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LETTER

Spectroscopic evaluation of native, milled, and functionalized β-TCP seeding into dental enamel lesions

Robert L. Karlinsey · Allen C. Mackey · Emily R. Walker · Katherine E. Frederick

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Infrared (IR) spectroscopy has been used extensively to probe chemical and structural aspects of synthetic calcium phosphate-based mineral and biological hard tissue, including bone and enamel, due to the IR active modes pertaining to phosphate and/or carbonate [1–5]. Distinction among these vibrational modes allows one to identify and contrast tricalcium phosphate and apatite mineral phases, [2] for instance, or the similarities between prehistoric and extant reptile enamel [6]. The apatite in tooth enamel manifests both carbonate and hydroxyl groups, which are susceptible to demineralization, or weakening, when exposed to acidic events that naturally arise during eating events, for example [7]. The body's natural repair mechanism is saliva, which contains critical minerals, such as calcium and phosphate, that can help repair weakened tooth structure [8]. Because the mineral content in saliva may be too low to adequately remineralize weakened enamel, studies have shown the use of fluoride, calcium, and other minerals can help restore the lesions created through the loss of enamel mineral (i.e., calcium, hydroxyls, carbonate, and phosphate) [9–11]. Of considerable interest from an efficacy point of view is the ability to obtain structural information demonstrating inclusion of the seed mineral into enamel lesions. For instance, enamel uptake of fluoride, the most clinically renowned anti-cavity remineralizing agent, [12] can be performed by making measurements on biopsies extracted from enamel lesions using a fluoride-sensitive electrode [13] as outlined in the United States Food and Drug Administration's 'Anticaries Drug Products for Over-the-Counter Human Use' monograph [14]. The effects of calcium phosphate (CaP) seeding into enamel tissue are particularly challenging and have been primarily limited to atomic absorption spectroscopy [15, 16] or ^{45}Ca radiolabeling [17]. These methods, however, do not provide chemical and structural insight into enamel remineralization stimulated by a given CaP salt/mineral (i.e., calcium lactate, calcium phosphate, etc.); hence, there exists a fundamental disconnect in how CaP agents affect enamel tissue. Recently, we reported on the novel solid-state synthesis and dental application of a functionalized tricalcium phosphate (fTCP) mineral comprised of β-TCP and sodium lauryl sulfate (SLS) in remineralizing weakened enamel (98% β-TCP + 2% SLS) [18]. In efforts to further our understanding of fTCP and its remineralizing effects on enamel, in this letter we use IR spectroscopy to probe the ensuing enamel structure when weakened enamel is seeded with native β-TCP, milled β-TCP, and fTCP. Our observations reveal significant structural differences in enamel arising from each β-TCP system and confirm the novelty of fTCP as a promising mineralizing agent. While we show this approach resolves modifications in dental tissue structure when seeded with CaP minerals, we also believe this can be extended to growth processes in the mineralization of teeth and bone.

Preparation of enamel specimens was performed as follows. 3 mm enamel cores were drilled from clean bovine molars [19] and mounted in acrylic rods. The cores were then serially ground with 600, 1000, and 1500 grit sandpaper, then polished with 9, 3, and 1 μm polycrystalline diamond suspension (Buehler). After sonication and rinsing with distilled (DI) water, artificial caries-like lesions were formed in the bovine enamel specimens by immersion for 26 h at 37 °C in a solution (pH = 5.0) containing 1 wt% of 100,000 M.W. poly(acrylic acid), 0.1 M lactic acid, 1.45 mM Ca^{2+} (calcium chloride

R. L. Karlinsey (✉) · A. C. Mackey · E. R. Walker · K. E. Frederick
Indiana Nanotech, 351 West 10th Street, Suite 309, Indianapolis, IN 46202, USA
e-mail: rkarlins@gmail.com

dihydrate, $CaCl_2{\cdot}2H_2O$), and 5.4 mM P_i (potassium phosphate monobasic, KH_2PO_4). The acids were purchased from Sigma Aldrich and the salts were obtained from Fisher Scientific. The caries-like lesions produced by this solution have a dense surface mineral zone approximately 15 μm thick, while lesion depth extends to about 70 μm, as determined with reflective microscopy. Specimens were then subjected to a single 30-min seeding period in solutions or suspensions containing SLS, β-TCP, milled β-TCP (mTCP), and fTCP. Both mTCP and fTCP were prepared using a solid-state mechanochemical process described previously [18].

