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Novel rechargeable nano-CaF_2 orthodontic cement with high levels of long-term fluoride release

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ABSTRACT

Objectives: Fluoride-containing orthodontic cements are used to combat white spot lesions (WSLs) in enamel. However, the fluoride (F) ion releases from these cements are relatively low and short-term. The objectives of this study were to develop a novel rechargeable orthodontic cement with nanoparticles of calcium fluoride ($nCaF_2$) to provide long-term and high levels of F release, and to investigate F recharge and physical and cytotoxic properties.

Methods: The $nCaF_2$ with a mean particle size of 58 nm were synthesized using a spray-drying method. Pyromellitic glycerol dimethacrylate (PMGDM), ethoxylated bisphenol A dimethacrylate (EBPADMA), 2-hydroxyethyl methacrylate (HEMA) and bisphenol A glycidyl dimethacrylate (BisGMA) were used to prepare the cements (denoted PE and PEHB resins). A resin-modified glass ionomer (RMGI) served as control. Enamel shear bond strength (SBS), cytotoxicity, and F ion recharge and re-release were evaluated.

Results: $nCaF_2$ cements had good SBS and excellent biocompatibility that were comparable to RMGI ($p > 0.1$). After a recharge for 1 min, the F re-release from PEHB + 30%$nCaF_2$ cement was 80% higher than RMGI ($p < 0.05$). Increasing $nCaF_2$ content from 20% to 30% greatly increased the F ion re-release ($p < 0.05$). The F ion re-release of $nCaF_2$ cements did not decrease with increasing the number of recharge and re-release cycles ($p > 0.1$).

Conclusions: A novel F ion-rechargeable orthodontic cement containing $nCaF_2$ was developed with clinically acceptable enamel SBS, good biocompatibility, and sustained F ion recharge and re-release that were 1.8 folds that of a commercial RMGI.

Clinical Significance: Novel rechargeable $nCaF_2$ orthodontic cement is promising to provide the needed long-term and high levels of F ion releases to inhibit WSLs in orthodontics.

1. Introduction

Orthodontic treatments are becoming increasingly popular due to growing desire for esthetics. However, white spot lesions (WSLs) are a common drawback in fixed orthodontic treatments [1]. WSLs are defined as subsurface enamel porosities from carious demineralization and clinically characterized by chalky areas adjacent to orthodontic appliances [1]. WSLs occur in up to 97% of all orthodontic populations and decrease their quality of life by compromising the esthetics [2]. Constant endeavors have been made to develop interventions that could prevent WSLs during orthodontic treatment [3].

To combat tooth structure demineralization, fluoride (F) is a widely

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recognized anti-caries agent. The correlations between caries inhibition and F ions in saliva and drinking water have been identified by previous investigations [4,5]. F ions reduce caries by acting as a glycolytic enzyme inhibitor and transmembrane proton carrier which inhibit the cariogenic bacterial metabolism [6]. Furthermore, F ions could decrease the enamel solubility by forming fluoroapatite (FAP) and protect the demineralized enamel by promoting remineralization [7,8].

Several F-containing products have been developed to prevent enamel demineralization, including dentifrice, rinse, varnish and composite [9–13]. A common problem for these products is that the release of F ions is short-term [14]. A cochrane review found that, among the many types of F therapies, the periodic application of F-containing varnish is the only effective F-based therapy to prevent WSLs during fixed brace treatment [15]. This review also suggested that F-based treatments which do not need patient compliance are more promising in preventing WSLs [15]. Indeed, the patient compliance required by the periodic F-based treatments often leads to unsatisfactory effects [16].

Therefore, it is highly desirable to develop novel antibacterial and remineralizing orthodontic cements that do not rely on patient compliance to prevent WSLs [17]. Resin-modified glass-ionomer cements (RMGIs) are used in orthodontic practice due to their acceptable shear bond strength and the ability to release F ions [18]. However, the F ion release from RMGIs is relatively short-term, with an initial burst followed by a rapid decrease in F ion release [19]. Indeed, several clinical trials identified that the concentration of F ions released from RMGIs were too low to inhibit the biofilm metabolism in oral cavities in the long-term [20,21]. Therefore, currently, there is a need to develop a new orthodontic cement that can repeatedly be recharged to re-release high levels of F ions to provide a sustained prevention of WSLs during orthodontic treatments.

Regarding the ion recharge property, RMGIs were shown to be able to act as a reservoir for topical fluoridation and then re-release the F ions [22]. However, the re-release of F ions from RMGIs after the recharge lasted only 2–3 days, with a low level of F ion re-release [19]. Recently, novel composites and cements were synthesized using a matrix resin that consisted of pyromellitic glycerol dimethacrylate (PMGDM) and ethoxylated bisphenol A dimethacrylate (EBPADMA), showing a strong recharge capability of calcium (Ca) and phosphate (P) ions with re-releases lasting 14 days after one recharge [23,24]. The PMGDM-EBPADMA resin can chelate with inorganic ions from solutions thus achieving an effective ion recharge [25]. However, to date, there has been no report on the use of the PMGDM-EBPADMA resin together with nanoparticles of calcium fluoride for F ion recharge and re-release.

Nanotechnology has revolutionized health care industry and its application in dentistry has drawn great attention in past decades. Nanoparticles are more effective to suppress bacterial adherence and biofilm formation with stronger antibacterial property than traditional particles [26–28]. The size-dependent effects also apply to remineralization-promotion [26]. Recently, we synthesized nanoparticles of calcium fluoride ($nCaF_2$), and greatly improved the antibacterial and remineralization capabilities of a RMGI by $nCaF_2$ incorporation [29]. The addition of $nCaF_2$ at a relatively low mass fraction resulted in a high level of F ion release from the orthodontic cement, which was mainly attributed to the small particle size and the resultant high surface area of $nCaF_2$ [29–31]. However, previous studies on $nCaF_2$ did not investigate the F ion recharge capability.

