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Preventive Dentistry Techniques in the Treatment of Dental Caries and Biofilm Control: A Review

Tyler R. Oatmen

Abstract: This study reviews the biological mechanisms and recent advances in preventive dentistry therapies that prevent the formation dental caries, covering both traditional and novel techniques. The techniques covered prevent the formation of dental caries through two therapeutic approaches, either promoting the remineralization of the hard tooth surface or regulating the level and composition of the oral biofilm present in the mouth. Traditional therapies covered include fluoride and calcium treatments that promote the remineralization of the tooth surface, and antibiotic therapies that attempt to regulate oral biofilm levels, such as chlorhexidine, essential oils, systemic antibiotics, and salivation stimulating therapies. Novel applications covered are therapies that utilize antimicrobial peptides, immunizations, and probiotics that may become important to dentistry in the near future. Dental caries management is a complex and dynamic process, but by reestablishing optimal oral health through preventive techniques, dentists can preserve tooth structures and avoid costly and extensive restorative treatments.

Key Words: Carious Lesions, Hydroxyapatite, Acidogenic, Phosphopeptide, Periodontitis, Xerostomia

Background

Commonly referred to as cavities or tooth decay, dental caries is a disease where the hard tissues of the teeth have been damaged, creating cavities in the tooth. Although the prevalence of dental caries has steadily declined over the last 20 years due to dental hygiene practices and the increased use of fluoride-containing products, health surveys still estimate the percentage of adults with decayed, missing, or filled tooth surfaces to be 98.3%. (Brown) While the disease is multifactorial in nature, the mechanism for caries formation is well understood. Four main components exist in the process of caries formation: a tooth surface, acidogenic bacteria, fermentable carbohydrates, and time.

Enamel and dentin are the tooth's two outermost surfaces, and are primarily composed of a crystallized calcium phosphate called: hydroxyapatite. The tooth's hydroxyapatite crystal lattice exists in a constant equilibrium of demineralization and remineralization, which is a pH dependent process. When the pH drops below a critical value (pH 5.4 to 5.5 for enamel), the acid dissolves hydroxyapatite minerals into solution. Demineralization of the tooth surface causes areas of tooth decay called lesions, which can progress into clinical dental caries. Demineralization at the tooth surface is a reversible process, however, and when the pH levels recover within the mouth, the recrystallization of minerals upon the tooth surface occurs. Remineralization using calcium and phosphate

minerals present in saliva is a slower process than the dissolution process, but promoting remineralization is a key action in preventing dental caries. Fortunately, caries is not a disease that develops rapidly and it takes time for lesion initiation to produce a clinical result. It may take up to three or four years to develop a cavitation and as a result, there exists ample time to interrupt the caries formation process through preventive techniques. (Peters)

Caries formation is a pH dependant process, and the fluctuation of pH within the oral cavity is a daily occurrence due to the presence of certain cariogenic (caries promoting) bacteria and fermentable carbohydrates. The presence of microbes within the mouth is natural within the oral cavity, and bacteria are the most common and numerous. (Aas) When bacteria such as mutans streptococci or lactobacilli colonize the oral cavity as a result of environmental shifts that make these bacteria more competitive, namely a lowered pH level, plaque formation occurs. Oral biofilm (or plaque) is the accumulation of cariogenic bacteria that resides directly on the tooth surface in a diseased state. During the metabolism of fermentable carbohydrates these acid producing (acidogenic) bacteria produce byproducts of fermentation that lower the pH of the mouth, and the dissolution of the tooth surface proceeds. Both the mutans streptococci and lactobacilli families produce lactic acid and these two bacterial groups, either separately or together, are primary causative agents of dental caries. Acidogenic bacteria promote the formation of caries, but the anticariogenic properties of saliva is also well established. Saliva contains oxidative enzymes that function as antimicrobial agents. Saliva also acts as a pH buffer, and contains calcium and phosphate ions used in the remineralization process. Both increasing saliva production and mediating plaque levels in the mouth are also approaches used in preventive dental caries formation.

