

# Size Dependence of Particulate Calcium Phosphate Fillers in Dental Resin Composites

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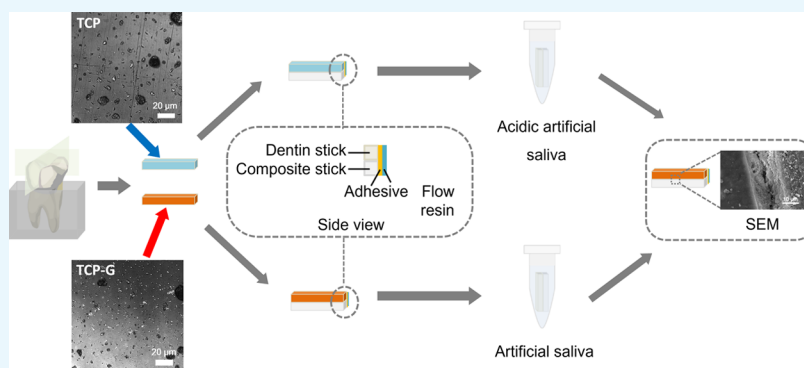
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**ABSTRACT:** Resin composites that consist of polymeric resins and functional fillers are commonly used as restorative materials for dental caries. Various types of calcium phosphates (CaPs) are studied as remineralizing fillers in the formulation of dental resin composites, which are generally inhibitory to demineralization of teeth, but the performance of resin composites has not yet been investigated comprehensively with respect to the size of CaP particles. In this study, the same tricalcium phosphate (TCP) particles within two different size ranges, the as-received TCP particles (TCP) and those resulted from grinding (TCP-G), were tested to determine the size dependence of CaP fillers in dental resin composites. The buffering capability, mechanical properties, ion release, antibacterial performance, and remineralization effect of TCP/TCP-G-containing composites were experimentally characterized and compared against two other commercial dental materials. The integration of micrometer-sized TCP particles resulted in a similar buffering effect and  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  release behaviors compared to the resin composite containing much smaller TCP-G particles. The flexural strength of the TCP-G resin composite was lower than that of the TCP composite after immersion in water for 30 days. However, the TCP-G composite facilitated crystal deposition toward better gap-closing performance at the dentin–composite interface. This study explored detailed insights about the size effect of CaP fillers, which is useful for the development of functional dental resin composites and their clinical translation.

## INTRODUCTION

Dental caries is one of the most common oral diseases. The cavity restoration treatment costs ~46 billion dollars per year in the US.<sup>1</sup> Resin composites are the main materials used in direct restorations at present, due to their adequate esthetics and mechanical performance.<sup>2,3</sup> However, most of the resin composites have limited service lives.<sup>4</sup> Two challenges remained critical to the development of dental resin composites, secondary caries and bulk fracture. Secondary caries is caused by cariogenic bacteria, such as *Streptococcus mutans* (*S. mutans*), which ferment carbohydrates and produce organic acids into the microgaps at the composite–tooth interface,<sup>3,5,6</sup> dissolve the hard tissue, and shift the calcium phosphate balance.<sup>7</sup> Therefore, a mechanically robust composite with caries-inhibition capability is highly desirable for dental restorations.

Dental caries occurs when the extent of bacterial acid demineralization is greater than natural salivary remineraliza-

tion in the mouth.<sup>8</sup> It has been acknowledged that the buffering capacity of resin composites might contribute to compositional shift in the overlying plaque toward lower cariogenicity and less susceptibility to secondary caries.<sup>9</sup> Special requirement for composite restoration is to quickly neutralize the local acids and increase the cariogenic pH from 4 to 5.5 or above. Previous reports showed that resin composites containing various types of calcium phosphates (CaPs) are promising for combating caries, due to their capability of neutralizing acid.<sup>10,11</sup> Furthermore, the compositional  $\text{Ca}^{2+}$

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and  $\text{PO}_4^{3-}$  can be released from the composites to remineralize the remnant of tooth lesions.<sup>10,11</sup> However, the CaP fillers are usually incompatible with the polymeric resins, which leads to severe phase separation and mechanical instability.<sup>12,13</sup> For example, the biaxial flexural strength (FS) of a reported CaP composite only exhibited half of that of its corresponding unfilled resin.<sup>13</sup> Therefore, how to improve the mechanical performance of the  $\text{Ca}^{2+}/\text{PO}_4^{3-}$ -releasing restoratives became a major challenge in CaP filler selection, in addition to the anti-demineralizing/remineralizing capabilities.

CaP-containing dental composites have been actively developed and investigated regarding their mechanical properties,<sup>14,15</sup>  $\text{Ca}^{2+}/\text{PO}_4^{3-}$ -release capability,<sup>14,16</sup> buffering ability,<sup>17</sup> enamel remineralization effect,<sup>16,18</sup> antibacterial activity, and so forth.<sup>16–18</sup> Among all the reported CaP fillers, nano-CaP particulates have drawn much attention, due to their significant specific surface area (ratio of surface area/volume). For instance, dicalcium phosphate anhydrous (DCPA) and tetracalcium phosphate (TTCP) nanoparticles were synthesized and incorporated into dental resins, respectively.<sup>15,19,20</sup> Although these dental composites exhibited both stress-bearing and  $\text{Ca}^{2+}/\text{PO}_4^{3-}$ -releasing capabilities, mechanical reinforcement was only attributed to the additional nano-silica-fused whiskers in the composites, which was prohibitory to photopolymerization of the resins. However, amorphous calcium phosphate (NACP)-containing composites can be photopolymerized and  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  release was also evident, but silanized glass particles were also required for higher mechanical properties.<sup>14</sup> Meanwhile, it is worth noting that the incorporated nanoparticles tend to reduce their surface energy via agglomeration, resulting in variation of mechanical properties and buffering capability.<sup>21</sup> Domingo et al. demonstrated that micrometer-sized hydroxyapatite (HA) fillers provided higher FS than nanoparticulate HAs.<sup>22</sup> In another study, the FS of DCPA-containing composites reduced with decreasing filler size.<sup>15</sup> Overall, how does the size of particulate fillers influence the comprehensive characteristics of dental materials, including mechanical properties, buffering effect, and remineralization of the CaP-containing resin composites? The conclusions from the current publications remain controversial. Therefore, more detailed and systematic assessments are highly desirable for future restorative applications of CaP composites.

