Fluoride Plus Functionalized β -TCP: A Promising Combination for Robust Remineralization

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ABSTRACT

With more than 50 years of clinical success, fluoride serves as the gold standard agent for preventing tooth decay. In particular, the action of fluoride facilitates saliva-driven remineralization of demineralized enamel and alters solubility beneficially. Still, tooth decay remains problematic, and one way to address it may be through the development of new mineralizing agents. Laboratory and clinical studies have demonstrated that the combination of fluoride and functionalized β -tricalcium phosphate (*f*TCP) produces stronger, more acid-resistant mineral relative to fluoride, native β -TCP, or *f*TCP alone. In contrast to other calcium-based approaches that seem to rely on high levels of calcium and phosphate to drive remineralization, *f*TCP is a low-dose system designed to fit within existing topical fluoride preparations. The functionalization of β -TCP with organic and/or inorganic molecules provides a barrier that prevents premature fluoride-calcium interactions and aids in mineralization when applied *via* common preparations and procedures. While additional clinical studies are warranted, supplementing with *f*TCP to enhance fluoride-based nucleation activity, with subsequent remineralization driven by dietary and salivary calcium and phosphate, appears to be a promising approach.

Despite advances in understanding, recommended hygiene regimens, and dental preparations, dental decay still affects the majority of the world's population (Dye *et al.*, 2007). The role of fluoride in reducing dental decay has been well-established since its discovery, around 1900, as a potential cariostatic agent (Schamschula *et al.*, 1981). Over the years, numerous studies have evaluated and/or confirmed fluoride's efficacy in a variety of fluoride-containing dental formulations (Muhler *et al.*,

1954; Marinho *et al.*, 2003). Although some believe that alternative therapies or reducing the fluoride concentrations in dental preparations may be warranted, the Cochrane Oral Health Group recently reviewed the scientific literature and concluded that a daily oral regimen incorporating at least 1,000 ppm F likely provides the greatest anti-caries protection (Walsh *et al.*, 2010). While fluoride's benefits mostly relate to the caries experience, dental erosion, which can be defined as the chemical dissolution of dental hard tissue by acids of non-microbiological design (e.g., through consumption of acidic beverages and foods, or through acid-reflux or vomiting), is gaining prominence. Dental erosion is relatively difficult to treat, since, while relatively lower fluoride concentrations may provide anti-caries benefits, higher fluoride concentrations are needed to address dental erosion (Amaechi and Higham, 2005). Hence, the continued prevalence of caries and the limitations of fluoride for dental erosion contribute to the need for improved practices and/or therapies. The clinically proven success and acceptance of fluoride for prevention and/or maintenance of dental decay suggest that one way of improving efficacy might be through improving fluoride's activity (Pfarrer and Karlinsey, 2009). In this paper, we describe our approach in enhancing fluoride's benefits through promising functionalized tricalcium phosphate (*f*TCP) ingredients. These fluoride-compatible ingredients are designed to support and enhance fluoride's activity on the tooth and depend critically on the nature of the dental preparation. In the following paper, we review the science of *f*TCP as well as recent studies supporting the development and efficacy of fluoride plus *f*TCP.

FUNCTIONALIZED β -TRICALCIUM PHOSPHATE

*f*TCP is the resultant material derived through the coupling of β -tricalcium phosphate (β -TCP) with organic and/or inorganic moieties, such as carboxylic acids and surfactants (Karlinsey and Mackey, 2009; Karlinsey *et al.*, 2010b,c). β -TCP serves as a bioactive source of mineralizing components and is an appealing calcium phosphate system due in part to its limited solubility relative to other calcium salts and minerals, which has implications for fluoride compatibility in water-based preparations. In surgical practice, β -TCP is considered a Class II device used in facilitating bone remodeling in maxillofacial procedures (FDA, 2005) and orthopedic applications (FDA, 2003).

Key Words

caries, erosion, regeneration, enamel, dentin, preventive dentistry.

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Figure 1. Schematic of the two symmetry sites, C₃ and C₁, of the β-TCP unit cell.

