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Materials Used in Remineralization of Initial Caries

A Project Submitted to College of Dentistry at the University of Baghdad, department of Pedodontics & Preventive dentistry in Partial Fulfilment of the Requirement of B.D.S degree

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Certification of the Supervisor

I certify that this project entitled **" Materials used in Remineralization of Initial Caries "** was prepared by the fifth-year student **Mohammed Ahmed Naji** under my supervision at the College of Dentistry/University of Baghdad in partial fulfilment of the graduation requirements for the bachelor's degree in Dentistry.

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List of abbreviations

IGA	**Immunoglobulin A**
ACFP	**Amorphous Calcium Fluoride Phosphate**
ACP	**Amorphous Calcium Phosphate**
ADS	**Arginine deiminase pathway**
Ca	**Calcium**
CPP	**Caisen Casein Phospho-peptide**
F	**Fluoride**
FAP	**Fluro-Agitate**
FHAP	**Fluro-Hydroxy apatite**
GIC	**Glass ionomer cement**
HAP	**Hydroxy apatite**
HCA	**Hydroxy carbonate apatite**
Mg F/Kg	**Milligram of Fluoride per Kilogram**
nmol F/mg	**Nano mol of fluoride per milligram**
OH	**Hydroxide**
P	**Phosphorus**
Po4	**Phosphate**
Ppm	**Parts per million**
TCP	**Tricalcium phosphate**
Wt	**Wight**

Introduction

Dental caries is a main oral health disease which is initiated by demineralization of hard tissue structure of the tooth through organic acids produced from carbohydrates fermentation by cariogenic bacteria in the dental plaque. The presence of sufficient ions of calcium and phosphate in the environment enables the reconstructing of partly dissolved apatite crystals in a process called remineralization (Thakkar *et al.*, 2017).

Efforts to prevent or control caries tend to focus on limiting the growth of cariogenic bacteria as well as inhibiting demineralization as well as promoting re-mineralization. This is done by application of remineralizing agents to incipient caries to control demineralization and promote remineralization. Remineralizing agents make a super saturated environment around the decayed lesion, thus, it will prevent mineral loss and concentrate the phosphate and calcium ions in the vacant areas (Ten Cate, 2009; Ten Cate, 2012).

The demineralization process involves loss of minerals at the advancing front of the lesion, at a depth below the enamel surface, with the transport of acid ions from the plaque to the advancing front and mineral ions from the advancing front toward the plaque (Robinson and Shore, 2000).

The action of 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 is called remineralization. Fluoride is known to promote remineralization but is dependent on calcium and phosphate ions from saliva to accomplish this. The goal of modern dentistry is to manage non-cavitated carious lesions non-invasively through remineralization to prevent disease

progression, and to improve strength, esthetics, and function of teeth (Goswami *et al.*, 2012).

Aims of the Review

The aim of this study is to understand the mechanism of action of the materials that has been used for remineralization of the early incipient carious lesions, to know the better materials in the present time according to their characteristics.

Chapter one: Review of literature

1.Dental caries

Dental caries is one of the most common diseases among humans, causing pain and disability that can lead to acute infection and tooth loss at any age (Ismail *et al.*, 2001; CDCP, 2005).

it is now understood as a multifactorial disease process including host susceptibility, acid produced by cariogenic bacteria which metabolize the sugar and carbohydrate that ingested by the host, sugar rich diet advocating bacterial activity and time (Alkattan *et al.*, 2018).

These bacterial organic acids resulted from the fermentation of carbohydrates, might induce changes in the biofilm, capable of forming the cariogenic bacterial dental plaque. As previously mentioned, exposure time is another determinant factor in the intensity of the disease. Besides, it's paramount to contemplate the behavioral factor, as cultural, educational, and Socioeconomic status of population with the dental caries (Fejerskov, 2004; Costa *et al.*, 2012; Cummins, 2013; Al-Meedani and Al-Dlaigan, 2016).

Before the cavitation, a partial demineralization in a subsurface of the lesion is present, but it could be remineralized under a good condition, while a natural remineralization could control a small amount of dental caries attack. When the bacterial acid product is more, a natural remineralization is insufficient (Liang *et al.*, 2017).

With this greater understanding of the disease comes an opportunity to promote ‘preventive’ therapies that encourage the remineralization of non-cavitated lesions resulting in inactive lesions and the preservation of tooth structure, function, and aesthetics. Central to this vision is the ability to detect caries lesions at an early

stage and correctly quantify the degree of mineral loss, ensuring that the correct intervention is instigated (al-Khateeb *et al.,* 1997).

