl phosphate block (MOEP) and a hydrophobic methyl methacrylate block (MMA), was designed as a novel non-fluoride agent to prevent tooth erosion under acidic conditions. The structure of the polymer, synthesized by reversible addition-fragment transfer (RAFT) polymerization, was confirmed by gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR). While the hydrophilic PMOEP block within the amphiphilic block copolymer strongly binds to the enamel surface, the PMMA block forms a hydrophobic shell to prevent acid attack on tooth enamel, thus preventing/reducing acid erosion. The polymer treatment not only effectively decreased the mineral loss of hydroxyapatite (HAP) by 36–46% compared to the untreated control, but also protected the surface morphology of the enamel specimen following exposure to acid. Additionally, experimental results confirmed that low pH values and high polymer concentrations facilitate polymer binding. Thus, the preliminary data suggests that this new amphiphilic diblock copolymer has the potential to be used as a non-fluoride ingredient for mouth-rinse or toothpaste to prevent/reduce tooth erosion.

Introduction

Dental caries and dental erosion are two major problems in oral health ^{1,2}. With the development of caries prevention strategies during the past five decades, the incident ratio of untreated dental caries has been significantly reduced. As an example, the incident ratio for

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†Electronic Supplementary Information (ESI) available: [details of synthesis and characterization of polymethyl methacrylate block polyacrylic acid (PMMA-b-PAA), FTIR spectra of PMMA-b-PAA treated enamel, relative intensity ratio of (I₁₇₃₀/I₁₀₂₂), UV-Vis spectra of PMMA-b-PAA before and after binding with HAP]. See DOI: 10.1039/b000000x/

‡Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

adolescents (6–19) in the United States has been reduced to 15.6% (2007–2010) from 54.7% in the 1970s³. In contrast, dental erosion, i.e. mineral loss due to acidic food or soft drinks^{4, 5}, is becoming a major dental problem. Because of the strikingly increased consumption of soft drinks⁶, tooth erosion caused by external acids is becoming more of an issue, especially for young people². According to a recent report, enamel loss caused by drinking soda pops could be as high as 3 mm per year⁷. The situation is even worse for those people experiencing gastroesophageal reflux, because the acid regurgitated from the stomach into the mouth is a lower pH, stronger acid which could erode the teeth to a far greater extent⁸. However, in comparison to dental caries, the impact of dental erosion is significantly underestimated both by the dentist and by the patient. Often, dental erosion isn't realized as a problematic situation until the condition clinically presents itself later in the form of increased dentin exposure and tooth sensitivity.

Currently, the most common treatment for the inhibition of tooth erosion is the use of fluoride, which can be included in toothpastes, mouth-rinses, and varnishes. Fluoride can protect and remineralize the tooth's surface via the formation of fluoroapatite or protection of the enamel from dissolution by adsorbing to crystal surfaces^{9–12}. However, over exposure to fluoride may lead to potential risks such as fluorosis and bone fracture^{13–15}. Therefore, development of non-fluoride materials for tooth erosion prevention and remineralization is an alternative route for tooth care.

As a representative example, casein phosphopeptide stabilized amorphous calcium phosphate (CPP-ACP)^{16, 17} has been found to be able to protect enamel from acid erosion in some in vitro and in situ studies. Similarly, other natural or synthetic proteins^{18–26} and peptides^{27–30} (e.g. salivary proteins such as mucins, statherin, and proline rich proteins) were reported to be able to bind on the tooth surface and protect the tooth from acid attack or aid in the remineralization of the tooth^{18, 31, 32}. While proteins or peptides can avoid the negative effect, the costs of producing these materials are high^{27, 33, 34} and they may have the risk of allergic reactions for certain populations. Therefore, it is of great importance to develop an alternative non-fluoride agent which is able to effectively protect the tooth against acid erosion and/or enhance tooth remineralization safely at low cost.