Therefore, the objectives of the present study were to develop a novel $nCaF_2$-containing orthodontic cement and investigate F ion recharge and re-release properties for the first time. The following hypotheses were tested: (1) The new rechargeable $nCaF_2$ orthodontic cement would have an enamel-bracket shear bond strength comparable to the commercial RMGI; (2) The $nCaF_2$-containing cement would have greater F ion recharge and re-release than the commercial RMGI; (3) The level of ion recharge and re-release would increase with increasing $nCaF_2$ mass fraction; (4) The recharge and re-release of F ions from the $nCaF_2$ cement would be sustainable and not decrease with increasing the number of recharge and re-release cycles.

2. Materials and methods

2.1. Synthesis of $nCaF_2$

A spray-drying approach was used to prepare the $nCaF_2$ as previously described [29–31]. A two-liquid nozzle (ViscoMist, Lechler, St. Charles, IL, USA) was used to allow two different solutions to be fully mixed at the time of atomization. In this study, calcium hydroxide [$Ca(OH)_2$] and ammonium fluoride (NH_4F) were dissolved in distilled water respectively, and were simultaneously fed to the nozzle at a calcium/fluoride molar ratio of 1:2. Two solutions were sprayed into the heated chamber of the spray-dryer to produce the nanopowder. The reaction of $Ca(OH)_2$ and NH_4F lead to the formation of calcium fluoride (CaF_2), ammonia (NH_3) and water (H_2O). The NH_3 and H_2O vapor were removed with air flow. The nanopowders were collected using an electrostatic precipitator (MistBuster, Minneapolis, MN, USA). The nanopowders were confirmed to be $nCaF_2$ by X-ray diffraction and had an average particle size of 58 nm in previous studies [29,30]. In the present study, the $nCaF_2$ was examined using a transmission electron microscopy (TEM, 3010 HREM, JEOL, Peabody, MA, USA).

2.2. Fabrication of orthodontic cements

Two types of matrix resins were prepared to formulate the experimental orthodontic cements. The first consisted of PMGDM (Hampford, Stratford, CT, USA) and EBPADMA (Esstech, Essington, PA, USA) at a mass ratio of 1:1, which was rendered light-curability with 0.2% (all weight) camphorquinone (CQ; Sigma-Aldrich, St Louis, MO, USA) and 0.8% ethyl 4-N, Ndimethylaminobenzoate (4E; Sigma-Aldrich). PMGDM is an acidic monomer that can chelate with inorganic ions [25,32]. Recently, the combined use of PMGDM and EBPADMA has been found to recharge calcium and phosphate ions [24]. Our preliminary study observed a strong recharge and re-release ability of F ions with this resin. This matrix resin is referred to as the PE resin.

To formulate the second matrix resin, 10% of 2-hydroxyethyl methacrylate (HEMA) (Esstech) and 5% of bisphenol A glycidyl dimethacrylate (BisGMA) (Esstech) were added into the PE resin, yielding a resin with 42% PMGDM, 42% EMPADMA, 10% HEMA, 5% BisGMA, 0.2% CQ and 0.8% 4E. The aim of adding HEMA and BisGMA was to improve the cross-linkage of monomers and tooth-bonding ability of the cement. The mass fractions of BisGMA and HEMA were determined following a previous study in which the combined use of BisGMA and HEMA successfully improved the bond strength [33]. The second matrix resin is referred to as the PEHB resin.

The $nCaF_2$ were incorporated into PE and PEHB at mass fractions of 0%, 20% and 30%. Mass fractions of $nCaF_2$ higher than 30% were not used because of the reductions in enamel bond strength in our preliminary study.

A commercial RMGI (GC Ortho LC, Fuji, Aichi-ken, Japan) was used as commercial control. GC is used as a bracket-bonding cement in orthodontic practice due to its acceptable enamel bonding strength and ability to release F ions [18]. According to the manufacturer, GC contained fluoroaluminosilicate glass, hydroxyethyl methacrylate and polyalkenoic acid. Seven cements were tested:

(1) GC ortho LC (referred to as GC control)
(2) 40% PE resin + 0% $nCaF_2$ + 60% glass (PE control)
(3) 40% PE resin + 20% $nCaF_2$ + 40% glass (PE + 20%$nCaF_2$)
(4) 40% PE resin + 30% $nCaF_2$ + 30% glass (PE + 30%$nCaF_2$)
(5) 40% PEHB resin + 0% $nCaF_2$ + 60% glass (PEHB control)
(6) 40% PEHB resin + 20% $nCaF_2$ + 40% glass (PEHB + 20%$nCaF_2$)
(7) 40% PEHB resin + 30% $nCaF_2$ + 30% glass (PEHB + 30%$nCaF_2$)

2.3. Enamel shear bond strength (SBS) testing

Human premolars that were extracted for orthodontic reasons were collected with the informed consent of patients and the approval by Institutional Review Board of the authors' affiliations. The teeth were stored in 0.1% thymol solution at 4 ℃. Eight-four teeth were randomly divided into seven groups, with 12 teeth per group.