Herein, commercialized CaP particles (tricalcium phosphate, TCP) were ground into smaller particles (TCP-G). The TCP and TCP-G powders were mixed with a commonly used resin formulation to achieve CaP dental composites with different sized fillers but without the addition of any other reinforcement materials (whiskers or glass particles). The buffering capability, mechanical properties, ion-release ability, antibacterial activity, and remineralization effect of these resin composites were extensively characterized, compared to a commercial resin composite (SonicFill 2) and glass ionomer cement (GIC). Overall, the size dependence of the CaP fillers was comprehensively evaluated in this study, which might provide an adequate experimental basis for future application of such remineralizing dental composites.

## MATERIALS AND METHODS

**Alkalinity of Different Fillers.** TCP (Kermel, Tianjin, China),  $\beta$ -TCP (Sigma-Aldrich, St. Louis, MO, USA), TTCP (Macklin, Shanghai, China), and bioactive glass SM520 (BAG, Schott AG, Standort Landshut, Germany) were weighed 0.05 g

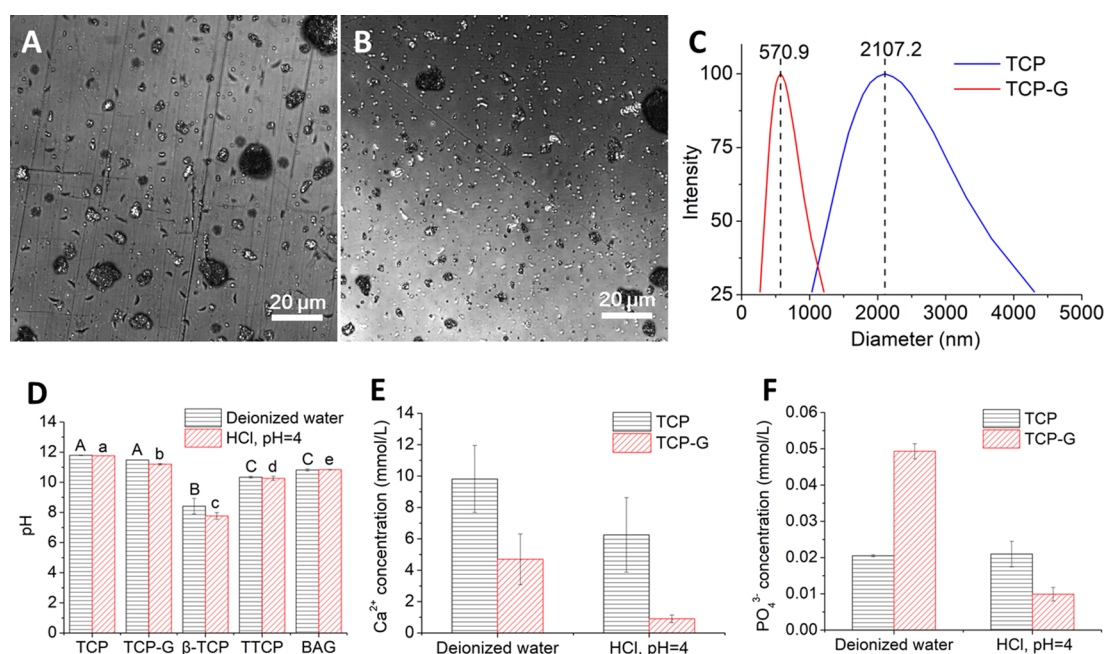
each and placed in a 15 mL centrifuge tube, respectively. A total of 13 mL of deionized water or HCl (pH = 4) was added into each tube. The tubes were agitated for 1 h and then centrifuged at 3000 rpm for 5 min. The pH of the supernatant was measured using a pH meter (S400-K, Mettler Toledo, USA).

**Grinding and Size Measurement of TCP Particles.** The as-received TCP powder was ground using a freezing ball mill (JXFSTPRP-CL, Jingxin, Shanghai, China; frequency = 60 Hz) for 30 min at  $-10\text{ }^\circ\text{C}$ ; the ground TCP particles were denoted as TCP-G. Both TCP and TCP-G particles were observed using a laser confocal microscope (LSM700, Carl Zeiss, Germany). The size and distribution of both TCP and TCP-G particles were determined using dynamic light scattering (DLS). The TCP and TCP-G particles were dispersed in anhydrous ethanol at 0.1 mg/mL. Then, the particle size was measured using a Brookhaven Zeta BI-PALS zeta-sizer at  $25\text{ }^\circ\text{C}$ . Alkalinity of the TCP/TCP-G powder was measured as described above. The  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  concentrations of the supernatant were examined using a microplate reader (SynergyH1, BioTek, USA) using a calcium colorimetric assay kit (MAK022, Sigma-Aldrich, USA) and a phosphate colorimetric assay kit (MAK030, Sigma-Aldrich, USA), respectively.

**Preparation of Resin Composites.** Bisphenylglycidyl dimethacrylate (Bis-GMA) and *N,N'*-dimethyl aminoethyl methacrylate (DMAEMA) were purchased from Aladdin (Aladdin Biochemical Technology, Shanghai, China). Triethylene glycol dimethacrylate (TEGDMA) and camphorquinone (CQ) were purchased from Sigma-Aldrich (St Louis, MO, USA). Bis-GMA and TEGDMA were mixed at mass ratio = 1:1; then, 0.7% DMAEMA and 0.7% CQ were added as photoinitiators (the resulting resin composite is denoted as the BT resin). The TCP and TCP-G particles were mixed with BT resin to form TCP- and TCP-G-containing resin composite, respectively, at a weight fraction of 15%. Both composites were carefully blended until the particles were homogeneously distributed in the BT resins. GIC (Fuji IX, GC, Japan) and a commercial resin composite (SonicFill 2, Kerr, USA; denoted as SF) were used as the control groups.

