

## Prevention of Dental Caries beyond Fluorides

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### Abstract

Dental caries is a pathologic process which is multifactorial and causes the destruction of the dental tissues and produces local and general complications. Fluoride involves inhibition of tooth demineralization, promotion of incipient lesion remineralization. Even though fluoride had a profound effect on the level of caries prevalence but non-fluoride agents can enhance fluoride action. There are various steps that have been taken in the prevention of dental caries, out of which fluoride tops the list. Non-fluoride agents like, arginine, plant extracts, probiotics, novamin, dentrifiers, antimicrobials, CPP- ACP etc are commonly used in prevention of dental caries. This paper reviews about these non-fluoride agents in the prevention of dental caries.

**Keywords:** dental caries, fluoride, non-fluoride agents, prevention

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### I. Introduction

Dental caries is a pathologic process depending on several etiologic factors, which cause the destruction of the dental tissues and produces local and general complications. The term "caries" originates from Latin for "rot" or "rotten" prompted original researchers of the past two centuries to develop methods to counter this process of tooth decay. Data from the National Health and Nutrition Examination Survey (NHANES) conducted between 1999 and 2004 revealed that 28 % of children ranging from 2 to 5 years had one or more primary tooth affected and 51 % had one or more primary tooth affected by ages 6 to 11.<sup>[1]</sup> The caries result from the interplay of three main factors over time- dietary carbohydrates, cariogenic bacteria within dental plaque and susceptible hard tooth surfaces.<sup>[2]</sup> These factors contribute in lowering pH in the oral cavity resulting in demineralization of tooth surface as low pH does not allow remineralization of tooth. Traditionally, it was recommended to remove the caries and replace it with a restorative material.

In 1901, Dr. Fredrick Mckay of Colorado (U.S.A) accidently discovered that many of his patients had an apparently permanent stain on their teeth which was referred to as "COLORADO STAIN" by the locals. Mckay at this stage failed to relate this unsightly stain with any factor and he named it "Mottled enamel". In

1934, Dean developed a standard classification of mottling- the Mottling Index. It was modified between 1936 and 1938 as Dean's index of fluorosis. In 1942, an important milestone discovery was made by Dean et al that at 1 ppm of F in drinking water, 60% reduction in caries experience were observed. The discovery of fluoride in dentistry has revolutionized treatment modalities with a new aspect of prevention and conservation of tooth structure. Since then, there has been a lot of research on both topical and systemic fluoridation in an overzealous attempt to control the most debilitating dental problem of caries. Although topical fluoride is still being widely used as a preventive measure for dental caries, systemic administration of the fluoride has gained major criticism worldwide due to the low margin of safety of fluoride.

Fluoride's anti-caries efficacy is well-proven and may arise from multiple modes of action, i.e., inhibition of tooth demineralization, promotion of incipient lesion remineralization. Even though fluoride had a profound effect on the level of caries prevalence but in most instances high fluoride strategy cannot be followed to avoid potential adverse effects. Since 1970s, researches started to search for non-fluoride agents for the prevention of caries and they also tried to see whether these products can work alone or can be synergistic to the effect of fluoride.

### WHY TO GO NON- FLUORIDE STRATEGIES?

- Although fluoride has had a profound effect on the level of caries prevalence, it is far from a complete cure. <sup>[3]</sup>
- Fluoride alone is not sufficient for the prevention of dental caries. <sup>[4]</sup>
- Fluoride is highly effective on smooth-surface caries, but its effect is limited on pit and fissure caries. <sup>[5]</sup>
- Toxicity of fluoride increases with inadequate nutrition. <sup>[3]</sup>
- A high-fluoride strategy cannot be followed because over-exposure of fluoride can lead to fluorosis. <sup>[6]</sup>