An important feature of β-TCP is that it manifests lattice defects, notably the under-bonded CaO₃ polyhedra residing in the crystal C₃ symmetry site (Fig. 1) (Karlinsky *et al.*, 2010b), that may allow for crystal modification (Karlinsky and Mackey, 2009). In contrast, the C₁ symmetry site does not possess such defects within the calcium and phosphate polyhedra. Because the issue of compatibility with fluoride is paramount in the design of improved anti-carries therapies (Pfarrer and Karlinsky, 2009), the possibility of modifying, or *functionalizing*, β-TCP provides a prospective avenue in the research of bioactive and fluoride-compatible mineralizing agents. The purpose of functionalizing β-TCP with organic and/or inorganic molecules in this case is two-fold: First, it creates barriers that prevent premature fluoride-calcium interactions, and, second, it facilitates targeted delivery (Karlinsky *et al.*, 2009b) when applied to the teeth *via* common dental preparations (*e.g.*, dentifrice, mouthrinse, etc.).

Mechanochemical methodology is used to prepare *f*TCP (Karlinsky and Mackey, 2009). In the process, interfacing among the components can proceed entirely in the solid-state phase, and this is essential to maintaining the integrity of the β-TCP framework, including lattice defects. Although mechanochemical processing can be used for producing diminutive, amorphous particles, the parameters used to functionalize β-TCP are such that micron-sized particles are produced (comparable with dental silica abrasives), and β-TCP crystallinity is largely preserved (Karlinsky *et al.*, 2010b,c).

Because *f*TCP is tailored to the dental preparation and intended purpose, many possible functionalizing agents exist, and we have focused on those that are generally recognized by the

Table 1. IR Analysis of Peak P-O Vibrations in White-spot Lesion (WSL) Enamel Treated with β-TCP, Milled (*i.e.*, not functionalized) β-TCP (mTCP), and Functionalized β-TCP (*f*TCP) (Karlinsky, 2009b)

Enamel Substrate Group	Peak Intensity (A.U.)	Peak Position (cm ⁻¹)
Sound enamel (no treatment)	1.094 ^d	1030.4 ^A
WSL (baseline)	0.758 ^c	1031.2 ^A
WSL + 100 ppm β-TCP	0.504 ^a	1031.2 ^A
WSL + 100 ppm mTCP	0.684 ^b	1033.6 ^B
WSL + 100 ppm <i>f</i> TCP	0.754 ^c	1037.6 ^C

Significant differences ($p < 0.05$) in peak P-O intensity and position are indicated with letters, with $a < b < c < d$ and $A < B < C$.

FDA as safe. One example is β-TCP functionalization with sodium lauryl sulfate (SLS) for purposes of improving remineralization of white-spot lesions with water-based fluoride preparations; in this instance, the 12-carbon anionic surfactant was selected, in part, based on its reactive sulfate head group, which is known to stimulate surface modifications and mediate crystal nucleation and growth (Rodriguez-Hornedo and Murphy, 2004). In another example, where a high-fluoride, non-aqueous, and extended-release varnish was desired, fumaric acid was selected based on its high thermal stability, relatively slow dissolution, and strong calcium chelation tendency (Goldberg *et al.*, 1991). In a final example, where anti-erosion benefits may be desired from a water-based rinse or dentifrice, β-TCP is prepared with silica, which may provide linking opportunities with hard-tissue defects under acidic conditions (Phan *et al.*, 2003), and urea, which can permeate throughout enamel without attacking the interprismatic organic material (Arends *et al.*, 1984), which may encourage greater calcium, phosphate, and fluoride uptake in erosive lesions.

Each *f*TCP ingredient is designed to supplement fluoride to enhance fluoride-based nucleation activity, with subsequent remineralization driven by dietary and salivary calcium and phosphate. It is a tenet that the level of *f*TCP is lower than the fluoride level of the dental preparation. Furthermore, because it is fluoride-compatible, *f*TCP shares the same compartment as fluoride, to ensure optimal delivery of fluoride and *f*TCP. It is important to stress that *f*TCP is *not* a substitute for either fluoride or saliva. Rather, it is an agent that works in synergy with fluoride to create stronger, more acid-resistant mineral relative to that achievable with fluoride, β-TCP, or *f*TCP alone. These observations are supported with laboratory and intra-oral clinical evaluations and are discussed below.

