Focus on the cariogenic process: microbial and biochemical interactions with teeth and oral environment

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This paper aims to describe the biochemical interactions between teeth and the oral environment that occur during the caries process, hence it covers all the steps related to physico-chemical reactions, including the most up-to-date theoretical basis in the clinical application for the prevention and treatment of caries. The terms 'demineralization' and 'remineralization' that characterise this process were analysed, as well as the role of the microbiota in its interaction with the hard surface of the teeth. The biochemical mechanisms that lead to the onset of carious lesions and those that occur during the healing and repair of such lesions are listed.

Dental caries is the destruction of the dental structure and occurs on any dental surface where dental plaque grows for a long time. The creation of one of the dental plaques is an example of a biofilm that occurs through a natural process and is a community of interacting microbes such as *Lactobacillus* spp, *Candida* spp (1-3). The crown of the teeth can be further divided into separate areas and special ecological sites, each of which are characterized by higher or lower specific risk for the development of caries. Some of these sites host microbial communities that produce acids and can therefore withstand an acid environment. The composition of the dental microbiome is influenced not only by the specific position of each tooth in the mouth and its proximity to the flow of saliva (from neighbouring ducts), but also by the anatomy and physiology of the tooth surface and surrounding teeth (1). Most caries lesions occur in: (a) the cavities and fissures of the chewing surfaces of the posterior teeth; (b) the buccal and palatal cavities of the posterior and anterior teeth; (c) the adjacent surfaces, as well as from the point of contact of the teeth; (d) the cervical area of the teeth near the tooth gap; and (e) in the ossified root surface of the gums from gingivitis (1,4) (Fig. 1).

A change in pH (beyond the physiological value) can affect the integrity of the tooth surface through

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biomolecular mechanisms and physico-chemical reactions. Some oral microbes could produce acids by metabolizing dietary carbohydrates, thus creating a lowering of the pH in the surrounding environment. The humid environment created mainly by the secretions of the oral cavity and maintained through the salivary glands with the secretion of saliva has a protective role, because it has the ability to neutralize acids and increase the pH and recover the ions lost by the teeth (5-7). Furthermore, the part of the teeth exposed to this humid environment causes the formation of a solid-liquid phase system between the hard tissues of the tooth and the saliva, which is governed by biochemical laws, and is in a state of constant dynamic physic-chemical equilibrium. Under

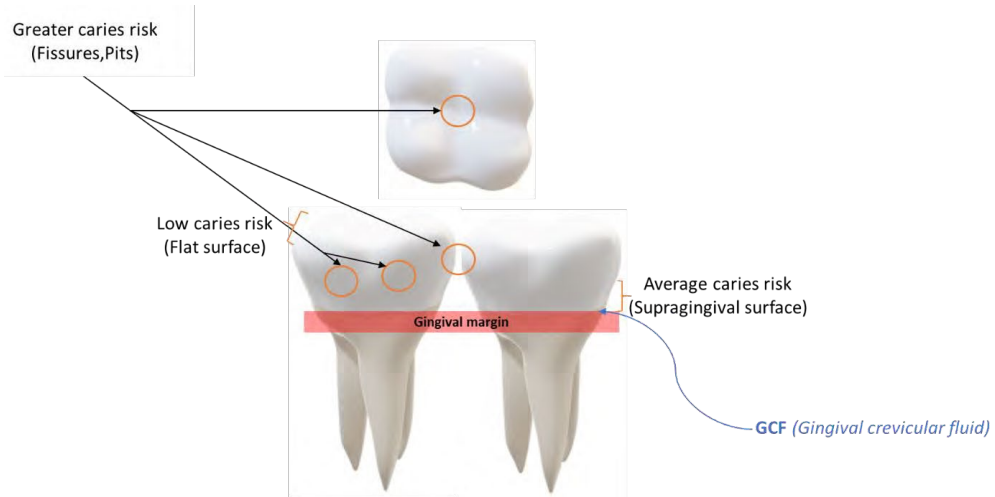

Fig. 1. *The main sites where caries lesion occurs. GCF: Inflammatory exudate of the gingival crevicular fluid is composed of serum that contains degenerated waste tissues from the periodontal areas, inflammatory mediators, and antibodies against dental plaque bacteria.*