### RECENT ADVANCES IN PREVENTION OF CARIES

<b>Plant Extracts</b>	Arginine, AzadirachtaIndica, Ocimum Sanctum, PrunusMume Camellia Sinensi, HumulusLupulus Apigenin and ttFarnesol Oleic Acid, Linoleic Acid, Epicatechin polymer Proanthocyanidins, Phenolic Acids, Flavonols Twigs of SalvadoraPersica, Xylitol
<b>Animal Extracts</b>	Propolis
<b>Biological</b>	Probiotics, Vaccines
<b>Synthetic</b>	Nano-Hydroxyapatite CPP-ACP
<b>Natural</b>	Essential Oils Trace Elements
<b>Chemical Agents</b>	Novamin Chlorhexidine Chemoprophylactic agents Anti-microbial Peptides Dicalcium phosphate dehydrate Bioactive Glass Material

### Arginine

Arginine is a semi-essential amino acid which is found in human body and it is broken down in the oral cavity by oral microorganism to acid. Some of less aciduric microorganism (*Streptococcus sanguinis* and *Streptococcus gordonii*) are associated with protection from plaque acidification by hydrolyzing urea or arginine to ammonia by expressing urease enzyme or arginine deiminase system.<sup>[7]</sup> Urea and arginine can be rapidly metabolized by oral microorganism which evoke rise in environmental pH which is a key environmental factor affecting the physiology, ecology and pathogenicity of the oral biofilms. In oral biofilms, arginine is mainly metabolized by the arginine deiminase system (ADS) of oral bacteria, which yields citrulline, ornithine, CO<sub>2</sub>, ATP and ammonia.<sup>[8]</sup> Bijle et al found that daily use of 2% arginine in NaF toothpaste might provide a synergistic anti-caries effect.<sup>[9]</sup>

### PLANT EXTRACTS

#### Azadirachta indica (neem)

Pre-treatment of saliva-conditioned hydroxyapatite with the Neem stick or gallotannin-rich extract prior to exposure to bacteria yielded significant reductions in bacterial adhesion. The Neem stick extract and the gallotannin-enriched extract from *Melaphis chinensis* inhibited insoluble glucan synthesis.<sup>[10]</sup> Wolinsky et al.

found the inhibitory effects of aqueous extracts derived from the bark-containing sticks of *Azadirachta indica* upon bacterial aggregation, growth, adhesion to hydroxyapatite and production of insoluble glucan which may affect in vitro plaque formation.<sup>[11]</sup>

#### **Ocimum sanctum (tulsi)**

*Ocimum sanctum* (Tulsi) is a tested premier medicinal herb which has antimicrobial property and maximum antimicrobial potential is achieved at 4% concentration level. Agarwal et al. demonstrated anti-microbial potential of *ocimum sancti* at 4% against a variety of microorganisms like *Staphylococcus aureus*, *Klebsiella*, *Candida albicans*, *E. coli* and *Proteus* sp.<sup>[12]</sup>

#### **Prunus mume**

*Prunus mume* is a common fruit in China and Seneviratne CJ et al found the potential to control or prevent oral diseases associated with oral pathogenic bacteria like *Streptococcus mutans*, *S. Sobrinus*, *S. Mitis*, AA etc.<sup>[13]</sup>

#### **Camellia sinensis (green & black tea)**

The extract of green and black tea leaves contain polyphenolic components which can inhibit the growth of gram negative and gram positive bacterial species. The assam tea with higher concentration of galloylated catechins may assist in prevention of dental caries as compared to green tea whose inhibition is offset by pectins. Ferrazzano et al. concluded that coffee and tea extracts are beneficial in the prevention of dental caries.<sup>[14]</sup>

#### **Humulus lupulus (hop plant)**

Tagashira et al. demonstrated the inhibition of *S. Mutans* and other oral streptococci, by the antimicrobially active ingredients of hop plant. They found that all tested hop constituents inhibited the streptococci with minimum inhibitory concentration at pH 7.5 ranged from 2 to 50 µg/ml. It has been found that hop plant contains hop bract polyphenols (HBP) which inhibits cariogenic effect of streptococci.<sup>[15]</sup>