Recently, a new block copolymer containing carboxylate groups has been synthesized and was proved to have the effect to effectively prevent tooth erosion³⁵. Inspired by the tooth binding and erosion capability of the phosphate group from phosphopeptides^{20, 36}, a new polymer was synthesized utilizing phosphate monomers in order to improve binding capability toward tooth. In addition, a hydrophobic block was incorporated in order to form a hydrophobic barrier which can prevent acid attack to the tooth and/or calcium release from tooth enamel. In the meantime, the phosphate groups within the hydrophilic block can form the binding layer to immobilize the polymer onto the surface of enamel, thus enhancing its retention time. The main goal of this paper is to describe the synthesis and performance characteristics of a synthetic amphiphilic diblock copolymer, which contains a methacryloyloxyethyl phosphate (MOEP) block and a hydrophobic methyl methacrylate (MMA) block, and to scrutinize its effectiveness as a new protective material for preventing tooth erosion. In order to obtain a block copolymer with well controlled molecular weight but avoid the potential cytotoxicity of the copper catalyst usually used for atom transfer

radical polymerization (ATRP), reversible addition-fragment transfer (RAFT) polymerization was employed to synthesize the block copolymer. FTIR and NMR spectroscopy were used to characterize the chemical structure of the block co-polymer and its binding capacity to hydroxyapatite (HAP) and enamel. The block co-polymer's efficacy of acid erosion prevention was also examined. The potential mechanism of erosion prevention is discussed.

Materials and Methods

Materials

Methyl methacrylate (Acros, 99%) was purified through a silica column before use. Hydroxyapatite (HAP) powder was purchased from Sigma-Aldrich. 2,2'-azobisisobutyronitrile (AIBN) was recrystallized in ethanol before use. Monoacryloxyethyl phosphate (MOEP, Polysciences, 97%) and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid (Sigma-Aldrich, 97%) were used as the chain transfer agent (CTA) without further purification. Bovine teeth were purchased from Southeastern Dental Research Corp (Baton Rouge, LA). The center buccal section of the tooth was cut into 6×6 mm sections using a Horico diamond saw. The surface of the enamel block was flattened using a 15u diamond polishing disk followed by polishing with Metadi 6u diamond suspension and Masterprep 0.05u polishing suspension (Buehler, IL), respectively.

Synthesis of PMMA-*b*-PMOEP

Because reversible addition-fragment transfer (RAFT) polymerization is highly versatile for tailoring polymer structures and endowing them with varieties of functionalities^{37–39}, the proposed amphiphilic diblock copolymer was synthesized by a RAFT polymerization, which is illustrated in Scheme 1. A hydrophobic monomer, methyl methacrylate (MMA), and a phosphate containing monomer, methacryloyloxyethyl phosphate (MOEP), were utilized in constructing the hydrophobic segments and the hydrophilic segments of the final block copolymers, respectively. One-pot RAFT polymerization, in which the two monomers were introduced into the same reaction subsequently, was performed to understand how feeding ratios of monomer/CTA/initiator, temperature and time affected the molecular weight of the polymer^{40–43}. Typically, 10 mmol MMA, 0.25 mmol 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid and 0.1 mmol AIBN were dissolved in 1,4-dioxane. Under Argon atmosphere, the system was heated to 70 °C and lasted for around 2–3h. The molecular weight of PMMA block was monitored by gel permeation chromatography (GPC). Five mmol MOEP in 10 mL 1,4-dioxane was then introduced into the flask by syringe and the reaction was kept for another 4 h to polymerize the second block into the block copolymer. The block polymer was purified by precipitation and vacuum dried. The composition of the block copolymer was characterized by ¹H NMR spectroscopy.

Binding of phosphate block copolymer onto enamel

Before polymer treatment, the surface of the enamel was preconditioned by immersing in citric acid solution (1%, pH=3.8) for 5 min. Polymer solution with different concentrations (0.2 and 1.0 g/L) and pH values (3.1, 4.2 and 7.0) were used to treat the enamel surface for 5

min at 50 rpm in a water bath. Then, the treated enamel blocks were washed with phosphate buffer solution ($[\text{NaCl}]=0.15 \text{ mol/L}$, $[\text{Na}_3\text{PO}_4]=0.01 \text{ mol/L}$, $\text{pH}=7.0$). Polymer-treated enamel was then subjected to acid etching cycle, which includes acid challenging (1% citric acid, $\text{pH}=3.8$ for 2 min) and washing with PBS buffer for 5 min. The preconditioned, treated and etched enamel blocks were characterized by FTIR spectroscopy after air drying.

