Novel rechargeable calcium phosphate nanoparticle-filled dental cement

Xianju XIE1,2,*, Lin WANG2,3,*, Dan XING2,4, Manlin QI3, Xiaodong LI5, Jirun SUN6, Mary Anne S. MELO2, Michael D. WEIR2, Thomas W. OATES2, Yuxing BAI1 and Hockin H. K. XU2,7,8

1 Department of Orthodontics, School of Stomatology, Capital Medical University, Beijing, China
2 Department of Advanced Oral Sciences and Therapeutics, University of Maryland Dental School, Baltimore, MD 21201, USA
3 VIP Integrated Department, Stomatological Hospital of Jilin University, Changchun, China
4 Department of Dentistry, China Rehabilitation Research Center, Beijing, China
5 Department of Oral Medicine, School of Stomatology, Zhejiang University, Hangzhou, Zhejiang, China
6 Volpe Research Center, American Dental Association Foundation, National Institute of Standards & Technology, Gaithersburg, MD 20899, USA
7 Center for Stem Cell Biology & Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA
8 University of Maryland Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore 21201, USA

Corresponding authors, Hockin H. K. XU; E-mail: hxu@umaryland.edu, Yuxing BAI; E-mail: byuxing@263.net

The objectives were to develop a novel rechargeable cement containing amorphous calcium-phosphate nanoparticles (nanoACP) to suppress tooth decay. Five cements were made with: (1) 60% glass particles (experimental control); (2) 40% glass+20% nanoACP; (3) 30% glass+30% nanoACP; (4) 20% glass+40% nanoACP; (5) 10% glass+50% nanoACP. Groups 1–4 had enamel bond strengths similar to Transbond XT (3M) and Vitremer (3M) (p>0.1). The nanoACP cement had calcium and phosphate ion release which increased with increasing nanoACP fillers. The recharged cement had substantial ion re-release continuously for 14 days after a single recharge. Ion re-release did not decrease with increasing recharge/re-release cycles. Groups 3–5 maintained a safe pH of medium (>5.5); however, control cements had cariogenic pH of medium (<4.5) due to biofilm acid. Therefore, nanoACP cement (1) had good bond strength to enamel, (2) possessed calcium and phosphate ion recharge/re-release capability, and (3) raised biofilm pH to a safe level to inhibit caries.

Keywords: Dental cement, Demineralization, Calcium phosphate nanoparticles, Rechargeable, Long-term ion release

INTRODUCTION

Tooth decay is a prevalent problem which constitutes a heavy economic burden worldwide. For example, one area that is prone for demineralization is teeth with orthodontic appliances, which make it difficult for patients to clean. This leads to enamel demineralization which appears as an opaque, white color and is referred to as white spot lesions4). Recent investigations suggested that the incidence of white spot lesions during orthodontic treatments with fixed appliances was as high as 50 to 70%2,3). Such lesions could eventually lead to cavities4). Studies also showed that white spot lesions took only one month to develop5). Therefore, there is a need to develop a bioactive cement that can help regenerate tooth minerals. In addition, bioactive cements with remineralizing capabilities to regenerate tooth minerals would be useful as crown cements, inlay/onlay cements, pit and fissure sealants, bases, liners, and tooth root coatings to combat caries and protect tooth structures.

Incorporating calcium phosphate (CaP) filler particles into resins represents an effective approach for tooth mineral regeneration9-11). Dental resins containing CaP particles were shown to release calcium (Ca) and phosphate (P) ions9-12). They act as Ca and P ion reservoirs in plaque and tooth surfaces, which can be released during an acidic challenge to prevent mineral-loss and facilitate mineral regeneration9,11,12). However, conventional CaP-containing resins used CaP particle sizes of 1 to 55 μm and had relatively low mechanical properties, with flexural strength of about half of that for unfilled resin9,11,12). In addition, their Ca and P ion releases lasted for only a couple of months, too short to be effective for most dental applications9,12).

Novel nanocomposite was developed with amorphous calcium phosphate nanoparticle (nanoACP) having a mean particle size of 116 nm13). The nanoACP composite released Ca and P ions similar to conventional CaP composites, but with a 2-fold increase in mechanical properties for load-bearing restorations13,14). The nanoACP composite could regenerate minerals in enamel lesions, reaching a mineral regeneration efficacy that was 4-fold that of a commercial fluoride-releasing composite15). In another human in situ study, the enamel mineral loss at the margins around nanoACP composite under biofilm acids in situ in human participants was reduced to 1/3 that of a control composite without nanoACP16). However, these Ca and P ion releases were short-term, with no report of Ca and P ion recharge capabilities in those studies9-12).