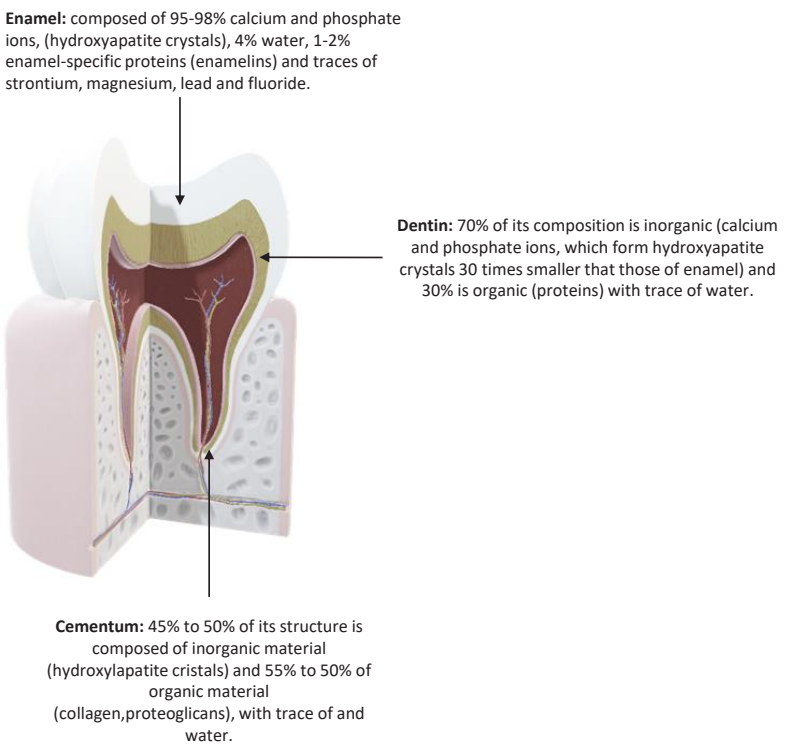

Fig. 2. *Hard tissues of human teeth and their chemical composition*

normal conditions, saliva contains an important number of various ions present also in the salts of the inorganic portion of the hydroxyapatite of the enamel. Thanks to this balance, the hard tissue of the tooth maintains its natural conformation and structural integrity (4, 5, 8) (Fig. 2).

ORAL MICROBIOME AND ITS ROLE IN DENTAL CARIES

The microbial risk factors that develop dental caries

Caries is mainly linked to lifestyle (e.g., diet, alcohol, tobacco, abuse of drugs such as cocaine, methamphetamine and others), socio-economic and behavioural factors such as poor oral hygiene (9, 10). The consumption of large quantities of carbohydrates causes an excessive production of acids that saliva is no longer able to regulate, leading to a continuous decrease in pH. This acidic environment in the oral cavity induces the production of protein enzymes causing changes in the qualitative and quantitative composition of the microbiota, and this also leads to the survival of microorganisms growing in a low pH (acidic) environment (11, 12). Hence, these microorganisms (such as *Streptococcus mutans*, *Lactobacillus* spp) also ferment sugars that produce more lactic acid. This leads to keeping the pH much more lower, thus creating favorable conditions for the damage of hard tissue of the teeth. It has been found that people with or without tooth decay share about 50% of germs. For many decades since the 1950s, *S. mutans* has been held solely responsible for tooth decay, however, several recent studies to determine whether the causative agent of the disease has produced vague results (13). *S. mutans* is often over-represented in white patches but is also present in some healthy people in small colonies, however, it appears to be associated only with the onset of caries and not with the development of caries. Furthermore, it appears to have the characteristics of a pathogen that is affected by dietary changes. In some patients with caries, *Lactobacillus* spp. and *S. mutans* are low or undetectable, suggesting that the onset and progression of lesions cannot be attributed to *S. mutans*. *S. mutans* has several virulence factors that allow it to establish itself in the oral cavity such as the cell surface localized the Ag I/II family proteins (**P1**, SpaP, AgB or **PAc**). Hence, the expression of P1 protein is necessary for the adhesion to hydroxyapatite which occurs during aggregation of **S**. **mutans** to saliva-coated materials. This is the first step towards its colonization in the tooth surface and the formation of dental plaque. During this phase, there is a change in the initial composition of the microbiome with the loss of a part of the microbial populations useful for eubiosis, leading to the development of caries (14, 15). Thus, organic acids derived from the hydrolysis of disaccharides, such as sucrose, and associated with caries also influence enzymatic reactions. Caries, in turn, reduces the diversity of microbes in relation to periodontal disease, possibly due to the acidic environment, thus limiting microbial growth in organisms that do not grow in an acidic environment. The bacteria that cause tooth decay use an enzyme, glucansucrase (or glucosyltransferase), to build long, sticky sugar chains called glucans that use the same sugars we introduce with food as the starting material. These glucans make bacteria adhere to the surface of the teeth, forming and fixing the biofilm. Glucansucrase catalyses two reactions: in the first one the enzyme reacts to sucrose to produce glucose and fructose; and in the second it releases fructose, and the enzyme binds glucose to the glucan chain. Glucan sucrase is a large protein with many sites of action that serve to anchor it to other glucan chains, however, these reactions take place in the central part of the enzyme. It has been noted that the synthesis of extracellular glucan largely depends on the part of *S. mutans*. Hence, glucansucrase catalyses glucan, instead there are proteins that bind glucan known as the glucan-binding proteins (Gbps). The functions of Gpbs include dextran-dependent aggregation, dextranase blockade, plaque adhesion, and possibly cell wall synthesis (16-18). The properties of the GBPs are related to the biological functions of *S. mutans* in the oral cavity. *S. mutans* also has other virulence factors such as collagen binding proteins (CBP) which are the Cnm and Cbm gene. These proteins, along with SpaP and WapA, are important cell surface antigens also linked to its adherence to collagen tissue. Furthermore, CBP + strains of *S.*