The failure to detect early caries, leaving those detectable only at the deep enamel, or cavitated stage, has resulted in poor results and outcomes for remineralization therapies. It can also be argued that practitioners have often failed to embrace prevention because its efficacy cannot be easily monitored. Therefore, the ability to monitor early lesions and determine if they have indeed arrested or stabilized is also key to ensuring that effective prevention can become commonplace in general dentistry (Ismail *et al.,* 2007).

2. Saliva

Saliva is an extracellular fluid that produced and secreted by the salivary glands in the oral cavity. In human, saliva is consisting of 99.5% water with electrolyte, mucus, enzymes such as lipase and amylase, white blood cell, epithelial cell, antimicrobial agent such as secretory IgA, and lysozymes (Nosek and Thomas, 2016).

Saliva is a fluid that protect the oral cavity from harmful microorganism and irritants. It lubricates the oral tissue and has another function such as mastication, swallowing, speech and preserve the teeth and oral tissues (Farooq and Bugshan, 2020).

At physiological pH, normal saliva is supersaturated with respect to both ionic and non-ionic forms of calcium (phosphate and bicarbonate, hydroxyapatite, and fluorapatite), with ionic calcium comprising about 50% of the total calcium concentration (Fejerskov *et al.,* 2003).

The remineralization possibility of saliva is well certified, that saliva can transport Ca+ and PO4 ions to the dental hard tissue during development and maintaining it for life (Stookey, 2008; Cochrane and Reynolds, 2012).

Saliva is supersaturated with a phosphoprotein that stabilized Ca+ and PO4 ions at the physiological pH which maintaining the ions remain bioavailable for diffusing into mineral deficient lesion (Cochrane *et al.*, 2010).

3. Demineralization and Remineralization

Dental caries is a multifactorial disease determined by the cumulative result of consecutive cycles of demineralization and remineralization at the interface between the biofilm and the tooth surface (Fejerskow *et al.*, 2008).

Demineralization occurs at a low pH when the oral environment is under saturated with mineral ions, relative to a tooth's mineral content. The enamel crystal, which consists of carbonated apatite, is dissolved by organic acids (lactic and acetic) that are produced by the cellular action of plaque bacteria in the presence of dietary carbohydrates. Remineralization is the natural repair process for non cavitated lesions and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization as show in figure (1). These remineralized crystals are less acid soluble than the original mineral (Featherstone, 2008).

Demineralization and remineralization occur many times during the day, and it is the balance between these two processes over time that influences whether a caries lesion will progress, reverse, or stay the same (Ismail *et al.*, 2007).

Strategies to prevent caries should aim to limit exposure to demineralizing factors, where possible, and to promote remineralization (Reynolds, 2008).