After the treatments, the bovine enamel cores were drilled under water cooling using a Power Glide 5-speed bench top drill press with a 7/64″ bit, and the resulting enamel powder was collected and dried in a Fisher Scientific Isotemp Model 280A vacuum oven at 80 °C under normal pressure. The enamel powder was mixed with dried KBr powder (Buck Scientific) in a ratio of 1:100, and then finely ground with an agate mortar and pestle. A total of 55 mg of this mixture was added to the barrel of a KBr pellet press (Buck Scientific). The press was secured in a vise, tightened to 40 ft-lbs using a torque wrench, and then left under pressure for 10 min. The bolts were carefully removed and the pellet was scanned from 4000 cm^{-1} to 600 cm^{-1} using a Buck Scientific Model 500 Infrared Spectrophotometer (2 cm^{-1} resolution, 3 min scan time, zero scan gain, 0.8 pen response). Acceptable pellet windows had percent transmission between 70 and 75% at 4000 cm^{-1}. A background scan was subtracted from the sample scan, and the spectrum was analyzed using graphing software (Microcal Origin 6.0). The pellet preparation method was standardized so that the absorbance values of different samples could be quantitatively compared. This process was repeated for sound enamel and caries-like lesioned enamel with and without native β-TCP, mTCP, and fTCP. Reproducibility (the error in absorbance intensity units (A.U.) was about ±0.05 at 1032 cm^{-1}) was confirmed in triplicate for sound and lesioned enamel.

Figure 1 shows the IR spectra pertaining to pure hydroxyapatite (HAP), sound bovine enamel, lesioned bovine enamel, and native β-TCP. Since we standardized the KBr: sample loading ratio comprising the pellet, these spectra can be qualitatively and quantitatively compared. The presence of carbonate between 1400 and 1500 cm^{-1} confirms that bovine enamel consists at least partially of carbonate apatite. The lesioned and sound enamel spectra are qualitatively similar, indicating the acidic softening does not significantly alter orthophosphate character in the enamel structure; alternately, the reduction in intensity is significant for the lesioned enamel and likely results from the reduction in mineral constituents through loss of CO_3^{2-}, OH^-, Ca^{2+}, and/or PO_4^{3-} during the acid challenge. This

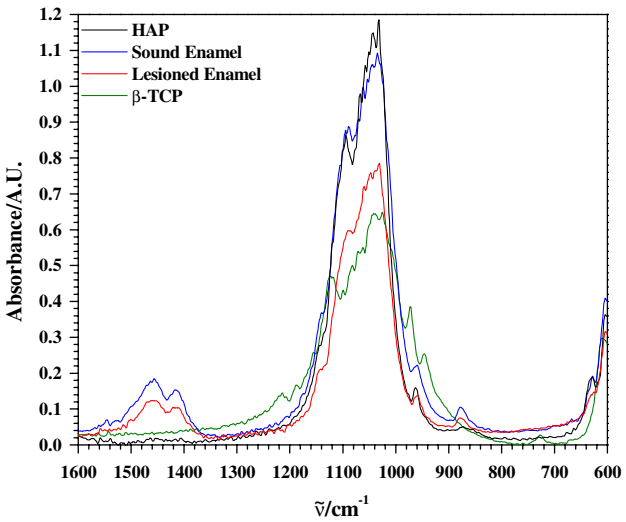

Fig. 1 IR absorbance spectra of native hydroxyapatite (HAP), sound bovine enamel, lesioned bovine enamel, and native beta-tricalcium phosphate (β-TCP)

loss in mineral therefore introduces voids in the enamel structure and contributes significantly to structural weakening: surface microhardness measurements (200 gF, 15 second dwell time) show a reduction in Vickers Hardness Number (VHN) from about 330 VHN for sound enamel to about 50 VHN for lesioned enamel (data not shown). The clear resolution of native β-TCP spectral features compared to the apatites can be attributed to the distinctly different distribution of orthophosphate groups in its mineral structure, [4] which when altered with small molecules may help facilitate remineralization of weakened enamel [18]. Evidence of this is shown in Fig. 2, where lesioned enamel has been treated with native β-TCP, mTCP, and fTCP. Significant spectral differences among the treated enamel groups are observed, with native β-TCP contributing the least amount of mineralization relative to the mTCP and fTCP. The principle P–O peak positions arising from PO_4^{3-} vibrational stretching and their corresponding intensities for lesioned enamel by itself and treated with β-TCP, mTCP, and fTCP are 1031.2 cm^{-1} (0.758 A.U.), 1031.2 cm^{-1} (0.504 A.U.), 1033.6 cm^{-1} (0.684 A.U.), and 1037.6 cm^{-1} (0.754 A.U.), respectively. We observe that not only does fTCP provides significantly more mineralization relative to native β-TCP and mTCP, but it also instigates a shift from relatively unencumbered P–O stretching from the PO_4^{3-} groups towards stronger P–O bonding. This shift suggests the quality of the minerals seeded into the enamel lesions favors lattice reconstruction. That the quality and not the quantity of the calcium phosphate seeds may play a dominant role in the mineralization of hard tissue is a most interesting result of this work. Separately, we have conducted solubility experiments and found that milling β-TCP by itself increases β-TCP solubility about five times, while