**Mechanical Characterization.** Five groups of dental composites, including the 15% TCP- and 15% TCP-G-containing BT resins, BT resin alone, GIC, and SF, were subjected to a three-point bending mechanical test (span = 20 mm) using a universal testing machine (AG-I 10KN, Shimadzu, Japan) at crosshead speed = 1.00 mm/min, and the load cell capacity was 250 N. The FS and flexural modulus (FM) were obtained according to the ISO 4049: 2009 standard. Prior to the mechanical tests, 16 specimens per group were prepared using a customized 2 mm  $\times$  2 mm  $\times$  25 mm split mold. Each photocurable sample was irradiated under a visible light-curing unit (Elipar S10, 3M ESPE, Germany) at an averaged irradiance of 1800 mW/cm<sup>2</sup> for 2 min (1 min each side). While the GIC samples were fabricated by following the manufacturer's introduction in the same mold. The setting time of the GIC specimen was over 48 h so that the sample was completely cured before the mechanical tests. Eight specimens in each group were subjected to mechanical tests under dry conditions. The other eight specimens in each group were first immersed in 30 mL of deionized water at  $37\text{ }^\circ\text{C}$  for 30 days; deionized water was replaced every week.

**Long-Term Buffering Effect and Ion Release.** The 15% TCP, 15% TCP-G, and GIC composites were fabricated into



**Figure 1.** (A) Alkalinity (pH values) of different particulate fillers when immersed in deionized water and HCl (pH = 4), respectively. Different upper or lower case letters indicate statistical differences between groups immersed in deionized water or HCl ( $p < 0.05$ ). Representative micrographs of TCP particles (B) before and (C) after grinding using laser confocal microscopy. (D) Size distribution of TCP and TCP-G particles, characterized by DLS analysis. Concentrations of (E)  $\text{Ca}^{2+}$  and (F)  $\text{PO}_4^{3-}$  in the solutions after immersing TCP and TCP-G in deionized water and HCl (pH = 4) for 1 h, respectively.

disc-shaped samples (thickness = 2 mm, diameter = 9 mm,  $n = 3$ ) and then polished with dry 600-, 2000-, and 5000-grit SiC papers to remove edge defects, respectively. Each sample was first exposed to 1700  $\mu\text{L}$  of HCl or lactic acid solution (pH = 4) in 24-well plates at 37  $^\circ\text{C}$ . The pH values of the solutions were measured at 1, 2, 3, 24, 48, and 72 h. Then, solutions were replaced after pH measurement every day until either 30 days or when the obtained pH value reached a steady state. For the ion-release assessment, each specimen was stored in an individual plastic container with 1700  $\mu\text{L}$  of deionized water or HCl solution (pH = 4) at 37  $^\circ\text{C}$  for 24 h. Then, the soaking solution was collected and replaced with the same volume of fresh deionized water or HCl solution (pH = 4). The  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  concentrations of the soaking solutions were measured using a microplate reader (SynergyH1, BioTek, USA) using a calcium colorimetric assay kit (MAK022, Sigma-Aldrich, USA) and a phosphate colorimetric assay kit (MAK030, Sigma-Aldrich, USA), respectively. The 15% TCP and 15% TCP-G groups were tested for 12 days, and the GIC group was examined for 30 days. The experiments were implemented in triplicate.

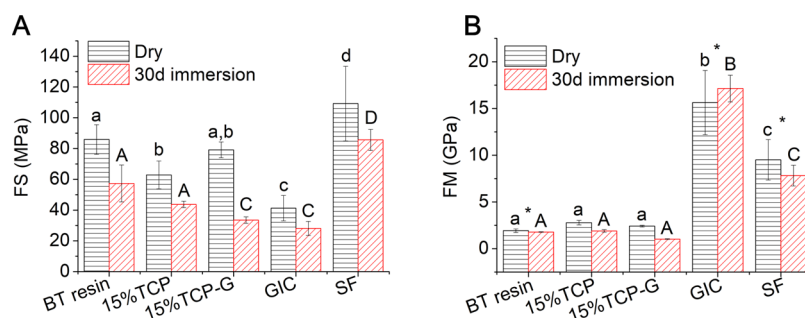
**Antibacterial Test.** The prepared disc-shaped samples (thickness = 2 mm, diameter = 7 mm,  $n = 4$ ), including 15% TCP- and 15% TCP-G-containing resin composites, pristine BT resin, GIC, and SF, were sterilized by ultraviolet light before bacteria incubation. *Streptococcus mutans* (*S. mutans*, UA159) were cultured in brain heart infusion medium overnight. The inoculum was adjusted to  $1 \times 10^7$  colony-forming unit counts (cfu/mL). Each sterilized specimen was placed in a 48-well plate, and 400  $\mu\text{L}$  of bacterial suspension was added. After 24 h of anaerobic incubation at 37  $^\circ\text{C}$ , bacterial suspension from all wells was mixed with a pipette, and 200  $\mu\text{L}$  of the blended bacteria suspension was then

transferred to a 96-well plate; the absorbance was measured at wavelength = 630 nm using a microplate reader.

