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LETTER

Solid-state preparation and dental application of an organically modified calcium phosphate

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Mechanochemical (MC) ball milling is a unique type of milling that has been in use as early as 1922 and smashes elements, oxides, ceramics, powders, etc. together to form new alloys or composites, entirely in the solid state [1–3]. This destructive process deforms components through powerful ball–particle, particle–wall, and particle–particle collisions, creating significant grain boundaries at the nanoscale where components have fractured and fused [4, 5]. In order to generate the energy required for such fracturing and welding, typically the vessel containing the balls and material are rotated opposite to the direction of the rotating platform on which the vessel is placed. The resultant hybrid materials formed from this physical and chemical process have enabled material synthesis to extend beyond the usual wet laboratory synthetic procedures and open up a myriad of new opportunities. Examples include the extraction of elements from fluorescent powder for energy conservation, improvement of nitrogen absorption characteristics of getter materials, and the synthesis of nanoscale graphite platelets as an alternative to carbon nanotubes [6–8]. A major benefit of the MC ball milling process is that it is readily scaleable and is currently used to create pigments for coatings, lotions, and paints [4]. Recently, surfactants have been incorporated into MC ball milling events to expedite particle size reduction [9], but the application of MC ball milling to produce functionalized hybrid materials tailored for specific health-related applications has not yet been demonstrated until now.

Calcium phosphates are important minerals found in bone and dentition. Beta-tricalcium phosphate (β-TCP), a unique form of partially insoluble calcium phosphate, is a known precursor to hydroxyapatite formation and is commonly included in bioceramics and in coating or filling materials to improve osseointegration of implants [10, 11]. Due to drawbacks with β-TCP and other calcium phosphates such as cracking or delamination from an implant, it is desirable to modify these materials (through electrochemical or precipitation reactions, for example) with the goal of stimulating cellular and/or antimicrobial activity for improved tissue response [12, 13]. Within the field of preventive dentistry, the ability to combine calcium phosphates with fluoride (i.e., *the* clinically proven anticaries agent) in a simple dental format (e.g. toothpaste) is extremely appealing as research has shown this combination can produce higher levels of enamel remineralization (or repair) relative to fluoride or calcium phosphates alone [14, 15]. Formulating a singular vehicle comprising soluble calcium and fluoride has been extremely challenging, however, due to CaF_2 formation, which inevitably reduces bioavailable fluoride and therefore impairs dental product performance.

With respect to functionalized materials, there are few economical chemical processes that allow for industry scale-up. Existing techniques include hydrothermal/solvothermal synthesis, micellar templates, and controlled growth in hot volatile solvents [16]. Although these and other chemistries readily create hybrid materials, many of these ultimately are commercially limited due to one or more of the following reasons: need for volatile solvents to effectuate dissolution of one or more phases, reduced-pressure systems to alleviate oxygen or water contact with reagents, expensive templating molecules, or multi-step syntheses. With these limitations serving as an opportunity to investigate potential scale-up synthetic possibilities, here we report on the feasibility of creating functionalized β-TCP using a solid-state MC ball milling process. And as

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an example of how the resulting 'functionalized' calcium phosphate may find applications in the life sciences, we demonstrate its synergistic performance with fluoride (i.e. NaF(aq)) to provide superior remineralization of weakened enamel relative to fluoride alone.

The β-TCP crystal structure manifests several reactive sites, including lattice defects, under-bonded CaO_3 clusters, and larger calcium clusters (e.g. CaO_7 and CaO_8) connected via shared vertices and edges [17]. Presumably, these sites enable mineral transformation to hydroxyapatite, but also leave the β-TCP mineral susceptible to, for example, cracking and delamination, or reaction with ionic fluoride. As these sites may be prone to reaction, we propose the modification of β-TCP with the 12-carbon chain anionic surfactant, sodium lauryl sulfate (SLS), which manifests a sulfate head group: SLS stimulates surface modification of metals, polymers, electrolytes, etc., mediates crystal nucleation and growth, and is largely responsible for producing the observed 'foaming' effect in cosmetic formulations [18, 19]. Such modification may protect sensitive calcium environments in β-TCP from CaF_2 formation when combined in an NaF(aq) dental product (e.g. mouthrinse or toothpaste); then at the time of product use, calcium and fluoride would be available for delivery to the dentition.

The IR spectra (Fig. 1) generated from pressed KBr pellets clearly demonstrate the modification of β-TCP with SLS. Spectra in Fig. 1b and c were generated from materials synthesized by loading 25 g of β-TCP (98 wt.%) and SLS (2 wt.%) plus four 12-mm diameter balls into 250 mL vessels and rotated at 400 rpm in a planetary ball mill (Retsch) for 2 h. As shown in Fig. 1, the vibrational intensity shift from 1,120 cm^{-1} to 1,040 cm^{-1} indicates the relative increase in 'free' phosphate (PO_4^{3-}) environments from 'bound' orthophosphate and is particularly prominent when SLS is milled with β-TCP; in this case, the milling event apparently facilitates modification of under-bonded calcium environments with SLS as well as calcium–phosphate bonding networks as depicted in the hypothetical mechanism described in Scheme 1. In this Scheme, the sodium cation may act as a network modifier to disrupt calcium–phosphate bonding, while the sulfate anion interacts with CaO_n (i.e. CaO_3, CaO_7, and/or CaO_8) clusters. A most interesting aspect of this synthetic process worth mentioning, but will be discussed in detail elsewhere, is the retention of crystalline features (e.g. vibrations near 974 and 945 cm^{-1}) plus an overall lineshape narrowing in Fig. 1c relative to those in both Fig. 1a and b, despite the powerful collisions experienced during the milling event: these differences further suggest SLS chemically modifies calcium and phosphate coordination as opposed to simply facilitating physical breakdown of β-TCP structure.