Binding of phosphate block copolymer onto HAP powder

The binding of polymer with HAP, as well as the effect from varied pH, concentration and functional group, was evaluated by UV-Vis spectrophotometer based on the absorption of the thiocarbonyl ($\text{C}=\text{S}$) moiety⁴⁴. Five mL of polymer solutions with different concentrations (0.06, 0.12, 0.25, 0.5 and 1.0 g/L) and different pH values were mixed with 100 mg of HAP powder for 2 h at room temperature. After centrifuging the mixture for 10 min at 10,000 rpm, the supernatant was tested using UV-Vis spectrophotometer. The UV-Vis absorbance of the polymer solution before and after binding with the HAP powder was utilized to calculate the adsorbed polymer onto HAP powder. In order to compare the binding capability between phosphate and carboxylic groups, a poly(acrylic acid) based block copolymer with similar chain length was synthesized (see supplementary information). Similar experiments were performed to compare the binding strength between phosphate groups and carboxylic groups.

Anti erosion test

Atomic absorption (AA) spectrometry was used to evaluate the released calcium from sintered HAP discs (Hitemco Medical Applications Inc., NY) surface. Typically, HAP discs were conditioned by immersing in citric acid (1%, $\text{pH}=2.5$) for 15 min at room temperature. Then, the HAP discs were mounted in 6 well plates using wax (Kerr, CA, Part No. 00429) with 3 HAP discs in each well and then the discs were then exposed to citric acid (1%, $\text{pH}=3.8$) for another 15 min at 37 °C. After being washed with DI water, the discs were then challenged with citric acid (1%, $\text{pH}=3.8$) for another 15 min at 37 °C. The solution was collected and the calcium concentration, as $[\text{Ca}]_{\text{ref}}$, was tested by atomic absorption (AA) spectroscopy. After being treated with the polymer solution (1.0 g/L at various pH values: 3.1, 4.2, or 7.0) for 2 min, the HAP discs were again challenged by citric acid (1%, $\text{pH}=3.8$) for another 15 min. The calcium concentration was measured by AA and designated as $[\text{Ca}]_{\text{treat}}$. Relative calcium levels (Ca level), $[\text{Ca}]_{\text{treat}}/[\text{Ca}]_{\text{ref}} \times 100\%$, were calculated to assess the calcium release or protecting efficacy against acid erosion. For statistical analysis, sample number is 5. In the analysis of variable (ANOVA), *t*-Test was used to evaluate the statistical significance among different treatments.

Preparation of enamel blocks for SEM observation

The surface of enamel block was pre-conditioned by immersing in citric acid solution (1%, $\text{pH}=3.8$) for 5 min. Polymer solution with a fixed concentration of 1.0 g/L at $\text{pH}=4.2$ was used to treat the enamel surface for 2 min at room temperature. After washing using PBS solution for 5min, the polymer treated enamel block was then subjected to acid challenging by citric acid (1%, $\text{pH}=3.8$) for 15 min in a water bath of 37 °C at a shaking speed of 50

rpm. Both the pre-conditioned enamel and polymer treated enamel were air dried for SEM observations without sputtering gold.