mutans have been observed to adhere early and are associated with systemic diseases such as infective endocarditis and others. Finally, other factors associated with the development of caries can be various immune and genetic factors (19-21).

Saliva microbiome and teeth interactions

The oral cavity secretes saliva that contains minerals, organic ingredients (amino acids, mucoproteins, carbohydrates, and others) and antimicrobial action proteins (such as lysozyme, lactose, cysteines, histamines, galactoxin, lactoperoxidase and calicidin). Therefore, the host's defenses against plaque occur through antimicrobial proteins together with immune ones, such as antibodies that reach the oral cavity through the salivary ducts. These factors play an important role in the formation and synthesis of the oral microbiome. The mucous membranes on the surface of the oral cavity and teeth are constantly immersed in saliva. The proteins and glycoproteins present in saliva lubricate, contribute to the sense of taste, facilitate chewing for the formation of the food bolus and support the formation of bio membranes (22). Microorganisms do not directly colonise the surfaces of the teeth. Teeth are always covered with a cell-free protein coating, the pellicle, which forms on the tooth surface. The acquired enamel pellicle formed by selective absorption of glycoproteins, phosphoproteins, lipids and bacterial derivate molecules on the surface of the enamel (23) (Fig. 3).

When there is no food in the oral cavity, microorganisms can break down these specific glycoproteins which are the source of their nutritional needs. The germs of saliva are those that come from the microbiota of the tissues of the oral environment. All epithelial surfaces peel off, releasing relevant germs in the saliva, however, the saliva microbiome is mostly enriched by microorganisms from the biomembrane of the tongue, such as *Prevotella*