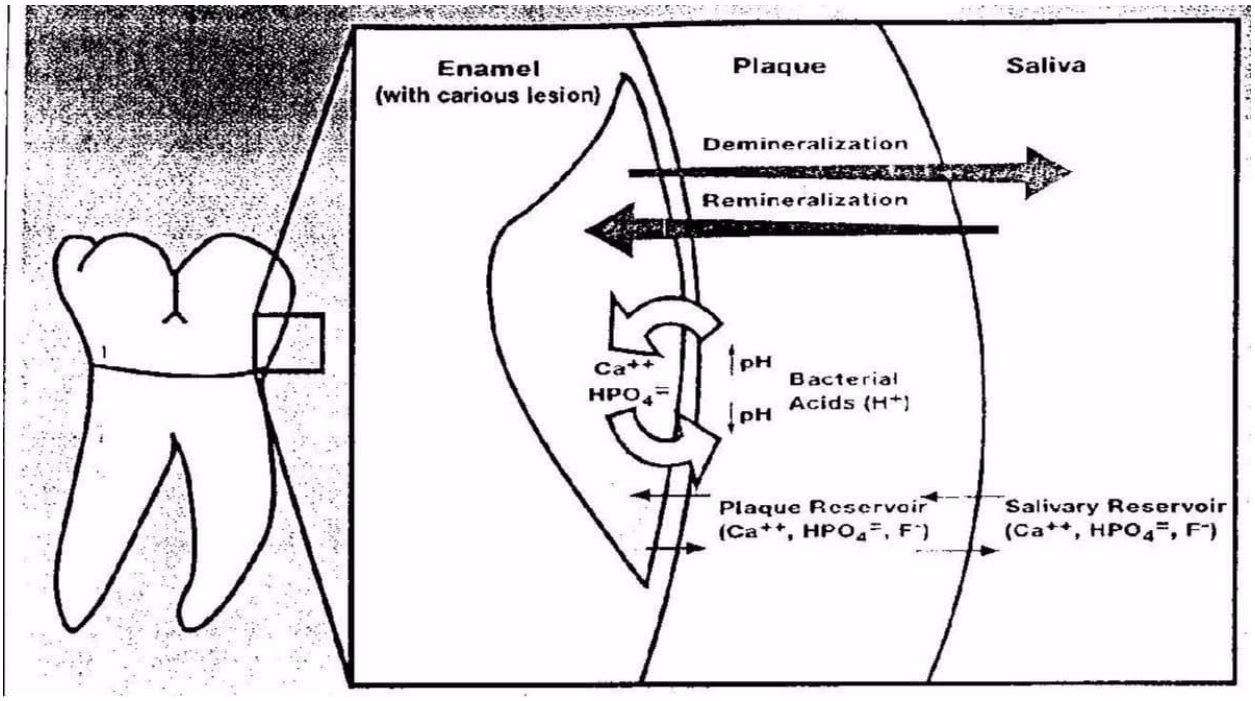

Figure 1: Cycle of demineralization and remineralization in enamel (Featherstone, 2008).

For remineralization of enamel to occur the following six conditions or events must occur at the same time (Pradeep and Kumar, 2011):

1. Sufficient mineral must be present in the saliva.

2. A molecule of carbonic acid must be produced.

3. The carbonic acid molecule must be produced in proximity to a mineral molecule.

4. This all must occur in proximity to a demineralized spot in the hydroxyapatite (HAP) latticework.

5. That spot of the tooth must be clean, so that the mineral deficient spot is accessible.

6. The carbonic acid must convert to carbon dioxide and water before any of the above circumstances change.

4. Remineralizing agents

Flouridated and Non-flouridated agents cause the remineralization of the carious lesions. Flouridated agents increases caries resistance may arise from both systemic and topical applications of fluoride and can be broadly grouped by increased enamel resistance, increased rate of maturation, remineralization of incipient caries, interference with microorganisms and improved tooth morphology. (Tyagi *et al.*, 2013).

4.1 Ideal requirements of a remineralizing agent

The ideal requirements include the following (Brunton *et al.*, 2013):

• Should deliver calcium and phosphate into the subsurface

• Should not deliver any excess of calcium

• Should not favor calculus formation

• Should work at an acidic pH so as to stop demineralization during a carious attack

• Should work in xerostomic patients also, as saliva cannot effectively stop the carious process

• Should be able to boost the remineralizing properties of saliva

4.2 Remineralizing Agent Types

Remineralizing agents includes (Arifa *et al.*, 2018):

-Fluoride

-Non-fluorinated remineralizing agents

- Amphoras calcium phosphate
- Casein Phosphuretted amphoras calcium phosphate
- Sodium calcium phosphor-silicate
- Xylitol
- Dicalcium dehydrate (DCDP)
- Theobromine
- Poly (Amide Amine) Dendrimers
- Arginine
- Self-assembling Peptides

-Nano particle materials for remineralization

- Calcium Fluoride nanoparticles
- Calcium phosphate-based nanoparticles
- Nano hydroxyapatite particles
- Amphoras calcium phosphate nanoparticles
- Nano bioactive glass materials

5. Fluoride

An important adjunct in the prevention of dental caries, fluorides are introduced into the oral environment via personal (eg, dentifrices, rinses) or professional (eg, varnishes, foams, gels, fluoride releasing restorative materials) applications. Fluoride levels of about 3 parts per million (ppm) in the enamel are required to shift the balance from net demineralization to net remineralization (Summit, 2001).

5.1 Mechanism of action of Fluoride

The fluoride ion can exchange with hydroxyl group in apatite crystals forming fluorapatite which is more stable and less soluble crystal, The fluoride enters the void space on apatite crystals which provides stability by additional bond, it contributes in the remineralizing of the early caries lesion, it can act as antimicrobial agent and Fluoride Inhibit enzyme essential for bacterial growth and metabolism (Venkatesan and Ranjan, 2014)

Remineralization may be enhanced by providing low levels of calcium and phosphate in conjunction with minimal amounts of fluoride. The difference that a very small amount of fluoride (< 1 ppm) has on demineralization and remineralization is remarkable. This is because fluoride acts as a catalyst and influences reaction rates with dissolution and transformation of various calcium phosphate mineral phases within tooth structure and plaque adjacent to tooth surfaces. Incorporating minimal amounts of fluoride into hydroxyapatite (HAP) yields fluorohydroxyapatite (FHAP), which resists demineralization at a level similar to fluoroapatite (FAP) (Hicks *et al.,* 2004).

The concentration of fluoride applied intraorally also appears to have a significant influence on the degree of remineralization of advanced (> 150 microns)

enamel lesions. This suggests treatments with 5000 ppm F—the concentration found in a fluoride varnish—significantly enhance remineralization and inhibit demineralization when compared to treatments with 1500 ppm F. (Buijs, 2008).