Preventive dentistry aims to stop the progression of dental caries by promoting daily habits and clinical therapies that either promote the remineralization of the tooth surface or prevent the formation of the oral biofilm responsible for lowering the oral pH levels in an attempt to prevent cavity formation. Mineral based treatments include fluoride or other ions that attempt to replace lost hydroxyapatite. Methods that focus on biofilm control include local and systemic antibiotics or interventions that artificially increase saliva production. This review will look at the mechanisms and clinical efficacy of several traditional preventive dentistry measures and also describe new technology that shows promising results in preventing dental caries.

Preventing Demineralization/ Promoting Remineralization

Fluoride Treatment

The use of fluoride therapy in dentistry aims to replace lost hydroxyapatite material with fluoroapatite in the remineralization process by increasing the amount of available fluoride ions in saliva. Fluoroapatite is the product of a reaction between fluoride and a calcium phosphate, producing a calcium halophosphate mineral that combines well with the hydroxyapatite structure of hard tooth structures. Ten calcium ions and six phosphate ions form one fluoroapatite unit ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$). These fluoroapatite crystals are relatively insoluble in comparison the hydroxyapatite crystals and as a result, repeated remineralization in the presence of fluoride ions results in a more caries resistant tooth surface. During periods of “acid challenge” the fluoride treated tooth surface actually becomes more resistant to demineralization and subsequent lesion formation. Fluoride has become an important tool in preventive dentistry, and fluorinated water has been called one of the greatest health achievements of the 20th century by the Center for Disease Control.

Topical at-home fluoride uses such as tooth pastes, mouth rinses, and gels remain a cost-effective way to retain a sufficient therapeutic concentration of fluoride within the mouth. A recent systematic review of at-home fluoride treatments concluded that the benefits of daily use for preventing dental caries is firmly established. On average, in a population with an average of 2 DMFS (Decayed, Missing, and Filled Surfaces) per year, topical fluoride agents prevented up to 0.74 DMFS per year. (Marinho) Professionally applied fluoride in the form of foam, gel, and varnish has also been extensively studied, and the American Dental Association has recently published guidelines for the clinical use of fluoride treatments. The report concluded that children and adults with any risk factors or existing cavitations should receive clinical fluoride application at a minimum of six month intervals. (JADA) The ADA cautions against the overuse and unnecessary application in children to prevent fluorosis, but concludes that fluoride application is beneficial in both the treatment and prevention of dental caries.

An alternate method of delivering fluoride concentrations to the tooth-biofilm interface at a consistent concentration over time are slow-release systems. The slow-release systems utilize a dental device that is placed upon a molar tooth surface that provides a controlled sustained release of fluoride ions. Historically slow-release systems have either been a copolymer design or a glass device. Copolymer devices consist of sodium fluoride ions surrounded by a HEMA/MMA copolymer membrane that provides a controlled rate of fluoride release as the materials become hydrated and granulated NaF are released. (Pessan) Glass devices, which were innovated initially from animal husbandry devices that delivered trace elements to animals with deficiencies, utilize a dome or disk shaped glass material containing fluoride ions. Attachment via a plastic mounting bracket allows the glass device to dissolve fluoride ions into solution when moistened. Slow-release systems are typically applied to only high risk patients that

experience problems with compliance to an at-home fluoride routine. Each individual slow-release system is typically rated based on fluoride retention rates intraorally, as a fluoride level of 0.1 ppm in saliva have been identified as an optimal therapeutic level. (Featherstone) Both copolymer and glass devices are effectively able to achieve this level, with some studies concluding that beneficial levels of up to 0.69 ppm are achieved.