Recently, CaP-rechargeable resins were developed for the first time17,18). The nanoACP was mixed into a resin
of pyromellitic glycerol dimethacrylate (PMGDM) and ethoxylated bisphenol-A dimethacrylate (EBPADMA)\textsuperscript{17}. Ca and P ions could be repeatedly recharged into the specimens for re-release, exhibiting no decrease in ion re-release after multiple cycles of recharge and re-release\textsuperscript{17,20}. The novel Ca and P ion rechargeable resins are promising to provide long-term ion release and cavity-fighting benefits. However, to date, there has been no report on the effects of nanoACP filler level (0–50%) in dental cement on Ca and P ion rechargeability, re-release of ions, and oral biofilm pH.

The objectives of this study were to develop a novel Ca and P ion-rechargeable cement and investigate the effect of nanoACP filler level on ion recharge and biofilm pH for the first time. The following hypotheses were tested: (1) Bioactive cement could be formulated to recharge Ca and P ions, without decreasing the enamel bond strength; (2) The nanoACP filler level would determine the Ca and P ion recharge and re-release efficacy; (3) Ca and P ion re-release from the cement would be durable and not decrease with repeated cycles of recharge and re-release; (4) The nanoACP cement would neutralize biofilm acids and increase the biofilm medium pH.

**MATERIALS AND METHODS**

**Development of CaP rechargeable cement**

The resin matrix consisted of 44.5% (all mass% in this article) of PMGDM (Hampford, Stratford, CT, USA), 39.5% of EBPADMA (Sigma-Aldrich, St, Louis, MO, USA), 10% of 2-hydroxyethyl methacrylate (HEMA) (Esstech, Essington, PA, USA) and 5% of bisphenol-A-glycidyl methacrylate (Bis-GMA) (Esstech), following a previous study\textsuperscript{17}. One percent of phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide (Sigma-Aldrich) was added for photo-polymerization\textsuperscript{17}. PMGDM and EBPADMA had a low cytotoxicity similar to other dental dimethacrylates\textsuperscript{19}. Being an acidic adhesive monomer, PMGDM can chelate with Ca ions in the ion recharging solution to enable the ion recharge to occur\textsuperscript{17,20,21}. HEMA was added to improve the flowability and hydrophilicity, following a previous study\textsuperscript{22}. Bis-GMA was added because it could improve the cross-link and enhance the bond strength to teeth\textsuperscript{15}. This resin is referred to as PEHB and the compositions are shown in Table 1.

A spray-drying method was used to synthesize nanoACP (Ca\(_3\)(PO\(_4\))\(_2\)) as previously described\textsuperscript{13,15}. Briefly, calcium carbonate and dicalcium phosphate were first dissolved in an acetic acid solution. The Ca and P ion concentrations were adjusted to be 8 and 5.333 mmol/L, respectively, to yield a Ca/P molar ratio of 1.5. The solution was sprayed into a heated glass column to dry and evaporate the water and volatile acid. This technique produced the dried nanoACP, which were silanated with 4% 3-methacryloxypropyltrimethoxysilane and 2% n-propylamine\textsuperscript{19}. Five cements were formulated:

1. PEHB+60% glass fillers (referred to as "PEHB+60% nanoACP" experimental control);
2. PEHB+20% nanoACP+40% Glass (referred to as "PEHB+20% nanoACP");
3. PEHB+30% nanoACP+30% Glass (referred to as "PEHB+30% nanoACP");
4. PEHB+40% nanoACP+20% Glass (referred to as "PEHB+40% nanoACP");
5. PEHB+50% nanoACP+10% Glass (referred to as "PEHB+50% nanoACP").

For measuring the bond strength to enamel, a commercial control Transbond XT (3M, Monrovia, CA, USA) was used and designated “TRBD control”. TRBD was used as an orthodontic cement, and, according to the manufacturer, consisted of silane-treated quartz (70–80%), bisphenol A diglycidyl ether dimethacrylate (10–20%), bisphenol-A-bis(2-hydroxyethyl) dimethacrylate (5–10%), silane-treated silica (<2%) and diphenyliodonium hexafluorophosphate (<0.2%).