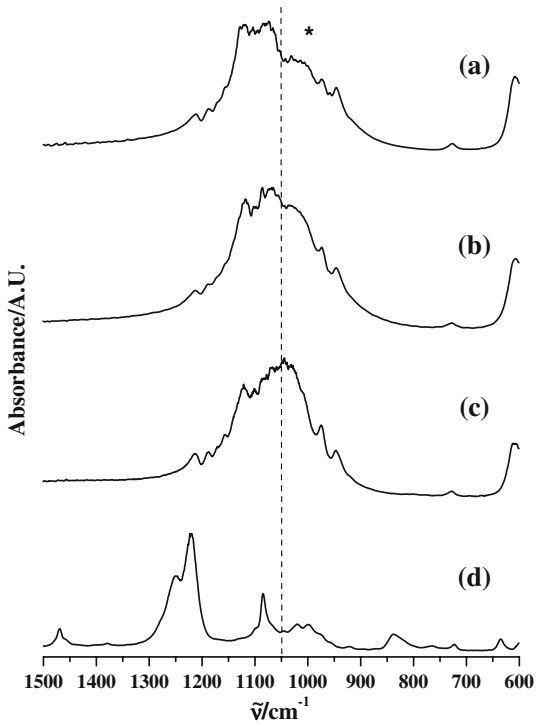
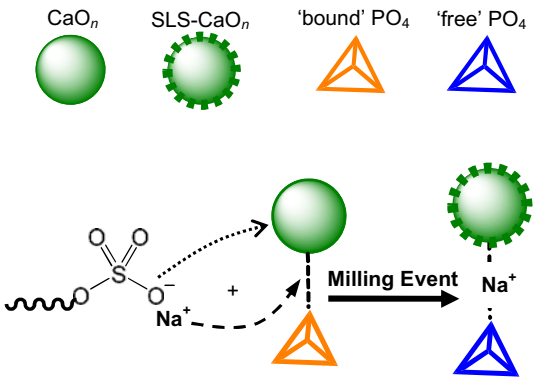


Fig. 1 IR spectra for (a) β-TCP, (b) mTCP, (c) $TCP_{98}SLS_2$, and (d) SLS. The asterisk marks the relative vibrational position corresponding to 'free' phosphate. The dashed line at 1,050 cm^{-1} is shown to improve distinction between 'bound' and 'free' phosphate environments



Scheme 1 Hypothetical mechanism demonstrating the ability of SLS in modifying calcium and phosphate environments in β-TCP to produce 'functionalized' calcium and 'free' phosphate

Since soluble calcium will readily react with ionic fluoride to form CaF_2 (which is undesirable in the development of dental products such as a NaF dentifrice), fluoride compatibility studies were performed to assess the nature of the SLS-TCP interactions. Milled β-TCP (mTCP) and $TCP_{98}SLS_2$ materials combined with NaF(aq) at several loading levels were incubated for 7 days at 37 °C to assess the extent of available ionic fluoride. Measurements were then performed in a 1:1 ratio of TISAB II with each

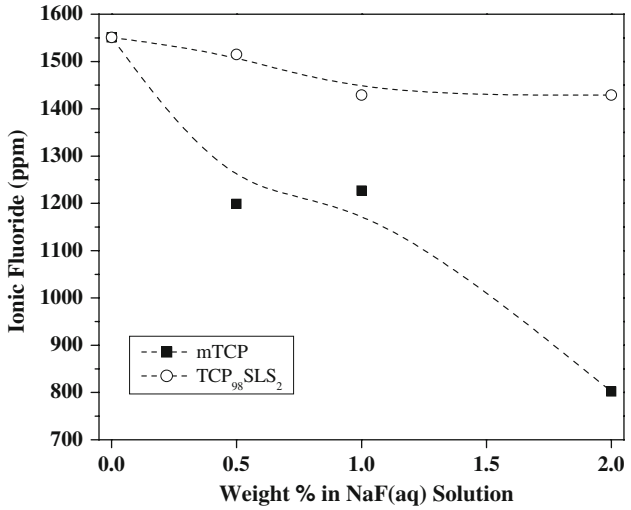


Fig. 2 Ionic fluoride determined using fluoride-sensitive electrode for various loading levels of mTCP and $TCP_{98}SLS_2$ in 1,550 ppm F solutions

system using a fluoride-sensitive electrode calibrated to fluoride standards (Fig. 2). Based on these data, a strong contrast in the level of ionic fluoride is observed for mTCP and $TCP_{98}SLS_2$, especially at 2 wt.% loading level which leads to a 48% and 8% reduction in F^-, respectively. This result bears directly on the feasibility of formulating a stable dental formulation comprising both fluoride and calcium (one that complies with the FDA monograph for fluoride dentifrices, for example). Additionally, these data also reveal the protective effect of SLS on calcium gained due to its solid-state processing with β-TCP.