Calcium Phosphate Treatments

Fluoride alone cannot achieve remineralization, the presence of calcium and phosphate ions are also required. Increasing concentrations of calcium and phosphate ions in saliva while effective fluoride levels are present has become a clinical strategy for non-invasive remineralization of the tooth surface. The calcium and phosphate ions can become the limiting factor for remineralization, especially in cases of xerostomia (dry mouth) where low saliva levels exist. Past systems that have attempted to deliver calcium and phosphate ions have experienced troubles with the solubility of these ions at neutral pH ranges. A number of commercialized systems have claimed to increase the intraoral calcium and phosphate ion concentrations, and a recent report has examined the anticariogenic efficacy of these systems. (Reynolds)

One commercialized system that has displayed superior efficacy is the casein phosphopeptide stabilized system. This technology utilizes phosphopeptides from the casein family of proteins to stabilize a large amount of calcium phosphate at the tooth surface by binding the phosphopeptide to the plaque-enamel interface. The casein phosphopeptide contains the cluster sequence –Ser-Ser-Ser-Glu-Glu-, which stabilizes the amorphous calcium phosphate into nanoclusters that do not perform nucleation or crystallization, thus keeping the calcium phosphate soluble in the delivery solution. (Cross) When delivered, the casein phosphopeptide amorphous calcium phosphate complex (CPP-ACP complex) attaches to the tooth surface, and the amorphous calcium phosphate is free to participate in the remineralization process. The anticariogenic properties of this system have been confirmed in a recent clinical trial. (Morgan) The CPP-ACP complex is typically incorporated into a commercial product such as chewing gum, milk, mouthrinse, or a paste for at home use. Other systems are being developed and have shown efficacy within animal models or *in vitro* trials, but have yet to achieve clinical trial results.

Controlling Biofilm/ Plaque Levels

In addition to focusing on the remineralization of the tooth surface as a way to combat dental caries, preventive dentistry also seeks to control the bacteria that make demineralization possible. Current treatments within preventive dentistry most often seek to kill the microbes within the mouth with antibacterial agents delivered locally, through the use of systemic antibiotics, or through the stimulation of saliva production. Typically

antimicrobial agents target both supra-gingival plaque, and more importantly sub-gingival plaque buildup. Sub-gingival plaque is difficult to remove with regular dental hygiene habits, and the ability to control the levels and growth of bacteria at the sub-gingival level is an important part in controlling root caries formation. It is important to note however that the simple elimination of microbes within the mouth may not produce the best outcome for the dental patient. The mouth is a rich mixture of microorganisms that, when present at the right composition within the mouth, provide benefits to their human hosts. Therefore, preventive dentistry methods should try to control the levels of bacteria within the mouth to achieve a level of plaque that is consistent with optimal oral health. Clinical decisions to deliver antimicrobial agents exist in a balance between delivering relevant and clinically measurable amounts, but at the same time not disrupting the natural ecology of the mouth which provides protection from opportunistic pathogens and the overgrowth of exogenous microbes. A closer look into the mechanism of action, delivery methods, and retention in the mouth of these antimicrobial agents is essential in making preventive dentistry treatment decisions.

Topical/Local Methods for Biofilm Control

Chlorhexidine: Currently mouthrinses that contain 0.12% chlorhexidine are marketed within the United States. 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. Chlorhexidine is typically utilized because of its great retention within the plaque coated enamel surface, and studies report that 30% of the delivered chlorhexidine is retained in the mouth after use. (Marsh) The efficacy of the chemical has come under scrutiny as some clinical trials have failed to produce significant level of caries reduction, and side effects such as staining and an altered taste sensation have been reported. (Autio-Gold) Chlorhexidine's retention rate and broad spectrum antibacterial action make it a great candidate for the control of plaque, but the recent questionable nature of the long-term clinical evidence for caries prevention and possible undesirable side effects should be considered before clinical recommendation.