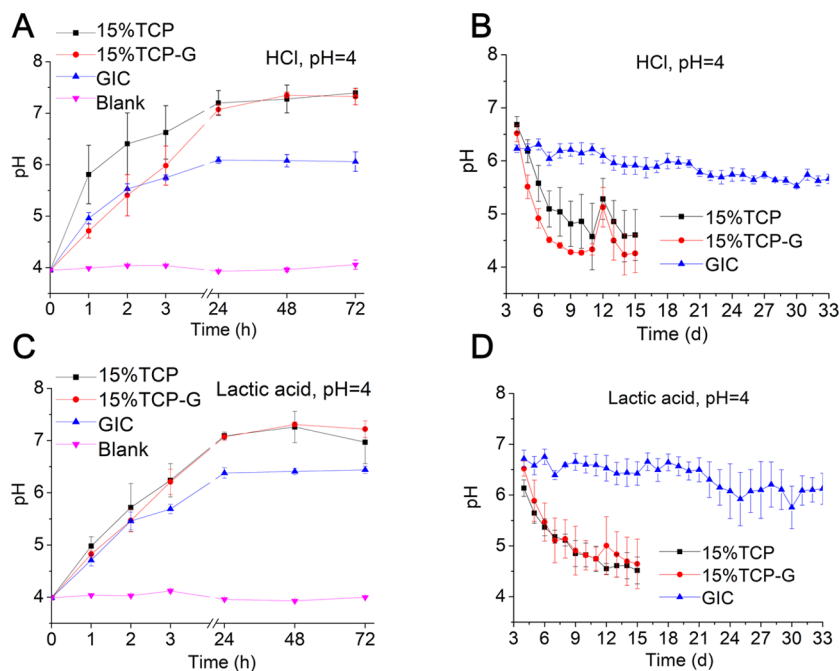
**Remineralization Effect.** To investigate the remineralization capability of the dental composites, artificial saliva (pH = 6.8, Solarbio Life Science, Beijing, China) was used, which was a multi-ionic solution consisting of deionized water, sodium chloride (NaCl), potassium chloride (KCl), ammonium chloride ( $\text{NH}_4\text{Cl}$ ), and so forth. The acidic artificial saliva was prepared by buffering artificial saliva with lactic acid (Macklin, Shanghai, China) to pH = 4. Wisdom teeth, devoid of any lesions and/or restorations, were obtained from 18- to 28-year-old healthy patients who underwent routine extractions at the Hospital of Stomatology. All experiments followed the protocol guidelines by the Hospital of Stomatology Ethics Committee. Written consent was obtained from all patients. The teeth were disinfected in 0.5% chloramine and used within 6 months post-extraction. Teeth were vertically sectioned in both “x” and “y” directions, parallel to the long axis of the tooth, in a cutting machine (WYQ-50S, Weiyee, Guangzhou, China) yielding rectangular sticks with a cross-sectional area  $\sim 4 \text{ mm}^2$ . The sticks were obtained by sectioned perpendicularly to the long axis of each tooth and then trimmed by a fine diamond bur to 5 mm length. Four groups, including 15% TCP, 15% TCP-G, GIC, and SF, were tested.

Two different treatments were employed to investigate the remineralization effect. In one approach, dentin sticks were used directly without any surface treatments. Each testing composite specimen was placed as close as possible to the undemineralized dentin stick and fixed with an adhesive (Single Bond Universal, 3M ESPE, USA) and a flow resin (Beautiful Flow Plus F00, Shofu Inc., Japan). The tooth-composite specimens were immersed in 400  $\mu\text{L}$  of acidic artificial saliva at 37  $^\circ\text{C}$  for 3 days; the artificial saliva was changed every day. In the other approach, the surface of each





**Figure 2.** Flexural mechanical properties of the dental composites. (A) FSs and (B) FMs of BT resin alone, 15% TCP, and 15% TCP-G resin composites, the commercial GIC, and SF composites, at the dry state (black bars) and after 30 days of immersion in deionized water (red bars), respectively. Different lowercase letters indicate statistical differences between groups without immersion ( $p < 0.05$ ). Different uppercase letters indicate statistical differences between groups after immersion ( $p > 0.05$ ). \* indicates no significant statistical differences between values before and after immersion ( $p > 0.05$ ).



**Figure 3.** Buffering capacity of 15% TCP and 15% TCP-G resin composites and GIC specimens. (A) Specimens were immersed in HCl solution (pH = 4) for 72 h and then (B) immersed for a much longer term with HCl solution being changed every day. (C) Specimens were immersed in lactic acid solution (pH = 4) for 72 h and then (D) immersed for a much longer term with lactic acid solution being changed every day. “Blank” denotes the acidic solutions alone without specimen immersion.

dentin stick was pre-demineralized with 37% phosphoric acid gel for 15 s and then rinsed with distilled water. The composite specimen was placed and fixed next to the demineralized dentin stick as close as possible. The tooth–composite specimens were immersed in 400  $\mu$ L of artificial saliva at 37  $^{\circ}$ C for 10 days.