Characterization

The FTIR spectra were recorded on a Nicolet 6700 FT-IR Spectrometer by using a Smart Orbit accessory. ^1H NMR and ^{31}P NMR spectra of the phosphate block copolymer were performed on a Bruker NMR instrument (400 MHz) in CDCl_3 . When performing ^{31}P NMR spectroscopy, tri-*n*-octylphosphineoxide (TOPO) was used as the external standard in order to calculate the phosphorus content within the block copolymers. GPC was conducted using a PL-GPC 50 plus (Agilent Tech) at 1.0 mL/min and 35 °C with tetrahydrofuran (THF) as the mobile phase and polystyrene as the calibration reference. UV-Vis spectroscopy was completed using a Cary 5000 UV-Vis-NIR spectrometer (Agilent Tech). Atomic absorption (AA) spectroscopy was performed on an AAnalyst 800 atomic absorption spectrometer (Perkin Elmer) with the calcium hollow cathode lamp (422.7 nm) as the radiation source. SEM images for preconditioned, treated and etched enamel blocks were collected using a Zeiss Auriga FIB-SEM workstation. The specimens were not sputtered by gold or any other metal, but an InLens detector at an accelerating voltage of 2 kV was employed in order to obtain high resolution images.

Results and discussion

Synthesis of block copolymers

As two of the most utilized controlled radical polymerization approaches, RAFT polymerization was chosen for synthesis of phosphorus acid or carboxylic acid containing block copolymer for its mild requirement, high controllability and good biocompatibility⁴⁵. In order to investigate the potential influence of polymerization conditions, different monomer ratios, CTA/AIBN ratios, and polymerization times were used. ^1H NMR (Figure 1A) and FTIR spectra (Figure S1, see Supplementary Information (SI)) were used to determine the composition of the synthetic copolymers. The ^1H NMR peaks shown at 6.0–6.5 ppm (P-OH), 4.0–4.5 ppm (POO-CH₂-C-), 3.4–3.6 (O-CH₃), 1.8–2.0 (P-O-C-CH₂-), 0.9–1.2 (-CH₂-), 0.7–0.9 (-CH₃) confirmed the expected structure of block copolymer^{46,47}. The strongest and sharpest FTIR peak at 1728 cm⁻¹ attributed to the carbonyl group (C=O) in both PMMA and PMOEP blocks. The characteristic groups such as 1077 cm⁻¹ (P=O), 1022 cm⁻¹ (P-O), and 977 cm⁻¹ (P-O-H) confirmed the presence of phosphate units in the block copolymers³⁹. The C-O group in the MOEP unit is found at 1118 cm⁻¹. Methyl groups are indicated by the absorption at 2889 and 2952 cm⁻¹. Methylene groups are also indicated by the peaks around 1450, 2854 and 2918 cm⁻¹. With the assistance of an external standard containing phosphate from ^{31}P NMR spectrum (Figure 1b), the composition of the phosphate block copolymer was calculated (detailed calculation method, SI). Another carboxylic block copolymer with acrylic acid as the hydrophilic block was also similarly synthesized via RAFT polymerization (see SI). Both the compositions of block copolymers, e.g. PMMA-*b*-PMOEP and poly(methyl methacrylate)-*b*-poly(acrylic acid) (PMMA-*b*-PAA), are listed in Table 1.