In addition, a resin-modified glass ionomer cement (RMGI) (Vitremer, 3M) served as another control (referred to as RMGIV control). RMGIV was selected because RMGIs have been used as orthodontic cements that can bond to enamel\textsuperscript{20}. RMGIV contained fluoroaluminosilicate glass and a light-sensitive, aqueous polyalkenoic acid. Indications include Class III, Class V and root-caries restorations, and Classes I and II restorations in primary teeth. A powder/liquid mass ratio of 2.5/1 was used according to the manufacturer.

**Testing of cement shear bond strength (SBS) to enamel**

The aforementioned five PEHB cements and two commercial controls were tested for enamel shear bond strength\textsuperscript{20}. The use of extracted human teeth was

| Table 1 | Compositions (Mass%) of CaP rechargeable cement PEHB |
|---------|-----------------------------------------------|
| **Components** | **PMGDM** | **EBPADMA** | **HEMA** | **Bis-GMA** | **BAPO** |
| **Proportion** | 44.5% | 39.5% | 10% | 5% | 1% |

PMGDM: pyromellitic glycerol dimethacrylate (Hampford, Stratford, CT, USA).
EBPADMA: ethoxylated bisphenol-A-dimethacrylate (Sigma-Aldrich, St, Louis, MO, USA).
Bis-GMA: bisphenol-A-glycidyl dimethacrylate (Esstech, Essington, PA, USA).
HEMA: 2-hydroxyethyl methacrylate (Esstech).
BAPO: phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide (Esstech).
approved by the University of Maryland Baltimore Institutional Review Board (IRB # HP-00075835). Seventy extracted human first premolars were randomly divided into seven groups of ten teeth each. Teeth were selected with intact buccal enamel that had not been pretreated with chemical agents, no visible cracks, and no enamel irregularities24). Teeth were immersed in 0.01% thymol solution at 4°C and tested within 60 days from extraction. Each tooth was placed in a self-polymerizable acrylic polymer (Lang Dental, Wheeling, IL, USA) taking into account of the buccal axis of the clinical crown, so that their labial surfaces were parallel to the load during enamel shear bond strength measurement24). The coronal portion was submitted to prophylaxis with pumice and rubber cups for 10 s.

For TRBD control, the buccal surfaces of teeth were etched for 30 s with 35% phosphoric acid (Scotchbond, 3M), then washed and dried until a frosty white appearance occurred. Transbond XT primer was placed on the etched surfaces in a thin, uniform coat. TB control paste was applied to the base of premolar metal orthodontic brackets (Ormco Series 2000, Sybron Dental, Orange, CA, USA) for a total of 40 s. The curing was done for the bracket was placed and bonded to the enamel 27,29). The RMGIV paste was applied to the bracket base, and the RMGIV was photo-polymerized for 40 s in the same manner as described above27,29).

After the bonding procedures, the tooth-bracket sets were incubated in distilled water at 37°C for 24 h prior to mechanical testing28-30). SBS was measured following previous studies29,31). A chisel on a mechanical testing machine (MTS, Eden Prairie, MN, USA) was positioned on the upper part of the bracket base and parallel to the bonded interface. A 0.019×0.025 inch stainless steel wire was ligated into each bracket slot to reduce deformation at failure/bracket bonded surface area, following previous studies24).

Each tested tooth surface was examined using a stereomicroscope (Leica Zoom 2000, Leica Microsystems, Wetzlar, Germany). The adhesive remnant index (ARI) was scored to assess the remaining cement material on the enamel using the following criteria24: 0=no cement remained on enamel; 1=less than half of the cement remained on enamel; 2=more than half of the cement remained on enamel; and 3=all the cement remained on enamel.