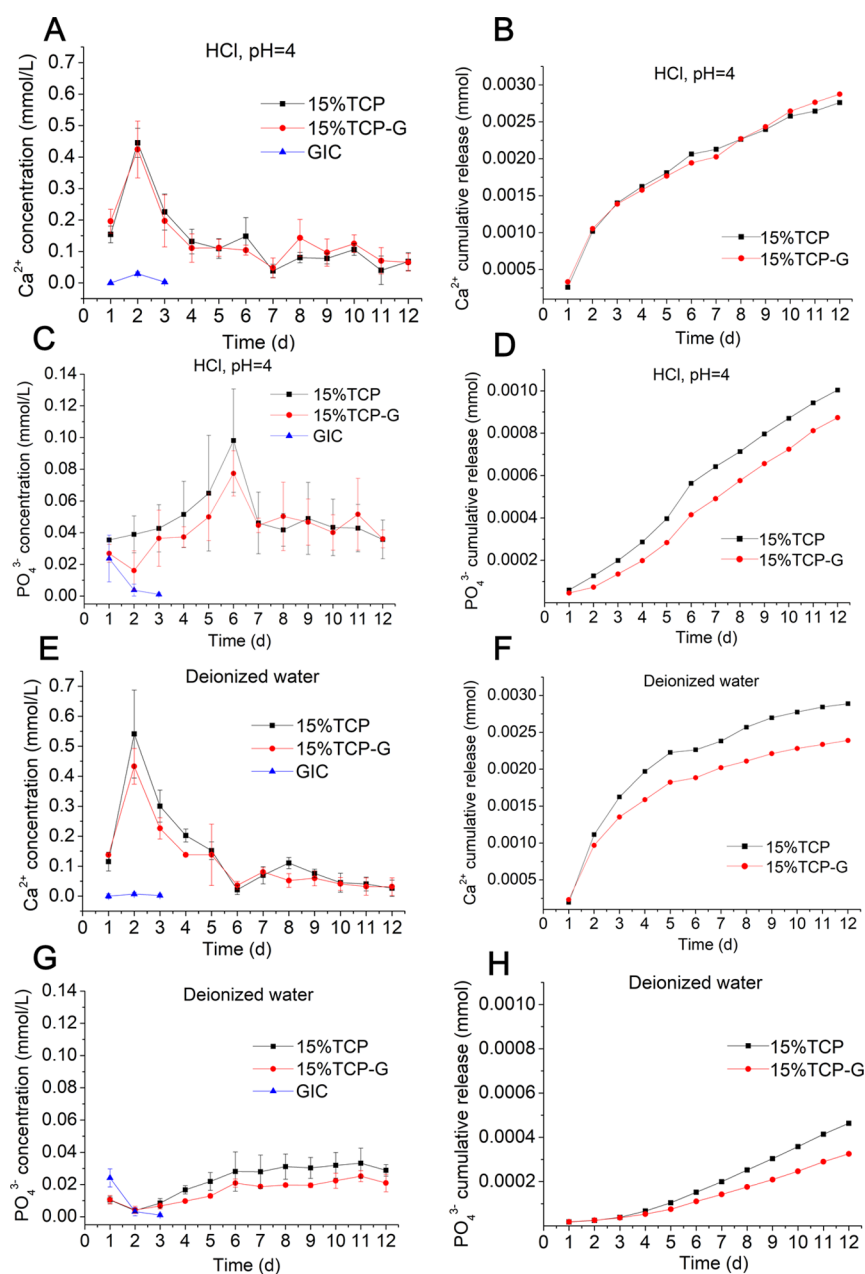
After immersion, specimens were dehydrated with gradient ethanol; then, the surface without an adhesive and flow resin was sputter-coated with gold prior to scanning electron microscopy (SEM, S-4800, Hitachi, Japan).

**Statistical Analysis.** The experimental results were analyzed using one-way analysis of variance (ANOVA) at a significance level of 0.05 with SPSS software (IBM SPSS Statistics for Windows, Version 20.0, IBM, Armonk, NY, USA). Subsequent multiple comparisons were made with the use of Turkey’s post hoc analysis.

## RESULTS

**Physical Properties of the Fillers.** All the TCP,  $\beta$ -TCP, TTCP, and BAG fillers/particles exhibited a significant buffering capacity after soaking in either deionized water or HCl solution (pH = 4) for 1 h (Figure 1A). The buffering capacity of TCP particles was the most significant, while the pH only resulted in 7–9 after immersing  $\beta$ -TCP for 1 h. Therefore, the TCP particles were chosen and used in the following experiments.

To prepare CaP fillers with different sizes, the commercially available TCP particles were ground using a freezing ball mill. It was noted that the size of the particles was significantly reduced after grinding (Figure 1B,C). DLS analysis (Figure 1D) showed that the size of TCP particles before grinding distributed mainly in the micrometer range (median diameter = 2107.2 nm, ranging from 1032 to 4303 nm), while the TCP-G particles were much smaller than the TCP particles and with



**Figure 4.**  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  release of 15% TCP and 15% TCP-G resin composites and GIC specimens. (A) Daily measurement of  $\text{Ca}^{2+}$  concentration in HCl (pH = 4). (B) Cumulative Ca ion release in HCl (pH = 4) within 12 days. (C) Daily measurement of  $\text{PO}_4^{3-}$  concentration in HCl (pH = 4). (D) Cumulative  $\text{PO}_4^{3-}$  release in HCl (pH = 4) within 12 days. (E) Daily measurement of  $\text{Ca}^{2+}$  concentration in deionized water. (F) Cumulative Ca ion release in deionized water within 12 days. (G) Daily measurement of  $\text{PO}_4^{3-}$  concentration in deionized water. (H) Cumulative  $\text{PO}_4^{3-}$  release in deionized water within 12 days.

much narrower size distribution (median diameter = 570.9 nm, ranging from 270 to 1208 nm).

The pH values of both TCP- and TCP-G-soaked solutions reached above 11 in both water and HCl (Figure 1A). Interestingly, the TCP/TCP-G particles showed different  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  ion-releasing profiles when immersed in water or HCl (Figure 1E,F). When exposed to deionized water (pH  $\sim$  7), the TCP particles released more  $\text{Ca}^{2+}$  than that released from TCP-G. Conversely, the  $\text{PO}_4^{3-}$  concentration in TCP-soaked water was much less than that in TCP-G/water. However, the releasing profile followed the same trend when immersed in the acidic solution, the  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  concen-

trations were higher in TCP/HCl solution rather than the TCP-G/HCl solution after 1 h immersion.

**Mechanical Properties.** Considering the results of short-term buffering effect and flexural properties using different dosages of TCP particles in BT resin composites (weight fraction = 5, 10, and 15%, Figure S1), the most significant increase in the pH value was evident using the 15% TCP composite after immersion within 3 days, while the FSs of the 15% TCP composite were significantly lower than that of the BT resin; a weight fraction of 15% was selected as the concentration of TCP and TCP-G in the resin composites for the following experiments.