#### **Apigenin and ttFarnesol**

Apigenin and ttFarnesol are two naturally occurring agents that affect the development of cariogenic biofilms. Koo H et al found inhibition activity of glucosyltransferases in solution and on the surface of saliva-coated hydroxyapatite beads and demonstrated modest antibacterial activity of tt-Farnesol against biofilm.<sup>[16]</sup>

#### **Oleic acid, Linoleic acid, epicatechin polymer (Cacao bean husk)**

Osawa K et al found out the inhibitory effect on water-insoluble substances, polymer glucan synthesis, adherence, acid production by *mutans* streptococci. The cacao bean husk has been shown to possess two types of cariostatic substances- anti-glucosyltransferase (GTF) activity and antibacterial activity.<sup>[17]</sup>

#### **Proanthocyanidins, phenolic acids, flavonols (Cranberry)**

These agents show antimicrobial activity against *mutans* streptococci by disrupting acidogenic/aciduric properties of planktonic and biofilm cells of *S. mutans*. Yamanaka A, revealed inhibitory effect on glucosyltransferase activity and adherence by *mutans* streptococci resulting in reduction of microorganism.<sup>[18]</sup>

#### **Twigs of salvadorapersica**

The stick showed very strong antibacterial effect against the microorganisms on embedding above agar plate. The findings suggest that miswak may have a selective inhibitory effect particularly against several oral streptococci species.<sup>[19]</sup> Almas K et al conducted a study and concluded that there was a marked reduction of *Streptococcus mutans* and it has an immediate antimicrobial effect.<sup>[20]</sup>

#### **Propolis**

Propolis or bee glue is a resinous mixture that honey bees produce by mixing saliva and beeswax with exudate gathered from tree buds, sap flowers or other botanical sources.<sup>[21]</sup> Studies shown that mouthwash containing propolis extract significantly reduced the concentration of bacteria in saliva. Ramakrishna et al concluded that it can be used as a prevention option against cariogenic bacteria.<sup>[22]</sup>

#### **Xylitol**

Xylitol is a sugar alcohol used as a sugar substitute. Xylitol is naturally occurring in small amounts in plums, strawberries, cauliflower, and pumpkin. Arends J, suggested that xylitol have an ability to inhibit enamel dissolution in vitro.<sup>[23]</sup> Milgrom P et al demonstrated that Xylitol pediatric topical oral syrup to prevent dental caries.<sup>[24]</sup>

### Probiotics

Probiotics are live microorganisms intended to provide health benefits when consumed and are considered generally safe to consume. Probiotics can create a biofilm in the oral cavity which acts as a protective lining for oral tissues against oral diseases.<sup>[25]</sup> Cheese might be the ideal vehicle for administering probiotics to humans.<sup>[26]</sup> Ahola A et al suggested that cheese enhances remineralization and prevents demineralization of enamel.<sup>[27]</sup> Caglar E et al demonstrated a short-term daily ingestion of lactobacilli-derived probiotics delivered via medical device containing probiotic lozenge reduced the levels of salivary mutans.<sup>[28]</sup>

In addition to probiotics, another measure that competitively reduces the pathogen composition in the oral flora has emerged with the advances in gene engineering and DNA recombination technology. This method is the so-called replacement therapy. To prevent an infection using replacement therapy recently referred to as probiotic therapy, a natural or genetically modified effector strain is used to intentionally colonize the sites in susceptible host tissues that are normally colonized by a pathogen. From a standpoint of replacement therapy for caries prevention, implantation of an effector strain would best be achieved in children immediately after tooth eruption and before the acquisition of a caries-inducing strain.