While fluoride is highly effective on smooth-surface caries, it is less so on pit and fissure caries (Goswami *et al.*, 2012).

5.2 Fluoride-Releasing Dental Materials

Fluoride-releasing dental restorative materials can provide an additional benefit in preventive dentistry. Preliminary studies indicate that glass ionomer cement and fluoride-releasing resin composite have synergistic effects with fluoride rinses and fluoridated dentifrices in the remineralization of incipient enamel caries (Marinelli *et al.*, 1997).

The materials could act as a fluoride delivery system. Upon exposure to additional external fluoride, the material surface undergoes an increase in fluoride. This fluoride is subsequently released and has demonstrated inhibition of demineralization and even the occurrence of remineralization at the adjacent tooth structure (Bynum and Donly, 1999).

5.3 Effects of Fluoride on Teeth (fluoride toxicity)

Ingestion of excessive amount of fluoride may cause harmful and toxic effect. The main cause of fluoride toxicity came from oral hygiene products like toothpaste followed by fluoride mouth wash then fluoride supplement. More than (80) percent of fluoride intoxication was reported in pediatric age group less than six years old (Table 1).

Table (1): Toxic and lethal doses of fluoride (Martinez, 2012).

Doses of Fluoride	
Optimal dose Fluoride (for children & adults)	0.05 – 0.07 mg F/kg body wight
Toxic does of Fluoride (for children & adults)	5 mg F/kg body wight
Lethal dose of Fluoride (children)	16 mg F/kg body wight
Lethal dose of Fluoride (adults)	32 mg F/kg body weight

5.3.1 Clinical and Histological Features of Enamel fluorosis

Enamel fluorosis is a mottling of the tooth surface that is attributed to fluoride exposure during tooth formation. The process of enamel maturation consists of an increase in mineralization within the developing tooth and concurrent loss of early-secreted matrix proteins. Exposure to fluoride during maturation causes a dose-related disruption of enamel mineralization resulting in widening gaps in its crystalline structure, excessive retention of enamel proteins, and increased porosity. These effects are thought to be due to fluoride's effect on the breakdown rates of matrix proteins and on the rate at which the byproducts from that degradation are withdrawn from the maturing enamel (Aoba and Fejerskov, 2002).

Enamel in the transitional or early maturation stage of development is the most susceptible to fluorosis (DenBesten and Thariani 1992).

For most children, the first 6 to 8 years of life appear to be the critical period of risk. For anterior teeth, which are of the most aesthetic concern, the risk period appears to be the first 3 years of life (Evans and Stamm, 1991).

Because the severity of fluorosis is related to the duration, timing, and dose of fluoride intake, cumulative exposure during the entire maturation stage, not merely during critical periods of certain types of tooth development, is probably the most important exposure measure to consider when assessing the risk of fluorosis (DenBesten, 1999).

5.3.2 Fluoride health Issues and Clinical Treatment

There appears to be general acceptance in today's dental literature that enamel fluorosis is a toxic effect of fluoride intake that, in its severest forms, can produce adverse effects on dental health, such as tooth function and caries experience. For example:

• The most severe forms of fluorosis manifest as heavily stained, pitted, and friable enamel that can result in loss of dental function (Burt and Eklund, 1999).

• In more severely fluorosed teeth, the enamel is pitted and discolored and is prone to fracture and wear (ATSDR, 2003).

• With increasing severity, the subsurface enamel all along the tooth becomes increasingly porous. The more severe forms are subject to extensive mechanical breakdown of the surface (Aoba and Fejerskov, 2002).

• With more severe forms of fluorosis, caries risk increases because of pitting and loss of the outer enamel (Levy, 2003).

5.3.3 Clinical Treatment

Severe enamel fluorosis is treated to prevent further enamel loss and to address the cosmetic appearance of teeth. Treatments include bleaching, micro-abrasion, and the application of veneers or crowns. Bleaching and micro-abrasion are typically used with the mild to moderate forms of enamel fluorosis. Bleaching is

the least invasive procedure but, does not eliminate the dark stains associated with severe enamel fluorosis. Micro-abrasion involves the controlled abrasion of enamel to remove superficial stains. This technique has been reported to be minimally invasive and successful in treating single-line or patched opacities but, was not effective in treating defects that extend deeper into the enamel (Wong and Winter, 2002).

It has been found that while micro-abrasion improved the appearance of all degrees of enamel fluorosis, severely fluorosed teeth exhibited more defective surfaces following treatment. Pits and fissures can be filled with flowable composites. Partial veneers, composite veneers, and crowns provide the best aesthetic results for very severe enamel fluorosis but are the most invasive treatments. Crowns are usually used as a last resort because they can be a threat to tooth vitality (Train *et al.*, 1996).