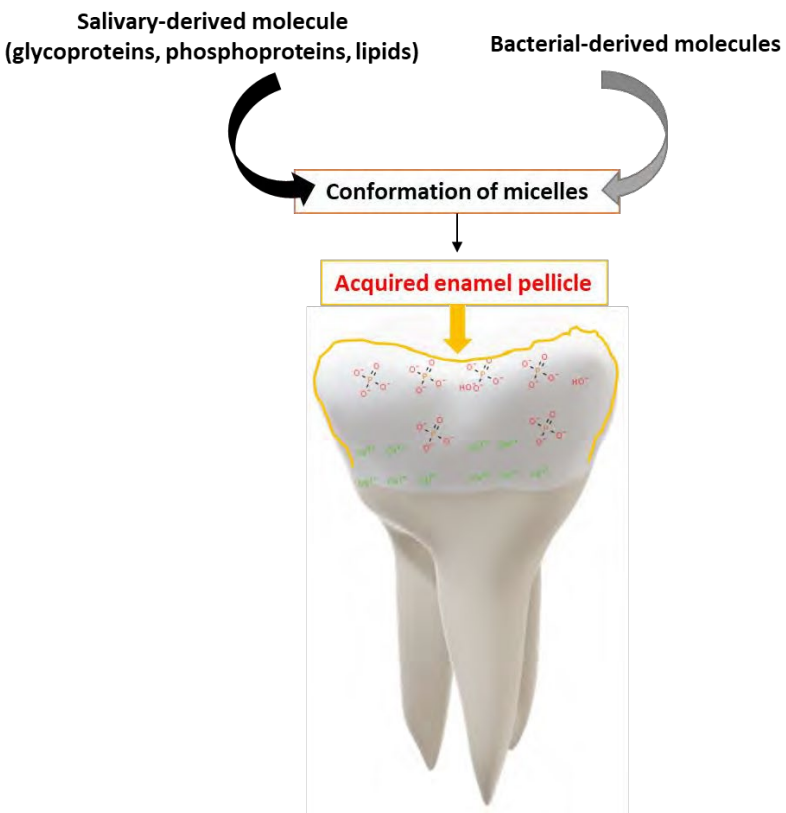

Fig. 3. *Example of the biochemical approach of the adsorption of salivary components to hydroxyapatite crystals and the formation of the enamel pellicle.*

and *Streptococ*cus (24, 25). Bacteria associated with caries, such as *Lactobacillus* spp, *S. mutans*, *Bifidobacterium* spp, *Atopobium* spp., have not been detected in subjects with healthy teeth. If the dental plaque is not removed by frequent brushing, the sugar-eating plaque bacteria release acids that destroy tooth enamel to form caries (26-29). Other genera have a presence of around 1% and are considered in any case transient or non-native microorganisms, thus saliva proteins and glycoproteins influence the formation of the bio membrane. These proteins can promote microbial adhesion because the salivary membrane and its constituent proteins overlap the teeth and mucous membranes. As already mentioned, saliva also contains antimicrobial proteins that can affect bioavailability by limiting the growth of other microorganisms (30). The tooth surfaces provide a stable attachment position for long-term bio membrane development. The tooth enamel is covered with a sialic membrane and the roots are coated with saliva and whey proteins. Consequently, the microbial populations of the plaque formed in the enamel that is supra gingival and above the gum line differs from the community below the gum line. As community development extends along the root and away from the salivary environment, the biofilm contains more serum, the environment becomes more anaerobic and the cleaning of the surface reduced. The microbial community of the tooth surface (crown) has a somewhat different composition from that of the saliva (such as for **Streptococcus** spp. and **Veillonella spp.**) (28, 31, 32).

Oral dysbiosis

As already stated, a disturbance in the balance of the oral and salivary microbiota leads to a condition of eubiosis to an irregular increase in certain bacterial strains that can cause tooth decay. This is the condition of oral microbiota dysbiosis. A community in microbial conditions of dysbiosis can deflect the host's immune response, resulting in local induction of nonspecific inflammatory reactions and subsequent tissue damage. This inflammatory process has as a consequence an increase of Gingival crevicular fluid (GCF) on small local haemorrhagic ulcers that, in turn, will deprive area of oxygen. This encourages the development of anaerobic bacteria (optional and mandatory) present in the gum cracks. This produces a worsening of the microbiota dysbiosis, with a consequent formation of numerous colonies of certain bacterial strains such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivali*s (associated with periodontitis), *Scardovia* spp., *Actinomyces* spp., *S. mutans*, and *Bacteria mutans,* as well as fungi such as *Candida albicans* (33-36). Finally, oral dysbiosis may have effects on other organs of the human body or even systemic consequences. It has been noted that the increase of some bacterial strains such as *P. gingivalis* have altered the composition of the intestinal microbiota qualitatively and quantitatively, resulting in inflammatory reactions in other parts of the body. This is because the intestinal microbiota is one of the most important metabolic bio-stations and helps the host to an effective immune response. There is not only a relationship between oral and gut but there is also a communication between other organs which are called the cross-talking axes, Gut/lug, Gut/skin, and Gut/brain axes. Oral bacterial natural flora can play an important role in the pathogenesis of various systemic diseases and carcinogenic conditions (e.g., inflammatory bowel diseases, cardiovascular, rheumatoid arthritis, diabetes, colon cancer). The periodontal disease is known to have a negative effect on blood sugar control in diabetes mellitus, and healthy individuals have been found to have a higher risk of developing type II diabetes (37-43).