Measurement of initial Ca and P ion release from PEHB+nanoACP cements

Ca and P ion release was measured with samples submerged in a NaCl solution. The solution pH was adjusted to pH 4 using 50 mmol/L lactic acid, simulating a cariogenic low pH condition39). As in previous studies10,13), three samples of dimensions of 2×2×12 mm were submerged in 50 mL of solution to have a sample volume/solution ratio of 2.9 mm³/mL. This ratio was consistent with a previous study using a sample volume per solution ratio of about 3.0 mm³/mL40). Ca and P ion releases from the cements were determined at 1, 3, 5, 7, 14, 21, 28, 35, and 42 days13). At each time period, an aliquot of 0.5 mL was taken, and a fresh NaCl solution of 0.5 mL was added. The solution pH was measured via a combination pH electrode (Orion, Cambridge, MA, USA). 50 mmol/L lactic acid was used to adjust the solution to pH 414). A spectrophotometric method (DMS-80 UV-visible, Varian, Palo Alto, CA, USA) was used to analyze the Ca and P concentrations via known standards and calibration curves13). These data represented the virgin ion release from the cements and were designated as the “initial release”. After subsequent recharge, the ions were denoted “re-release”.

Ion recharge and re-release of PEHB+nanoACP cements

The PEHB cement samples were submerged in the pH 4 solution for 42 days as described above to determine the initial ion release. After that, the samples were placed in 100 mL of a new NaCl solution at pH 4 for 30 days to ensure that the ions were depleted. Then, the samples were treated with ultrasonication for 30 min in water. Then, these samples were submerged in a new pH 4 solution to determine the Ca and P ion release for 7 days; this verified that, indeed, the ions were depleted as there was no more ion release. These depleted specimens were used to do Ca and P ion recharge test. For Ca ion recharge, the solution had 100 mmol/L of CaCl₂ and 50 mmol/L of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer16,32). For P ion recharge, the solution had 60 mmol/L of KH₂PO₄ and 50 mmol/L of HEPES buffer. They were adjusted to pH 7 by KOH at 1 mol/L16,32). Three depleted bars of dimensions of 2×2×12 mm were placed into 5 mL of the Ca or P ion recharge solution and agitated for 1 min using a mixing machine (Analog Vortex Mixer, Fisher Scientific, Waltham, MA, USA). This immersion and agitation process mimicked the actions in the mouth-rinsing process when calcium phosphate mouth-rinses could be used17). Then the samples were rinsed with water to remove any loose deposits on surfaces. In this way, only the ions recharged into the inside of the cement were determined in the re-release test. This recharge was repeated in one day for a total of three times at 9:00 am, 1:00 pm and 5:00 pm. Then the next day, the ion re-release test was started for 14 days. After recharging, the cements were placed...
in 50 mL of pH 4 solution to measure Ca and P ion re-release using the method described above. The ion re-release was measured continuously for 14 days without any additional recharge. This constituted “cycle 1” of ion recharge and re-release. Then the samples that had been releasing ions for 14 days were taken out of the solution, rinsed with water, recharged again as described above, and tested for ion re-release for another 14 days, as cycle 2. Therefore, each recharge treatment was followed by 14 days of continuous ion release. This measurement was repeated for 3 cycles in the present study.

Human saliva collection and the pH of dental plaque microcosm biofilm medium

The in vitro oral biofilm model was approved by the University of Maryland Baltimore Institutional Review Board. Saliva is ideal for growing microcosms biofilms in vitro, with the advantage of maintaining much of the complexity and heterogeneity of dental plaque in vivo33. Saliva was collected from ten healthy donors having natural dentition without active caries, and not having used antibiotics within the past three months. The donors did not brush teeth for 24 h and abstained from food and drinks for 2 h prior to donating saliva34. Ten donors participated, and an equal volume of saliva from each donor was mixed to form the saliva for subsequent experiments. The saliva was diluted in sterile glycerol to 70% concentration and stored at ~80°C35.

The saliva-glycerol stock was added, with 1:50 final dilution, into a McBain artificial saliva growth medium as inoculum34. This medium contained mucin (Type II, porcine, gastric) at a concentration of 2.5 g/L; bacteriological peptone, 2.0 g/L; tryptone, 2.0 g/L; yeast extract, 1.0 g/L; NaCl, 0.35 g/L; KCl, 0.2 g/L; CaCl2, 0.2 g/L; cysteine hydrochloride, 0.1 g/L; hemin, 0.001 g/L; vitamin K1, 0.0002 g/L, at pH 735. Two percent sucrose was added to this medium, and 24-well plates were used.