Essential Oils: Essential oils are by definition hydrophobic liquids that are extracted from plants, typically bearing some distinctive scent or 'essence'. The two oils typically used in mouthrinses are thymol and eucalyptol. Mouthrinses containing both chlorhexidine and essential oils are approved through the American Dental Association, and essential oil therapy does not exhibit the unwanted side effects of chlorhexidine use, potentially increasing patient compliance with the treatment. Additionally, essential oils have not shown any production of antimicrobial resistance over long term use, which

makes a prolonged treatment period possible. The mechanisms of action against bacteria are complex. At high concentrations the oils are lethal, disrupting the cell wall and causing precipitation of cell proteins, and at low concentrations the oils inactivate essential bacterial enzymes. The oils have been shown to penetrate the plaque biofilm and exert a bactericidal activity in embedded bacteria, effectively reducing the bacterial load and slowing plaque mass and pathogenicity. (Stoeken) The plaque reducing properties of both essential oils and chlorhexidine therapies is proven, but these therapies should never be used exclusively to prevent caries. Plaque buildup is only one portion of the etiology for dental caries, and prevention techniques should be used in concert to ensure the best patient outcome.

Systemic Antibiotics

Systemic antibiotics are antibiotic drugs delivered orally that affect the mouth as well as the whole body. The most common forms of antibiotics used are tetracyclines, penicillins, metronidazole, macrolides, clindamycin, and ciprofloxacin. The literature does not provide a clear indication of the superiority of one antibiotic regimen over the other, and because various antibiotics target various species of microbes, the treatment decision should be made on an individual basis. Systemic antibiotics have been advocated in the control of subgingival plaque levels only in accompaniment with non-surgical debridement and regular periodontal maintenance. Typically patients with some level of periodontal disease, the disease that affects the supporting tissues of the mouth, are clinically considered for systemic antibiotic therapy. Systemic antibiotics have severe limitations in that long term use or overuse of antibiotics can lead to bacterial resistance and that the affected areas of the body cannot be limited to just the mouth. Therefore the use of systemic antibiotics has been confined to patients with existing levels of plaque that are causing some form of periodontitis. A recent study confirms the effectiveness of systemic antibiotics when used in conjunction with mechanical debridement of plaque. (Heitz-Mayfield) While systemic antibiotics are used in concert with other methods on a short term basis to achieve a level of plaque that is consistent with optimal oral health, the method should be utilized with care as to not produce bacteria that are antimicrobial resistant.

Altering Saliva Production

Saliva is a major protective factor in the oral cavity, and a reduction in saliva output can occur from numerous systemic diseases and pharmaceutical interventions. The importance of saliva output in caries prevention is a relatively new topic in preventive dentistry, but pharmaceutical interventions have commonly treated the xerostomia that accompanies many drug therapies (eg. chemotherapy). Loss of antimicrobial capacity, pH buffering, and remineralization properties of saliva may lead to rapid and severe caries

formation, and may also leave the mouth susceptible to infections, mucosal pain, and difficulties chewing and swallowing. By increasing salivary function and its natural protective factors, these oral complications can be avoided and, when combined with other caries prevention techniques, can provide a synergistic approach in prevention of caries formation.

Clinical attempts at increasing secretory output first start at a local level within the mouth, and typically begin by increasing oral activity. Salivation physiologically increases in response to chewing or taste, particularly sour and bitter tastes. The use of sugar-free gums and lozenges will increase salivary output for a patient, and still remains the mainstay therapy for patients suffering from xerostomia (dry mouth). The use of sugar-free methods of increasing oral activity must be stressed, as the additional sugar 'coating' would only increase dental caries risk, negating the potential benefits of increased saliva production. Other topical therapies include artificial salivas that attempt to mimic the composition and abilities of natural saliva in the forms of oral rinses, gels, and flavored mouthwashes. These topical treatments are limited however by their transient nature, and stimulating saliva output over a prolonged period of time would be more desirable.

