Non-Cavitated Caries Lesions:  
A New Approach to Medical Treatment

Carmen Llena¹, Ana María Leyda² and Leopoldo Forner¹

¹Department of Stomatology, Universitat de València, 
Primary Dental Care, Valencian Health Service, 
²Private Practice in Pediatric Dentistry, Valencia, 
Spain

1. Introduction

Dental caries is a highly prevalent disease, although, in most developed countries, its prevalence has declined, but the disease remains a major public health problem (Selwitz et al., 2007). Signs of the caries process cover a continuum from the first molecular changes in the apatite crystals of the tooth to a visible white-spot lesion, continuing with dentin involvement and eventual cavitation. Progression through these stages requires a continual imbalance between pathological and protective factors that results in the dissolution of apatite crystals and the loss of calcium, phosphate, and other ions from the tooth (demineralization).

A goal of modern dentistry is to manage non-cavitated caries lesions non-invasively through remineralization in an attempt to prevent disease progression and improve aesthetics, strength, and function (Rao & Malhotra, 2011). Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain. The term ‘void’ is used to define any accessible space in a crystal caused by ion loss from the demineralization process. This definition of remineralization therefore includes any crystal repair to bring about net mineral gain to an enamel subsurface lesion, but does not extend to precipitation of solid phases onto enamel surface (Cochrane et al 2010). At physiological pH, un-stimulated and stimulated parotid, submandibular, and whole saliva are supersaturated with respect to most solid calcium phases. However, precipitation of calcium phosphate phases in saliva normally does not occur, due to the presence of salivary proteins, particularly statherin and proline-rich phosphoproteins The proposed mechanism of action is that the segments of the proteins containing phosphoseryl residues, in particular the statherin sequence, bind to calcium and phosphate ion clusters, preventing growth of the ion cluster to the critical size required for precipitation and transformation into a crystalline phase (Hay & Moreno, 1989). This critical stabilization of calcium and phosphate ions by salivary phosphoproteins ensures that the ions remain bioavailable to diffuse into mineral deficient lesions to allow for remineralization of demineralized crystals, while preventing surface deposition in the form of calculus.
Fluoride is the cornerstone of the non-invasive management of non-cavitated caries lesions, but its ability to promote net remineralization is limited by the availability of calcium and phosphate ions (Reynolds et al., 2008). Fluoride ions can drive the remineralization of extant non-cavitated caries lesions if adequate salivary or plaque calcium and phosphate ions are available when the fluoride is applied. For fluorapatite or fluorhydroxyapatite to form, calcium and phosphate ions are required, as well as fluoride ions.

Several authors have now shown that enamel remineralization in situ and the retention of fluoride in plaque are dependent on the availability of calcium ions (Vogel et al., 2008). Hence, on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for fluoride retention and net enamel remineralization to occur, and this is highly exacerbated under hyposalivation conditions (Reynolds et al., 2008). When adequate levels of calcium and phosphate ions are together with the fluoride ions, it has been shown in vitro that this combination can produce substantial remineralization of lesions of enamel and even those penetrating the underlying dentin in pH-cycling experiments (ten Cate et al., 2008). Therefore, the challenge now is to achieve this clinically, since salivary remineralization of enamel promoted by topical fluoride (particularly high concentrations) has been shown to give rise to predominantly surface remineralization (Willmot, 2004). Ideally, a remineralization system should supply stabilized bioavailable calcium, phosphate, and fluoride ions that favor subsurface mineral gain rather than deposition only in the surface layer.

2. Calcium and phosphate based enamel remineralizing systems

The fundamental difficulty with the clinical application of calcium and phosphate remineralization systems is the low solubility of the calcium phosphates, particularly in the presence of fluoride ions. Numerous authors have investigated various calcifying solutions in an attempt to remineralize demineralized tooth structure. Normally, these solutions have contained between 1 and 3 mM calcium ions with phosphate ions in the ratio of 1:1 (Koulourides et al., 1961; Wefel & Harless, 1987) or 1.66:1 (Koulourides et al., 1961; ten Cate & Arends, 1977; Silverstone et al., 1981; Iijima et al., 1999), often with the addition of 1 ppm fluoride ions.

Clinical remineralization systems have been developed using a single low-concentration calcium and phosphate solution. These systems can be classified into three types:

- Crystalline calcium phosphate
- Unstabilized amorphous calcium phosphate
- Stabilized amorphous calcium phosphate

New products utilizing these three types of delivery systems are now commercially available, and the manufacturers claim that these products provide new avenues for the remineralization of noncavitated caries lesions.