For dental plaque microcosm biofilm tests, each cement paste was filled into disk molds of 9 mm in diameter and 2 mm in thickness. They were light-cured for 1 min on each open side of the mold. The cured disks were demolded and immersed in distilled water at 37°C for 24 h to leach out any uncured monomers. Then, the cements with attached biofilms were placed in new 24-well plates with new medium. At this point the pH was not measured for the initial 0–24 h of culture, because the planktonic bacteria in the medium would interfere with the pH. By placing the cement with attached biofilms in new wells with fresh medium and measuring the pH from 24 to 72 h, it enabled the pH to be related to the biofilms on the cement36. The pH was determined once per hour from 24 to 72 h. Then, the pH was determined the next morning every two hours from 48 to 60 h of culture. The last pH value was measured in the next morning at 72 h of culture.

Statistical analysis

Kolmogorov-Smirnov test and Levene test were carried out to verify the normality and equal variance of the results of bond strength, ion release, ion re-release, and pH values. One-way analyses of variance (ANOVA) were employed to analyze the bond strengths. Two-way ANOVA were employed to analyze the ion release, re-release and pH data. Post hoc multiple comparisons were performed using the Tukey’s honestly significant difference test. Rank sum test was carried out for the ARI scores. Statistical significances were preset at p<0.05, using the SPSS 14.0 software package (SPSS, Chicago, IL, USA).

RESULTS

The shear bond strengths to enamel are plotted in Fig. 1 (mean±SD; n=10). PEHB+0% nanoACP, PEHB+20% nanoACP, PEHB+30% nanoACP and TRBD control had similar bond strengths (p>0.1). PEHB+40% nanoACP and RMGIV control had similar bond strengths (p>0.1). PEHB+50% nanoACP had the lowest bond strength (p<0.05). The ARI scores were listed in Table 2. Most of the specimens failed at the bracket-cement interface. PEHB+50% nanoACP had the lowest score in all groups (p<0.05). A lower score indicates more failures at the cement-enamel interface.

The cements were tested for initial Ca and P ion release, with results shown in Fig. 2: (A) Ca ion release, and (B) P ion release (mean±SD; n=6). The released ion concentrations became greater from 1 to 28 days; after 28

![Fig. 1 Enamel shear bond strengths of cements (mean±SD; n=10) after storage in water for 24 h. Different letters indicate significantly different values (p<0.05).](image-url)
Table 2  ARI scores of dental cements bonding to enamel

| Cement groups          | ARI scores and the number of samples in each group that scored at a particular ARI score | SIG# |
|------------------------|------------------------------------------------------------------------------------------|------|
|                       | 0  | 1  | 2  | 3  |                                      |      |
| PEHB+0% NACP           | 1  | 2  | 7  | 0  | A                                       |      |
| PEHB+20% NACP          | 1  | 3  | 6  | 0  | A                                       |      |
| PEHB+30% NACP          | 2  | 2  | 6  | 0  | A                                       |      |
| PEHB+40% NACP          | 4  | 4  | 2  | 0  | B                                       |      |
| PEHB+50% NACP          | 6  | 4  | 0  | 0  | C                                       |      |
| TRBD Control           | 1  | 2  | 7  | 0  | A                                       |      |
| RMGIV Control          | 3  | 6  | 1  | 0  | B                                       |      |

*Adhesive Remnant Index (ARI) for the debonded specimens using the following scales: 0=no cement left on the tooth surface; 1=less than half of the cement was left on the tooth surface; 2=half or more of the cement was left on the tooth; 3=the entire cement was left on the tooth surface.

SIG refers to statistical significance, with different letters (A, B) indicating significant differences in the ARI scores ($p<0.05$). Each group had ten samples ($n=10$).

Days, the ion concentration increase was slowed. There were significant differences among all the materials at 42 days ($p<0.05$). The ion release concentration at 42 days showed the order of PEHB+50% nanoACP>PEHB+40% nanoACP>PEHB+30% nanoACP>PEHB+20% nanoACP>PEHB+0% nanoACP ($p<0.05$).