The use of friendly bacteria called probiotics is recommended. Probiotics fight the spread of pathogens, strengthen the microbiota and contribute to the creation of a strong immune system, which helps to heal a disease in a natural way. The concept of probiotics was first used by Parker (1974) to describe the type of food which has a positive effect on the host thus contributing to the eubiosis of the gut microbiota. The Food and Agriculture Organization/Word Health Association (FAO/WHO) in 2001 identified probiotics as live microorganisms which, when assumed in sufficient quantities as part of a diet, have a beneficial effect on the host (44, 45). The most used probiotic species are *Lactobacillus* and *Bifidobacterium*. Probiotics restore the dysbiosis by introducing good bacteria that fight the bacterial

proliferation of the not-friendly ones. Probiotics help balance the pH in the mouth and protect the microbiota from acids produced by certain bacteria that destroy tooth enamel, and reduce sulphur-producing bacteria found mainly in the back of the tongue, thus preventing bad breath. Finally, probiotics help to restore the natural and healthy production of saliva in the mouth thus preventing xerostomia (46-48).

Regarding the goal of non-invasive caries treatment in addition to probiotics, many strategies have been studied to date in order to prevent caries. Several meta-analyses suggest that sealants or resin infiltrations with 5% sodium fluoride (NaF) toothpaste, or sodium fluoride gel (from 5.000 ppm F = 1.1% NaF) are valid for halting non-cavitated and cavitated carious lesions (9, 49). The use of chlorhexidine in caries prevention, such as a non-invasive management, represents another approach, however, its use for such prevention is controversial and several studies confirm the reduction of persistent plaque from *S. mutans* spp. by chlorhexidine varnish. Since dental caries is a disease with a multifactorial aetiology, it is increasingly appropriate to use evidence-based treatments, such as fluoride applications, complete oral hygiene, and an appropriate diet (50, 51). As already pointed out, chronic oxidative stress, the acidic pH on the enamel surface, together with the dissolution (demineralization) of calcium (Ca) and magnesium (Mg) can contribute to the development and progression of dental caries. It has been noted that zinc (Zn) plays a fundamental role in the bioavailability of Ca and Mg in the oral cavity as it helps the deposition of the two ions in the enamel (remineralization). It has been noted that the use of oral hygiene products such as toothpaste and mouthwash based on zinc salts (zinc chloride, zinc sulphate, zinc citrate, zinc lactate) can reduce the formation of plaque and block the formation of tartar. Finally, several studies are underway to develop vaccines. This means development of antibodies against various *S. mutans* antigens against caries formation (51, 52). Several *S. mutans* cell surface antigens have been pressed to create antibodies such as glucosyltransferase (GTF), AgA/C /D, AgI/II, AgIII, lipoteic acid (LTA), Gbps and dextrasucrase. Dextransacrase antibodies appear to reduce biofilm formation and thus plaque growth in the presence of *S. mutans* (53, 54).

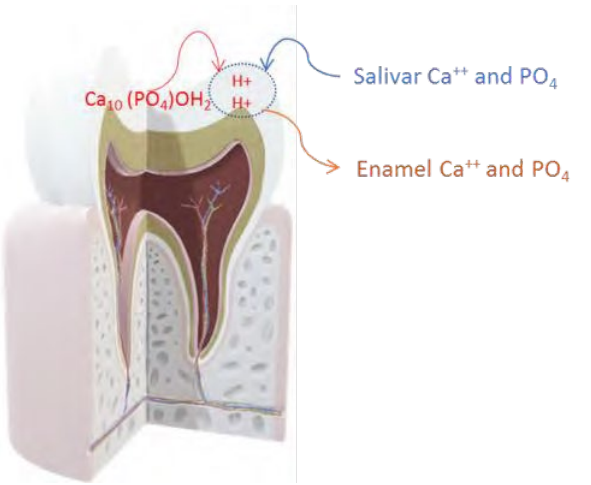

Fig. 4. *In the chemical imbalance of saliva surrounding the enamel (hyper saturation) there is a movement of calcium and phosphorus ions from the enamel into the oral environment (saliva). In restoring the chemical balance of the saliva surrounding the enamel (supersaturation), there is a movement of calcium and phosphorus ions from the oral environment (saliva) to the enamel. In restoring the chemical balance of the saliva surrounding the enamel (supersaturation), there is a movement of calcium and phosphorus ions from the oral environment (saliva) to the enamel.*

PHYSICO-CHEMICAL INTERACTIONS OF TEETH AND THE ORAL ENVIRONMENT

Crystal dissolution process

According to the chemical proprieties, all salt crystals show intrinsic and stable solubility in water at a given temperature. Their dissolution in water is initially rapid, but subsequently slows down when the released ions are concentrated in higher number in the saliva, resulting in the saturation of those ions. In this state of equilibrium, a slow ion exchange remains between the crystals and the solution (Fig. 4) (55).