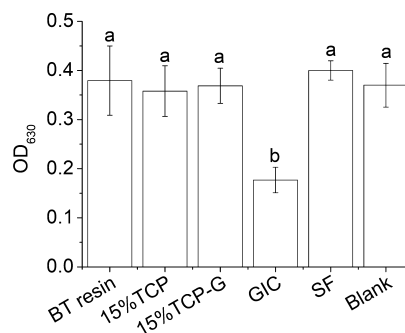
The FSs and FMs were characterized using three-point bending mechanical assessments. Regardless of the dry state or after 30 days of immersion in deionized water, the incorporation of TCP particles into the BT resin significantly decreased the FSs of the resin composites but exhibited little impact on the change in the FMs ( $p > 0.05$ ) (Figure 2). Interestingly, the FS of the 15% TCP-G resin composite was greater than that of the 15% TCP composite under dry conditions. However, the bending strength of the 15% TCP-G resin composite decreased dramatically after immersion in water for 30 days, which was lower than those of the BT resin and 15% TCP resin composite ( $p < 0.05$ ). The GIC composite exhibited the smallest FS but the greatest FM among all dental composites. Relatively better mechanical performance was reached using the commercialized SF resin composite, which showed the greatest FS and the second-highest FM under both dry and wet conditions.

**Long-Term Buffering Capability and Ion Release.** The buffering capabilities of both 15% TCP and 15% TCP-G resin composites were stronger than that of GIC within 3 days, when immersed in HCl and lactic acid (both pH = 4), respectively (Figure 3). After the first 3 days, the immersion solution was replaced every day, it was noticed that the buffering capability of both TCP- and TCP-G-containing composites decreased rapidly. Meanwhile, the GIC continuously elevated the pH in both acidic solutions at pH > 5.5 and lasted for at least 30 days.

The released  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  from 15% TCP and 15% TCP-G integrated resin composites were assessed and presented by daily concentrations and cumulative release, respectively (Figure 4). The daily release of  $\text{Ca}^{2+}$  had burst to a peak value after two days and then kept decreasing during the first week until reaching a minimal and steady release state (Figure 4A,E). No significant difference was identified between the cumulative  $\text{Ca}^{2+}$  release profiles from TCP and TCP-G composites when immersed in acidic solution (HCl, pH = 4, Figure 4B). When immersed in deionized water, the cumulative  $\text{Ca}^{2+}$  released from the 15% TCP composite was slightly more than that released from 15% TCP-G (Figure 4F). Unlike  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$  was hardly released from the resin composites. When immersed in acidic HCl solution, the daily release of  $\text{PO}_4^{3-}$  reached a peak concentration after 6 days and then decreased to a steady state (Figure 4C). The cumulative  $\text{PO}_4^{3-}$  release from the 15% TCP-G composite was slightly lower than that from 15% TCP (Figure 4D,H). In contrast to the TCP-containing resin composites, GIC released little-to-no  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  during 3 days of HCl immersion (pH = 4).

**Antibacterial Performance.** The antibacterial capability of the BT resins with or without the TCP/TCP-G fillers was evaluated by *S. mutans* incubation and assessed by the optical density (OD) measurement at a wavelength of ~630 nm, with resin composites immersed in the culture media, and compared to GIC/SF. It was noted that only the GIC extract exhibited an inhibitory effect on the growth/proliferation of *S. mutans* (Figure 5). Otherwise, all the TCP- and TCP-G-containing composites, BT resin alone, and the SF had little impact on the bacterial proliferation.

**Remineralization.** The remineralization effect was examined by specimens that underwent two different pretreatments with in-process variations (illustrated in Figure 6A). First, each composite specimen was placed next to an un-demineralized dentin stick without any surface treatments. The attached



**Figure 5.** ODs of *S. mutans* when incubated with different composites immersing in the culture medium, including BT resin alone, 15% TCP and 15% TCP-G resin composites, the commercial GIC, and SF composites. Blank denotes the culture medium alone.

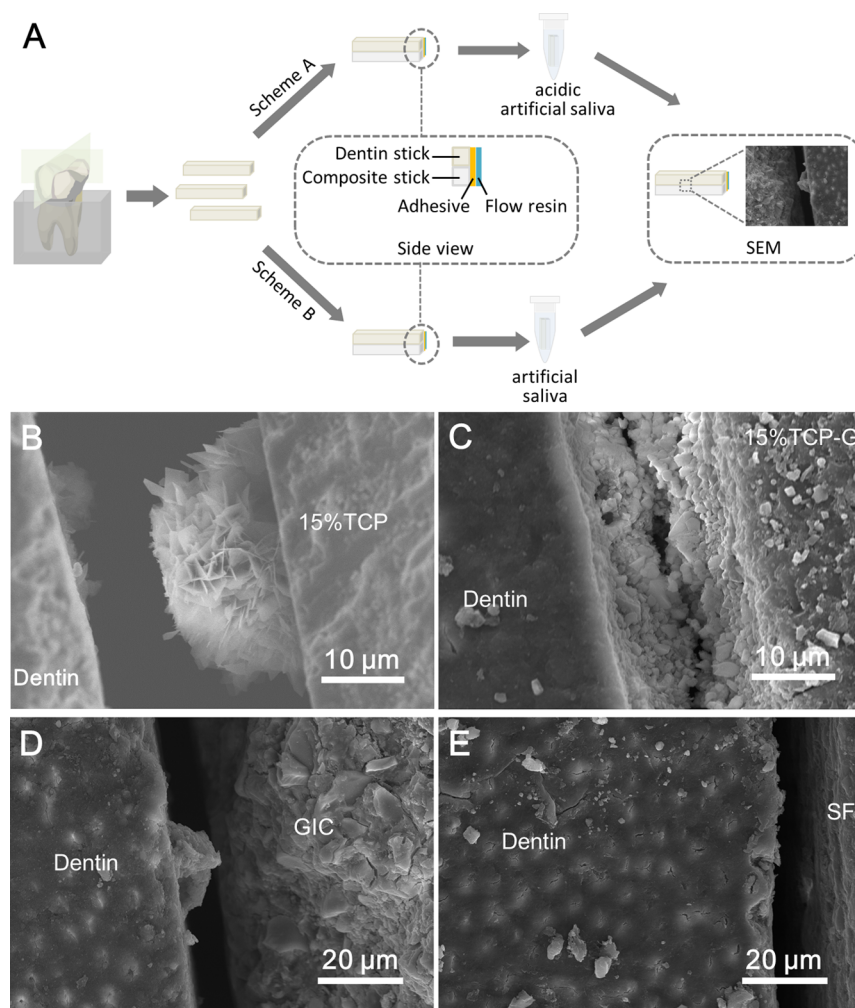
specimens were then immersed in acidic artificial saliva at 37 °C for 3 days. The tooth–composite interface was observed using SEM. The mineral precipitate was identified at the small gap between the dentin stick and TCP/TCP-G resin composites, as well as the dentin/GIC interface. At the interface between dentin and 15% TCP-G composite, a large number of crystals were observed on both sides with almost full surface coverage, which led to a closure of the gap (Figure 6C), while mineral precipitate or particulate fillers were scattered and isolated at the dentin/15% TCP and dentin/GIC interfaces (Figure 6B,D). Mineral deposition was hardly identified at the dentin/SF interface (Figure 6E).