Binding of block copolymer to enamel and HAP powder

FTIR spectroscopy was utilized to investigate the binding interaction between enamel and the PMMA-*b*-PMOEP copolymers. Because HAP is the dominant composition of enamel (96wt%)^{48, 49}, HAP disc, HAP powder and enamel block with polished surface were used for different test. Polished enamel block with flat surface were used for FTIR study. Prior to FTIR measurement, polymer treated enamel was washed with PBS buffer in order to remove the physically adsorbed polymers. The FTIR spectra are shown in Figure 2 where the influence of polymer concentration and pH on the binding properties was included. The absorption peaks at 1452, 1407, and 869 cm⁻¹ could be assigned to the carbonated HAP present on the surface^{39, 50, 51}. The peak at 1730 cm⁻¹ could be ascribed to the characteristic absorption peak of C=O in block copolymers bonded on the enamel surface (Figure 2A). However, exposing polymer treated enamel to acid challenge led to a weaker absorption at the same wave-number. When the polymer concentration was increased from 0.2 (Figure 2B) to 1.0g/L (Figure 2A), the relatively stronger peak of 1730 cm⁻¹ can be observed. In addition to the concentration effect, the influence of pH on binding efficacy was also evaluated. When the pH value increased from 3.1 (Figure 2A) to 4.2 (Figure 2C), the characteristic peak at 1730 cm⁻¹ decreased. When the pH value further increased to 7.0, no characteristic peak could be tested (data not shown). It indicates that both high polymer concentration and low pH values can facilitate the binding of the phosphate block copolymer on the enamel surface. This binding effect is consistent with other materials such as bovine serum albumin²⁰, dentine proteins⁵², and amino acids⁵³. Although electrostatic interactions were generally explained as the possible mechanism, there are substantial debates on the dominance of the multi-factors involved in this dynamic process^{20, 36, 50, 53}. There are a number of explanations to describe the binding results. First, the electrostatic interaction between the negatively charged phosphate groups from the polymer and positively charged calcium sites on the surface of enamel could facilitate the binding process. Alternatively, acidic solutions with low pH values will slightly etch the enamel surface, increasing the effective surface area and active sites, thus facilitating polymer adsorption. Hence, the polymers equilibrated under low pH conditions appeared to have enhanced binding to HAP. A third explanation could involve the increased acidity increasing, the absolute magnitude of the negative zeta potential of the HAP surface⁵⁴. This effect would reduce the repulsion force between the phosphate groups from polymers and the negatively charged HAP surface, activating the binding process of the negatively charged phosphate block copolymer onto the surface of HAP⁵⁴.

Sonication of the polymer treated enamel was employed to evaluate the binding strength between the phosphate block copolymer and the enamel. A typical polymer solution with a concentration of 1.0 g/L was used to evaluate the binding strength between phosphate block copolymers and enamel. An additional sonication treatment of 2 min in PBS solution was employed to check whether the bonded molecules could be easily displaced from the enamel surface. As shown in curve d in Figures 2A–2C, the characteristic peak at 1730 cm⁻¹ can be observed after acid challenge. Particularly, this characteristic peak in curve e in Figure 2D still can be observed even after 2 minutes sonication. In order to compare the relative binding capability with other functional groups such as the carboxylate group, another amphiphilic block copolymer containing carboxylate groups (PMMA-*b*-PAA) was

synthesized (See Scheme S1 and Figure S2). The enamel treated by the carboxylate block copolymers was also tested by FTIR spectroscopy (Figure S3, SI). No peak at 1730 cm^{-1} could be observed for the carboxylate block copolymer treated enamel.

The binding affinity between the enamel substrate and the adsorbent must be sufficiently strong to provide a large enough retention time of the polymer on the enamel surface for effective protection. As shown in Figure 2D, the characteristic group (C=O) was still observed after the exposure to the acid and sonication, implying that a strong chemical interaction exists between the phosphate block copolymer and the enamel surface. As suggested above, chemical bonding between phosphoric acid groups and HAP could be responsible for the high binding strength⁵⁰.

By calculating the relative FTIR intensity ratio (I_{1730}/I_{1022}) of the peak at 1730 cm^{-1} for C=O group from the polymer to the peak at 1022 cm^{-1} for phosphate group from the enamel, polymer retention on the surface of enamel could be approximately estimated. The ratios of blank enamel, polymer treated enamel and polymer-treated enamel with multi-acid challenges are compared in Figure S4 in SI. While the blank enamel showed a small ratio of (I_{1730}/I_{1022}) probably due to the noisy or other organic components, the greater relative ratios for polymer-treated enamels after different acid challenge cycles (curves b, c, d, and e) than that of blank enamel (curve a) confirmed the polymer adsorption and retention onto the enamel. The reduced relative ratio with acid challenge (curve c) in comparison with the polymer treated enamel (curve b) indicated that acid challenge could remove certain amount of polymer from the enamel surface. After 2 cycles of acid challenge and 1 cycle of sonication treatment, the characteristic peak of polymer can be still clearly observed in curve e, indicating that the block copolymer can be strongly adsorbed onto the surface even after acid challenge with additional sonication. By comparing the relative intensity ratios from Fig S4, approximate 70%, 57% and 29% of polymer were retained on the enamel surface after one challenge (curve c), two challenges (curve d) with an additional two minutes sonication (curve e). Both the acid challenge and sonication provided evidences that the prepared block copolymer could strongly bind to enamel surface and the polymer had a good retention on the surface of enamel even after acid challenge.