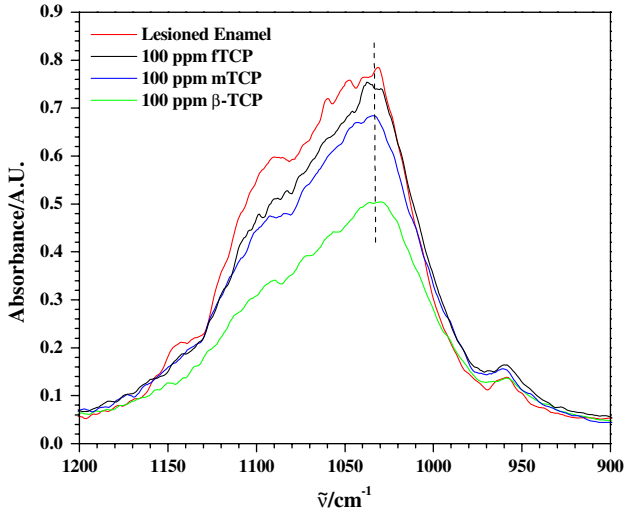

Fig. 2 IR absorbance spectra of lesioned enamel, native beta-tricalcium phosphate (β-TCP), milled β-TCP (mTCP), and SLS-functionalized β-TCP (fTCP). *Dashed line* is shown to improve distinction in the nature of P–O vibrational stretching of PO_4^{3-} groups

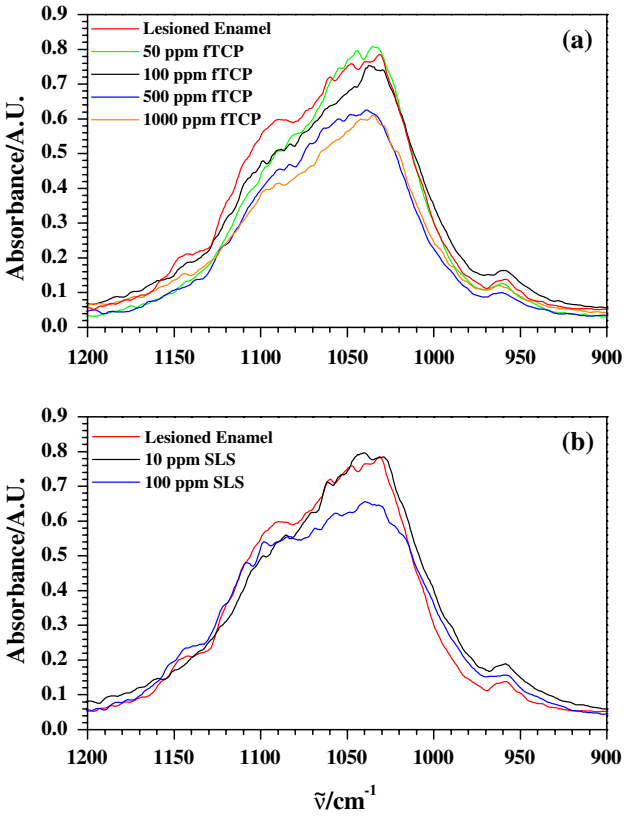

Fig. 3 Enamel lesion dose response of fTCP (**a**) and SLS (**b**)

milling with SLS reduces β-TCP solubility by about 10 times. Thus, the data in Fig. 2 show that mineralization of lesioned enamel is not controlled by the quantity of available Ca^{2+} and PO_4^{3-} ions in solution. Apparently native β-TCP promotes relatively poor bioavailability into enamel tissue, as verified with the broad, low intensity spectral features, while fTCP promotes improved nucleation capability presumably through the formation of intricate TCP-SLS seeding clusters since its solubility is lower than native β-TCP and mTCP.

