Chapter 2

REMINERALIZATION AND CARIES PREVENTIVE AGENTS

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Remineralization and Caries Preventive Agents

Hydroxyapatite is a crystalline mineral, a complex form of calcium phosphate, and is the main inorganic component of bones and teeth (1). In the demineralization process, mineral ions are removed from the hydroxyapatite crystals of hard tissues such as enamel, dentin, cementum and bone. Re-acquisition of these mineral ions by hydroxyapatite crystals is called remineralization. Demineralization is a reversible process; therefore, repair can occur if partially demineralized hydroxyapatite crystals in teeth are exposed to an environment that promotes remineralization (2).

It has been reported that there is a continuous ion exchange in a dynamic balance between the enamel surface and plaque and salivary fluid throughout the day. It is known that remineralization is the natural repair process of our body against caries lesions located under the surface and not yet cavitated (3-4).

Chemical demineralization of teeth is caused by acid attacks in two main ways: dietary acid consumed through food or drink and microbial attack by bacteria in the mouth. The critical pH value at which demineralization takes place in the enamel is 5.2-5.5. During an acidic attack or a typical demineralization process, chemical dissolution of both organic and inorganic matrix components takes place (4-6).

It has been determined that the acid produced by the plaque bacteria is buffered by the saliva and the pH increases and becomes neutral. It has been explained that when the pH of the plaque exceeds the critical pH, the dissolved minerals begin to precipitate and remineralization occurs through saliva with the effect of the minerals dissolving from the enamel (7). In contrast to demineralization, it has been reported that passive transport during remineralization is not by H^{+} ion transfer, but by passive transport from saliva and plaque to the lesion body where the concentrations of Ca^{+2} and $PO4^{-3}$ ions are low (4). During the remineralization period, minerals accumulate in the crystal cavities formed during demineralization, and it is known that the lost minerals are compensated. It has been stated that since the repaired crystals may be smaller or larger than the actual crystal size, the permeability of the enamel decreases and it becomes more resistant to acid attacks (8). Saliva, fluoride therapy, dietary control, and probiotic bacteria are defined as preventatives for tooth demineralization (2, 3, 9).

It is known that saliva is a solution that takes part in remineralization. It has been determined that Ca^{+2} and $PO4^{-3}$ in the salivary structure provide remineralization of the crystal structure in the demineralized areas by diffusing in the enamel structure with the help of the F^{-} ion, which acts as a catalyst. It is known that this new structure contains fluoride hydroxyapatite, that is, fluorapatite. It has been emphasized that this new structure is more resistant to acid attacks than the first structure (8).

Low carbohydrate consumption, low number of cariogenic bacteria in the plaque, high saliva buffering capacity and flow rate, high concentration of inorganic ions in saliva, low carious lesion depth and inactivity, effective mechanical cleaning and the use of remineralization agents It has been reported to be among the factors that accelerate the process (10).

In this review, researches on Fluoride, Propolis and Chitosan will be discussed when remineralization agents and caries preventive agents are examined.

Remineralization Agents and Caries Preventive Agents; Fluoride, Propolis and Chitosan

Propolis

Propolis is a resinous hive product collected by bees from various plants and processed with bees' enzymes. Its color can vary in shades of green, red and dark brown. It has a characteristic odor and shows sticky properties as it interacts with proteins and fats in the skin (11).

Composition; It varies depending on the plant it collects, the region, the season and the colony. However, it consists of an average of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen, 5% other substances and organic residues (11, 12).

Propolis collection is carried out by worker honey bees. Going on a propolis expedition, the bee first pulls the propolis off the plant with its mandibles. It makes pellets by moistening, softening and adding some enzymes in its mouth, and transfers the pellet to the pollen basket on its hind legs using its front legs. While the worker bee, which comes to the hive loaded with propolis, clings tightly to the honeycomb using its feet; Other worker bees in the hive hang with their mandibles and take the propolis from the pollen basket of the carrier bee. They use propolis in the hive by accumulating propolis behind the bottom board, frame edges and entrance hole (13).