2.1 Crystalline calcium phosphate remineralizing systems

Calcium phosphate can exist in one of numerous crystalline phases: brushite, dicalcium phosphate dihydrate, β-tricalcium phosphate, octacalcium phosphate, hydroxyapatite, fluorapatite. Each of these crystalline phases has different solubilities, and many have been
tested as potential methods of delivering calcium and phosphate ions to subsurface enamel lesions. The problem with applying crystalline material to the oral cavity to promote enamel remineralization is the poor solubility of the calcium phosphate phases, such that the calcium and phosphate ions are unavailable for remineralization. These crystalline calcium phosphate phases must be released from the product on contact with saliva and then dissolve in that fluid to liberate ions capable of diffusing into the enamel subsurface lesion. The dissolution of the calcium phosphate phase in saliva requires that saliva be undersaturated with respect to that crystalline phase. Based on some typical concentrations of calcium, phosphate, and fluoride ions in saliva, at the normal salivary pH range, these crystalline calcium phosphate phases would not dissolve (Larsen & Pearce, 2003).

Brushite has been added to products such as dentifrices (Zhang et al., 1995; Sullivan et al., 1997) in an attempt to enhance the remineralization of enamel subsurface lesions. Brushite is one of the more soluble crystalline calcium phosphate phases; however, remineralization of subsurface lesions in vivo and slowing of caries progression in clinical trials have not been shown.

Tricalcium phosphate (TCP) has recently been added to dental products that are claimed by the manufacturers to remineralize white-spot lesions. Interestingly, the TCP is referred to as “functionalized”, since it has been altered by ball milling with sodium lauryl sulfate (Karlinsey & Mackey, 2009). We could not find clinical studies supporting its ability to remineralize enamel subsurface lesions.

A variation on the use of crystalline calcium phosphates is the use of solid calcium sodium phosphosilicates. For dental applications, this calcium sodium phosphosilicate glass is marketed under the name of Novamin. It has been studied in vitro and clinically as a treatment for dentin hypersensitivity, with the proposed mechanism being the physical occlusion of dentin tubules (Greenspan DC, 2010). Novamin has also been claimed by the manufacturers to have applications in enamel subsurface remineralization (Burwell AK et al., 2009) or for prevent dental erosion.

### 2.2 Unstabilized amorphous calcium phosphate systems

The amorphous calcium phosphate (ACP) technology is an unstabilized calcium and phosphate system that has been developed and commercialized as Enamelon. It is based on unstabilized amorphous calcium phosphate, where a calcium salt and a phosphate salt are delivered separately intra- orally or delivered in a product with a low water activity (Tung & Eichmiller, 2004). As the salts mix with saliva, they dissolve, releasing calcium and phosphate ions. The mixing of calcium ions with phosphate ions to produce an ion activity product for amorphous calcium phosphate that exceeds its solubility product results in the immediate precipitation of ACP or, in the presence of fluoride ions, amorphous calcium fluoride phosphate (ACFP). In the intra-oral environment, these phases (ACP and ACFP) are potentially very unstable and may rapidly transform into a more thermodynamically stable, crystalline phase (hydroxyapatite and fluorhydroxyapatite). However, before phase transformation, calcium and phosphate ions should be transiently bioavailable to promote enamel subsurface lesion remineralization.

Published papers suggest that the unstabilized ACP/ACFP technology may have efficacy in preventing caries progression. The unstabilized ACP/ACFP may transform to poorly
soluble phases in the mouth, and, in so doing, may act to promote dental calculus. The formation of fluoride-containing apatite intra- orally would sequester available fluoride ions, thereby reducing their ability to promote remineralization of subsurface enamel lesions. It is likely, though, that some of the ACP/ACFP phases that are produced intra-orally may be stabilized by the phosphoproteins in saliva, pellicle, and plaque that are not at full stabilization capacity. This may explain the bioavailability of these technologies in the presence of saliva and the positive in situ model results (Reynolds, 2008). However, long-term randomized controlled caries clinical trials of the unstabilized ACP/ACFP technologies need to be conducted to demonstrate efficacy in preventing caries and safety by the lack of dental calculus promotion with long-term use.

2.3 Stabilized amorphous calcium phosphate systems

Biological fluids containing high concentrations of calcium and phosphate ions also contain inhibitory ions such as pyrophosphate and proteins to ensure stabilization. Statherin is a phosphoprotein with strong affinity to calcium, enamel and other apatite surfaces. Its functions include the capacity to inhibit the precipitation and growth of calcium phosphate crystals (Vitorino R et al., 2005). Proline-rich proteins acts in a similar manner to statherin and adhere to the surface of calcium phosphate crystals preventing their growth. Together with citrate, these proteins adhere to a significant amount of the total calcium in saliva and help to maintain correct proportions of ionic calcium and phosphate. These proline-rich proteins are key components of biofilms and adhere strongly through their amino-terminal portion to the enamel. The carboxy-terminal portion is the adhesion site for some bacteria in the early stages of plaque formation and is also the adhesion site for dietary tannins (Drobni M, et al. 2006).