Alternatively, the surface of each dentin stick was pre-demineralized with 37% phosphoric acid gel. The composite specimen was then placed and fixed next to the demineralized dentin stick. The tooth–composite specimens were immersed in artificial saliva (neutral pH) for 10 days. No precipitation was identified at the interfaces between demineralized dentin and 15% TCP resin composite; a few needle-like particles were found at the dentin/15% TCP-G interface (Figure S3A,B).

## DISCUSSION

A common dental resin composite often consists of three components, namely, the mixture of resin monomers, photoinitiators, and inorganic fillers. In this study, a frequently used formulation of BT resin was employed,<sup>23,24</sup> which comprised Bis-GMA and TEGDMA as the resin monomers and DMAEMA and CQ as the photoinitiator system. Although enormous inorganic fillers have been integrated and studied as in dental composites, remineralizing CaP fillers have drawn much more attention due to their inhibitory nature against demineralization, as well as potential of remineralization at the composite–tooth interface.<sup>5</sup> Herein, CaP fillers were incorporated into the BT resin and compared to the commercialized GIC and SF. Different types of filling materials, TCP,  $\beta$ -TCP, TTCP, and BAG, were tested alone for alkalinity, respectively. Figure 1A shows that the TCP particles, including TCP and TCP-G, were the most alkaline, which therefore were chosen to be the major fillers for preparation of resin composites. After grinding, the alkalinity of TCP-G particles was similar to that of TCP.

Early dental resin composites labeled “microfill” composites denote those filled with large particles sizing from 10 to 50  $\mu\text{m}$ . However, nanoparticulate fillers termed “nanofill” have brought much more concerns during the last decade. Previous reports have been mostly focusing on the new material



**Figure 6.** Remineralization effect at dentin–composite interfaces. (A) Schematic illustration of the in vitro remineralization assessments. Scheme A: specimens without pretreatment were immersed in acidic artificial saliva for 3 days with the solution being changed every day; scheme B: specimens etched with 37% phosphoric acid gel were immersed in artificial saliva for 10 days. Representative SEM images of specimens of undemineralized dentin in contact with (B) 15% TCP, (C) 15% TCP-G, (D) GIC, and (E) SF composites in acidic artificial saliva for 3 days, when scheme A was followed.

compositions of the nanofillers; the size dependence of the filler particles has not been thoroughly discussed. Therefore, the main idea of this work was to understand the major differences between CaP fillers with different sizes as remineralizing fillers in BT resin comprehensively. To obtain TCP particles with different sizes, the commercially available TCP particles were either used directly in the resin composite (TCP fillers) or ground to smaller particles before use (TCP-G fillers). It was noted that the average size of the TCP-G particles was only 1/4 of that of the TCP particles. Over 90% of the TCP-G particles were smaller than 1  $\mu\text{m}$  with much smaller dispersity compared to the TCP particles. It is also worth mentioning that to minimize the variances caused by filler preparation, both TCP and TCP-G fillers were integrated directly in the BT resin without surface pretreatments (e.g., silane coupling).

Various aspects of material performance were examined to verify the size dependence of the TCP fillers, including the mechanical properties, antibacterial behavior, buffering effect, and remineralization. First, mechanical robustness is important for dental composites in load-bearing restorations. FS reflects how much bite force materials can resist, while FM reflects the

ability of materials to resist deformation under stress. The FM values of either BT resin alone or TCP-containing resin composites are all much lower than the commercial GIC and SF, and the differences between various compositions/sizes of the TCP fillers were negligible. Since the TCP particles were not premodified, the weak interfacial interaction between the resin and TCP fillers would lead to phase separation. Therefore, mechanical property reduction of the resin composites was expected and confirmed by the experimental results (Figure 2). The results also showed that the TCP-G particles improved the FS of the resin composite (15% TCP-G) compared to the same but much larger particulate fillers (15% TCP) at the dry state, which was consistent with previous studies.<sup>14,20</sup> However, the FS of TCP-containing composites, especially the 15% TCP-G, decreased significantly after 30 days of immersion. The weakening effect of using smaller CaP fillers was minimal at the dry state but became significant after immersing in water for a relatively long period. This interesting phenomenon might explain why the controversial results on mechanical properties were reported after CaP integration in previous studies. The filler/resin interface often served as pathways for ion and water diffusion;



smaller particles would possibly lead to exposure of larger interfacial areas and thus resulted in lower FS after long-term immersion. The resin composites with CaP fillers alone are usually weak in mechanical properties, especially when smaller fillers are used to improve the remineralization capability, more attention should be paid to reinforcement of the mechanical properties after long-term immersion. It is quite desirable to explore additional filling strategies to obtain dental composites with higher stress-bearing capabilities, such as the integration of reinforcing fillers.