A qualitative experiment on PMMA-*b*-PAA to HAP was performed in order to compare the binding strengths between carboxyl and phosphate groups (Fig S3). The FTIR adsorption of carboxylic groups could not be detected, indicating the relatively weaker binding capability of PMMA-*b*-PAA compared to that of PMMA-*b*-PMOEP copolymer.

Quantitative evaluation of the binding between the block copolymer and HAP powder was also evaluated by UV-Vis spectroscopy based on the characteristic absorption of the thiocarbonyl bond (C=S) within the polymer (Scheme 1)⁴⁴. The effects from two major factors including pH and polymer concentration as well as the functional groups to the binding efficacy were evaluated. The UV-Vis spectra of the phosphate block copolymer solution before and after binding with HAP powder are shown in Figures 3A through 3C for example. Those of PMMA-*b*-PAA block copolymer are exhibited in Figures S5–S7 (see SI). The absorbance of thiocarbonyl group at 306 nm was then used to calculate the amount of polymer adsorbed on HAP, which is plotted in Figure 3D. It can be seen that higher polymer

concentration led to more polymers adsorbed onto the HAP surface, but, at the tested concentrations and different binding time, no saturation was observed. This indicates that there is different adsorption behavior in comparison with proteins such as bovine serum albumin^{20, 30}.

The pH value of the solution also influenced the dynamics of adsorption. At lower pH values such as 4.0, more polymers were bonded to HAP, while fewer polymers were bound at higher pH values (e.g. pH=7.0). Comparing the two block copolymers either with phosphate groups or carboxylate groups, the total adsorption amount of PMMA-*b*-PMOEP was greater than that of the PMMA-*b*-PAA under the same conditions. However, the difference due to functional groups is varied depending on the pH and polymer concentration. As an example, when the polymer concentration and pH were correspondingly fixed as 1.0 g/L and 7.0, respectively, the adsorption amount of PMMA-*b*-PMOEP was around 6×10^{-3} mmol/g, while that of PMMA-*b*-PAA was only 2×10^{-3} mmol/g. This reveals that 3-fold more PMMA-*b*-PMOEP could be adsorbed onto the HAP surface compared to that of PMMA-*b*-PAA. However, when the polymer concentration and pH were correspondingly fixed at 1.0 g/L and 4.0, respectively, the adsorption amount of PMMA-*b*-PMOEP was around 10×10^{-3} mmol/g, while that of PMMA-*b*-PAA was near 7×10^{-3} mmol/g, less than a 2 fold difference. According to the qualitative and quantitative analyses above, the strong affinity of phosphate block copolymers to HAP could be highly promising in designing non-fluoride agents for dental protection with a prolonged effective time. There are few reports on quantitatively comparing the binding strengths for different functional groups. Moreno et al. have employed five small molecules (i.e., L-alanine, L-aspartic acid, L-serine, L-phosphoserine and succinic acid) to evaluate the binding strength of functional groups³⁶. Their analysis of the adsorption isotherm based on the Langmuir type model indicated that the binding affinity of the phosphate bond was 20 times higher than that of the carboxylate bond. This deduction, however, may not be universally applied to other adsorbates with higher order structure such as proteins³⁶.

UV-Vis measurements (Figure 3) quantitatively confirmed that the binding affinity of the phosphate-containing block copolymer was higher than that of the carboxylate block copolymer since the phosphate groups possess a greater affinity toward HAP due to formation of chemical bonds with HAP^{20, 36, 50}. UV-Vis spectra in Figure 3 indicate that, as the polymer concentration increased, more polymers adsorbed onto the HAP surface. However, no saturation was observed at the tested concentrations, possibly indicating a different adsorption behavior in comparison with proteins, such as bovine serum albumin^{20, 30}. In addition, the pH value of the solution also influenced polymer adsorption to HAP or enamel. At lower pH values, such as 4.0, more polymer molecules were bound to HAP, while fewer molecules were bound at higher pH values such as pH 7.0. This result was confirmed by the FTIR result, in which more polymers bound with HAP at lower pH (Figure 2), and is consistent with other materials, such as bovine serum albumin²⁰, dentine proteins⁵², and amino acids⁵³. These results are most consistent with the acid etching the enamel surface allowing for increased polymer mineral interactions. To definitively determine the mechanism of polymer binding, however, additional experiments must be completed.