UPTAKE STUDIES

In the spirit of fluoride soak experiments as outlined in FDA Test Method #40, 30-minute exposure experiments involving white-spot enamel lesions (WSL) and eroded enamel have been performed (Karlinsky *et al.*, 2009b, 2010e). With infrared spectroscopy (IR), the orthophosphate (PO₄) environments of enamel were shown to respond to the different mineralizing agents as summarized in Tables 1 and 2 (measurements for each spectral group were made in triplicate with ± 0.05 A.U. peak intensity

Table 2. IR Analysis of Peak P-O Vibrations in Eroded Enamel Treated with 225 and 1100 ppm F (NaF) with and without *f*TCP (Karlinsky *et al.*, 2010e)

Enamel Substrate Group	Peak Intensity (A.U.)	Peak Position (cm ⁻¹)	[F]
Sound enamel (no treatment)	1.094 ^c	1030.4 ^A	—
Erosive lesion (baseline)	0.525 ^a	1036.0 ^B	—
Erosive lesion + 225 ppm F	0.603 ^a	1038.4 ^{B,C}	0.13 ± 0.01 ¹
Erosive lesion + 225 ppm F + 20 ppm <i>f</i> TCP	0.812 ^b	1039.2 ^C	0.13 ± 0.01 ¹
Erosive lesion + 1100 ppm F	0.745 ^b	1035.2 ^B	0.27 ± 0.01 ²
Erosive lesion + 1100 ppm F + 200 ppm <i>f</i> TCP	0.751 ^b	1040.0 ^C	0.39 ± 0.04 ³

Significant differences ($p < 0.05$) in peak P-O intensity and position, as well as fluoride uptake, are indicated, with $a < b < c$ and $A < B < C$ and $1 < 2 < 3$.

reproducibility error, and $\pm 2 \text{ cm}^{-1}$ wavenumber error) (Karlinsky *et al.*, 2009b, 2010e). Orthophosphate near the enamel surface or loosely bound through weak bonding, is susceptible to dissolution by acids, and is especially observed at lower phosphate-oxygen (P-O) wavenumbers, such as near $1,030 \text{ cm}^{-1}$; in contrast, tightly bound orthophosphate environments are typically observed at higher wavenumbers. For white-spot lesions treated with native β -TCP, little structural change is observed, possibly due to the inherent poor solubility of β -TCP. However, when β -TCP is mechanochemically processed in the absence of a functionalizing agent (mTCP), greater surface areas are produced, releasing calcium and phosphate ions for mineralization (Karlinsky *et al.*, 2010b). In turn, this produces relatively stronger P-O bonding within enamel, and the peak intensity and position are increased. But the greatest mineral integration occurs for *f*TCP as observed in the shift to 1037.6 cm^{-1} . In this case, the population and strength of P-O bonding have increased significantly, demonstrating that the enamel framework has been affected. Importantly, this effect is observed despite the fact that mTCP has greater availability of soluble calcium relative to *f*TCP (Karlinsky *et al.*, 2010b). Thus, analysis of these data shows that functionalization of the β -TCP lattice imparts a significant role in mineralization, and is not driven solely by the magnitude of soluble ions.

For comparisons with fluoride with and without *f*TCP, a separate study involving erosive lesions was also performed, and the summary results are shown in Table 2. Consistent with biological FDA Test Method #40, analysis of these data shows that both 225 and 1100 ppm F (NaF) integrate in a dose-response manner within the enamel framework, with increases in both peak P-O population and position. Although the peak position shifts corresponding to 225 and 1100 ppm F are not significant, the populations are, and this is consistent with the dose-response results obtained from the companion fluoride uptake study. This mineralization activity significantly improves in the presence of *f*TCP (which was comprised of β -TCP, silica, and urea). With respect to 225 ppm F, *f*TCP helps increase the population of strong P-O bonds relative to 225 ppm F alone, and this appears independent of the uptake of fluoride. With respect to 1100 ppm F, *f*TCP appears to produce not only stronger P-O bonding but also P-F bonding (as evidenced in the blue-shift to 1040 cm^{-1} relative to the 1,100 ppm F and baseline lesion) relative to fluoride alone (Karlinsky *et al.*, 2010e). The promotion of P-F bonding environments can be supported with the