### Novamin

NovaMin (Calcium Sodium Phosphosilicate), an active ingredient used in professional and over-the-counter dental products, is a tasteless white powder that augments the natural protective and repair mechanisms for teeth. The formulation of bio-glass in the Sensodyne toothpaste—commercially called NovaMin—reacts with saliva in the mouth to form a protective layer of hydroxyapatite on teeth. Allaudin SS et al suggested that NovaMin is effective in improving the Ca/PO<sub>4</sub> ratio and hardness in a demineralized enamel as fluoride.<sup>[29]</sup>

### Chlorhexidine

Chlorhexidine is a broad spectrum antibiotic that kills Gram-positive and Gram-negative bacteria as well as yeasts at high concentrations. At lethal concentrations chlorhexidine causes irreparable damage to the cell membrane of target microbes and at sub-lethal concentrations chlorhexidine can interfere with the sugar transport and acid production of the cariogenic streptococci strains, providing a bacteriostatic effect. Jenkins et al. found considerable reduction in bacterial count which remained significant for three hours with triclosan and for 7 hours with sodium lauryl sulfate and chlorhexidine.<sup>[30]</sup>

### Essential oils

Essential oils have also been extensively studied for antimicrobial activity against caries-related bacteria. Overall, the main chemical group is primarily composed of terpenoids, followed by aromatic and aliphatic constituents. Shapiro S. et al demonstrated that thymol and eugenol inhibit the growth of a wide range of oral microorganisms including mutans streptococci.<sup>[31]</sup>

### Trace elements

Different trace elements has been investigated were zinc, tin, aluminium, copper, iron, strontium, barium, manganese and molybdenum, gold, lead etc. Aluminum, copper, and iron have the most commonly used as cariostatic agent. Glass RL et al found the significant effects of trace elements on growth of *S. mutans* and may confirm epidemiological evidence suggesting a role for certain trace metals in the incidence of dental caries.<sup>[32]</sup>

### CPP-ACP

The casein phosphor-peptides (CPP) are produced from a tryptic digest of the milk protein casein by aggregation with calcium phosphate and purification by ultrafiltration. The CPP have a remarkable ability to stabilize calcium phosphate in solution and substantially increase the level of calcium phosphate in dental plaque. The proposed mechanism of anti-cariogenicity for the CPP-ACP is that they localize ACP in dental plaque, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. Mazzaoui et al. used CPP-ACP with fluoride and demonstrated a synergistic remineralization potential.<sup>[33]</sup>

### Chemoprophylactic agents

Chemoprophylactic agents that are used in dental caries prevention include classical antibiotics such as penicillin and vancomycin. There are cationic agents which are helpful in caries prevention include chlorhexidine and cetylpyridinium chloride. Mezine, et al. demonstrated prevention of dental plaque accumulation through inhibition of GTF enzyme activity leading to reduction in caries-associated inflammation in the oral cavity by cyclooxygenase inhibition and provides a strong anti-oxidative capacity.<sup>[34]</sup>

### Antimicrobial peptides

Antimicrobial peptides (AMPs) are a heterogeneous group of molecules with unique antimicrobial characteristics that have great potential for controlling bacterial infections and modifying biofilms. Koczulla and Bals suggested that AMPs have a broad range of antibacterial, antiviral, and antifungal activity mediated by selectively interacting electrostatically with negatively charged components of cell membrane phospholipids, resulting in membrane permeabilization and disruption, leading to cell death.<sup>[35]</sup> Eckert et al., in 2012 found the specifically targeted antimicrobial peptide (STAMP) with 2 independent functional domains, consisting of a *Streptococcus mutans*-selective “targeting domain” designated as C16, and a “killing domain” designated as G2.<sup>[36]</sup>

### Vaccines

Another line of defence in human body that can be utilized against *S. mutans* colonization is the specific antibody production from adaptive immunity. Immune defense in dental caries is mediated mainly by secretory IgA (sIgA)

antibodies present in saliva and generated by the mucosal immune system.<sup>[37]</sup> Nicole H Lazarus suggested a broad and unifying role for MEC in the physiology of the mucosal IgA immune system.<sup>[38]</sup>

#### **Dicalcium phosphate dehydrate**

Dicalcium phosphate dehydrate is the mineral brushite which can be easily crystallized from aqueous solutions at pH <6.5. DCPD is added to toothpaste both for caries protection and as a gentle polishing agent.<sup>[39]</sup>