Each tooth was embedded vertically into a self-curing acrylic resin (Lang Dental Manufacturing, Wheeling, IL, USA). The positions of embedded teeth were adjusted to ensure the buccal axis of clinical crowns would be parallel to the mechanical load in SBS test. The buccal surface of each sample was etched with 37% phosphoric acid gel (3 M united, Monrovia, CA, USA) for 30 s, washed with distilled water and was then thoroughly dried by an air stream until a chalky appearance was observed. For GC control, a metal bracket (Shinye, Hangzhou, China) was bonded to the buccal center of each sample using the GC paste, following the manufacturer's instructions. The bonding procedures for the other six groups were the same as that for GC. A compressive force of 200 g was applied to the bracket for 5 s with a force gauge. After that, the specimen was photo-polymerized (VCL 401, Demetron, CA, USA) from all four sides of the bracket for 10 s on each side.

For each group, the 12 teeth were divided into two subgroups of six teeth each. One subgroup was used to measure the initial SBS to enamel, and the other subgroup was used to investigate the enamel SBS after a demineralization challenge. The demineralization challenge was used to simulate the biofilm-induced acidic environment around the brackets which could potentially degrade the bracket-tooth interface and decrease the bonding strength [33,34]. The specimens used for the initial SBS test were immersed in distilled water at 37 ℃ for 24 h, and then subjected to the SBS test. The specimens of the other subgroup were immersed in a demineralization solution of pH 4.0 for 30 days before the SBS test. The demineralization solution was prepared by buffering a sodium chloride solution (133 mmol/L) to pH 4.0 with lactic acid (50 mmol/L) [29]. The pH 4.0 value was used because the cariogenic bacteria could reduce the plaque pH to 4.0–4.5 after fermenting carbohydrates and producing acids [35]. During the challenge time, the solution was refreshed every three days and the pH was adjusted to 4.0.

To measure the enamel SBS, a chisel tester was connected to a universal testing machine (MTS System Corporation, Eden prairie, MN, USA). An occlusal-gingival load was applied to the bracket at a rate of 0.5 mm/min until the bracket was detached. The SBS was determined as the load divided by the bracket-tooth interfacial area [29].

2.4. Initial F ion release from the virgin cements

A 133 mmol/L sodium chloride solution was buffered to pH of 4.0 with 50 mmol/L lactic acid to simulate the cariogenic low pH condition (termed the lactic acid solution) [29]. The cement pastes of all seven groups were placed into a rectangular mold of 2 × 2 × 12 mm and polymerized by a light curing unit (Demetron) for 1 min. Each specimen was placed in a vial and immersed by 16.5 mL of lactic acid solution, yielding a specimen volume/solution ratio of approximately 2.9 mm^3/mL, following previous studies [29,30]. The lactic acid solution was refreshed daily. The F ions released from the specimens were measured daily during the first 28 days and then every third day in the following 42 days. For each measurement, 0.5 mL of sample solution was transferred into a test tube, and 0.5 mL of the Total Ionic Strength Adjustment Buffer (TISAB, Thermo Scientific, Waltham, MA, USA) was added to provide a constant background ionic strength [19]. The concentrations of F ions were assessed using a selective electrode (Thermo Scientific). The F ion release from the specimens during this 70-day period was termed the "initial release" from the virgin cement specimens, to be distinguished from the subsequent ion recharge and re-release.

To calculate the cumulative F ion release during the 70-day period, the total F ion release of each specimen was calculated. The average values of total releases from the six samples in each group were determined as the cumulative F ion initial releases.

2.5. F ion recharge and re-release of cement specimens

After the 70-day initial release, the same specimens were immersed in a fresh lactic acid solution for 1 day to determine the F ion release baseline. Then, the specimens were recharged by immersion for 1 min in a sodium fluoride solution with F ion concentration of 5000 ppm, following a previous study [36]. The sodium fluoride solution and its concentration were used because of its favorable effectiveness in F ion recharge [22,36]. The specimens were then rinsed with running distilled water for 1 min to remove any loosely attached deposits on specimen surfaces. After the recharge, the specimen was immersed in 16.5 mL lactic acid solution to re-release the F ions. The F ion re-release from the recharged specimens were measured daily for 7 days, during which the lactic acid solution was changed daily. Each recharge and the 7-day re-release constituted one cycle of recharge and re-release. This was repeated four times to investigate whether the F ion recharge and re-release level would decrease with increasing the number of cycles.

2.6. Cytotoxicity test of orthodontic cements

Since several dental monomers and F ions were reported to potentially have a low level of cytotoxicity [37], the cytotoxicity of GC control, PE + 20%nCaF_2, PE + 30%nCaF_2, PEHB + 20%nCaF_2 and PEHB + 30%nCaF_2 were tested. Each cement paste was placed into a circular mold with a diameter of 8 mm and a thickness of approximately 1 mm, and photo-cured (Demetron) for 1 min. The cement disks were transferred into distilled water and stirred by a magnetic bar at a rate of 100 rpm for 1 h to release the initial burst of any uncured monomers, following previous studies [29,38]. The cement disks were then sterilized with ethylene oxide and degassed for seven days (Anprolene AN 74i, Andersen, Haw River, NC, USA). Then each disk was placed in a 48-well plate and immersed by 500 μL of Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island, NY, USA) containing 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). This had a ratio of cement surface area/solution volume to be approximately 2.5 cm^2/mL, which was within the recommended range of the International Organization for Standardization (ISO) [39]. The cement disks were incubated at 37 ℃, and the culture medium was replaced daily. The extracts of day 1, 4 and 7 were collected and stored at -20 ℃ for cytotoxicity tests.

Human gingival fibroblasts (HGFs, ScienCell, San Diego, CA, USA) were seeded into 96-well plates with a cell density of approximately 10,000/well. The cells were cultured with the extracts plus 10% fetal calf serum (FCS, Gibco) at 37 ℃ for 24 h. The HGFs cultured with DMEM (Gibco) containing 10% FCS (Gibco) and 1% penicillin/streptomycin (Invitrogen) were used as the negative control. MTT assay was conducted to determine the cell density at 492 nm spectrophotometrically (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA). The optical density ratio of the experimental group to the negative control was calculated as a measure of the cell viability.