Bees add propolis to thin parts of honeycombs or to cavities where they live. Propolis is used to close holes and tears, to repair honeycombs, to thicken weak parts of honeycombs, to make air holes in the hive or to help hive defense. They also use propolis in the hive by mixing it with beeswax for polishing and sterilizing larval nests. Bees benefit from both the mechanical and biological activity of propolis. Depending on the source of the resin, the color of propolis can vary from yellowish green to red and dark brown, or it can be transparent. Its physical structure is hard, brittle in cold, sticky in heat. (13)

Propolis is slightly soluble in water and hydrocarbon solvents, and highly soluble in alcohols. Apart from ethanol, it can also be dissolved in solvents such as ether, glycol, methanol, oil. When solvents other than ethanol are used, it has been observed that the substances to be isolated from propolis differ and many components cannot be isolated. Therefore, the most commonly used solvent for propolis is 96% ethanol. Propolis extraction methods can change the effect of propolis. Because its solubility in different solvents and its extracted parts will differ according to the solvent. The most commonly used solvents in biological studies are mixtures of ethanol, water and methanol at different concentrations (14, 15).

The chemical content of propolis is very complex, more than 300 chemicals have been identified so far, and this content varies depending on the plant and flora from which the propolis is taken. Due to this change in propolis content, problems are experienced in its medical use and standardization (13, 15).

Propolis contains more than 300 compounds covering various chemical structures such as polyphenols (flavonoid aglycones, phenolic acids) and their esters, phenolic aldehydes, alcohols and ketones, secuiterpene quinones, coumarins, steroids, amino acids and inorganic compounds. The part containing resin and balsam contains substances such as terpenes, polysaccharides, caffeic acid. Beeswax, on the other hand, contains fatty acids, B vitamins, and vitamins C and E. Propolis; It has been determined that it contains B1, B2, B6, C, E vitamins and minerals, silver, cesium, mercury, lanthanum, antimony, copper, Mn^{+3}, Fe^{+3}, aluminum, Ca^{+2}, vanadium (12, 14).

Flavonoids (polyphenolic content), the most important part of the organic part, are the most studied substances and are responsible for a significant part of the biological activity of propolis. This part contains substances such as pinosembrin, pinostrobin, queracetin. Propolis also contains different mineral and oligo elements. Flavones and flavonoids are substances that give propolis antifungal, antiviral and antibacterial properties. The main chemical compounds it contains are; flavonoids, cinnamic acid and its derivatives are benzoic acid, synaptic and isopherulic acids, various aldehydes, ketones and trace elements, cleredon, diterpenes, sesquiterpenes and tripenes (14-17).

It has been determined that the main components of propolis are flavonoids. The flavonoid structure of propolis may also show some differences depending on the plant from which it is collected. The ratio of flavonoids from all components in propolis is over 25%. Flavonoids are polyphenolic compounds. Due to their free radical scavenging properties, they are antioxidants and inhibit lipid peroxidation. Alternatively, it is stated that they can be antioxidants because they form metal chelates (13-17).

It has been stated that the biological activity of propolis is formed by the synergistic effect of phenolic and other compounds in its resin (12). At the same time, it has been reported that mixtures of pinosembrin, galangin, and caffeic acid phenyl ester inhibit bacterial RNA polymerase and exert antimicrobial effects (16).

The pharmacological value of propolis, the preparation of its medicinal preparations and its antibacterial and antiviral value are due to the secondary metabolites it contains. Among these metabolites; phenolic acids (caffeic acid and cinnamic acid) and their esters, ketones, phenolicaldehydes, flavones and flavonoids (pinosembrin, pinobanksin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, campherolricetin, quinomenoids, and terpenestin), terrypenes, aromatic acid and its esters, amino acids, alcohols, aldehydes, aliphatic acid and its esters and some hydrocarbons can be counted (16, 17).

Use of Propolis in Dentistry

Propolis is a highly effective antimicrobial agent against oral microorganisms. Sonmez et al. (18) stated that propolis solutions prepared in appropriate proportions were highly effective against P. gingivalis, P. intermedia, C. rectus, F. Nucleatum, C. Parapsilosis, C. Krusei and C. Albicans and were not cytotoxic against gingival fibroblasts.