Polymer-induced inhibition of mineral loss

Generally, natural saliva plays an important role in regulating de/remineralization. Saliva has a complex composition that includes a number of inorganic ions, proteins, enzymes, lipids, etc ^{55–57}. Saliva may protect teeth from acid erosion via different mechanisms: a) the bicarbonate and proteins could buffer the pH value and decrease the acidity of the environment; b) the calcium and phosphate ions from saliva could increase the saturation of the solution, thus decreasing mineral loss and enhancing remineralization on the enamel surface, and; c) certain organic molecules, such as proteins, could bind with the enamel surface to form a protective coating, thus preventing acid diffusion towards the tooth surface and mineral release ¹⁸.

Anti-erosion efficiency of the phosphate block copolymer was quantitatively evaluated by atomic absorption spectrometry based on the calcium released from the HAP discs. As shown in Figure 4, the calcium level detected for the polymer adsorbed HAP is decreased from 61.2% (blank, non-treated sample) to 39.3%, 38.3% and 33.3% for polymer treatments having pH values of 3.1, 4.2 and 7.0, respectively. While all polymer treatments showed a significant capability to reduce calcium release, the mineral loss was reduced by 36%–46% in comparison with the blank. When varying the pH of the polymer solution from pH = 3 to pH = 7, there was no statistic difference on enamel erosion prevention amongst the different pH values (p value > 0.05). This phenomenon seems to be inconsistent with the quantity of adsorbed polymer to HAP powder. It may be ascribed to the ‘trade-off’ effect. On the one hand, lower pH value could facilitate polymer adsorption but also enhance the dissolving of mineral by etching the surface under acidic conditions. On the other hand, high pH value may result in a lower quantity of adsorbed polymer but also inactivate the mineral loss due to less etched effect.

Polymer-induced protection of enamel surface morphology

Since the phosphate block copolymer could reduce the mineral loss of HAP under acidic conditions, it would be expected that similar treatment on the enamel surface would protect the surface morphology and the integrity of enamel. SEM experiments were completed to capture the changes to the enamel surface morphology before and after acid erosion. The SEM images of the conditioned enamel and the polymer treated enamel in Figures 5A and 5B, respectively, show that the polymer treatment didn’t change the surface morphology of enamel. However, the polymer treatment shows a significant protecting effect against acid erosion. The SEM images for untreated and polymer treated enamel with the same acid erosion conditions are shown in Figures 5C and 5D, respectively. The enamel surface without treatment shows the increased dissolution of HAP mineral within the rod sheaths (Figure 5C). When the enamel surface was treated with the phosphate block copolymer, less mineral was dissolved, and there was an increase in the inter-crystalline spaces (Figure 5D). It clearly indicates that the phosphate block polymer could effectively protect the enamel from erosion. This observation was consistent with the results of changes in calcium leaching level as a function of treatment described above. A binding/anti-erosion mechanism was proposed in Figure 5E. The phosphate block copolymer could bind with HAP via the interaction between phosphate groups and the enamel. The binding could increase the polymer retention time on the tooth surface, thus prolonging the anti-erosion time. While the

phosphate groups facilitate the binding, the hydrophobic segments of the block copolymer assemble into a layer as a physical barrier, which reduces the acid attack on the enamel surface, and prevents calcium release from the enamel surface. In this aspect, the polymer acts very similarly to salivary pellicle lipids that associate with enamel and resist acid damage^{58, 59}.