Water molecules enter the crystallised inorganic surface and release its ions. This is mainly due to the high dielectric constant of the water, which causes a reduction in the forces of attraction between the various ions with opposite charges in the crystal lattice. According to the law of mass action, the rate of a chemical reaction is proportional to products of the masses of the reactive

components elevated to a force equal to the number of molecules participating in the reaction. When a specific mass of a solid hydroxyapatite dissolves, five calcium ions, three phosphate ions and one hydroxyl anion are released ($Ca_5 (PO_4) 3 (OH) \rightleftarrows 5\ Ca_2^{++}\ 3\ PO_4^{3-} + OH-$) (39,40). The main factors that influence the solubility product of a crystalline salt and consequently the dissolution are pH, temperature, and coexistence of other ions. Hence, the solubility of hydroxyapatite is higher in cold water. When hydroxyapatite dissolves, calcium and phosphate ions accumulate in the solution, and the dissolution of hydroxyapatite slows and stops as the solution becomes saturated. If found in an acidic pH environment, PO_4 ions 3- and OH- bind to H^+ and form HPO_4^{2-} and H_2O, respectively, resulting in reduction of PO_4 concentration 3- and OH- (50). The solution becomes saturated, and more hydroxyapatite dissolves to restore the chemical balance. When the solution reaches pH 5 of the solution surrounding the tooth, it is considered non-saturated, but when it reaches pH 7, the solution becomes oversaturated, and hydroxyapatite is formed. Subsequently the pH of a supersaturated solution gradually decreases, and the point where the solution becomes almost saturated is called the critical pH (Fig. 5) (56).

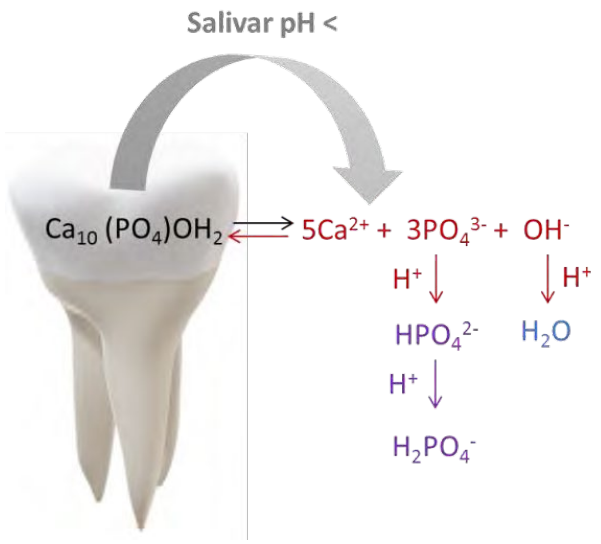

Fig. 5. *The dissolution of hydroxyapatite crystals is not isotropic. According to the equation shown in the figure, if there is an excess of one ion in the solution, less of the other ion is needed to achieve chemical equilibrium (this called the common ion effect). At normal pH (6.7-7.4), hydroxyapatite is the most stable form of calcium phosphate, however, after the eruption of the teeth, the hydroxyapatite of the outer surface of the enamel and dentin is exposed to a wide pH fluctuation due to dietary and metabolic conditions.*

Under favourable conditions, new crystals can therefore precipitate from the solution and these new and small crystals can increase in size in form of soluble salts or increase the pH. However, saliva contains elements that control and inhibit crystalline growth, such as pyrophosphates and various salivary glycoproteins such as statherin (STATH), acidic proline-rich proteins and stereochemical mechanisms e.g., in the enamel the hydroxyapatite crystals are so close together that there is insufficient space to grow and enlarge (4, 56, 57). Indeed, mesenchymal cell (MSC) research can be important for the treatment of various dental diseases. Mesenchymal stem cells of dental origin can be applied for regeneration and replacement of dental or oral tissues against caries, periodontitis, etc. Finally, the use of platelet-rich plasma and fibrin extracts in dental implant surgery may be useful.