The procedure requires the further removal of tooth enamel to allow for bonding of the crown, and sometimes requires replacement within a few years. The more invasive treatments should be used only in the most severe cases of enamel fluorosis (Christensen, 2005).

6. Sweetening Agents

In early toothpaste formulations, sugar, honey, and other sweeteners were used. Because these materials can be broken down in the mouth to produce acids and lower plaque pH, they can increase caries. They have been replaced with saccharin, cyclamate, sorbitol, and mannitol as primary noncariogenic sweetening agents. Sorbitol and mannitol serve a dual role as sweetening agents and humectants. Glycerin, which also serves as a humectant, adds to the sweet taste. A new sweetener in some dentifrices is xylitol. In laboratory studies, it is not metabolized by bacteria

to produce acid. In human studies, where it was placed in chewing gums and food, xylitol was noncariogenic and demonstrated an anticaries capability by facilitating the remineralization of incipient carious lesions. (Mass *et al.,* 1999 and Bader *et al.,* 2001).

6.1 Xylitol

This is a substitute of non-organic sugar that are found naturally. It cannot be modified or metabolized by bacteria in the oral cavity even if the administration continued for a long time (even years) (Tulsani *et al.,* 2014).

The levels of streptococcus mutans in the plaque and saliva was decreased by using xylitol for a short period. This agent was displayed in action through starvation of streptococcus mutans which was regarded as challenging cariogenic bacteria (Soldring *et al.,* 2014).

6.1.1 Mechanism of action Xylitol

Xylitol has the ability for inhibition plaque formation and is considered as a key limiting step of caries building. Streptococcus mutans is the initiator of dental caries. These bacteria supposed to be the target for xylitol (Söderling, 2009).

Xylitol interferes with the production of polysaccharides of streptococcus mutans in the plaque and saliva and has the ability to create a complex with calcium in high concentrations. This in turn facilitate calcium movement to the deepest layers in the demineralizing enamel (Lin *et al.*, 2016; Salli *et al.*, 2016 and Gargouri *et al.,* 2018).

This was cheated by this agent as a substitute to sucrose, this in turn prevent the fermentation and blocking the formation of acid which cause the demineralization of hard tissue of the tooth (Söderling, 2009).

It was known that xylitol used as sweetener agent in chewing gum free of sugar confirmed excellent properties as anti-biofilm and display potential for remineralization of tooth which was eroded. The outcome of the acidic environment is deminiralizatoin. This is caused by low pH as a consequences of food habits. Chewing gums free from sugar after meal decrease demineralization as it raises the saliva flow rate (Gargouri *et al.*, 2018).

Gums free of sugar and containing xylitol nowadays used as daily habits. This has the ability for interfere with dental caries and even dental erosions. These gums act as effective transporters that deliver therapeutic materials to the mouth as it prolongs the contact with the surface of the dentine (Konar, 2016).

7. Arginine

It is semi essential α amino acid that naturally present in plaque and saliva produced by metabolism of dietary protein. The end product of oral bacterial metabolism of arginine is Ammonia (Hajishengallis *et al.*, 2016).

The Ammonia increases arginolytic potency of oral micro-environment increases with arginine increases with arginine supplementation, alkalinization of plaque can be initiated by external arginine so the formation of biofilm will be inhibited and lead to ecological homeostasis (Bijle *et al.*, 2019).

The remineralization effect of fluoride will be enhanced by adding of arginine to flouride compound thus it affects the metabolic activity of biofilms. Therefore, the incorporation on arginine with sodium fluoride (NaF) (500) ppm solution was found to enhance uptake of fluoride which led to increase enamel resistance to caries (Cheng *et al.*, 2015).

It was proposed that the protonated positive charge group of arginine interact with negative charged fluoride ion to produce arginine fluoride complex in dental enamel

during remineralizing process. The acidogenic streptococcus Mutans was suppressed by synergistic effect of arginine and NaF (Zheng *et al.,* 2015).

The initial enamel carious lesion was effectively reversed by using 1.5 percent arginine and fluoride dentifrice (1450 ppm F) with sodium mono-fluorophosphate (MFP) in comparison to dentifrice containing fluoride alone (Wang et al., 2016).

The incorporation of 2 percent of arginine in 1100 ppm sodium fluoride dentifrice significantly enhancing the remineralization potential of Naf dentifrice (Bijle, *et al.,* 2018).

7.1 Mechanism of action of Arginine

When Arginine enters the oral cavity, the oral bacteria can metabolize it by arginine deiminase pathway (ADS) to produce ammonia with subsequent protonation of ammonia to form ammonium (Simeonov *et al.,* 2019).