Acidogenic bacteria in dental plaque, such as *S. mutans*, can metabolize carbohydrates to acids. In this study, we found that neither 15% TCP nor 15% TCP-G resin composites exhibited considerable impact on the growth of *S. mutans* (Figure 5). It was reported that composites containing NACP can moderately reduce bacteria growth,<sup>17</sup> probably due to the alkalinity of the CaP fillers. Conversely, Zhou et al. and Bhadila et al. found that there was no significant difference between the NACP-containing composites and their corresponding control groups using various characterizations of antibacterial performance (live/dead staining, colony counting, MTT metabolic assay, lactic acid/polysaccharide production evaluations, etc.),<sup>16,18</sup> suggesting that the addition of CaP fillers cannot endow the material with an antibacterial property. Our results agreed with the later conclusion that the integrated TCP particles made little contribution to the antibacterial effect, and similar results were also evident and reported by some other research studies.<sup>9,25</sup>

*S. mutans* and many other species of oral bacteria generate acids to induce tooth demineralization; once such demineralization is too severe to be reversed by natural salivary buffering in the mouth, caries occurs. Studies have reported that there is a critical pH value  $\sim 5.5$ , below which demineralization dominates, leading to a net mineral dissolution.<sup>26</sup> Therefore, it would be highly desirable for the local pH to remain greater than 5.5 to inhibit secondary caries at the restoration–tooth interface. The CaP fillers in resin composites have been proven to possess buffering and remineralization capability.<sup>11,17</sup> When TCP-containing composites were immersed in acidic HCl or lactic acid solution (pH = 4 or 5), they enable the pH value rapidly elevate to  $\sim 7$  within 24 h (Figure S1A–D). The neutralizing capability increased with more integrated TCP fillers, which was manifested by the higher pH that was reached at each time point. Since most previous studies paid little attention to the buffering performance of the CaP fillers, it was surprising to realize that no significant difference was evident for the particle-size dependence between 15% TCP and 15% TCP-G composites within 3 days or even longer immersion time in acids (Figure 3). Additionally, it was worth noting that even though the buffering effect of TCP-containing composites were superior to that of the GIC within the first 3 days, the buffering capability of GIC was more stable in long-term, in line with the literature studies shown in refs 27 and 28. However, the TCP-containing composites may be able to achieve long-term maintenance of buffering and ion-release performance through recharging.<sup>29</sup>

There are two advantages of remineralizing materials, one is to increase the pH to a safe range and the other is to promote remineralization by releasing  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$ . In this study, the assessment of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  release was quite different from the previous reports, in which the cumulative ion release was measured in a large amount of immersion solution.<sup>16,18</sup> Practically, liquid in the mouth remains at a small amount

and changes quickly. Therefore, a small volume of solution was applied for just immersing each resin composite and changed every day to better simulate the daily intake of acidic diet in this study. It was noted that the release of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  was neither in line with the trend of buffering capacity nor the intrinsic ratio of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  in TCP molecules ( $[\text{Ca}^{2+}]:[\text{PO}_4^{3-}] = 3:2$ ). In general, the concentration of  $\text{Ca}^{2+}$  was much greater than that of  $\text{PO}_4^{3-}$  in either acidic solution (Figure 4A–D) or deionized water (Figure 4E–H). The release of  $\text{Ca}^{2+}$  had increased significantly on the second day and then suddenly decreased to a steady state, while the release of  $\text{PO}_4^{3-}$  reached the peak value later than that of  $\text{Ca}^{2+}$ . This phenomenon was still observed even after the specimen surface area/solution volume was changed (Figure S2). These findings were experimentally demonstrated and noticed for the first time, its clinical relevance still needs to be explored. It is speculated that there was an individual equilibrium state for either  $\text{Ca}^{2+}$  or  $\text{PO}_4^{3-}$  in the solution, but the release of  $\text{Ca}^{2+}$  is more likely to play a critical role in solution buffering. The mechanism for ionic balance of released  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  also needs to be studied in the future. Furthermore, in most of the cases, the 15% TCP composites released more  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  than the 15% TCP-G, probably due to the larger local concentration difference at the particle–liquid interface.

Finally, to determine the remineralization effect of the dental composites without interference from other materials, the close-packed dentin–composite interface was prepared in the characterization with no additional adhesives, but the experiments were implemented by following two different approaches, that is, schemes A and B in Figure 6A. The rationale of scheme A was to simulate daily intake of acidic diet, while scheme B was to simulate the interface between the base material and demineralized dentin after treatment of caries. The 15% TCP-G composite in scheme A showed superior performance in gap-closing among all groups, implicating that TCP-G may effectively help in closing gaps that were formed by bonding failure (Figure 6B–E), while in a neutral environment, neither 15% TCP nor 15% TCP-G composite showed particle deposition within the gaps (Figure S3). Resin composites containing CaP fillers have been considered to serve as a base to remineralize remnants of caries, due to their  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  release.<sup>20</sup> In clinical practice, there should be much less liquid between the material and dentin compared to our in vitro analogy model; thus, the release of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  would be even further limited. Our results from scheme B in Figure 6A led to doubt about the viability of using the TCP-containing composites as the base materials. For further verification, detection of calcium and phosphorus contents on dentin surfaces shall be conducted in future studies.

## CONCLUSIONS

In the present study, two types of TCP particles, including the commercially available micrometer-sized particles (TCP) and the much smaller particles resulted after ball mill grinding (TCP-G), were integrated into BT resin so that the size dependence of these CaP fillers was evaluated by comparison between these two resin composites regarding their buffering capability, mechanical properties, ion release, antibacterial performance, and remineralization effect. It was realized that both TCP and TCP-G at the same composition (15 wt %) in the composites exhibited a similar buffering capacity and no significant antibacterial activity. The integrated TCP particles



were slightly beneficial for  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  release. Smaller particles (TCP-G) had a greater reducing effect on the long-term bending performance. Under the attack of acidic solution, the 15% TCP-G resin composite was more effective in promoting remineralization and gap-closing than the TCP particle containing the composite.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c05825>.

Materials and methods, formulation of the TCP-containing BT composites, buffering capacity of the dental composites,  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  release of 15% TCP and 15% TCP-G resin composites when immersed in deionized water and HCl, and representative SEM images of specimens of demineralized dentin in contact with 15% TCP, 15% TCP-G, GIC, and SF composites in artificial saliva (PDF)

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### Notes

The authors declare no competing financial interest.

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