companion study showing significantly increased fluoride uptake relative to 1100 ppm F alone, and this is consistent with the observed increased in peak intensity for eroded enamel treated with 1100 ppm F relative to the baseline lesion. Analysis of these data, overall, shows that *f*TCP improves fluoride-based mineralization of eroded enamel. The similarities in peak positions and populations reported in Tables 1 and 2 for enamel treated with *f*TCP with or without fluoride suggest that *f*TCP delivers calcium and phosphate similarly to the enamel framework, and is dependent on *f*TCP concentration (Karlinsky and Mackey, 2009; Karlinsky *et al.*, 2009b,c, 2010d,e). These studies show that *f*TCP promotes uptake of ions, including fluoride (when fluoride is present), into the enamel in a unique manner that depends on the nature of the enamel lesion, *f*TCP and/or fluoride concentration, and *f*TCP composition.

REMINERALIZATION EVALUATIONS

pH cycling studies have been performed to evaluate the quality of newly formed mineral. Designed to emulate the clinical setting, pH cycling models can provide critical assessments of promising formulations (White, 1992). Separately, intra-oral clinical studies offer the ability to collect data on tooth specimens that have been exposed to the natural oral environment in a relatively short time (White, 1992; Zero, 1995), and have been performed to provide clinically relevant information on the combination of fluoride plus *f*TCP. A summary of these studies involving fluoride and *f*TCP is discussed below.

In vitro pH cycling experiments designed to mimic remineralization of incipient enamel lesions have revealed that the combination of NaF (*i.e.*, 500, 950, 1100, or 5000 ppm F) plus *f*TCP (which was comprised of β -TCP and SLS) in a simple aqueous solution can produce significantly greater surface and subsurface rehardening of WSL relative to that achievable with fluoride alone (Karlinsky and Mackey, 2009; Karlinsky *et al.*, 2010b,d). Similarly, when added to a 0.05% NaF commercial mouthrinse and analyzed for enamel fluoride uptake and evaluated in a pH cycling model, *f*TCP provided significantly greater fluoride uptake and rehardening relative to a fluoride-free and control 0.05% NaF mouthrinse (Karlinsky *et al.*, 2010d). In silica-containing, water-based dentifrices having either 500 or 5000 ppm F (NaF), pH cycling studies have shown that *f*TCP improves fluoride uptake and surface and subsurface rehardening relative to control fluoride-only dentifrices (Karlinsky *et al.*, 2009a, 2010a, 2011b). Such

formulations affect lesion morphology as shown in Fig. 2, where the combination of fluoride and f TCP produces relatively large, densely packed crystals compared with the smaller and/or less dense crystals for the fluoride-free or fluoride-control specimens. Presumably, this fluoride plus f TCP combination also leads to the acid-resistant occlusion of demineralized dentin and, therefore, promising potential for hypersensitivity relief (Karlinsey *et al.*, 2011a). In addition to remineralization potential, f TCP was also observed to significantly inhibit lesion progression relative to clinically proven controls (3M ESPE, unpublished observations). Separately, a three-phase, 28-day, crossover *in situ* study involving 30 volunteers was performed to test the ability of 3 silica-containing NaF dentifrices to remineralize white-spot lesions. The surface and cross-sectional microhardness results showed that the combination of 500 ppm F plus f TCP produced greater remineralization relative to the 500-ppm-F control dentifrice and suggest that this combination may provide comparable anti-caries benefits relative to a clinically proven 1,100-ppm-F dentifrice (Mensinkai *et al.*, 2012). Another three-phase crossover intra-oral study has also been performed whereby 5,000 ppm F plus f TCP provided significantly greater remineralization benefits relative to placebo and 5,000-ppm-F control groups after 28 days (Amaechi *et al.*, 2012).