Conclusions

A bio-inspired amphiphilic block copolymer containing phosphate groups was designed as a novel non-fluoride agent for erosion prevention of teeth. The block copolymer was synthesized via a reversible addition-fragment transfer (RAFT) polymerization. According to the qualitative and quantitative analyses, the amphiphilic phosphate block copolymer was able to bind with HAP and enamel and form a protective coating on the tooth surface. The affinity of the phosphate block copolymer was stronger than that of the carboxylate block copolymer. The binding affinity was dependent on the polymer concentration and the pH of the solution. The hydrophobic block by forming a physical barrier on the surface of the enamel prevents acid attack and reduces calcium release from the enamel, thus reducing the acid erosion. Consequently, the presence of the phosphate block copolymer on the tooth surface effectively reduces mineral loss and protects the surface integrity of the enamel against acid erosion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was financially supported by the Colgate-Palmolive Company and partially supported by National Institutes of Health of the USA (NIH/NIDCR/R01DE021786). We also thank Dr. Earl M Kudlick for initiating this project, Dr Malaisamy Ramamoorthy on atomic absorption test and Dr Laurence C. Chow (ADAF Paffenbarger Research Center at NIST) for helpful discussions.

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Fig. 1. ^1H NMR (A) and ^{31}P NMR (B) spectra of phosphate block copolymer, the * marked peaks at 7.26, 2.05 and 0 ppm are from the CDCl_3 solvent, acetone and tetramethylsilane (TMS), respectively.

Fig. 2.

FTIR spectra of enamel treated with PMMA-*b*-PMOEP block copolymer at different pH and polymer concentrations [Poly] (A) pH=3.1, [Poly]=1.0 g/L; (B) pH=3.1, [Poly]=0.2 g/L; (C) pH=4.2, [Poly]=1.0 g/L; (D) pH=3.1, [Poly]=1.0 g/L. Each curve in the figures represents: curve a: enamel before polymer treatment; curve b: enamel after polymer treatment; curve c: polymer-treated enamel with acid challenge of 1 cycle; curve d: polymer-treated enamel with acid challenge of 2 cycles; curve e: polymer-treated enamel with acid challenge of 2 cycles and additional sonication of 2 min; insert in Figure 2A is the FTIR spectrum of PMMA-*b*-PMOEP block copolymer.

Fig. 3.

UV-Vis spectra of PMMA-*b*-PMOEP block copolymer (A), phosphate block copolymer solution after binding with HAP at pH=4 (B), phosphate block copolymer solution after binding with HAP at pH=7.0 (C), and the calculated polymer quantities adsorbed on HAP for different block copolymers (D). Polymer concentrations are indicated by different curves (a: 1.0 g/L, b: 0.5 g/L, c: 0.25 g/L, d: 0.12 g/L, e: 0.06 g/L) in Figures 3A, 3B, and 3C. The absorbance of polymer solution with 1g/L in Figure 3A was out of the linear range and so was not included. The vertical axis in Figure 3D indicates the amount of the adsorbed polymer per gram of HAP.

Fig. 4.
The influence of phosphate block copolymer (buffered at a pH of 3.1, 4.2, or 7.0) on the level of released calcium from HAP disc.

Fig. 5. SEM images of pre-conditioned blank (A), polymer-treated enamel before acid etching (B), enamel without polymer treatment with 15 min etching (C), and polymer-treated enamel with 15 min etching (D) and proposed mechanism of phosphate block copolymer on protecting enamel (E).

Scheme 1. Synthesis of PMMA-*b*-PMOEP by RAFT polymerization

Table 1

Composition of phosphate and carboxylic block copolymers

Polymer	[MMA] _x , ^a	[MOEP] _y or [AA] _y	Mn, kDa
PMMA- <i>b</i> -PMOEP	16.9	11.6 ^b	4.4
PMMA- <i>b</i> -PAA	16.5	35.1 ^c	4.6

^a the chain length of PMMA was measured by GPC;^b indirectly calculated by ³¹P NMR;^c indirectly calculated by ¹H NMR spectroscopy (Figure S2, SI).