Due to clinical relevance and agreement, in vitro pH cycling models designed to mimic the action of the oral environment during resting, eating, and hygiene events are typically employed to assess fluoride's performance in strengthening (i.e. remineralizing) weakened enamel [20, 21]. A short-term pilot study was performed to evaluate the remineralization efficacy of fluoride plus $TCP_{98}SLS_2$ versus fluoride alone. Bovine enamel specimens were ground and polished using standard methods and 'white-spot' (non-cavitated) lesions were subsequently formed in a hydroxyapatite-saturated carbopol-lactic acid solution (pH = 5). Baseline Vickers microhardness (VHN_{base}) was then measured (200 gf, 15 s dwell time) and the specimens were stratified into five groups ($N = 3$) consisting of one negative control (distilled water), two positive controls (500 and 1,100 ppm fluoride solutions), and two experimental groups (500 ppm F + 0.025% $TCP_{98}SLS_2$, and 1,100 ppm F + 0.05% $TCP_{98}SLS_2$). Each day for 5 days, the pH cycling regimen consisted of four 2-min treatments (pH ~ 7–8) and one 4-h carbopol-lactic acid challenge (pH = 5.0), with specimens immersed in artificial saliva (pH = 7.0) in between these events.

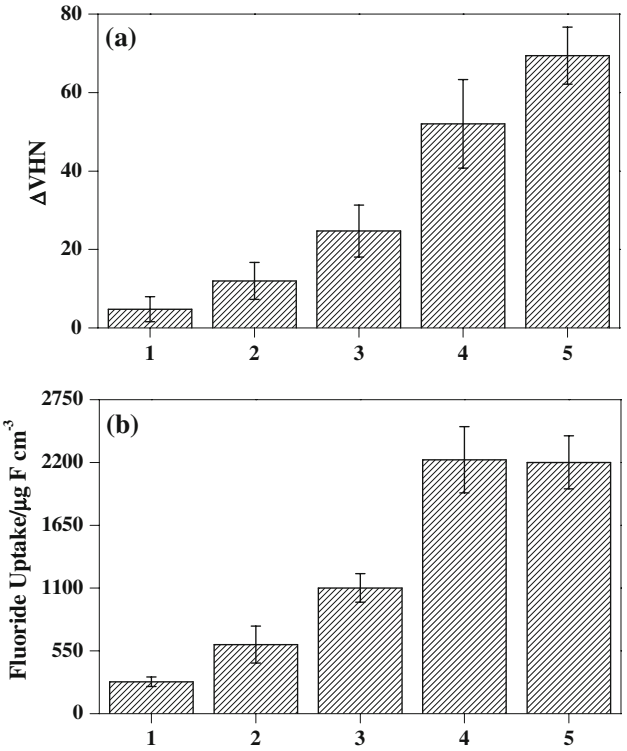


Fig. 3 Mean ± SEM data are shown for **a** the change in Vickers microhardness from baseline ($\Delta VHN = VHN_{post} - VHN_{base}$) and **b** bioavailable fluoride levels for enamel specimens after pH cycling for Groups **1**: distilled water (negative control); **2**: 500 ppm F; **3**: 500 ppm F + 0.025% $TCP_{98}SLS_2$; **4**: 1100 ppm F; **5**: 1100 ppm F + 0.05% $TCP_{98}SLS_2$

At the end of cycle, the specimens were examined for post Vickers microhardness (VHN_{post}) and enamel biopsies were extracted via microdrilling and measured for fluoride uptake using a fluoride-sensitive electrode calibrated to fluoride standards (Fig. 3). The results in Fig. 3 demonstrate a fluoride dose response consistent with clinical results [22] and therefore, confirm model validity. Importantly, the $TCP_{98}SLS_2$ material significantly boosts remineralization efficacy relative to fluoride alone. Increases in both ΔVHN and fluoride content observed for the 500 ppm F + $TCP_{98}SLS_2$ system demonstrate the synergistic effect of the multi-mineral treatment. Furthermore, the increase in ΔVHN for Group **5** relative to Group **4** is likely due to the presence of $TCP_{98}SLS_2$. While the SLS modifies the β-TCP structure and protects calcium from prematurely interacting with ionic fluoride while coexisting in solution, it appears that it also encourages enhanced tissue integration (via calcium and phosphate) in the presence of fluoride. Because SLS has a high affinity to hydroxyapatite, presumably the SLS molecule releases calcium for mineral integration when interfaced competitively with the enamel surface. The observed synergistic effect of combining fluoride plus a fluoride-compatible

'functionalized' calcium material is striking enough to warrant additional investigations into both the mechanism of action and the development of advanced dental formulations.

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