Because dental erosion manifests different characteristics relative to caries, a different composition of f TCP was designed to maximize anti-erosion benefits. In this case, β -TCP, silica, and urea were combined to form f TCP, and when this was added to a fluoride-containing preparation and evaluated in pH cycling models, significant surface enamel rehardening was observed relative to the control NaF systems (Karlinsey *et al.*, 2009c). The clinical relevance of this f TCP material combined with fluoride was evaluated in 2 intra-oral studies. In the first study, 20 volunteers participating in a crossover study each received 3 treatments over a 28-day period (Amaechi *et al.*, 2010). Treatments consisted of brushing with a fluoride-free dentifrice, followed by one of the following: one-minute 225-ppm-F rinse, one-minute 225-ppm-F plus f TCP rinse, or no rinse (saliva only). TMR assessment revealed that the combination rinse of 225 ppm F plus f TCP was able to remineralize initially eroded enamel (1% citric acid, pH = 2.5, 0.5 hr) significantly better than the 225-ppm-F control. The observation that the 225-ppm-F control did not produce significantly greater remineralization relative to saliva alone is consistent with limitations of fluoride and supports the difficulty in treating eroded enamel (Amaechi and Higham, 2005). In the second study, 80 volunteers participated in a four-leg parallel design (N = 20 *per leg*) over a 28-day period (Mathews *et al.*, 2012). The treatments consisted of brushing with a fluoride-free dentifrice, followed by one-minute rinsing with one of the following: 0 ppm F, 225 ppm F, 225 ppm F + f TCP, or 450 ppm F. Despite the limited effects fluoride may have on eroded enamel, the trending in the surface microhardness and TMR results demonstrated that the combination rinse of 225 ppm F plus f TCP produced greater remineralization of the initially eroded enamel (0.3% citric acid, pH = 3.75, 2 hrs) relative to the 225-ppm-F control and was comparable with 450 ppm F.

Although additional clinical studies are warranted, ongoing evaluations in models commensurate with fluoride and recommended by the FDA and ADA suggest that supplementing with f TCP enhances fluoride-based nucleation activity, with subse-

Figure 2. Scanning electron micrographs (100,000x) of white-spot lesions in bovine enamel treated with (a) fluoride-free, (b) 500 ppm F (NaF), and (c) 500 ppm F plus f TCP dentifrices via a 10-day *in vitro* pH cycling study.

quent remineralization driven by dietary and salivary calcium and phosphate. Though these models are designed for fluoride and not combinations of fluoride and other mineralizing systems, they may extend to the combination of fluoride plus f TCP. The design and purpose of f TCP supplements the role of fluoride and

is tailored to match the formulation and purpose of a given dental preparation. Although mechanistic understanding is ongoing, supplementing with *f*TCP does not appear to accelerate the kinetics of fluoride, but appears to promote uptake of ions, the nature of which depends on the lesion type, fluoride concentration, and *f*TCP composition to produce stronger, more acid-resistant mineral relative to fluoride alone.