2.7. Statistical analysis

SPSS 19.0 (SPSS Inc, Chicago, IL, USA) was used for the statistical analyses. All results were expressed as mean ± standard deviation (sd). Shapiro-Wilk analysis was adopted to test the normal distribution of all data. One-way analyses of variance (ANOVA) and Fisher's Least Significant Difference (LSD) post-hoc test were performed to evaluate the differences in F ion initial release, F ion re-release and cytotoxicity between the groups. Two-way ANOVA was used to investigate whether the demineralization challenge and type of matrix resin had effects on the SBS of cements. The differences with $p < 0.05$ were considered to be significant.

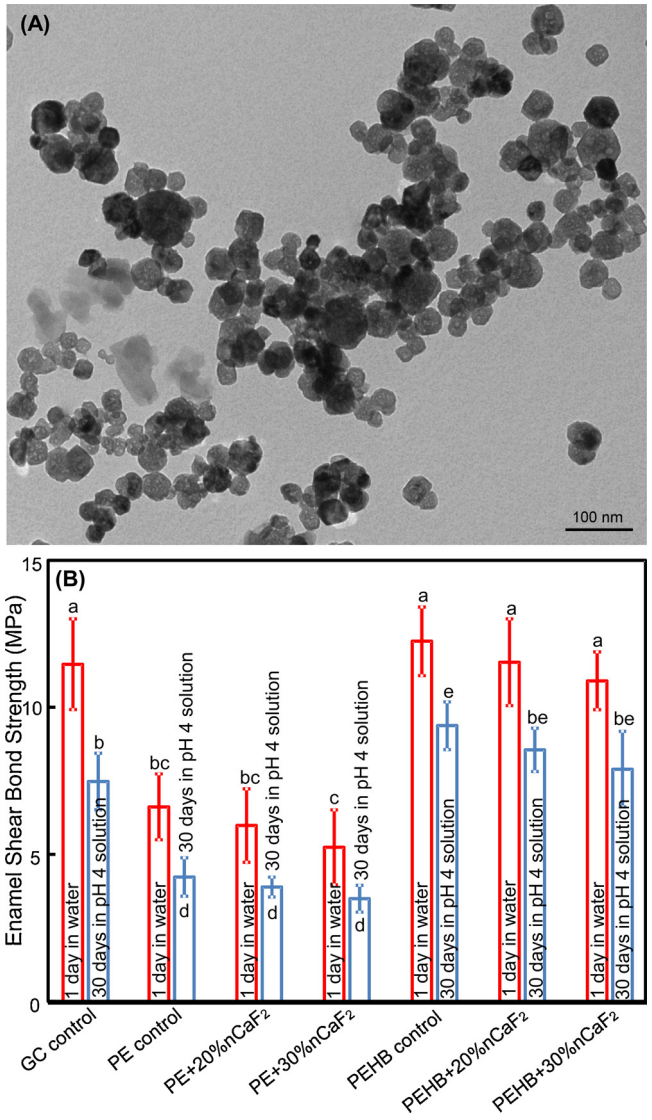

Fig. 1. CaF_2 nanoparticles and enamel shear bond strengths of orthodontic cements (mean ± sd; n = 6). (A) Typical TEM images of CaF_2 nanoparticles ($nCaF_2$). (B) The red and blue bars represent the specimens prior to and after 30-day demineralization challenge, respectively. The addition of 20% and 30% $nCaF_2$ had no significant effect ($p > 0.1$). The bonding strength of PEHB + 20% $nCaF_2$ and PEHB + 30%$nCaF_2$ were similar to that of GC control both before and after demineralization challenge ($p > 0.1$). The same letters indicate no statistical difference between the group ($p > 0.1$), while the different letters indicate a significant difference ($p < 0.05$). SBS: Shear bond strength. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3. Results

A representative TEM image of $nCaF_2$ from spray-drying is shown in Fig. 1A. The $nCaF_2$ powder consisted of fine particles with sizes of 10–20 nm, along with larger particles having sizes of approximately 100 nm. The larger particles appeared to be the agglomeration of numerous fine nanoparticles, which were formed in the spray-drying chamber before the fine particles were fully dried. The mean particle size was measured to be 58 nm.

The enamel bonding strengths of the cements are plotted in Fig. 1B. Both the type of matrix resin and the demineralization challenge had significant effects on the bond strength ($p < 0.05$); there was no significant interaction between the type of matrix resin and the demineralization challenge ($p > 0.1$). The initial SBS of GC control and