Then it neutralizes the glycolytic acid that contributed to rise in pH of dental plaque (Nascimento and Burne, 2014).

The oral bacteria benefit by ammonia which produced via ADS result in Environmental and cytoplasmic rise in PH by protecting them against acid killing, provide bioenergy by increase in PH and ATP synthesize and by Keeping relatively neutral environment that is not good for growing of cariogenic micro flora (Geraldeli *et al.,* 2017).

8. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP)

Casein is the dominant phosphoproteins in a bovine milk and account for 80 percent of its all proteins, at the beginning as a calcium phosphate stabilized complex. Casein phosphopeptide (CPP) comprise of amino acid cluster from casein,

has no taste, weak antigenicity and can purify as CPPACP Nano complexes by selective precipitation, ions exchanges or ultrafiltration. Casein phosphopeptide (CPP) can deliver ACP and assist ACP for binding with enamel. Casein phosphopeptide can also reduce the number of the Streptococcus Mutans that integrate in pellicle. Casein phosphopeptide can be stabilized the Ca and PO, which found in a solution of amorphous calcium phosphate, on dental plaque, it can interfere with growth of biofilm, reduce demineralization, and enhance remineralization by its buffering and antibacterial effect. (Farooq *et al*, 2013).

8.1 Mechanism of Action of CCP-ACP

8.1.1 Buffering action by the calcium phosphate reservoir

Reynolds in 1999 suggested that the anti-cariogenicity mechanism for CPP-ACP is that the ions of calcium and phosphate were substantially localized in the plaque, which brought about a soluble calcium phosphate ions reservoir on the surface of tooth. When the acidogenic bacteria presented in the dental biofilm they metabolize the dietary sucrose, the rapid decrement of pH takes place under the resting pH (7). The pH surrounding the teeth decreases because of this acid past a crucial level (pH=5.5), it is diffused potentially into enamel and leads to calcium phosphate mineral dissolution (Featherstone, 2000).

These acidic conditions allow the breaking down of the CPP-bound ACP and then they dissociate in order to release ions of calcium and phosphate. Enamel demineralization is perfectly prevented due to this mechanism, as it is sound that levels of plaque calcium and phosphate and experience of measured caries are inversely associated. Confocal laser microscopy and fluorescently labeled anti-CPP antibodies showed the presence of CPP inside a CPP-ACP remineralised enamel subsurface lesion (Cochrane *et al.,* 2010).

The weakly bound ions of calcium and phosphate are released by the CPP-ACP, immediately after the presence of CPP in the enamel subsurface lesion. Then, these ions would deposit into voids of crystal (Park *et al.*, 1998).

8.1.2 Inhibition of streptococci adhesion to the tooth surface

It was proposed that the CPP-ACP prevented the cariogenic streptococci adhesion to surface of tooth, resulting in the less cariogenic plaque formation. Also, CPP-ACP nano-complexes had been demonstrated to bind on the tooth surface to the adsorbed macromolecules, the intercellular plaque matrix components and the bacterial cells surface (Schüpbach *et al.,* 1996).

The hypothesis of the binding method of CPP-ACP into plaque is attributed to calcium cross-linking and/or hydrophobic and hydrogen-bond-mediated interactions (Reynolds *et al.*, 2003).

The investigation explained that there was a competition between CPP-ACP and calcium about the plaque calcium binding sites and therefore decreased the calcium binding degree between the pellicle and adhering cells and between the cells themselves. The incorporation of CPP-ACP into the pellicle and plaque led to change in the bacterial population ecology, which, together with the CPP-ACP had a remineralization ability. The potential of the plaque's cariogenic will be modified, leading to the formation of a less cariogenic plaque (Rose, 2000).

The complex could have a bactericidal and bacteriostatic effect that is affected by the maintenance of higher concentrations of extracellular non-bounding calcium (Cross *et al.*, 2005).

Transmission and scanning electron microscopy were used to demonstrate that the pellicle structure formed in vivo that could be substantially modified by milk containing different contents of fat. The structure of the formed pellicle is distinct globular not a uniform layer of protein. They suggested that "casein

glycomacropeptide and CPP adsorb to the pellicle surface and mask receptors on salivary molecules for these streptococci" (Nyvad, 1984).

In addition, it was realized that the formation of biofilm and the preferable nucleation and calcium phosphates crystallization, probably in form of apatitic, in matured biofilm are delayed due to the CPP-ACP agent (Tooth Mousse) presence (Rahiotis *et al.,* 2008).