Bovine milk is used to manufacture phosphopeptide based products for dentistry use in humans. There are three types of phosphopeptide based products: casein phosphopeptides (CPP), casein phosphopeptides with amorphous calcium phosphate (CPP-ACP), which contain 18% calcium ion and 30% phosphate ion in weight, and casein phosphopeptides with amorphous calcium fluoride phosphate (CPP-ACFP) (Aimutis WR, 2004).

2.3.1 Casein phosphopeptides

Dairy products are the most recognized anticaries active food group (Reynolds. 1998). Using in vitro, animal and in situ caries models, the components largely responsible for this anticariogenic activity have been identified as casein, calcium and phosphate (Reynolds & Black. 1989) The bovine milk phosphoprotein, casein, which is known to interact with calcium and phosphate (Reeves & Latour,1958) and is a natural food component, is an obvious candidate for an anticariogenic food and toothpaste additive, however this is precluded by organoleptic properties and the very high levels required for activity. Using a human intraoral caries model, Reynolds (1987) showed that digestion of caseinate with trypsin did not destroy the protein’s ability to prevent enamel sub-surface demineralization. Tryptic peptides of casein were found incorporated into the intra-oral appliance plaque and were associated with a substantial increase in the plaque’s content of calcium and phosphate. It was concluded that the tryptic peptides that were responsible for the anticariogenic activity were the calcium phosphate sequestering phosphopeptides.
2.3.2 CPP-ACP and CPP-ACFP

The casein phosphopeptides (CPP) are approximately 10% (w/v) of the protein casein. They are tasteless, have low antigenicity, and can be purified as CPP-ACP complexes from a casein enzymatic digest by filtration. CPP-ACP has been recognized as safe by the Food and Drug Administration of the United States of America and other regulatory bodies around the world and can be incorporated into oral care products and foods.

Four major bovine CPPs containing the sequence –Ser(P)–Ser(P)–Ser(P)–Glu–Glu–, where Ser(P), have been shown to stabilize high concentrations of calcium and phosphate ions in metastable solution supersaturated with respect to the calcium phosphate solid phases (at acidic and basic pH (Cochrane et al., 2008). A 1% CPP solution at pH 7.0 can stabilize 60 mM calcium and 36 mM phosphate. Additionally, stabilization of calcium phosphate phases by CPP has been shown in the presence of fluoride ions (Cochrane et al., 2008).

CPPs bind more calcium and phosphate ions than can be attributed to just the calcium-binding motif –Ser(P)–Ser(P)–Ser(P)–Glu–Glu–, indicating that other acidic residues of the phosphopeptide sequence contribute to the stabilization of calcium phosphate. This interaction prevents growth of the calcium and phosphate ion clusters to the critical size required for nucleation and phase transformations (Cross et al., 2005). This is similar to the properties of statherin; however, the capacity of the casein phosphopeptides is significantly greater than that of statherin, due to the higher content of phosphoseryl and other acidic residues. In solution, an equilibrium exists between free and CPP-bound calcium, phosphate, and fluoride ions. This equilibrium is dependent on environmental factors such as pH, ion concentration, and the presence of competing binding surfaces for the CPP (Cochrane et al. 2008). CPPs only weakly bind calcium and phosphate ions, thus allowing for a dynamic equilibrium between free and CPP-bound ions. This therefore provides a reservoir of bioavailable ions.

3. Scientific evidence for remineralization by CPP-ACP and CPP-ACFP

The evidence for remineralization efficacy has been shown with CPP-ACP and CPP-ACFP in a variety of vehicles in laboratory (Cochrane et al., 2008) and human in situ experiments (Shen et al., 2001; Cai et al., 2007; Reynolds et al., 2008), as well as in randomized, controlled clinical trials (Andersson et al., 2007; Morgan et al., 2008; Bailey et al., 2009; Rao et al., 2009).

The CPP-ACP literature has been reviewed by several authors (Reynolds, 1998; Llena et al., 2009; Azarpazhooh, et al 2008; Neuhaus, et al 2009, Yengopal & Mickenautsch, 2009). Yengopal and Mickenautsch (2009), in a systematic review, concludes that, within the limitations of the systematic review with meta-analysis, results of the clinical in situ trials indicate a short-term remineralization effect of CPP-ACP. Additionally, the promising in vivo randomized controlled trials results suggest a caries-preventing effect for long-term clinical CPP-ACP use. Further randomized control trials are needed in order to confirm these initial results in vivo.