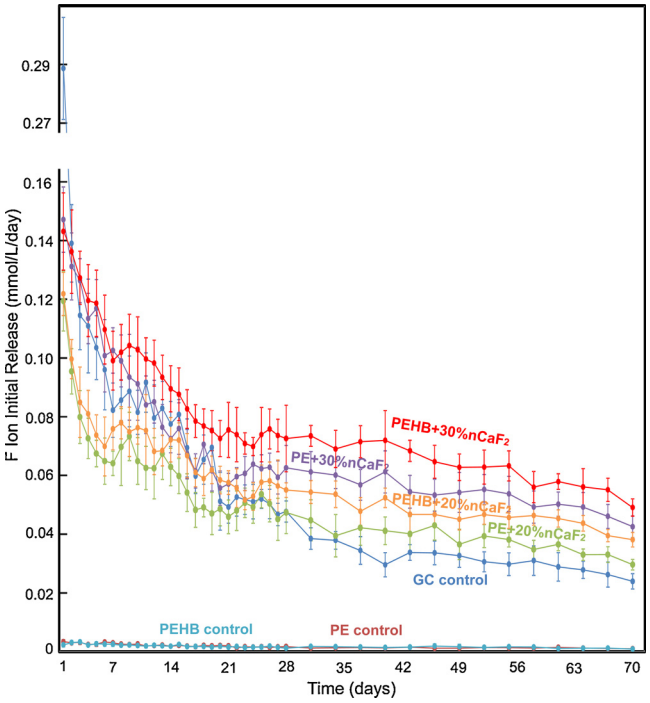

Fig. 2. Initial F ion release from orthodontic cements (mean ± sd; n = 6). The initial release indicates the 70-day F ion release in pH 4.0 lactic acid solution before recharge. The release of GC control was particularly high at day 1 ($p < 0.05$), but then drastically decreased. At the first 7–10 days of initial release, the releases from PEHB resin-based $nCaF_2$ cements were similar to that of PE cements with the same nCaF2 contents ($p > 0.1$). At the steady phase, PEHB + 20%$nCaF_2$ PEHB + 30%$nCaF_2$ had higher F ion release than their PE counterparts ($p < 0.05$).

PEHB control were similar ($p > 0.1$), both of which were higher than that of PE control ($p < 0.05$). The incorporation of 20% and 30% $nCaF_2$ into PE and PEHB resin had no adverse effects on the initial SBS ($p > 0.1$). The SBS of all seven groups were reduced significantly after the 30-day demineralization challenge ($p < 0.05$). For the SBS after challenge, the value of GC control was similar to that of PEHB + 20% $nCaF_2$ and PEHB + 30%$nCaF_2$ ($p > 0.1$). The addition of $nCaF_2$ had no adverse effects on the SBS after the demineralization challenge ($p > 0.1$).

The initial releases of F ions from the virgin cements are shown in Fig. 2. The F ion release of GC control was relatively high at day 1; its release then decreased rapidly in the first week and gradually declined to reach a lower long-term release rate. The release profiles of PE + 20%$nCaF_2$, PE + 30%$nCaF_2$, PEHB + 20%$nCaF_2$ and PEHB + 30%$nCaF_2$ were similar, with long-term release rates higher than that of GC control ($p < 0.05$). For the long-term release, PEHB + 30%$nCaF_2$ had the highest F ion release rate, which was approximately 60% higher than that of PE + 20%$nCaF_2$ ($p < 0.05$), and 110% higher than that of GC control ($p < 0.05$).

The cumulative F ion initial releases of orthodontic cements are presented in Fig. 3. The release was significantly enhanced when the mass fraction of $nCaF_2$ was increased from 20% to 30% ($p < 0.05$). PEHB + 20%$nCaF_2$ and PEHB + 30%$nCaF_2$ had higher releases than their PE counterparts ($p < 0.05$).

The F ion recharge and re-release of the cements are plotted in Fig. 4. In each recharge and re-release cycle, the re-release of GC control increased substantially right after the recharge ($p < 0.05$), but then decreased rapidly to a baseline level within 3–4 days ($p > 0.1$). However, the re-releases of PE + 20%$nCaF_2$, PE + 30%$nCaF_2$, PEHB + 20%$nCaF_2$ and PEHB + 30%$nCaF_2$ were higher than their baseline levels even at the 7th day ($p < 0.05$). PEHB + 30%$nCaF_2$ had

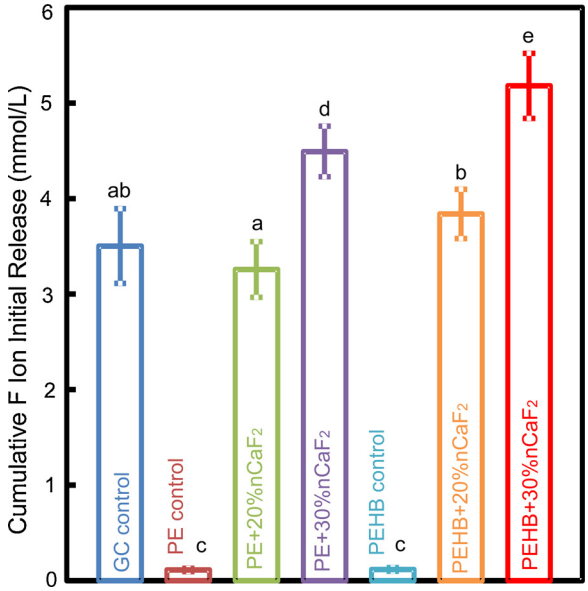

Fig. 3. Cumulative initial F ion release from orthodontic cements (mean ± sd; n = 6). The amount of F ions released from GC control was similar with that of PE + 20%$nCaF_2$ and PEHB + 20$nCaF_2$ ($p > 0.1$). Increasing the $nCaF_2$ mass fraction from 20% to 30% greatly improved the cumulative F ions release from $nCaF_2$ cements ($p < 0.05$). With the same $nCaF_2$ content (20% and 30%), PEHB resin-based $nCaF_2$ cements had higher cumulative initial F ion release than that of PE counterparts ($p < 0.05$).