S. mutans is the most important bacteria in caries formation. These bacteria produce organic acids that demineralize the enamel and synthesize glucans that provide adhesion of other cariogenic bacteria to the tooth surface. Therefore, bacterial control is important in preventing caries formation. In studies, it has been determined that propolis is very effective in destroying or reducing the effectiveness of bacteria that are effective in the formation of dental caries. (19, 20). Park et al. In their study examining the effect of propolis on the proliferation and enzyme activities of S. mutans, they showed that propolis highly inhibited bacterial growth and glucosyltransferase synthesis (21).

Propolis has been used successfully in the treatment of inflammatory lesions of gingival-periodontal tissues and buccal mucosa (such as gingivitis, periodontitis, aphthous ulcers, stomatitis, glossitis) without being exposed to the side effects of other drugs (19, 22).

Al-Shaher et al. reported that aqueous solutions of propolis prepared at 4mg/ml or lower concentrations showed minimal toxicity against periodontal ligament cells and pulp fibroblasts, whereas calcium hydroxide at the same concentrations was highly toxic (23). In one study, it was stated that propolis stimulated the healing process and reduced tissue inflammation in the treatment of direct pulp capping (24). Silva et al. reported that propolis can be easily used as an intracanal drug in endodontic treatments (25).

Propolis has started to be used for prophylactic purposes for caries and periodontal diseases by adding it to toothpastes, mouthwashes, dental floss and gums. It has been reported that toothpastes with propolis are very good plaque cleaner, prevent plaque formation and have anti-inflammatory effects (26). While the topical application of propolis accelerates epithelial healing after tooth extraction, it has not been found to have an effect on socket wound healing (27).

It has been reported that the teeth can be stored in propolis solutions in order to maintain the vitality of the periodontal ligament cells until the treatment procedures are started in cases of avulsion, and that the best alternative to storage media such as milk and saliva is propolis solutions (28).

In the study of Dualibe et al. (2007), in their study with propolis-containing mouthwash, it was reported that the amount of S. mutans was 49% lower in saliva samples collected before and after mouthwash use (29). Netto et al. (2013), in a study in which propolis-containing mouthwashes were compared with chlorhexidine-containing mouthwashes, it was found that propolis-containing mouthwashes were more effective than chlorhexidine in reducing the number of S. mutans and Lactobacillus (30).

Chitosan

Chitosan has been defined as a natural biopolymer with a chemical structure closest to cellulose, which is common in nature. It is stated that it is obtained from the cell walls of arthropods, crustaceans, fungi and yeasts. It is known that chitin is a natural cationic polysaccharide found in the shells of crustaceans, fungal cell walls, and insect cuticle, similar to chitosan. It has been reported that it has some biological activities such as antimicrobial and hemostatic activity (31).

It has been reported that many years ago, Koreans ground the shells and bones of sea creatures into powder and applied them to bone and skin wounds. It has been emphasized that chitosan is a useful derivative of chitin. (32).

Antimicrobial activity of chitosan on certain fungi and bacteria has been described, depending on the type, degree of polymerization, physical and chemical properties of chitosan. It has been determined that chitosan with short chain length has reduced antimicrobial activity, and partial depolymerization of chitosan increases its antibacterial properties. It is stated that the degree of deacetylation affects the activity of chitosan. It was emphasized that chitin constitutes the majority of the dry weight in the exoskeleton of crustaceans. It has been reported that chitosan is found in nature in lesser amounts than chitin (32, 33).

It is emphasized that the main interest in chitosan derivatives stems from their cationic structure in acidic solutions. It is known that this structure allows chitosan to be used in other water-based treatments. It has been determined that chitin can dissolve in the soil without harming the environment and is biocompatible. It has been reported that chitin has biological properties such as cell viability and antitumor activity (33-35).

Chitosan; It has been stated that it has properties such as promoting wound healing, absorbing heavy metals and absorbing dangerous oral microorganisms. It has been determined that the effect of chitin on wound healing is accelerating. Chitin derivatives. It has been stated that the glycosaminoglycan components of the scar tissue have a role in the restructuring of the newly formed collagen in the granulation tissue of the healing wound. It has been reported that another mechanism by which chitin derivatives affect wound healing is related to macrophages. Along with these, chitosan and its derivatives; It has been stated that it has healing-accelerating effects during tissue regeneration and is also bactericidal (33, 34).