Chemical changes in enamel during the caries process

Enamel crystals differ from pure hydroxyapatite as they also contain other inorganic ions. The structure of the enamelled hydroxyapatite allows the flow of external ions to sites that would normally be occupied by calcium ions, phosphates ions and hydroxyl groups. In some crystals, calcium ions are replaced by sodium ions, hydroxyl ions are replaced by fluoride ions, and phosphate ions are replaced by bicarbonate ions. Other ions can be chloride ion (Cl^-) and magnesium ion (Mg^{++}). These elements influence the biochemical properties of the structured crystals such as the solubility of the enamel. The presence of bicarbonate ions causes hydroxyapatite to be more soluble, while fluoride ions cause the opposite effect (62). It is important to mention that fluoride ions, when present in solution, drastically reduce the amount of Ca released by the enamel at acidic pH, so low concentrations of fluoride are effective in reducing tooth decay. Enamel crystals are small and have a relatively large surface area, so they have a high capacity to receive various foreign ions. All ions are absorbed on the surface, or near it, through a layer of water, consequently forming the hydration

zone. Enamel crystals are not only heterogeneous on the surface but also in their mass. Near the outer surface, they contain more fluoride and less carbonate ions than inside and its outer layer is less soluble. Saliva has a significant degree of phosphate (PO_4^{3-}) and calcium (Ca^{++}) ions and is usually also saturated with salts from the enamel (Fig. 6) (4, 63). Enamel does not dissolve at normal oral pH. In supersaturated conditions, the enamel crystals do not continue to grow or form new crystals on the tooth surface because saliva contains proteins that inhibit the growth of hydroxyapatite crystals. These proteins are statherin (a phosphoprotein), a protein with peptides rich in tyrosine and proline, that covers the enamel and protects it from the precipitation of limestone and the consequent crystalline growth. Dental plaque covers the enamel and generally prevents the tooth surface from coming into contact with saliva. This film contains biological fluid of the plaque and its chemical composition differs from saliva (the concentrations of calcium and phosphorus ions are 2-3-fold higher than in saliva). This supersaturated solution is beneficial for the remineralization of the underlying caries lesions but also creates the conditions for the growth of tartar (64-66). The microbial plaque is subjected to significant pH fluctuations, due to the metabolic activities of its microbial flora. When the pH value of the solution surrounding the tooth falls below the critical pH value, the liquid phase ceases to saturate in respect to the surface salts of the enamel with which it comes into contact. This condition involves imbalance between saliva and enamel, so initiating the process of salts from the enamel to the liquid phase (demineralization of the enamel). The average critical pH of the enamel which corresponds to the layer of liquids which are in direct contact with it is around 5.3-5.5. The increase in pH, which occurs with the intake of fermentable carbohydrates, keeps below the critical pH for a period of approximately 19-39 minutes, and the restoration of the pH is achieved by removing acids or neutralizing them from the saliva buffer systems. A certain amount of the acid produced by microbial plaque penetrates the enamel (67, 68). The balance comes about by melting some enamel and reshaping the hydroxyapatite if the supersaturation conditions are suitable. This means that in the tooth surface there is a continuous cycle of demineralization and