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REFERENCES

- Amaechi BT, Higham SM (2005). Dental erosion: possible approaches to prevention and control. *J Dent* 33:243-252.
- Amaechi BT, Karthikeyan R, Mensinkai PK, Najibfard K, Mackey AC, Karlinsky RL (2010). Remineralization of eroded enamel by a NaF rinse containing a novel calcium phosphate agent in an *in situ* model: a pilot study. *Clin Cos Invest Dent* 2:93-100.
- Amaechi BT, Karthikeyan R, Mensinkai PK, Najibfard K (2012). *In situ* remineralization of a new high-fluoride dentifrice. *Gen Dent* (in press).
- Arends J, Jongebloed WL, Goldberg M, Schuthof J (1984). Interaction of urea and human enamel. *Caries Res* 18:17-24.
- Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, et al. (2007). Trends in oral health status: United States, 1988-1994 and 1999-2004. National Center for Health Statistics. *Vital Health Stat* 11(248):1-92.
- Food and Drug Administration, HHS (2003). Class II Special Controls Guidance Document: Resorbable Calcium Salt Bone Void Filler Device. *Fed Regist* 68:32761-32762.
- Food and Drug Administration, HHS (2005). Reclassification of Tricalcium Phosphate Granules and Classification of Other Bone Grafting Material for Dental Bone Repair. *Fed Regist* 70:21947-21950.
- Goldberg I, Peleg Y, Rokem JS (1991). Citric, fumaric, and malic acids. *Biotechnol Food Ingrid* 74:349-374.
- Karlinsky RL, Mackey AC (2009). Solid-state preparation and dental application of an organically-modified calcium phosphate. *J Mater Sci* 44:346-349.
- Karlinsky RL, Mackey AC, Stookey GK, Pfarrer AM (2009a). *In vitro* assessments of experimental NaF dentifrices containing a prospective calcium phosphate technology. *Am J Dent* 22:180-184.
- Karlinsky RL, Mackey AC, Walker ER, Frederick KE (2009b). Spectroscopic evaluation of native, milled, and functionalized β -TCP seeding into dental enamel lesions. *J Mater Sci* 44:5013-5016.
- Karlinsky RL, Mackey AC, Walker ER, Frederick KE, Fowler CX (2009c). *In vitro* evaluation of eroded enamel treated with fluoride and a prospective tricalcium phosphate agent. *J Dent Oral Hyg* 1:52-58.
- Karlinsky RL, Mackey AC, Walker ER, Amaechi BT, Karthikeyan R, Najibfard K, et al. (2010a). Remineralization potential of 5,000 ppm fluoride dentifrices evaluated in a pH cycling model. *J Dent Oral Hyg* 2:1-6.
- Karlinsky RL, Mackey AC, Walker ER, Frederick KE (2010b). Surfactant-modified β -TCP: structure, properties, and *in vitro* remineralization of subsurface enamel lesions. *J Mater Sci: Mater Med* 21:2009-2020.
- Karlinsky RL, Mackey AC, Walker ER, Frederick KE (2010c). Preparation, characterization and *in vitro* efficacy of an acid-modified β -TCP material for dental hard-tissue remineralization. *Acta Biomater* 6:969-978.
- Karlinsky RL, Mackey AC, Walker ER, Frederick KE (2010d). Enhancing remineralization of subsurface enamel lesions with functionalized β -TCP. In: Biomaterials developments and applications. Bourg H, Lisle A, editors. New York, NY: Nova Science Publishers, pp. 353-374.
- Karlinsky RL, Mackey AC, Walker ER, Walker TJ, Fowler CX (2010e). Mineralization of eroded dental enamel seeded with fluoride and a tricalcium phosphate ternary biomaterial. *Adv Sci Technol* 76:42-47.
- Karlinsky RL, Mackey AC, Schwandt CS, Walker TJ (2011a). SEM evaluation of demineralized dentin treated with professional-strength NaF topical pastes *in vitro*. *Am J Dent* 24:357-362.
- Karlinsky RL, Mackey AC, Walker TJ, Frederick KE, Blanken DD, Flaig SM, et al. (2011b). *In vitro* remineralization of human and bovine white-spot lesions by NaF dentifrices: a pilot study. *J Dent Oral Hyg* 3:22-29.
- Marinho VC, Higgins JP, Logan S, Sheiham A (2003). Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews* 4:CD002782.
- Mathews MS, Amaechi BT, Ramalingam K, Ccahuana-Vasquez RA, Chedjieu IP, Mackey AC, et al. (2012). *In situ* remineralization of eroded enamel lesion by NaF rinses. *Arch Oral Biol* 57:525-530.
- Mensinkai PK, Ccahuana-Vasquez RA, Chedjieu I, Amaechi BT, Mackey AC, Walker TJ, et al. (2012). *In situ* remineralization of white-spot enamel lesions by 500 and 1,100 ppm F dentifrices. *Clin Oral Investig* [Epub ahead of print 7/13/2011] (in press).
- Muhler JC, Radike AW, Nebergall WH, Day HG (1954). The effect of a stannous fluoride-containing dentifrice on caries reduction in children. *J Dent Res* 33:606-612.
- Pfarrer AM, Karlinsky RL (2009). Challenges of implementing new remineralization technologies. *Adv Dent Res* 21:79-82.
- Phan PV, Grzanna M, Chu J, Polotsky A, El-Ghannam A, Heerden DV, et al. (2003). The effect of silica-containing calcium-phosphate particles on human osteoblasts *in vitro*. *J Biomed Mater Res A* 67:1001-1008.
- Rodriguez-Hornedo N, Murphy D (2004). Surfactant-facilitated crystallization of dihydrate carbamazepine during dissolution of anhydrous polymorph. *J Pharm Sci* 93:449-460.
- Schamschula RG, Barmes DE (1981). Fluoride and health: dental caries, osteoporosis, and cardiovascular disease. *Ann Rev Nutr* 1:427-435.
- Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X (2010). Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews* 1:CD007868.
- White DJ (1992). The comparative sensitivity of intra-oral, *in vitro*, and animal models in the 'profile' evaluation of topical fluorides. *J Dent Res* 71(Spec Iss):884-894.
- Zero DT (1995). *In situ* caries models. *Adv Dent Res* 9:214-230.