Use of Chitin and Chitosan Derivatives in Dentistry

It has been stated that chitosan is used in the prevention of dental caries due to its bacteriostatic and bactericidal properties. At the same time, it has been stated that chitosan can buffer the effects of organic acids that lower the pH values in the mouth (36).

The role of S. mutans, which is the most important etiological factor in dental caries, in the early stage of dental caries has been defined and attributed to its ability to colonize the tooth surface. It has been reported that the basis of the proposed approach for the prophylaxis of caries at an early stage is the inhibition of HAP binding and colonization of S. mutans. It has been emphasized that chitosan, which is an N-deacetylation derivative of chitin, is important because it protects against the harmful effects of organic acids, stimulates the regulated regeneration of oral soft tissues, and shows bactericidal properties against certain pathogens (32, 37).

It has been disclosed that chitosan-modified chitosans have a negative logarithm of the acidity constant Ka (pKa) of 6.3. It has been reported that this pH value is suitable for buffering at high oral pH value and is sufficient to protect against the destructive effect of organic acids. It has also been shown to have a bactericidal effect against many pathogens, including S. mutans. Chelation of basic metal ions and formation of polyelectrolyte complexes with bacterial surface components, enzyme inactivation have been reported to be among the mechanisms of its bactericidal effect (35-37).

Antibacterial compounds; It has been reported that host sub-minimal inhibitory concentration (sub-MIC) impairs the production and function of bacterial adhesins, as well as inhibits the attachment and colonization of bacteria to host tissues. Therefore, considering that chitosan sub-MICs can be obtained in the oral cavity when mouth rinses and toothpastes containing these polymers are used, it has been discussed whether the binding of S. mutans to HAP can be reduced by chitosan sublethal concentrations (36, 37).

Ong et al. (2017) evaluated the role of chitosan-propolis nanoparticles for their antimicrobial and anti-biofilm properties against E. faecalis. With this formulation, E. faecalis inhibited biofilm formation and it was concluded that it reduced the number of bacteria in the biofilm. They reported that the formulation not only reduced bacterial numbers, but also physically disrupted the biofilm structure, as observed by scanning electron microscopy. It was determined that exposure of biofilms to formulation containing chitosan-propolis nanoparticles changed the expression of biofilm-related genes in E. faecalis. As a result, it is thought that chitosan-propolis nanoformulation can be considered as a potential anti-biofilm agent in resisting infections involving biofilm formation such as chronic wounds and surgical site infections (38).

Fluoride

Fluoride is widely used in the prevention of dental caries. The effect of fluorine on preventing tooth decay occurs in different ways. Fluor prevents demineralization of enamel as a result of acid attacks and provides remineralization. In addition, it inhibits bacterial metabolism, prevents acid production and increases plaque pH (7, 39). Fluor ions replace the hydroxyl (OH^-) group in apatite crystals, forming fluorapatite crystals with lower solubility and more resistance to caries. In topical fluorine applications, CaF_2, a loosely bound fluorine compound, is formed on the enamel surface. CaF_2, which accumulates in tissues such as plaque and saliva, helps remineralization by providing fluorine ions in case of enamel demineralization (40, 41).

It is known that the acid that emerges during the activities of cariogenic bacteria lowers the oral pH below the critical level of 5.5, and then the demineralization process begins. Initially, it was stated that this process is reversible. If there is an F- ion in the plaque fluid, it has been reported that this ion participates in the crystal structure of the enamel and prevents the dissolution of Ca_2^+ and $PO4^{-3}$ from the tooth enamel. It is emphasized that when the pH rises above the critical level, F^- ions join the HAP structure and form a more durable crystal, fluoroapatite. In this way, it was stated that remineralization took place. At the same time, it is stated that F^- ions contribute to the prevention of demineralization indirectly by disrupting the microbial cell physiology (42).