Ca and P ion recharge and re-release data for the cements are plotted in Fig. 3: (A) Ca and (B) P ion re-release (mean±SD, $n=3$). The recharged samples were submerged in a new pH 4 solution and ion re-release was determined for 14 days, which was referred to as one cycle. Three such cycles of recharge and re-release were plotted. For all groups, after each recharge treatment, the ion release concentration increased rapidly in the first week, then the release slowed. The ion re-release concentrations at the end of each cycle (14, 29 and 44 days, respectively) had the order of PEHB+50% nanoACP>PEHB+40% nanoACP>PEHB+30% nanoACP>PEHB+20% nanoACP>PEHB+0% nanoACP ($p<0.05$). Furthermore, for each group, there was no significant difference in the re-released ion concentrations at 14, 29 and 44 days with repeated recharge and re-release from 1 to 3 cycles ($p>0.1$). This demonstrated that the cements could be repeatedly recharged without losing the recharge and re-release efficacy.

In order to examine the relationship between ion re-release and nanoACP filler level, the Ca and P ion concentrations at the end of the 3rd cycle are plotted vs. nanoACP mass fractions in Fig. 4: (A) Ca and (B) P ion concentrations (mean±SD, $n=3$). Linear best fit through the origin yielded for released Ca ion concentration: $Ca=1.04\times N$, where $N=nanoACP$ mass fraction in the cement (for example, for 40% nanoACP, $N=0.40$). A high correlation coefficient $R^2=0.959$ was achieved. The phosphate ion concentration $P=0.46\times N$, with $R^2=0.9853$.

The pH of culture medium with biofilms on cement disks is plotted in Fig. 5 (mean±SD; $n=6$). Two-way ANOVA showed significant effects of material type and time ($p<0.05$), with a significant interaction between
the two variables (p<0.05). The culture medium started with a pH of 7 and then the pH decreased with time due to biofilm acid production. For biofilms on the samples of TRBD control and PEHB+0% nanoACP, the pH decreased with increasing time and reached a steady-state pH of close to 4. For RMGIV control, the biofilm medium had a steady-state pH of approximately 4.2. In contrast, the pH was 4.5 for PEHB+20% nanoACP, 5.5 for PEHB+30% nanoACP, 5.7 for PEHB+40% nanoACP, and 5.8 for PEHB+50% nanoACP. The steady-state pH values of all the groups are significantly different from each other (p<0.05).

DISCUSSION
The average orthodontic treatment time is usually about two years. However, previous CaP-containing dental resins were capable of releasing Ca and P ions lasting for only a couple of months, then the amount of ion release became minimal\(^{9-12}\). Such short-term release would not be sufficient for orthodontic applications to suppress white spot lesions. Therefore, it is important to develop a rechargeable CaP cement that can be repeatedly recharged to provide long-term Ca and P ion releases. The present study developed a novel rechargeable nanoACP cement. The hypotheses were proven that the bioactive cement could be recharged with Ca and P ions without decreasing the enamel bond strength, and the nanoACP filler level determined the Ca and P ion recharge and re-release efficacy. Furthermore, the Ca and P ion

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**Fig. 3** Re-release from the exhausted and recharged PEHB cements: (A) Ca ion re-release, and (B) P ion re-release (mean±SD; n=3). Three recharge/re-release cycles were tested, and each re-release was measured for 14 days. The ion re-release increased with increasing time and nanoACP filler level. There was no decrease in the ion re-release amount with increasing the number of recharge/re-release cycles from 1 to 3 (p>0.1).

**Fig. 4** The relationship between Ca and P ion re-release and nanoACP filler level. Ion concentrations at 14 days of the third cycle were plotted: (A) Ca ion re-release, and (B) P ion re-release (mean±SD; n=3). N is defined as the nanoACP mass fraction in the cement (for example, for 40% nanoACP, N=0.40). The equation in each plot indicates the linear best fit through the origin with a high correlation coefficient R\(^2\), demonstrating that the ion recharge efficacy was directly proportional to nanoACP filler level.
Biofilms produced acids causing the pH to decrease from the initial pH 7. The pH of biofilm medium with PEHB+30% nanoACP, PEHB+40% nanoACP and PEHB+50% nanoACP was maintained at above 5.5 during the 72 h of culture. The pH of the biofilm medium with TRBD was cariogenic and at about pH 4.

Importantly, the new cement greatly increased the pH of the biofilm culture medium, reaching a safe pH>5.5 at≥30% nanoACP, while TRBD control had a cariogenic pH of 4 due to biofilm acid production. Therefore, this new cement is promising to have long-term Ca and P ion release and acid neutralization to suppress enamel mineral-loss and inhibit caries.