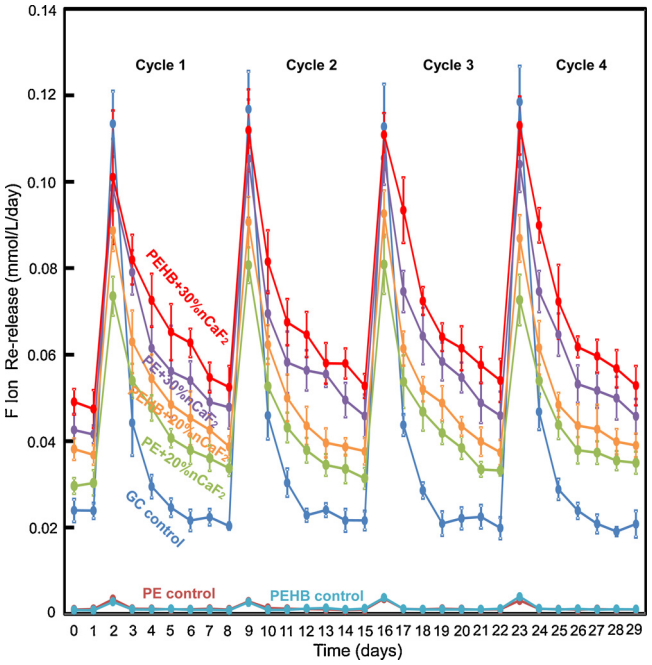

Fig. 4. The recharge and re-release behaviors of orthodontic cements after the 70-day initial release (mean ± sd; n = 6). The recharge and re-release cycles were repeated for four times. The release at day 0 was the last day of the initial release. The release at day 1 was determined to serve as the F ion release baseline before the F ion recharge.

the highest F ion re-release among all groups ($p < 0.05$).

The cumulative F ion re-release in the four cycles are displayed in Fig. 5. The amount of F ions re-released from GC control was only 62% and 55% of that from PE + 30%$nCaF_2$ and PEHB + 30%$nCaF_2$, respectively ($p < 0.05$). Increasing the mass fraction of $nCaF_2$ from 20% to 30% greatly increased the re-release ($p < 0.05$). Both PEHB + 20% $nCaF_2$ and PEHB + 30%$nCaF_2$ had higher levels of re-releases than their PE counterparts ($p < 0.05$).

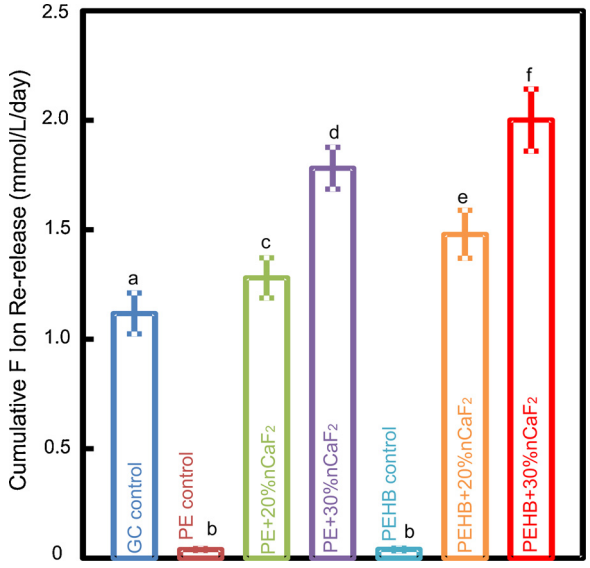

Fig. 5. Cumulative F ion re-release from orthodontic cements (mean ± sd; n = 6). The re-release of F ions from GC control was lower than that of PE + 20% $nCaF_2$ and PEHB + 20$nCaF_2$ ($p < 0.05$). Increasing the $nCaF_2$ mass fraction from 20% to 30% greatly improved the cumulative F ions re-release ($p < 0.05$). At the same $nCaF_2$ filler level, PEHB-$nCaF_2$ cement had higher cumulative F ion re-release than that of PE-$nCaF_2$ cement ($p < 0.05$).

The viabilities of HGFs against eluents of orthodontic cements are plotted in Fig. 6. The viabilities of HGFs cultured with the extracts of GC control were similar to that of PEHB + 20%$nCaF_2$ and PEHB + 30% $nCaF_2$ ($p > 0.1$), but slightly lower than that of PE + 20%$nCaF_2$ and PE + 30%$nCaF_2$ for day 1 and 4 ($p < 0.05$). The cell viability of PEHB + 30%$nCaF_2$ was 76.6%, 80.7% and 85.5% for day 1, 4 and 7, respectively, all of which exceeded the 70% viability requirement of ISO [39].

4. Discussion

The present study represents the first report on $nCaF_2$ dental cement showing high levels of F ion release and much greater recharge and re-release than commercial RMGI control. The prevention of WSLs has been a critical need in orthodontics that has not been met for decades. Due to the favorable anti-caries effects of F ions, several F-releasing orthodontic cements have been developed. Unfortunately, the releases of F ions from these materials are short-lived, thus cannot effectively protect the enamel from demineralization during the long course of orthodontic treatment [15]. In this study, we developed novel F ion-rechargeable orthodontic cements by incorporating $nCaF_2$ into PE and PEHB resin. The hypotheses were proven that incorporating the tailored mass fractions of $nCaF_2$ into PEHB resin yielded orthodontic cements that had comparable enamel SBS to a commercial RMGI; the initial F ion releases of the $nCaF_2$ cements were higher and longer-lasting than that of commercial RMGI; the recharge ability of the $nCaF_2$ cements was stronger with much higher levels of F ion re-release than that of commercial RMGI control; and the recharge and re-release capability of the $nCaF_2$ cements was sustained and did not decrease with increasing the number of recharge and re-release cycles.