High to moderate evidence has been reported that fluoride technologies and fissure sealants are primarily effective in the prevention of dental caries. In terms of secondary caries formation, the evidence has been shown to be weaker. It has been reported that the role of fluoride in caries prevention is undisputed today. It has been announced that fluoride is the only compound accepted as a caries preventative by the Food and Drug Administration (FDA) (43).

Fluoride Application Methods

Systemic Fluoride Applications

It is known that systemic fluoride applications have been used around the world for many years. Although systemic fluoride applications have decreased with the widespread use of fluoride toothpastes in recent years, the use of systemic fluoride supplements is still recommended by the European Academy of Pediatric Dentistry (EAPD) (2009). Accordingly, it has been emphasized that the use of systemic fluoride supplements should be considered if the daily intake of fluoride with drinking water is less than one unit per 0.6 million (ppm) (44).

Fluoridation of Drinking Water

Fluoridation of drinking water; It is expressed as bringing fluoride, which is naturally present in waters, to an optimal level for dental health. Fluoridation of water has been reported as an effective method for caries prevention (44).

Fluoridation of Milk

The EAPD shows that fluoridated milk has caries-reducing activity, according to evidence-based systematic reviews on milk fluoridation in 2009. It has been stated that the optimal fluoride concentration in milk should be 2.5-5.0 ppm (44).

When the effectiveness of different concentrations of fluoridated milk on enamel remineralization was investigated, it was found that milk containing 1.0 ppm fluoride was clearly effective in remineralization. It was found that the remineralization efficiency increased up to 5.0 ppm (45).

Fluoridation of Salt

It has been reported that fluoridated salt is used in more than 30 countries in the world and such use of systemic fluoride is recommended by the World Health Organization (WHO). It is stated that the salt is fluoridated to contain 250 milligrams (mg) of fluoride per kilogram. Although fluoridation of salt has a caries-preventing effect, it should be noted that children in the younger age group may not be able to benefit from this effect sufficiently because they consume less salt (44).

Fluoride Tablets and Drops

The American Academy of Pediatric Dentistry (AAPD) (2012) stated that the use of fluoride tablets should be considered for children older than 6 months whose daily fluoride intake dose is below 0.6 ppm. The daily fluoride intake dose of the child should be determined by taking into account the drinking water and diet; Accordingly, it was emphasized that it is necessary to calculate the required fluoride tablet dose. It has been stated that it is recommended that the child chew or suck the tablets in order to obtain a topical effect in addition to the systemic effect (46). In 2009, EAPD stated that if the daily amount of fluoride taken is between 0.3 and 0.6 mg, there will be no need for additional fluoride tablet/drop support for the use of fluoride toothpaste in the 2-3 age group. It has been reported that 0.25 mg fluoride support can be used in higher age groups (44).

Topical Fluoride Applications

In 2012, AAPD stated that before deciding on the frequency of professional topical fluoride application, it is necessary to determine which risk group the patient is in. Every 6 months for patients in the intermediate risk group; it has been reported that topical fluoride application is required every 3-6 months for patients in the high-risk group (46).

Fluoride Varnishes, Gels and Mouthwashes

The effectiveness of fluoride gels and mouthwashes on primary teeth is not certain, but they have a caries preventive effect on permanent teeth; It has been emphasized that gels and mouthwashes should not be used in children younger than 6 years of age due to the risk of swallowing. Fluoride varnishes, on the other hand, have caries-preventing effects on primary and permanent teeth; It has been reported that it can also be used in children younger than 6 years old (44).

Fluoride Toothpastes

EAPD stated in 2009 that one of the main reasons for the significant decrease in dental caries rates in recent years is the use of fluoride toothpastes. Use of fluoride toothpaste; It is an ideal public health method in terms of ease of use, prevalence, cheapness and traditionality. One of the harms of using fluoride toothpaste is the risk of swallowing by young children. Children under 3 years of age should be careful in this respect. Parents should be informed that only a pea-sized amount of paste should be used and the child should be accompanied while brushing teeth until at least seven years of age. In order to prevent a possible risk, the use of paste containing less fluoride in children may be considered, but it has been reported that the paste must contain at least 500 ppm fluoride in order to have anti-caries effectiveness (44).

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