One randomized, controlled caries clinical trial of CPP-ACP assessed the impact of CPP-ACP in sugar-free gum relative to a control sugar-free gum. This trial demonstrated that the CPP-ACP gum significantly slowed progression and enhanced regression of caries compared with the control sugar-free gum (Morgan et al., 2008).
Three randomized, controlled clinical trials of post-orthodontic white-spot lesion regression by a CPP-ACP dental cream have been reported by Andersson et al. (2007), Bailey et al. (2009) and Bröchner et al. (2011). Andersson, investigate and compare the effects of a dental cream containing complexes of casein phosphoprotein-amorphous calcium phosphate (CPP-ACP) and fluoride mouthwashes on the regression of white spot lesions, after debonding of fixed orthodontic appliances. Clinical scoring and laser fluorescence assessment suggested that both regimens could promote regression of white spot lesions. The visual evaluation suggested an aesthetically more favorable outcome of the amorphous calcium phosphate treatments.

A randomized, double-blind, crossover studies were conducted to investigate the potential of CPP-ACP added to hard candy confections to slow the progression of enamel subsurface lesions in an in situ model. Consumption of the control sugar confection resulted in significant demineralization (progression) of the enamel subsurface lesions. However, consumption of the sugar confections containing CPP-ACP did not result in lesion progression, but in fact in significant remineralization (regression) of the lesions. Remineralization by consumption of the sugar + 1.0% CPP-ACP confection was significantly greater than that obtained with the sugar-free confection (Walker, et al., 2010).

Other studies are not conclusive regarding the ability of remineralization products based on CPP-ACP. Beerens et al., (2010) analyzed the effect of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up, and concluded that no clinical advantage was observed for use of the CPP-ACFP paste supplementary to normal oral hygiene over the 12 week follow up.

The CPP-ACP technology has also been demonstrated to significantly increase the levels of calcium and phosphate ions in supragingival plaque when delivered in a mouthrinse and to promote the remineralization of enamel subsurface lesions in situ. In fact, in a mouthrinse clinical study, the CPP-ACP technology was shown to be superior to other forms of calcium phosphate including un-stabilized ACP (Reynolds et al., 2003). These studies highlight the importance of the CPP in stabilizing the high levels of calcium and phosphate ions but also in delivering the ions to the tooth surface. Electron microscopic analysis of immunocytochemically stained thin sections of supragingival plaque samples showed that the CPP-ACP nanocomplexes were localized in the plaque matrix and on the surface of bacterial cells (Rose, 2000).

### 3.1 Mechanism of action for CPP-ACP

The mechanisms of action of CPP-ACP need to be considered at a location inside the enamel subsurface lesion, as well as at the surface of that lesion. The CPP-ACP and CPP-ACFP have been determined to be amorphous electroneutral nanocomplexes with a hydrodynamic radius of $1.53 \pm 0.04$ nm and $2.12 \pm 0.26$ nm, respectively (Cross et al., 2005). It would be expected that they would enter the porosities of an enamel subsurface lesion and diffuse down concentration gradients into the body of the subsurface lesion (Cochrane et al., 2008; Reynolds et al., 2008). Recently, it has been shown, with confocal laser microscopy and fluorescently labeled anti-CPP antibodies, that CPP was present inside a CPP-ACP remineralized enamel subsurface lesion. Once present in the enamel subsurface lesion, the
CPP-ACP would release the weakly bound calcium and phosphate ions (Park et al., 1998; Cross et al., 2005; Cochrane, et al.2008), which would then deposit into crystal voids. The release of the calcium and phosphate ions would be thermodynamically driven. The CPPs have a high binding affinity for apatite; hence, on entering the lesion, the CPPs would bind to the more thermodynamically favored surface of an apatite crystal face. Interestingly, the CPPs have been shown to prefer binding to the (100) and (010) faces of hydroxyapatite crystals, such that crystal growth would be allowed to continue only at the hydroxyapatite (001) plane or along the c-axis, which is the pattern of crystal growth during amelogenesis (Huq et al., 2000). Hence, the CPPs, once bound to apatite crystals in the enamel subsurface lesion, may have an important role in regulating anisotropic crystal growth and also inhibiting crystal demineralization.

When CPP-ACP is provided with a low background of fluoride, electron microprobe analysis has shown that the mineral formed in the enamel lesion is consistent with hydroxyapatite, and when fluoride is present, the mineral is consistent with fluorapatite or fluorhydroxyapatite (Cochrane et al., 2008). The diffraction patterns of this newly formed mineral were consistent with those of apatite.