Several systemic agents that can stimulate saliva production can have a more profound and consistent effect. Pilocarpine is the drug with the most extensive background of clinical evidence, as it has been a treatment for dry mouth for over 100 years. Pilocarpine was initially utilized in Sjogren's syndrome and postradiation therapy to stimulate salivary gland production. Pilocarpine is a parasympathomimetic agent with a mild β -adrenergic stimulating property that can be utilized without serious adverse side effects, although treatment for patients with cardiovascular disease is not advised. The majority of the studies have used dry mouth symptoms as primary outcome variables, but recently oral dryness and salivary secretion has been measured in clinical trials. Studies find that pilocarpine maximally stimulates salivary secretion approximately 1 hour after a dose and increases in baseline output are found for 3-4 hours afterwards. (Wiseman) Although pilocarpine has not been shown to increase baseline salivary secretion, the increased salivary output is dose-related and consistent. Typically an orally ingested form of pilocarpine HCL is delivered 3 times a day, and common side effects include excess sweating and urination. Cevimeline is a drug that has recently gained clinical backing in increasing salivary output. (Petroni) The pharmacological profile is very similar to Pilocarpine, and the important properties of the drug are the later onset time and longer duration of the action. A therapy using a combination of both Pilocarpine and Cevimeline is under review, that could potentially deliver a longer lasting, consistent increase in saliva production.

Novel Technology

The aforementioned therapies have all been identified as useful for preventive dentistry and are at various stages of clinical trials. To look into the future of preventive dentistry measures, the literature concerning promising advances in potentially useful technologies for the prevention of dental caries and the control of biofilm was reviewed. The following is a description of potential technologies that may become important in the advancement of preventive therapies, including antimicrobial peptides, immunizations, and probiotics.

Antimicrobial Peptides

An important issue with chemotherapies and antimicrobial therapies today is the proliferation of antibiotic-resistant organisms that lessen the efficacy of conventionally utilized antibiotics. Just recently antimicrobial peptides (AMPs) have come forward as agents that exhibit a wide spectrum antimicrobial activity against bacteria, including drug-resistant strains. Antimicrobial peptides vary in their peptide sequence and posttranslational modifications, but the majority of AMPs are amphipathic mixtures of α -helical and β -sheet structures with a cationic charge. (Pazgier) The action of AMPs typically involves binding to the negatively charged functional groups of microbial membranes (eg. lipopolysaccharides) and creating a disruption by insertion into the membranes, although it has been suggested that a number of AMPs translocate intracellularly and are lethal via a different mechanism. (Zasloff)

Commercial applications of AMPs have stalled due to the difficulty and expense of their manufacture, and their relatively short half-lives due to enzyme degradation. (Marr) Several solutions have recently been proposed to make AMP therapies less expensive, one being the use of exogenous modifiers to increase AMP transcription levels rather than directly using the peptide. The 25-dihydroxyvitamin D3 vitamin is a biologically active form of vitamin D that has recently been discovered to induce the expression of the Cathelicidin antimicrobial peptide family in the human genome without a large amount of host immune response. (Wang) Another solution that may become useful are non-peptidic polymers that resemble natural AMP compounds, adopting the amphiphilic structure similar to AMPs and exhibiting the same antimicrobial activity. These compounds are much cheaper to manufacture, and a recent study showcased the ability to act specifically against bacterial families. A pheromone produced by *S. Mutans*, a cariogenic bacteria, was used to deliver lethal nanomolar concentrations of synthetic AMP directly to the *S. Mutans* bacteria and did not affect other streptococci that were present. (Eckert) Researchers linked an AMP peptide sequence to the *S. Mutans* pheromone peptide sequence using a chain of amino acids, termed the linker region. The functionality of both the pheromone and the AMP remained consistent, and the resulting peptide showed specific lethality to the

S. Mutans bacteria. This pheromone-guided 'smart' antimicrobial peptide potentially achieves a level of clinical specificity that avoids the clearance of all bacteria within the domain, protecting the host from opportunistic pathogens. Specifically targeted antimicrobial peptides (STAMP's) could be delivered in current oral care products such as mouthwash, toothpaste, or dental floss and could help with the suppression of cariogenic bacteria. Recently, reports of AMPs that are down to simply 10 peptides long have been published, and in consideration of the relative stability and cost to produce peptides commercially, smaller peptides do hold promise. Researcher's knowledge of antimicrobial peptides is a rapidly advancing, and AMPs certainly have the potential to become ideal antimicrobial agents in the future of dentistry.

