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Mechanisms of Action of Fluoride for Caries Control

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Abstract

Fluoride was introduced into dentistry over 70 years ago, and it is now recognized as the main factor responsible for the dramatic decline in caries prevalence that has been observed worldwide. However, excessive fluoride intake during the period of tooth development can cause dental fluorosis. In order that the maximum benefits of fluoride for caries control can be achieved with the minimum risk of side effects, it is necessary to have a profound understanding of the mechanisms by which fluoride promotes caries control. In the 1980s, it was established that fluoride controls caries mainly through its topical effect. Fluoride present in low, sustained concentrations (sub-ppm range) in the oral fluids during an acidic challenge is able to absorb to the surface of the apatite crystals, inhibiting demineralization. When the pH is re-established, traces of fluoride in solution will make it highly supersaturated with respect to fluorhydroxyapatite, which will speed up the process of remineralization. The mineral formed under the nucleating action of the partially dissolved minerals will then preferentially include fluoride and exclude carbonate, rendering the enamel more resistant to future acidic challenges. Topical fluoride can also provide antimicrobial action. Fluoride concentrations as found in dental plaque have biological activity on critical virulence factors of *S. mutans* in vitro, such as acid production and glucan synthesis, but the in vivo implications of this are still not clear. Evidence also supports fluoride's systemic mechanism of caries inhibition in pit and fissure surfaces of permanent first molars when it is incorporated into these teeth pre-eruptively.

The multifactorial disease dental caries is caused by the simultaneous interplay of different factors – dietary sugars, dental biofilm and the host – within the context of the oral environment. The complex and long-lasting interactions of these factors and how they lead to caries was already described half a century ago [1]. With our current understanding, the most obvious way to fight caries is to control the causal agents by removing the dental biofilm and reducing sugar consumption. These approaches form the basis of comprehensive protocols to control the disease, but have been proven insufficient to lead to a desired level of prevention because they strongly rely on patient compliance. Even before a complete understanding of the etiology of dental

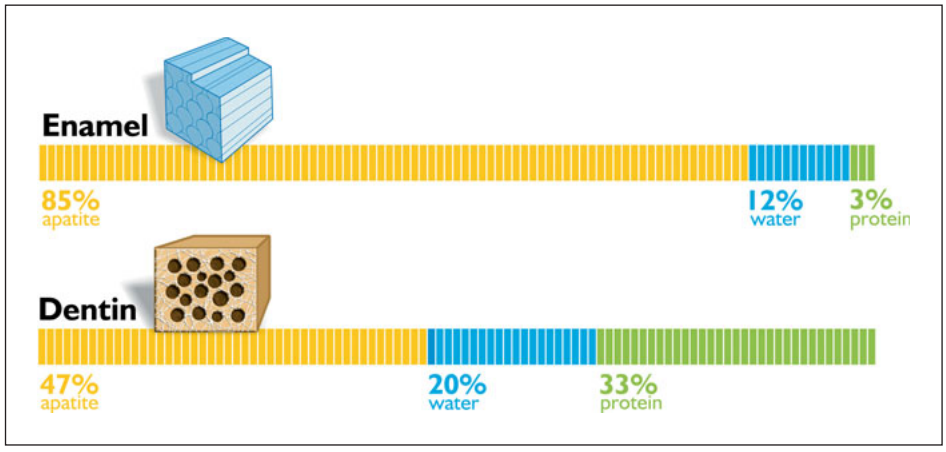

Fig. 1. General composition of dental enamel and dentin.

caries was reached, fluoride had emerged as a pivotal adjunct to combat the disease [2]. Fluoride is currently recognized as the main factor responsible for the significant decline in caries prevalence that has been observed worldwide [3]. On the other hand, excessive fluoride intake during the period of tooth development may cause dental fluorosis, the only proven side effect of the use of fluoride of dental relevance [4]. An increase in the prevalence of dental fluorosis has been reported concomitantly with the decrease in caries [5–7]. Although most of this fluorosis is mild or very mild, and has little or no impact on quality of life of affected people [8], a judicious use of fluoride to avoid moderate and severe fluorosis is needed. Thus, in order that the maximum benefit of fluoride for caries control can be achieved with a minimum risk of side effects, it is necessary to have a comprehensive understanding of the mechanisms by which fluoride promotes caries control.

Biochemistry of Caries Development

Dental caries is the net result of consecutive cycles of de- and remineralization of dental tissues at the interface between the biofilm and the tooth surface, with demineralization being caused by the production of acids by oral bacteria after sugar consumption [9]. To understand how the acids can attack the dental tissues, it is fundamental to know their biochemical properties.