The CPP-ACP nanocomplexes have also been demonstrated to bind onto the tooth surface and into supragingival plaque to significantly increase the level of bioavailable calcium and phosphate ions. A randomized, doubleblind, cross-over in situ study was conducted to measure calcium and phosphate incorporation into plaque after 5 days of rinsing with either water, 2% CPP-ACP, 6% CPP-ACP, or unstabilized calcium and phosphate. The plaque calcium and phosphate levels following rinsing with the water and the unstabilized calcium and phosphate rinses were similar, whereas both CPP-ACP rinses resulted in significantly higher incorporation of calcium and phosphate ions. When the plaque CPP levels were determined with competitive ELISA, it was found that 3 hours post-exposure to CPP-ACP, there remained a 4.6-fold higher peptide content than baseline levels. Electron microscopic analysis of immunocytochemically stained thin sections of supragingival plaque samples (Reynolds et al., 2003) showed that the CPP-ACP nanocomplexes were localized in the plaque matrix and on the surfaces of bacterial cells, confirming the work of Rose (2000a,b), who showed the CPP-ACP nanocomplexes bound to Streptococcus mutans and model plaque to produce a reservoir of bioavailable calcium ions. These results are also consistent with those of Schupbach et al. (1996), who showed the CPP to inhibit binding of mutans streptococci to saliva-coated enamel in vitro and in animal studies. These authors suggested that CPP-ACP incorporates into pellicle and plaque and results in an ecological transition of the bacterial population, which, together with the remineralizing capacity of the CPP-ACP, modifies the plaque’s cariogenic potential.

The method of binding CPP-ACP into plaque has been hypothesized to be due to calcium cross-linking (Rose, 2000; Reynolds et al., 2003), and/or hydrophobic and hydrogen-bond-mediated interactions (Reynolds et al., 2003). Using acid and alkali extraction, to help distinguish between these mechanisms of binding, investigators found that the majority of the bonds localizing CPP in the plaque were hydrophobic and/or hydrogen-bond-mediated interactions between the CPP and bacterial cell/pellicle surfaces. The surface-bound CPP molecules display a hydrophobic patch on the surface of the nanocomplex that may be responsible for the binding and localization of the complexes at the tooth surface.
In a randomized, controlled, mouthrinse trial, a rinse containing 2.0% CPP-ACP nanocomplexes plus 450 ppm fluoride significantly increased supragingival plaque fluoride ion content to $33.0 \pm 17.6 \text{ nmol F/mg}$ when compared with $14.4 \pm 6.7 \text{ nmol F/mg}$ attained by the use of a rinse containing the equivalent concentration of fluoride ions (Reynolds et al., 2008). Although marked increases in plaque calcium, phosphate, and fluoride were found, calculus was not observed in any of the study participants, indicating that the plaque calcium, fluoride, and phosphate remained stabilized at the tooth surface by the CPP as bioavailable ions and did not transform into a crystalline phase. The release of calcium, phosphate, and fluoride ions by the CPP localized in plaque would be driven thermodynamically, this process in dental plaque would be promoted by low pH. As the pH of plaque decreased by bacterial acid production, then this would facilitate the release of calcium, phosphate, and fluoride ions from the complexes.

4. A controlled clinical research

4.1 Objective

The aim of this study was to investigate the in vivo effects of commercially available CPP-ACP (GC Tooth Mouse), CPP-ACFP (MI-Paste Plus) on the remineralization of enamel white spot lesions (WSLs), assessed by laser fluorescence (Diagnodent, KaVo), in a randomized controlled clinical trial during a 3-month period.

4.2 Materials and methods

The study was performed as a blinded, prospective, randomized clinical trial, to determine the effect of a CPP-ACP, CPP-ACFP paste versus a control group on remineralization of enamel WSLs. The medical ethical committee at the University General Hospital Foundation of Valencia (Spain) approved the research protocol.

4.2.1 Subjects

A sample of children aged 7 to 14 years, attending for the first time to a dental office from Primary Dental Care was assessed. The subjects fulfilled the following requirements: healthy male and female subjects, one or more WSLs in their permanent teeth seen with or without prolonged air drying as a distinct visual change in enamel and or localized enamel breakdown without clinical visual signs of dentinal involvement, no systemic diseases, no syndromic abnormalities, and no proven or suspected milk protein allergy and/or sensitivity, or allergy to benzoate preservatives, as both are components of the CPP-ACP product. Informed consent was obtained from the parents/guardians. None of the subjects lived in an area where the community water was fluoridated.

4.2.2 Study outline

The study group consisted of 60 participants (33 males and 27 female) with a mean age of 9.5 yr. Subjects, complying with the inclusion criteria, were then randomly assigned to either the CPP-ACP, CPP-ACFP, or control group, as determined by a computer-randomization scheme that was created and locked before the start of the study (20 participants in each group).
The subjects received neutral-coloured toothpaste tubes marked A, B or C, which contained either CPP-ACP, (GC Toorh Mousse 35 ml, Recaldent -GC, Leuven, Belgium-), CPP-ACP + sodium fluoride (0.2% w/w; 900 p.p.m.) (MI Paste Plus 35 ml, Recaldent -GC, Leuven, Belgium-) or a non-fluoridated toothpaste. Subjects were informed that they could receive CPP-ACP paste, CPP-ACFP paste or a non-fluoridated toothpaste for home use.

All participants received the same verbal hygiene instruction. They were advised and informed how to brush properly, twice a day with a fluoridate tooth paste, either with a hand toothbrush or an electric toothbrush for at least 2 min. The participants were advised not to administer additional fluoride mouth rinses or professional application, during this investigation.