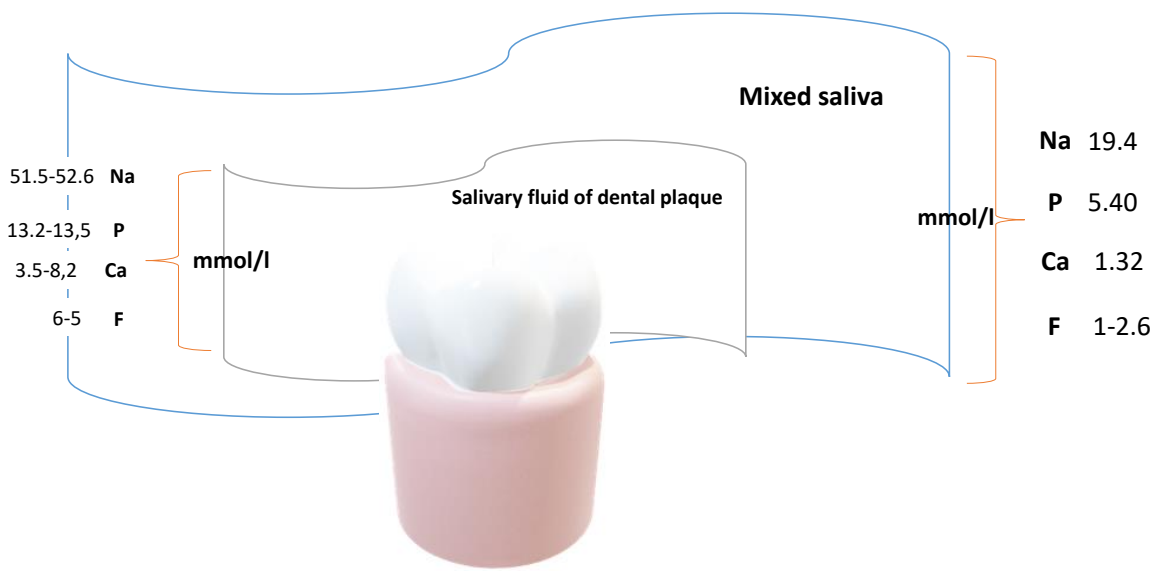

Fig. 6. *During metabolic activity, the salivary fluid of dental plaque has concentrations of minerals higher than in the mixed saliva. It should be noted that the concentrations of Ca and P minerals are 2-3 times higher. Resting mixed saliva is supersaturated compared to pure enamel hydroxyapatite and these concentration levels steadily increase when saliva flow is stimulated.*

remineralization, speed- and duration-dependent. The decrease in pH affects the solubility of the hydroxyapatite crystals and the critical pH of the environment decreases, resulting in in an important modification of its solubility. Hence, the solubility of hydroxyapatite will increase approximately 10 times for each pH unit decrease. During the formation of caries there is partial dissolution of the hard tissue, leaving a sub-superficial lesion body with a loss of 30-50% of minerals (which progresses deep into the enamel or dentin) and a well-defined 20-50 μm-thick calcified layer. When the pH decreases in oral fluids, due to thermodynamic and chemical kinetic properties of the crystals, bruxite appears on the surface of the enamel (67-69). Furthermore, the microbial plaque fluid remains supersaturated for fluor-hydroxyapatite (less soluble) and unsaturated for hydroxyapatite. Under these conditions, hydroxyapatite dissolves on the surface, and bruxite with fluorohydroxyapatite forms a layer on the enamel surface which is responsible for the long-term integrity of the superficial structure. Diffusion of H^+ ions in carious lesions is a slow layering process because the surface of the calcified lesion protects from both demineralization and remineralization. This external enamel layer, after remineralization, presents a disorganized structure (amorph tissue), in opposition to the normal differentiated crystalline structure of hydroxyapatite (65, 66). According to the salivary theory of Leimgruber, endogenous dental tissues are assimilated to a semipermeable membrane, thus dividing the saliva from the blood and interstitial pulp fluids. Bacterial plaque consists of a corpuscular fraction (bacteria, flaking epithelial cells) and an amorphous fraction (deriving from salivary glycoproteins and sugars introduced with the diet) which promotes bacterial adhesion to the tooth surface). We distinguish a supragingival plaque (responsible for caries and influenced by diet) and a subgingival plaque (responsible for periodontal disease). The presence of supragingival plaque exoenzymes, such as glycosyltransferases, glucosidases, pyrophosphatases and amino peptidases, facilitates dental damage. If the plaque is not removed, there is the formation of deposits of calcium and phosphorus salts, which transform it into tartar (70).

Chemical changes in dentin

Dentin has a chemical behaviour like enamel. The difference of dentin lies in the hydroxyapatite crystals, as they are smaller which makes the dentin surface more vulnerable to decay. Dentin has a high percentage of ions in the form of impurities and contains an important percentage of organic matter, mainly collagen. The acid produced by the germs has no effect on the collagen of the dentin. The degradation of its proteins begins with the action of proteolytic enzymes (proteinases or proteases) produced by cariogenic microbes. Another class of proteases that are activated during the caries process are metalloproteinases which are activated at acid pH and cause collagen degradation (64, 69).

CONCLUSION

The approach to dental pathologies such as caries has always concerned prevention, aetiology, histopathology and the diagnostic means, while the treatment has mainly concerned dental surgery. However, tooth decay is a disease with characteristics common to other diseases. It is assumed that an in-depth knowledge of the aetiology of caries and of all the biomolecular/biochemical mechanisms on which the appearance and development of damage depends is required for a better approach. In particular, the microbiota of the teeth and saliva plays an important role because it regulates the health of the hard surface of the teeth and protects it from "bad" bacteria. Probiotics could prevent dental diseases via the beneficial properties of oral cavity commensal bacteria. Prevention and diagnosis in the earliest stages is crucial. Indeed, the treatment of caries, in addition to prevention and restoration of the lesion, now include new approaches with non-invasive therapies

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