A major advantage of using nanoACP was that, due to the small size and high surface area of nanoparticles, less nanoACP fillers in the cement were needed to achieve substantial ion release. This made room in the polymer matrix for reinforcing (but non-releasing) glass fillers to provide mechanical integrity. In the present study, to enable a direct comparison of these cements, the filler level of 60% was selected to form a cohesive and flowable paste suitable as an orthodontic cement. The cements with 20 and 30% nanoACP matched the SBS of TRBD control. The cement with 40% nanoACP had a SBS of 10.7 MPa that was similar to that of RMGIV control. The recommended bond strength of metal brackets to enamel is ≥6 MPa in order to provide adequate adhesion to enamel29). Therefore, the cement with 40% nanoACP having a SBS of 10.7 MPa would provide adequate bracket-enamel bond strength. The cement with 50% nanoACP had a SBS of 6 MPa, which may still be acceptable but is at the lower end of the recommended bond strength range, and therefore may risk accidental debonding during treatment in the clinic. These results indicate that the cement with 40% nanoACP would be optimal to provide the greatest recharge and re-release of Ca and P ions, while still achieving sufficient bracket-enamel bond strength.

The Ca and P ion rechargeability resulted from the use of the PEHB resin. As an acidic monomer with bonding capability, PMGDM can chelate with Ca ions because of the carboxylate groups20). EBPADMA is a monomer employed in composites, with a relatively flexible structure, smaller vinyl group concentration and less viscous compared to Bis-GMA22). Both HEMA and Bis-GMA are conventional monomers commonly employed in dental resins23). Bis-GMA has a core chemical structure being bisphenol A; this makes Bis-GMA a stiff molecule to yield a polymer that is strong mechanically. Furthermore, Bis-GMA has two pendant hydroxyl groups to achieve strong hydrogen bonds with hydroxyl groups on neighboring Bis-GMA molecules. In addition, Bis-GMA has a relatively high molecular weight, which can improve mechanical properties24,25). Nonetheless, Bis-GMA-rich and HEMA-rich adhesives could undergo modest hydrolysis and resulting in potential failure of the bonded interface26,27). Therefore, the Bis-GMA and HEMA amounts should be limited because they may affect the long-term bond integrity. This was why the current study employed only 5% of Bis-GMA and 10% of HEMA to avoid compromising the long-term bond integrity.

This PEHB resin composition yielded the best CaP rechargeability as shown previously in comparison with several other resin systems28). In the present study, this resin yielded a cement with durable release of Ca and P ions, with great potential to inhibit tooth mineral loss. The rechargeability of PEHB cement likely depended on the following mechanism24,25). PMGDM contains carboxylate groups that can chelate with Ca ions in the recharge solution. The solution used to recharge the samples (simulating a mouth-rinse) was made with pH 7. The cement contains PMGDM, and when immersed in the recharge solution, it can chelate with the Ca ions diffusing from the solution into the cement, thus causing the ion recharge to occur. After the recharge and during the re-release process, the samples were immersed in a pH 4 solution to simulate an acidic challenge. The bond between Ca ions and PMGDM could be severed in the pH 4 solution, thereby re-releasing the ions to combat the cariogenic challenge.

The present study showed that increasing nanoACP mass fraction increased the Ca and P ion release, recharge and re-release. There are three factors that may contribute to this phenomenon. First, the nanoACP fillers provided the ion source which contributed to the ion release. Increasing the filler level produced more ion source and therefore more ion release. Second, when the nanoACP content in the resin was greater, it...

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**Fig. 5** Effect of nanoACP mass fraction in cement on culture medium pH using a dental plaque microcosm biofilm model (mean±SD, n=6).
not only increased the source of release, it also yielded greater interfaces in the resin. The interface between the nanoACP particles and the resin served as relatively easier passageways for water and ions to diffuse in or out. Third, after the initial release of Ca and P ions which depleted the ions, the positions that were previously occupied by the Ca and P ions would become available for the new Ca and P ions being recharged into the cement from the recharge solution. Therefore, for a CaP-containing resin, the resin with a greater initial release of Ca and P ions is anticipated to have a higher ability of recharge and re-release of Ca and P ions. This is in agreement with previous results on F-releasing dental materials, exhibiting that the samples with more initial release of F ions also achieved higher ability of F ion recharge and re-release\(^{41-43}\).