The participants were instructed to use their respective paste once a day at bedtime, during 3 months. Verbal and written instructions were given to the patient. They were informed that the paste should be applied to the tooth surfaces using a clean, dry finger. A sufficient amount of paste was to be applied to the upper and the lower teeth. A pea-size amount for each arch was the minimum amount required. The pastes were to be kept in the mouth for as long as possible. Subjects were asked not to rinse afterwards. Compliance was checked by questions asked on each visit about the frequency of tooth brushing and application of the study paste and how often, and when these were forgotten. Furthermore, subjects were asked to bring their study paste on each visit.

4.2.3 Clinical performance

At baseline, the teeth were prophylactically cleaned. Using dental light reflector, visual inspection of tooth surfaces for white-spot lesions was undertaken with tooth surfaces initially wet and again after 5 sec of drying with a gentle air stream, lesions were diagnosed. Multiple lesions could be recorded per tooth. Each lesion was scored according to ICDAS II (Topping & Pitts, 2009) for severity. Activity was assessed according to the Ekstrand et al., 2009 (Table 1). Lesions scored by codes 1, 1a, 2, 2a and 3 where selected at the baseline visit.

At review visits (4, 8, and 12 weeks), the procedure was repeated for lesions identified at baseline. If a lesion ceased to fulfill the lesion criteria, it was scored 0 (sound enamel). Examinations were conducted by one dentist with prior clinical trial experience. Following two sessions of training and standardization with handouts, slides and live participants, the calibration examinations resulted in intra-examiner kappa values of 0.92.

Laser fluorescence (LF) examination was performed using DIAGNOdent (Kavo, Biberach, Germany). It is a laser-based instrument developed for detection and quantification of dental caries on smooth surfaces and occlusal surfaces. It operates with a diode laser having a wavelength of 655 nm and 1 mW peak power. The light is transmitted through a descendent optic fiber to a handheld probe with a bevelled tip with a fiber optic eye, signal is finally processed and presented on the display between 0 and 99. DIAGNOdent consists of two probes, probe A for occlusal caries detection and probe B for smooth surface. In this study, both probe were used, A for the occlusal WSLs and B for the smooth surfaces WSLs. The instrument was calibrated according to manufacturer’s instructions. Probe was place perpendicular to the test site and rotated along the WSL to completely scan the area, under cotton roll isolation and after air drying with an air syringe. Three measurements were taken and the mean of them was considered as a final base line value and at review visits (4, 8, and 12 weeks).
Contemporary Approach to Dental Caries

<table>
<thead>
<tr>
<th>code</th>
<th>Detection criteria (ICDAS II)</th>
<th>code</th>
<th>Activity criteria modified by Ekstrand et al. (2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(sound enamel)</td>
<td>0</td>
<td>(sound enamel)</td>
</tr>
<tr>
<td>1</td>
<td>Opacity or discolouration visible after air-drying &gt; 5 sec</td>
<td>1a</td>
<td>white or yellow spot rough and soft</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1i</td>
<td>white or brown spot smooth and hard</td>
</tr>
<tr>
<td>2</td>
<td>Opacity or discolouration distinctly visible without air-drying</td>
<td>2a</td>
<td>white or yellow spot rough and opaque</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2i</td>
<td>white or brown spot smooth and hard</td>
</tr>
<tr>
<td>3</td>
<td>Localized enamel breakdown with no visible dentin or underlying shadow</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Scores and activity criteria to assess coronal caries lesions on occlusal surfaces on permanent teeth

4.2.4 Statistical analysis

Data were analyzed using SPSS for Windows software, version 17. Quantitative data were described by means and confidence interval at 95%. Data were checked for normal distribution. ANOVA paired test was used in order to compare mean values of LF between baseline and 4, 8, and 12 weeks follow up. Unpaired t tests were used to compare LF and clinical findings.

4.3 Results

A number of 766 clinically identified caries lesions, with the above mentioned criteria, were evaluated at baseline, and at 4, 8 and 12 weeks. 256 (33.4%) where included in the CPP-ACP treatment group, 259 (33.8%) in the CPP-ACFP group and 251 (32.8%) in the non-fluoridated toothpaste group.

None of the analyzed groups showed clinically significant changes, according ICDAS II codes, throughout the study. Only in the group treated with CPP-ACP, 6 lesions coded as decay was coded as sound enamel at the end of the review period (Table 2).

In the CPP-ACP group we can see significant florescence changes after the 8th treatment week. The CPP-ACFP group had significant changes after the 4th week. While in the control group any significant change could be seen during the study. The CPP-ACFP group, showed significantly lower fluorescence values at the 4th week in comparison with the CPP-ACP group (Table 3).