The F ion release from GC control rapidly decreased to a relatively low level after the initial burst in our study, consistent with previous reports on RMGIs [19,40]. For GC, the F release was mainly attributed to the acid-base setting reaction between fluoroaluminosilicate glass powder and polyacid liquid, which liberated a large amount of F ions after the material set [41,42]. This mechanism and the surface wash-off

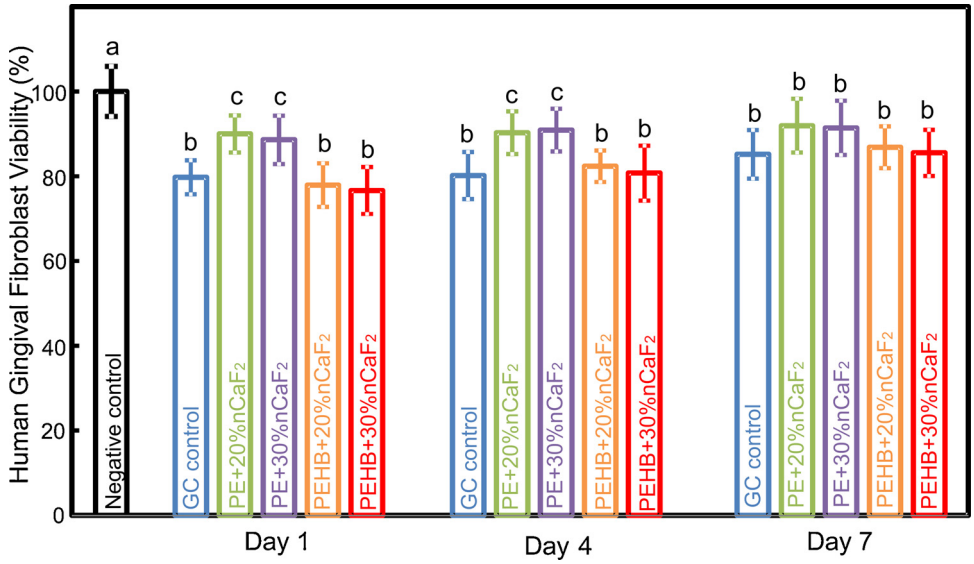

Fig. 6. The cell viabilities of human gingival fibroblasts cultured with extracts of orthodontic cements. PEHB-nCaF_2 cements had similar cell viability to GC control for all three time points ($p > 0.1$).

effect are the main reasons accounting for the initial profound F ion release from GC [22]. For nCaF_2 cements, the F ion release was mainly the result of diffusion of water-soluble F ions from the bulk material into the solution [43,44]. This explains the substantial difference in the F ion release between GC and the nCaF_2 cements. After the initial burst, the F ion release from GC also came from diffusion which was relatively slow and gradually reached a lower long-term release rate [45]. The particle size of nCaF_2 in this study was approximately 58 nm [29], which was much smaller than the glass particles in RMGIs ranging from 1 to 10 μm [46,47]. The smaller particle size of nCaF_2 yielded a much higher surface area, thereby providing more F ions leaving these surfaces [36]. This was likely the reason that the nCaF_2 cements had higher long-term release rate of F ion than that of GC.

The surface elution is the main mechanism for the initial rapid releases of F ions from cements and composites, during which the surface area of specimen and concentration gradient between specimen and solution are critical to the ion release behavior [36,40]. Therefore, in the first week of the initial release, the rates of F ion release were similar between the cements with the same nCaF_2 filler level. After the F ions at the surface and the superficial part of the cements were released into solution, a water diffusion process was required for the F ions to migrate from the inner part to the surface, before being released [36]. Due to the favorable hydrophilicity, the addition of HEMA improved the water sorption of cements thereby promoting the water diffusion into resin and enhancing the ion release [48,49]. This was likely that reason that the long-term release rate of PEHB-nCaF_2 cement was higher than that of the PE-nCaF_2 cement. Therefore, the PEHB + 30% nCaF_2 cement achieved the highest initial release, which was 48% higher than that of GC control.

It was suggested that the materials with higher initial F ion release would also have higher recharge capability [19]. However, PE + 20% nCaF_2 and PEHB + 20%nCaF_2 had similar initial F ion release to GC control, but much higher recharge and re-release of F ions than GC control. This was likely caused by the different recharge mechanisms between GC and the novel nCaF_2 cements. For GC, the space-occupying effect is the main recharge mechanism [19]. After the initial F ion release, the space in GC that was previously occupied by the released F ions received the incoming of F ions from the recharge solution. Therefore, higher initial F ion release from GC would produce more spaces, thereby storing more F ions and leading to more F ion recharge and re-release [19]. However, for nCaF_2 cements, the recharge mechanisms are more complicated. Besides the space-occupying effect, the carboxylate groups of PMGDM in the nCaF_2 cements could chelate with inorganic ions [25,32]. The chelation of F ions from recharge solution could contribute to the superiority of the recharge and re-release behaviors of the nCaF_2 cements. Moreover, the smaller particle size and high surface area of nCaF_2 provided more spaces in the cements after the initial release than the tradition particles in RMGIs [29,46,47], thus amplifying the space-occupying effect and resulting in more efficient recharge and re-release.

In the present study, the specimens were immersed in a sodium fluoride solution for 1 min once every seven days to be recharged. This method was used to mimic the use of F ion-containing mouth-rinses only once per week to recharge the orthodontic cement, which would be patient friendly. Sodium fluoride was selected because it was a common ingredient in commercial mouth-rinses and has been shown to be effective in recharging F ions [22]. We used a lactic acid solution of pH 4.0 to test the re-release, which was to simulate the local cariogenic acidic condition around the brackets [29]. It should be noted that the plaque pH could decrease to 4.0 to 4.5 after the bacteria consume carbohydrates in oral conditions [35]. This low pH would usually return to 5.5 or even higher due to the buffering effect of saliva in about 30 min [50]. We previously found that the F ion release from nCaF_2-containing orthodontic cements was greatly enhanced when the pH decreased from 5.5 to 4.0 [29]. Thus, the continuous immersion in the pH 4.0 lactic acid solution in this study represented an accelerated model of measuring the ion re-release when compared to the real oral environment. Therefore, the re-release from nCaF_2 cements after each recharge would likely last even longer than a week orally where the acid attacks are intermittent. Future studies are required to investigate the F ion recharge and re-release behavior and anti-caries effect of nCaF_2 cements in vivo.