8.1.3 Synergistic Effects of Fluoride and CPP-ACP

Casein Phosphopeptide-Amorphous Calcium Phosphate and fluoride have an additive anticariogenic effect, which may be attributed to the ACFP localization at the surface of tooth, which, in impact, ions of calcium, phosphate, and fluoride would co-localized (Reynolds, 1997).

It was clear that the consistency of hydroxyapatite with the mineral formed in the enamel lesion; depending on the analysis results of Electron microprobe, when CPP-ACP is provided with a low background of fluoride, and at the presence of fluoride, the consistency of the mineral with fluorapatite or fluorhydroxyapatite takes place (Cochrane *et al.,* 2008).

The required fluoride amount is reduced due to the utilization of the CPP-ACP alone, or together with fluoride, and this leads to a reduction in the fluorosis amount. It was concluded that the observation of the subsurface lesion's remineralization at all the tested pH values, and the maximum was with 5.5 PH. The CPP stabilized a significant amount of calcium, phosphate, and fluoride ions concentrations and with pH values ranging between 4.5 and 7. When the pH value was 5.5 and below, the remineralization produced by CPP-ACFP solutions was utmost than that of the CPP-ACP solutions. There was a consistency between the mineral formed in the subsurface lesions and HA, together with fluorapatite for remineralization with CPP-ACP and CPP-ACFP, respectively. The produced ammonia consequently increases pH, according to the presence of the plaque enzymes, such as phosphatases and

peptidases that cause a partial degradation in CPP-based products. This is considered other reason for the CPP-ACP and fluoride additive effect. (Vitorino et al., 2005).

According to a clinical trial, it was found that caries activity in the animals receiving 0.5% CPP-ACP plus 500 ppm fluoride was considerably lower than those receiving either fluoride or CPP-ACP alone (Reynolds *et al.,* 1995).

Sakaguchi in 2006 performed an investigation to demonstrate the CPP-ACP and fluoride synergistic impact, as available in "Tooth Mousse Plus™ (MI Paste Plus$_{TM}$)", in remineralizing of primary teeth. The comparison was between Tooth Mousse Plus$_{TM}$ and Tooth Mousse $_{TM}$, a placebo had no fluoride or CPP-ACP, and a paste with 950 ppm fluoride. The possible Tooth Mousse Plus$_{TM}$ (Tooth Mousse$_{TM}$ with 900 ppm fluoride) remineralization was larger than the Tooth Mousse$_{TM}$ and the 950 ppm fluoridated toothpaste groups additive effect. Enamel subsurface lesions remineralization of a paste with 2% CPP-ACP nanocomplexes plus 1,100 ppm F (CPP-ACPF) was superior (2.6 times) to that of toothpaste with only 1,100 ppm F (Reynolds *et al.,* 2008).

The fluoride was greater integrated into the subsurface enamel in the fluorapatite form because of the CPP-ACP Nano-complexes-plus-fluoride dentifrices. A controlled mouth rinse was randomly tried for supra gingival plaque fluoride ion that content a 33.0±17.6 nmol F/mg dry weight (wt.). The remineralization was significantly increased with a rinse of 2.0% CPP-ACP nanocomplexes plus 450-ppm fluoride comparing to that with 14.4±6.7 nmol F/mg dry wt of plaque gained by the utilization of a rinse containing an equal fluoride ions concentration (Chow *et al.,* 2000).

The efficacy of ACFP-forming dentifrice was tested in patients with significant risk of caries who underwent irradiation of head and neck, the dentifrice forming ACFP was found to be significant in lowering the root caries incidence in comparison to the dentifrice having only fluoride (Papas *et al.,* 2008).

9. Tricalcium phosphate (TCP)

Tricalcium phosphate has the chemical formula Ca3(PO4) 2, and exists in two forms, alpha and beta. Alpha TCP is formed when human enamel is heated to high temperatures. It is a relatively insoluble material in aqueous environments (2mg/100 mL in water) (Aminzadeh *et al.* 1999; Feuerstein *et al.,* 2005).

Crystalline beta TCP can be formed by combining calcium carbonate and calcium hydrogen phosphate and heating the mixture to over 1000 degrees Celsius for 1 day, to give a flaky, stiff powder. The average size of the TCP particles can then be adjusted by milling them. Typically, particles range from 0.01 to 5 microns in size. Beta TCP is less soluble than alpha TCP, and thus in an unmodified form is less likely to provide bio-available calcium (Döri *et al.,* 2005).