This study further showed that the new cement could neutralize acids and increase the biofilm pH. Oral acidogenic bacteria can ferment carbohydrates and produce organic acids including lactic, formic, acetic and propionic acids\(^{44}\). These acids can cause the oral biofilm plaque pH to drop to 4.5 or even 4 after a sucrose rinse\(^{45}\). Studies have indicated a critical pH, below which mineral-loss dominates\(^{46}\). This critical pH was shown to be about 5.5 for most people, although it could change with changing ionic concentrations in the oral fluid\(^{47}\). Therefore, it would be greatly beneficial for the cement to neutralize the local biofilm acids and raise the cariogenic pH from 4 to a safe pH of >5.5 or greater, thereby to avoid tooth mineral loss. In the present study, the PEHB+30% nanoACP and PEHB+40% nanoACP maintained the biofilm pH in the safe area of ≥5.5. This was likely because the nanoACP was alkaline and had an acid-neutralization capability\(^{13,19}\). In addition, the greater the nanoACP mass fraction, the greater the biofilm pH. Since PEHB+50% nanoACP had a relatively low bond strength, and PEHB+20% nanoACP had biofilm pH<5.5, this study suggests that PEHB+30% nanoACP and PEHB+40% nanoACP are promising bioactive cements for dental applications.

Teenagers constitute the major group of orthodontic patients, yet they may have poor compliance in hygiene and diet controls. Therefore, the CaP recharge for the PEHB+nanoACP cement should be user-friendly, requiring a minimal time to perform. The present study used a recharge of 1 min for 3 times a day, and it yielded continuous release of Ca and P ions for at least one week without further recharge. Therefore, the user could potentially use a mouth-rinse for 1 min each after breakfast, lunch and dinner on a Sunday, and do this only once per week.

In addition, the ion re-release was measured with specimens immersed in a pH 4 solution continuously as an accelerated test. Clinically, the Stephan Curve shows that the plaque pH, following a glucose intake, stays in the cariogenic area of about pH 4.5 for less than 30 min\(^{46}\). Then the pH increases back to a safe pH of 6 or higher, after the oral bacteria have finished their metabolization of the glucose and the saliva has nullified the acid\(^{45}\). Therefore, instead of being immersed at pH 4 for 24 h every day, the cement in vivo would experience a pH close to 4 for only a couple of hours per day, thus preserving the ion reservoir in the cement from being quickly exhausted. This means that the PEHB+nanoACP cement, after one recharge, could potentially re-release Ca and P ions for much longer than 2 weeks, perhaps even allowing the patient to do a recharge mouth-rinse only once per month. Further study is needed to investigate the re-release of Ca and P ions from the PEHB+nanoACP cement under a pH condition consistent with the Stephan’s Curve for the biofilm plaque pH, to determine the in vivo re-release duration after a recharge treatment, and to determine how often the patient needs to recharge.

The present study investigated the PEHB+nanoACP cement for orthodontic application as an example. Other potential applications of PEHB+nanoACP cement with tooth bonding capability could include crown cement, inlay cement, base, pit and fissure sealant, and other restorative and preventive applications. For example, PEHB+nanoACP cement could potentially be used in treating root caries and Class V restorations for senior patients with gingival recession and increased root exposures. The bioactive cement could neutralize biofilm acids in the root area, raise the local pH, inhibit mineral-loss and enhance tooth root mineral regeneration, with recharge capability to provide long-term protection to tooth structures. Further studies are needed to investigate various dental applications of the rechargeable PEHB+nanoACP dental cement.

CONCLUSIONS

This study developed a novel nanoACP cement and investigated the effect of nanoACP filler level on Ca and P ion recharge, long-term ion release and biofilm acid neutralization for the first time. Ca and P ion recharge and re-release was linearly proportional to nanoACP filler level. The nanoACP cement could be repeatedly recharged to have long-term ion release, which did not decrease with increasing cycles of recharge and re-release. The new cement increased the biofilm pH to a safe region of >5.5, while commercial cements had cariogenic pH<4.5. These results, together with previous studies showing that Ca and P ions inhibited caries and remineralized tooth lesions, indicate that the new cement is promising dental applications.

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