An adequate enamel SBS is a key requirement of orthodontic cements. In the present study, the initial SBS of PE control (6.62 ± 1.12 MPa) was significantly lower than that of GC control (11.47 ± 1.54 MPa). Therefore, we added BisGMA and HEMA into PE resin to improve the SBS. BisGMA is a frequently used monomer in cements and composites [51,52]. Due to its high molecular weight, the addition of BisGMA into cements could decrease polymerization shrinkage, accelerate polymerization and provide favorable mechanical properties [51,52]. As a hydrophilic monomer, HEMA could improve the hydrophilicity and flowability of orthodontic cement, thus facilitating its contact to tooth structure and promoting the formation of cross-linked interlocks between the cement and tooth tissues [51,53].

The addition of BisGMA and HEMA into PE resin improved the initial SBS of PEHB control and PEHB + 30%nCaF_2 to 12.25 ± 1.16 MPa and 10.90 ± 0.98 MPa respectively, which were comparable to that of GC control.

A common concern for HEMA-containing cements is that the HEMA-induced hydrolysis could degrade the bonded interface between the bracket and tooth structures, thereby reducing the bond durability [54]. In the present study, a pH 4.0 lactic acid solution was used to immerse the bracket-enamel bonded samples for 30 days to simulate an accelerated demineralization challenge, based on following reasons. First, lactic acid accounts for the majority of acids produced by the cariogenic bacteria [55]. Second, the local pH of plaque in oral cavities could reach 4.0 after the bacteria consume carbohydrates and produce organic acids [56], which is the reason for the wide use of pH 4.0 demineralization solution as an accelerated laboratory testing model in previous studies [34]. Third, the lactic acid immersion for 1 h is an approximate estimate of the accumulated acid challenge times orally per day [34,57]. Thus, the 30-day lactic acid challenge in the present study was equivalent to 720 days of acid challenge in the oral environment, which approximated the average orthodontic treatment time duration [58]. The SBS of PEHB-nCaF_2 cements were comparable to that of GC control after the 30-day demineralization challenge, indicating that the nCaF_2 cements had acceptable enamel SBS for clinical applications. It should be noted that commercial RMGI products usually included approximately 15–20% HEMA in the liquid phase, yielding the mass fraction of HEMA to be approximately 5% after mixing with the cement power [59]. The 10% HEMA in the PEHB resin also led the HEMA content in the final nCaF_2 cements to be 4% after mixing with glass and nCaF_2 in the present study. The similar HEMA contents in GC control and nCaF_2 cements could be part of the reasons for their similar SBS before and after the demineralization challenge.

Another potential concern for dental resins is that the uncured and released monomers could compromise the biocompatibility [60]. In the present study, the cell viabilities of PEHB-nCaF_2 cements were comparable to that of the clinically-used GC control, but slightly lower than that of the PE counterparts at day 1 and 4. However, no difference was detected among the five groups at day 7. This is consistent with a previous study that reported that the release of uncured monomers was drastically decreased after the initial burst, which occurred mainly in the first 24 h after polymerization [60] In addition, it was reported that the concentration of F ions released from resin was too low to decrease the biocompatibility [61]. Consistent with this finding, the increment of nCaF_2 mass fraction in the cement from 20% to 30% had no adverse effect on the cytotoxicity. The cell viability of PEHB + 30%nCaF_2 ranged from 76.6% to 85.5%, suggesting that the biocompatibility of the nCaF_2 cements would be acceptable according to the requirement of ISO [39]. Future in vivo studies are required to evaluate the biocompatibility of the nCaF_2 cements in an animal model.

There are two main limitations in the present study. First, although we repeated four recharge/re-release cycles to test the sustainability of F ion recharge and release, the 28-day duration was relatively short when compared to the long course of orthodontic treatment. Future studies should investigate the F ion rechargeability of PEHB-nCaF_2 cement with many more recharge and re-release cycles. Second, we used a sodium fluoride solution with an F ion concentration of 5000 ppm to recharge the cement, following previous studies [22,36]. However, most of the commercial mouth-rinses only contain 230 ppm or 900 ppm F ions for daily and weekly use, respectively [62]. Thus, future studies are required to investigate the recharge and re-release behavior of PEHB-nCaF_2 cement when recharged using commercial mouthwashes.

5. Conclusion

The present study developed the first rechargeable nCaF_2 orthodontic cement to provide long-term F ion release to inhibit enamel WSLs during orthodontic treatments. The novel nCaF_2 cements had enamel SBS comparable to commercial RMGI, with clinically acceptable biocompatibility. Increasing the mass fraction of nCaF_2 enhanced the F ion initial release, recharge and re-release of nCaF_2 cements. The initial F ion release of PEHB + 30%nCaF_2 cement was 48% higher than that of a commercial RMGI. After each recharge for 1 min, the nCaF_2 cements had continuous high levels of F ion re-release for at least 7 days. There was no decrease in the recharge and re-release efficacy of nCaF_2 cements with increasing the number of recharge and re-release cycles. In each cycle, the amount of F ions released from PEHB + 30%nCaF_2 was 80% higher than that of commercial RMGI. Therefore, the combined use of PEHB and nCaF_2 yielded an orthodontic cement with high levels of F ion recharge and re-release, as well as excellent SBS and biocompatibility. This new material is promising for orthodontic and other dental applications to inhibit caries and protect tooth structures.

Declaration of Competing Interest

The authors declare no conflict of interest

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