TCP has also been considered as one possible means for enhancing levels of calcium in plaque and saliva. Some small effects on free calcium and phosphate levels in plaque fluid and in saliva have been found when an experimental gum with 2.5% alpha TCP by weight was chewed, when compared to a control gum without added TCP (Vogel *et al.,* 1998)

A major problem with such uses of TCP is the formation of calcium-phosphate complexes, or if fluorides are present, formation of calcium fluoride, which would inhibit remineralization by lowering the levels of bioavailable calcium and fluoride. For this reason, TCP levels have to be kept very low, in the order of less than 1%. Alternatively, TCP can be combined with a ceramic such as titanium dioxide, or other metal oxides, to limit the interaction between calcium and phosphate, and make the material more stable in solution or suspension (Karlinsey and Mackey, 2009).

10. Nano-hydroxyapatite

Hydroxyapatite is the main biomineral component found in human hard tissues, i.e., tooth and bone. Its stoichiometry is represented by the formula (Ca10 (PO4) 6 (OH)). It is comprised of calcium and phosphorus present in the ratio (Ca/P) of 1.67 (Oliveira and Mansur, 2007).

It is the main mineral component of the enamel, comprising of more than 60% of tooth dentine by weight (Goenka *et al.,* 2012; Al-Sanabani *et al.,* 2013).

Research has emphasized the relevance and necessity of biomimetic oral health products containing nano-sized hydroxyapatite particles in modern preventive dentistry (Yamagishi *et al.,* 2005).

Hydroxyapatite has attracted much interest as a biomaterial for use in prosthetic applications due to its similarity in crystallography and chemical composition to that of human hard tissue (Ramli *et al.,* 2011).

Nano-HAP is considered one of the most biocompatible and bioactive materials, due to the close similarity to the basic structures of the enamel with a high surface area and strong affinity to the enamel surface (Ajami *et al.,* 2016).

Nano-HAP offer the ability to protect the teeth by creation of a new layer of synthetic enamel around the tooth, rather than hardening the existing layer with fluoride. Several studies on Nano-HAP as a biomimetic material, showed the potential to remineralize initial enamel lesions under pH-cycling model. The newly introduced Nano-HAP paste (Desensibilize Nano P) is a bioactive agent organized in crystalline form of nanocrystals smaller than 100 nm, which improved the agent bioactivity due to the increase in the superficial area and wettability of hydroxyapatite nanoparticles (Medeiros *et al.,* 2013).

The paste is incorporated with 9,000 ppm fluoride ions and indicated for desensitization and/or remineralization of the enamel. According to the manufacturer, maximum remineralizing effect can be observed by two professional applications of the paste, but the estimated number of sessions may vary according to the clinical judgment. This bio ceramic material has got a variety of applications which include: Bone tissue engineering; restoration of periodontal defects; edentulous ridge augmentation; orthopedic and dental implant coating, endodontic treatment like pulp-capping, repair of mechanical bifurcation perforations and apical barrier formation, fillers for reinforcing restorative glass ionomer cement (GIC) and restorative composite resin; desensitizing agent post bleaching; for treating early carious lesions and as a remineralizing agent in toothpastes (Munting *et al.,* 1990; Rigo *et al.,* 2004).

11 Calcium Sodium Phospho-silicate (Bioactive Glass)

Calcium sodium phosphor-silicate is an inorganic compound that reacts in aqueous environments to release calcium, sodium, and phosphate ions over time. Originally developed as a bone regenerative material, this compound has been shown to be effective at physically occluding dentinal tubules through the development of a hydroxyapatite-like mineral layer (Andersson and Kangasniemi, 1991; Hench *et al.,* 1993).

11.1 Composition and mechanism of action:

Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica (sodium calcium phosphor-silicate) which are all elements

naturally found in the body. When particles come in contact with saliva or water, they rapidly release sodium, calcium and phosphorous ions into the saliva which are available for re-mineralization of the tooth surface. These particles also attach to the tooth surface and continue to release ions and re-mineralize the tooth surface after the initial application. These particles have been shown, in in-vitro studies, to release ions and transform into HCA for up to two weeks. Ultimately these particles will completely transform into HCA which is the mineral our teeth and bones are made of and results in 80 % tubular occludance and desensitization. In a clinical trial on tooth hypersensitivity a bioactive glass containing toothpaste was shown to decrease sensitivity significantly greater than a strontium chloride toothpaste. They have also been shown to have significant anti-microbial properties and can kill up to 99.99% of oral pathogens associated with periodontal disease and caries. (Reynolds, 2008; Burwell, 2009).

Chapter two: Conclusion

The development of new remineralizing therapies could be attributed to the deep understanding of the process of dental caries and improved and more diverse methodologies to evaluate early demineralization and caries. Hence, the aim of the modern dentistry emerging is to use remineralization for managing non-cavitated carious lesions non-invasively with the purpose of preventing the progression of the disease and improving strength, esthetics, and function of teeth. This current therapeutic philosophy requires a fundamental and crucial element, which is providing new and greatly effective technologies to remineralize enamel.

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