Decayed lesions, clinically identified as active, showed higher fluorescence values in comparison to those coded as inactive, although without statistically significant differences in all groups. Only lesions clinically coded as 3 (localized enamel breakdown with no visible dentin or underlying shadow) showed significant higher LF values in comparison to all others. We could assess that initial values between 3 and 7 were related to a clinical status compatible with visible white spot (without air-drying) and having activity characteristics (white or yellow spot rough and opaque), and that values higher than 8 were related with localized enamel breakdown with no visible dentin or underlying shadow (Table 4).
### Table 2. Clinical changes by groups throughout the study period

<table>
<thead>
<tr>
<th></th>
<th>CPP-ACP group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1a</td>
<td>1i</td>
<td>2a</td>
<td>2i</td>
<td>3</td>
</tr>
<tr>
<td>baseline</td>
<td>31 (12.1%)</td>
<td>2 (8%)</td>
<td>75 (29.3%)</td>
<td>38 (14.8%)</td>
<td>110 (43%)</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>3 (1.2%)</td>
<td>29 (11.3%)</td>
<td>2 (8%)</td>
<td>75 (29.3%)</td>
<td>39 (15.2%)</td>
<td>108 (42.2%)</td>
</tr>
<tr>
<td>8 weeks</td>
<td>3 (1.2%)</td>
<td>26 (10.2%)</td>
<td>2 (8%)</td>
<td>74 (28.9%)</td>
<td>42 (16.4%)</td>
<td>109 (42.6%)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>6 (2.3%)</td>
<td>22 (8.6%)</td>
<td>2 (8%)</td>
<td>74 (28.9%)</td>
<td>44 (17.2%)</td>
<td>108 (42.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CPP-ACFP group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1a</td>
<td>1i</td>
<td>2a</td>
<td>2i</td>
<td>3</td>
</tr>
<tr>
<td>baseline</td>
<td>31 (12.1%)</td>
<td>5 (1.9%)</td>
<td>84 (32.4%)</td>
<td>55 (21.2%)</td>
<td>84 (32.4%)</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>32 (12.4%)</td>
<td>6 (2.3%)</td>
<td>82 (31.7%)</td>
<td>55 (21.2%)</td>
<td>84 (32.4%)</td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>31 (12.1%)</td>
<td>4 (1.5%)</td>
<td>82 (31.7%)</td>
<td>59 (22.8%)</td>
<td>83 (32%)</td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>30 (11.6%)</td>
<td>4 (1.5%)</td>
<td>80 (30.9%)</td>
<td>61 (23.6%)</td>
<td>84 (32.4%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1a</td>
<td>1i</td>
<td>2a</td>
<td>2i</td>
<td>3</td>
</tr>
<tr>
<td>baseline</td>
<td>34 (13.5%)</td>
<td>2 (8%)</td>
<td>82 (32.7%)</td>
<td>63 (25.1%)</td>
<td>70 (27.9%)</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>33 (13.1%)</td>
<td>4 (1.6%)</td>
<td>79 (31.5%)</td>
<td>63 (25.1%)</td>
<td>70 (27.9%)</td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>32 (12.7%)</td>
<td>4 (1.6%)</td>
<td>81 (32.3%)</td>
<td>64 (25.5%)</td>
<td>70 (27.9%)</td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>31 (12.4%)</td>
<td>2 (2%)</td>
<td>78 (31.1%)</td>
<td>66 (26.3%)</td>
<td>71 (28.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Changes in the DIAGNOdent measurements obtained by groups throughout the study period. Within a study group (column), means sharing the same uppercase letter are statistically different. Between study groups (rows), means sharing the same lowercase letter are statistically different.
### Table 4. Mean values of LF for each clinical score at baseline, 4, 8 and 12 weeks by groups.

Within a study group (column), means sharing the same uppercase letter are statistically different.

#### 4.4 Discussion

Caries prevention is critical in children, especially in developing countries where younger generations are acquiring more westernized dietary habits, which is expected to contribute to an increase in dental caries. Despite there is a reduction in prevalence of dental caries it is still a problem of great importance. The reduction of caries prevalence has not occurred uniformly for all dental surfaces.

Several studies have been undertaken with the objective of determining the most accurate and precise method for detection of carious lesions among conventional and new methods, in order to identify caries lesion at an early stage when medical treatment is still possible.

Therefore new procedures have been standardized (Sridhar et al., 2009). The clinical diagnosis of WSLs has been made primarily by using traditional methods, such as visual inspection after air drying and tactile examination by dental probing. However, the
subjectivity, lack of reproducibility, and prerequisite of the presence of a significantly advanced lesion have led to the introduction of several optical devices in recent decades. One such technique is laser fluorescence – DIAGNOdent- (Lusi et al., 1999). The results of studies suggest that this technique might be appropriate for the early detection and assessment of WSLs (Aljehani et al., 2006; Andersson et al., 2007). In this clinical study we can indicate that, over a period of 3 months, DIAGNOdent was useful as a method for quantitative evaluation of the remineralization of early carious lesions.