Immunizations

The body's natural lines of defense can also be utilized in the fight against cariogenic bacteria of the mouth. Recent work has focused on using *S. mutans* antigens to initiate an antibody secretion, and subsequently eliminate *S. mutans* colonization in the body. Immune defense from dental caries is mediated mainly by secretory IgA (sIgA) antibodies produced in the oral mucosa. Mucosal immunization with antigens at the local lymphoid tissues, including the gut-associated and nasopharynx-associated lymphoid tissue, results in the migration of IgA-producing B cells to the salivary glands.

The main type of *S. mutans* antigen involved for which specific sIgAs have been found is Antigen I/II. Antigen I/II is the surface adhesion protein of the bacteria and contains an alanine-rich tandem-repeating region, and a proline rich repeat region associated with adhesion activity. (Shivakumar) The antigen I/II specific sIgA is able to inhibit the adhesion of *S. mutans* to hydroxyapatite and their subsequent colonization on the tooth surface. (Hajishengallis) Animal models of antigen I/II's effect upon caries formation are promising, but more clinical trials are required before the effect can be translated to human populations. Other studies have focused on the binding of *S. mutans* to sugars within the oral biofilm. Vaccines focused on the glucosyltransferase enzymes and glucan-binding proteins of the bacteria have also been tested. Both of these methods have been shown to mediate an exaggerated immune response, inhibiting the aggregation of *S. mutans* in animal models to some degree. (Taubman) (Smith)

Probiotics

As mentioned before, treatment intending to wipe out the entire oral flora may have undesired consequences in the form of secondary infections. Today's efforts in caries research have begun to focus on strategies to selectively inhibit oral pathogens by outcompeting or killing the bacteria with additional bacteria. Stemming from the success that the probiotics field have seen in gastrointestinal diseases, probiotics are now being developed that disrupt the attachment of cariogenic bacteria via competition for binding

sites at the biofilm, and competition for nutrients within the biofilm. Probiotic strains are also being produced that can secrete antimicrobial compounds, potentially inhibiting pathogenic oral bacterium.

Some gastrointestinal strains of bacteria have been tested in clinical trials to control the growth of cariogenic bacteria. Recently a study concluded that long term consumption of milk containing a *L. rhamnosus* strain reduced the incidence of caries in kindergarten children.(Nase) Ingestion of other bacteria, namely strains of *lactobacillus*, have shown antibacterial activity against *S. mutans* as well as good oral mucus binding characteristics that successfully mediates levels of pathogenic bacteria via direct competition. (Nase) One of the most interesting avenues of probiotics comes in the form of DNA recombination therapy. Termed DNA replacement therapy, the technology has allowed researchers to mutate *S. mutans* into bacteria that lack enzymes to metabolize fermentable carbohydrates into organic acids. This significantly less cariogenic strain is genetically stable, never reverting back to acid producing bacteria in both *in vivo* and *in vitro* tests, and is currently undergoing human efficacy trials. (Hillman) DNA therapy that aims to replace cariogenic bacteria with less pathogenic clones of themselves could someday become a key tool in preventing caries formation.

Conclusion

The field of preventive dentistry will certainly benefit from new technology in the future as well as the ongoing research into optimizing traditional approaches. Determining the correct treatment plan should always be made on an individual basis, taking into account existing dental carious lesions and future caries risk of the patient. Preventive techniques are most often dependent upon the patient's compliance to the prescribed therapy, and motivating the patient to follow the treatment plan plays a key role in a successful outcome. An increased amount of technologies are aimed at promoting tooth remineralization, but fluoride remains the most widely agent in managing the formation of caries due to its strong levels of clinical evidence. When fluoride therapy is not enough to overcome the formation process, dentists should consider a synergistic approach at preventing caries. Combining therapies that promote saliva production, increase the intraoral calcium and phosphate ion concentrations, and provide antimicrobial therapy can lead to more pronounced results. Novel technologies should only be considered adjuncts to treatments when their caries-preventing properties can be demonstrated in randomized controlled clinical trials.

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