#### **Nano hydroxyapatite**

Nano-hydroxyapatite is a form of calcium crystal. It works by remineralizing and replaces missing sections of minerals. The toothpaste containing n-HAp showed higher remineralizing potential as compared to amine fluoride toothpaste with bovine dentine. Roveri N et al found that enamel and dentine remineralization by nano-hydroxyapatite toothpastes.<sup>[40]</sup> Najibfard K demonstrated that concentration of 10% nano-hydroxyapatite (nHA) is considered to be optimal for remineralization of early enamel caries.<sup>[41]</sup>

#### **Bioactive glass material**

Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica (sodium calcium phospho silicate), which are all elements naturally found in the body. Narayana et al demonstrated that bioactive glass can be considered as an effective remineralizing agent.<sup>[42]</sup>

## **II. Discussion**

Dental caries is a multi-factorial disease that depends on a balance between pathological and protective factors. The caries process is a continuous process resulting from an imbalance between many cycles of demineralization and remineralization rather than unidirectional demineralization process. The efforts have been made continuously to eradicate dental caries but it is still widespread. There are various methods in the prevention of caries. Fluoride application inhibits demineralization of the crystal structures inside the tooth and thereby enhancing remineralization. It is well established that demineralization and remineralization occur in the mouth which is governed by the degree of saturation of oral fluids with respect to apatite minerals. The point to be taken into consideration is that if carious lesion is non-cavitated, it can be reversed or arrested chemically. As a consequence of the trend towards caries prevention, rather than treatment, it is necessary to gain better understanding of mechanisms that can enhance remineralization or reduce demineralization.

The current review assessed the effect of non-fluoride agents in the prevention of dental caries. Arginine, chlorhexidine, CPP-ACP and xylitol are the non-fluoride agents which are very significant in the prevention of dental caries. The cacao bean husk showed anti-glucosyltransferase (GTF) and antibacterial activity which leads to reduction in the mutans streptococci.<sup>[17]</sup> Cranberry also showed inhibits adherence by mutans streptococci.<sup>[18]</sup> Xylitol have an ability to inhibit enamel dissolution in vitro [Arends J et al].<sup>[23]</sup> The study conducted by Caglar E et al (2006) concluded that ingestion of lactobacilli-derived probiotics helps in reduction of mutans streptococci.<sup>[26]</sup> NovaMin has the potential in improving the Ca/PO<sub>4</sub> ratio and it is effective in remineralization of early enamel lesions [Allaudin SS et al].<sup>[29]</sup> Nano-hydroxyapatites had the potential of remineralizing early enamel lesions.

Another alternative to the fluoride-antimicrobial approach is combining fluoride with agents that promote and encourages the growth of health-associated bacteria resulting in better long-term dental caries control. Koczulla and Bals demonstrated that anti-microbial peptides have a broad range of antibacterial activity and have great potential for controlling bacterial infections and modifying biofilms.<sup>[35]</sup> Mazzaoui et al. used CPP-ACP with fluoride and demonstrated a synergistic remineralization potential.<sup>[33]</sup> Featherstone et al. -2012 demonstrated that arginine lowered caries increment in high risk groups. Incorporation of 2% arginine in NaF toothpaste significantly increased the remineralization of enamel caries-like lesion [Bijle et al-2018].<sup>[9]</sup> The combinatory application of fluoride and arginine has a potential synergistic effect in maintaining a healthy oral microbial equilibrium [X. Zhenget al-2015].<sup>[43]</sup> The ecological approaches could be valuable tools in achieving long-term dental caries control, allowing the clinician to shift to a biological model for the management of the disease.

## **III. Conclusion**

There is no hesitation that fluoride will continue to be the backbone in the prevention of dental caries. However, fluoride's effectiveness could be enhanced when combined with non-fluoride agents. Non-Fluoride agents like arginine, CPP-ACP, xylitol, novamin, cranberry etc. have shown promising results in the prevention of dental caries. Since, dental caries is a multifactorial disease, these non-fluoride agents should be evaluated with further limited studies.

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