To determine if the fTCP mineral exudes a dose response effect on lesioned enamel, we performed a dose response study with fTCP and SLS and the results are shown in Fig. 3. Figure 3a demonstrates a growth-like curve dependence of fTCP concentration on mineral seeding into lesioned enamel, with 50 and 100 ppm fTCP promoting significantly more mineralization benefits relative to 500 and 1000 ppm fTCP. Separately, we performed dose response experiments for native β-TCP and mTCP at 100, 500, and 1000 ppm, but did not find any evidence of a dose response effect; in contrast, Fig. 3a reveals a dose response effect for fTCP. As SLS is known to adhere strongly to enamel surfaces, [20, 21] and impede uptake of mineralizing ions, such as fluoride, [22] we performed a separate study to determine if SLS interacts with the enamel surface and the results are shown in Fig. 3b. Since the weight fraction of SLS in fTCP is 2% and 100 ppm fTCP promoted good mineral seeding, we chose to evaluate 10 and 100 ppm SLS. As shown in Fig. 3b, IR absorbance becomes inhibited at SLS concentrations near 100 ppm. Presumably, with sufficient SLS coverage on the enamel surface, the highly electronegative sulfate head group of SLS may scatter impinging IR light, thereby reducing phosphate group IR excitation. Increases in the index of refraction have been observed previously in aqueous solutions containing SLS [23]. Coupled with the high affinity of SLS to enamel surfaces, these characteristics suggest SLS likely imparts a significant effect in our spectroscopic studies, especially at fTCP concentrations between 100 and 500 ppm (which pertains to 2 and 10 ppm SLS, respectively); nevertheless, at fTCP concentrations less than 100 ppm, the amount of SLS is sufficiently low and allows significant mineral seeding to occur. This assessment then helps identify fTCP concentrations for effective enamel mineralization.

In conclusion we presented a spectroscopic evaluation that is sensitive to mineral seeding into enamel lesions by three distinct β-TCP materials. These results show the quality of the bioavailable mineral is important to mineralization, with the functionalized form of β-TCP providing significant mineralization potential relative to native β-TCP and mTCP. While native β-TCP and mTCP did not produce a dose response, fTCP did, and this appears to depend on SLS content. Though we demonstrated utility of this model to identify seeding behavior among β-TCP-based minerals, the application of this model to assess

different nucleation agents, such as other calcium phosphate systems and/or fluoride, is very promising and may find further use in mineralization growth models for biological hard tissues.

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References

1. Corbridge DEC, Lowe EJ (1955) Anal Chem 27:1383
2. Koutsopoulus S (2002) J Biomed Mater Res 62:600
3. Gadaleta SJ, Paschalis EP, Betts F, Mendelsohn R, Boskey AL (1996) Calcif Tissue Int 58:9
4. Rey C, Shimizu M, Collins B, Glimcher MJ (1991) Calcif Tissue Int 49:383
5. Joris SJ, Amberg CH (1971) J Phys Chem 75:3172
6. Botha J, Lee-Thorp J, Sponheimer M (2004) Calcif Tissue Int 74:162
7. Margolis HC, Moreno EC (1994) Crit Rev Oral Biol M 5:1
8. Whelton H (2004) In: Edgar M, Dawes C, O'Mullane D (eds) Saliva and oral health, 3rd edn. British Dental Association, London, p 1
9. LeGeros RZ (1999) J Clin Dent 10:65
10. Dedhiya MG, Young F, Higuchi WI (1974) J Phys Chem 78:1273
11. Feagin F, Patel PR, Koulourides T, Pigman W (1971) Archs Oral Biol 16:535
12. Tavss EA, Mellberg JR, Joziak M, Gambogi RJ, Fisher SW (2003) Am J Dent 16:369
13. Zero DT, Zhang JZ, Harper DS, Wu M, Kelly S, Waskow J, Hoffman M (2004) J Am Dent Assoc 135:231
14. Food and Drug Administration (1995) Fed Regist 60:52474
15. ten Cate JM, Exterkate RAM, Buijs MJ (2006) Caries Res 40:136
16. Gibbs CD, Atherton SE, Huntington E, Lynch RJM, Duckworth RM (1995) Archs Oral Biol 40:879
17. Moran RA, Deaton TG, Bawden JW (1995) J Dent Res 74:698
18. Karlinsey RL, Mackey AC (2009) J Mater Sci 44:346. doi: 10.1007/s10853-008-3068-1
19. Edmunds DH, Whittaker DK, Green RM (1988) Caries Res 22:327
20. Cardenas M, Elofsson U, Lindh L (2007) Biomacromolecules 8:1149
21. Rodriguez-Hornedo N, Murphy D (2004) J Pharm Sci 93:449
22. Barkvoll P (1991) J Biol Buccale 19:235
23. Lee K, Kunjappu J, Jockusch S, Turro NJ, Widerschpan T, Zhou J, Smith BW, Zimmerman P, Conley W (2005) Proc of SPIE 5753:537