A new clinical index, the International Caries Detection and Assessment System (ICDAS), was developed as an internationally accepted caries detection system that would also enable the assessment of early enamel demineralization (Ismail et al., 2007). In the ICDAS I (2003) the visual examination was carried out on clean, plaque-free teeth after careful drying. The criteria were subsequently modified and the ICDAS II was created (Topping & Pitts, 2009). The improvement consisted of an exchange of codes to ensure that the system would reflect increased severity.

CPP-ACP has been shown to localize and stabilize calcium and phosphate ions at the tooth surface in a bioavailable form that can promote remineralization of enamel subsurface lesions in situ, restoring the white opaque appearance of the lesions to translucency, even in the presence of fluoride (Reynolds et al., 2003, 2008). CPP-ACP remineralized initial enamel lesions and showed a higher remineralizing potential when applied as a topical coating after the use of fluoridated toothpaste (Kumar et al., 2008). CPP-ACFP has been proven to restore mineral throughout the subsurface enamel with a mineral that is consistent with fluorapatite (Reynolds et al., 2008).

In the present study white spot lesions, in permanent teeth on children between 7 to 14 years, were followed longitudinally through a clinical evaluation using the ICDAS II criteria and by DIAGNOdent measurement over a period of 3 months. During this time, DIAGNOdent readings of carious lesions had a tendency to decrease, indicating remineralization of the lesions in both CPP-ACP and CPP-ACFP groups but not in the control group. These data are similar to those from other authors’ studies made during similar periods of time (Andersson et al., 2009; Altemburger et al., 2010, Ferrezano et al., 2011, Uysal et al., 2010). Both products showed more efficacy than the control paste, despite all of the groups received the same oral hygienic instructions. The CCP-ACFP group presented significant lower fluorescence values after the 4th week. In the CPP-ACP group significant differences could be seen after the 8th week (table 3). This major remineralization speed could be caused by the presence of fluoride in the formulation. Some experimental studies have assessed that CPP-ACFP solutions at a pH lower than 5.5 have higher remineralization capacity than CPP-ACP (Cochrane NJ et.al, 2008).

There was a clear trend that the rate of visual score obtained was reasonably comparable with the DIAGNOdent values. Lesions clinically identified as active, showed higher fluorescence values in comparison to those coded as inactive, but only lesions clinically coded as 3 (ICDAS II criteria) showed significant higher LF value in comparison to all others in every phase of the study, as you can see in table 4. The results agree with those from Aljehani et al. (2006) in their study on white spots appeared after taking away brackets followed along a year.
Another topics we were interested in was to assess if carious lesions had clinical signs of activity (white, opaque, rough surface) or arrested (smooth yellow-brownish appearance) (Ekstrand et al., 2009) and if it was possible to detect changes along the study. As can be seen in table 2, clinical appearance of lesions was not modified during the study period in any group. The group of patients treated with CPP-ACP was the only showing a small number of lesions that clinically disappeared after the treatment. This results are in agreement with those given by Altenburger et al., (2010) and Aljehani et al., (2006), as well as Baley et al., (2009) that, in a 3-months study, after the evaluation of progression, regression or stabilization in a post-orthodontic population, did not find clinically detectable differences between a group treated with CPP-ACP and a control one, although considering only lesions coded as 2 or 3 (ICDAS II) he found a 31% more regressed lesions in the CPP-ACP group in comparison to the control group. Likely, a period longer than 3 months may be necessary in order to observe visually detectable changes of white spot lesions.

4.5 Conclusion

This clinical study indicated that over a period of 3 months it was possible to use DIAGNOdent as a method for the longitudinal quantification of carious lesions. Furthermore, DIAGNOdent could be used as an alternative method for monitoring the outcome of caries-preventive programs in caries-susceptible individuals. There was a clear trend that the rate of visual score obtained was reasonably comparable with the DIAGNOdent values It was concluded that white spot lesions might be remineralized over a relatively short period of time, and both CPP-ACP and CPP-ACFP products had a similar efficacy in promoting remineralization of white spot lesions. We could not detect visual clinically changes in the lesions with any of the applied treatments along the study. A period longer than 3 months may be necessary in order to observe visual clinically significant changes of white spot lesions.

In view of the literature review and the results of the study, the authors considered a useful alternative, in clinical practice, for early carious lesions remineralization, the studied CPP-ACP and CPP-ACFP formulations. Long-term clinical studies are needed to analyze some other factors influencing the remineralization possibilities as presence of plaque, acidogenic bacteria levels, salivary pH, or dietary habits.

5. References


randomized controlled trial in 12- to 15-year-old high caries risk children in Bangalore, India. Caries Research, Vol.43, No.6, pp.430-435, ISSN 0008-6568.