Composition of Enamel and Dentin

Despite the presence of common constituents, enamel and dentin have different structures that will affect caries progression within these tissues as well as the reactivity of fluoride with them.

Permanent enamel is an acellular tissue composed chiefly of minerals (calcium-deficient carbonated hydroxyapatite, 85% in volume). Hydroxyapatite molecules are arranged in long and thin apatite crystals, which in turn are organized into the resulting enamel prisms (fig. 1). Despite the high mineral content, the space between the crystals is occupied by water (12% by volume) and organic material (3% by volume) [10, 11]. It is in this space filled with the enamel fluid that the de- and remineralization reactions take place. In brief, upon a cariogenic challenge, hydroxyapatite crystals are dissolved from the subsurface, while fluorapatite crystals are deposited at the surface, thus resulting in a subsurface

lesion. The dissolution process of enamel is therefore a chemical event.

On the other hand, permanent dentin contains (by volume) 47% apatite, 33% organic components and 20% water (fig. 1). The mineral phase is also hydroxyapatite, similar to enamel, but the crystallites have much smaller dimensions than those found in enamel. As a consequence, the ratio surface area/crystallite volume is larger, which makes the mineral phase more reactive. As a result, dentin surfaces are more susceptible to caries attack than enamel surfaces. The organic matrix is mainly composed of collagen (90%), but there are many non-collagenous components that determine the properties of the matrix and interfere with de- and remineralization reactions. Collagen forms the backbone of dentin and serves as a template for the deposition of apatite crystallites within the collagen helix. This kind of structure promotes a synergism between matrix and apatite: the mineral phase cannot be completely dissolved during an acid attack and the matrix does not undergo enzymatic degradation while its surface is still protected by apatite [11]. Dentin caries is thus a biochemical process characterized initially by the dissolution of the mineral, which in turn exposes the organic matrix to breakdown [12–15] by bacterial-derived enzymes as well as by host-derived enzymes such as matrix metalloproteinases present in dentin and saliva [16, 17]. It is also important to highlight that dentin is a cellular tissue and that upon exogenous challenges the pulpo-dentinal organ responds with mineral deposition [18]. This process, combined with the flow of dentinal fluid from the pulp, reduces the rate of lesion progression in dentin in vivo [19].

Dental Mineral Dynamics

The reason why caries progresses slowly is due to the high supersaturation of saliva with respect to enamel mineral under physiological conditions. This can be easily understood when the concentrations of free ions required to form hydroxyapatite normally available in saliva are compared with the concentrations that are necessary to reach saturation and form this mineral. The solubility product of enamel (KSP_{enamel}) which is related to the concentrations of Ca^{+2}, PO_4^{-3} and OH^- required for the formation of enamel crystals, has been calculated at 5.5×10^{-55} mol^9/l^9 at 37°C, slightly higher than that required to form hydroxyapatite (KSP_{HA} 7.41×10^{-60} mol^9/l^9). Under physiological conditions (pH 7.0), based on the salivary concentrations of free Ca^{+2}, PO_4^{-3} and OH^- that are available to form enamel crystals, the ion activity product of hydroxyapatite (IAP_{HA}) has been calculated at 6.1×10^{-48} mol^9/l^9 [11]. Therefore, if the IAP_{HA} in saliva under physiological conditions is higher than the concentrations required to form enamel crystals (KSP_{enamel}) this implies that enamel mineral does not dissolve in saliva (fig. 2a). Contrarily, enamel crystals would be expected to grow or new crystals would be expected to form at the biofilm-free tooth surfaces. This does not happen because saliva contains proteins that inhibit hydroxyapatite crystal growth, including statherin and many proline-rich proteins [20].

When a biofilm is covering the enamel surface, it reduces the access of saliva to the tooth. The relevant fluid phase in this case is the biofilm fluid which, under resting conditions, is also supersaturated with respect to enamel (IAP_{HA} 1.4×10^{-47}). This would favor remineralization of previously demineralized enamel or promote the formation of supragingival calculus (fig. 2b).

The characteristics of the plaque fluid microenvironment change considerably upon a sugar challenge. In this case, bacteria produce lactic acid that makes the plaque fluid pH fall (typically between 4.5 and 5.5). The driving force is then shifted to mineral dissolution. But why does this happen if saliva is continuously secreted with relatively stable Ca^{+2} and PO_4^{-3} concentrations, which would apparently maintain IAP_{HAP} unaltered?

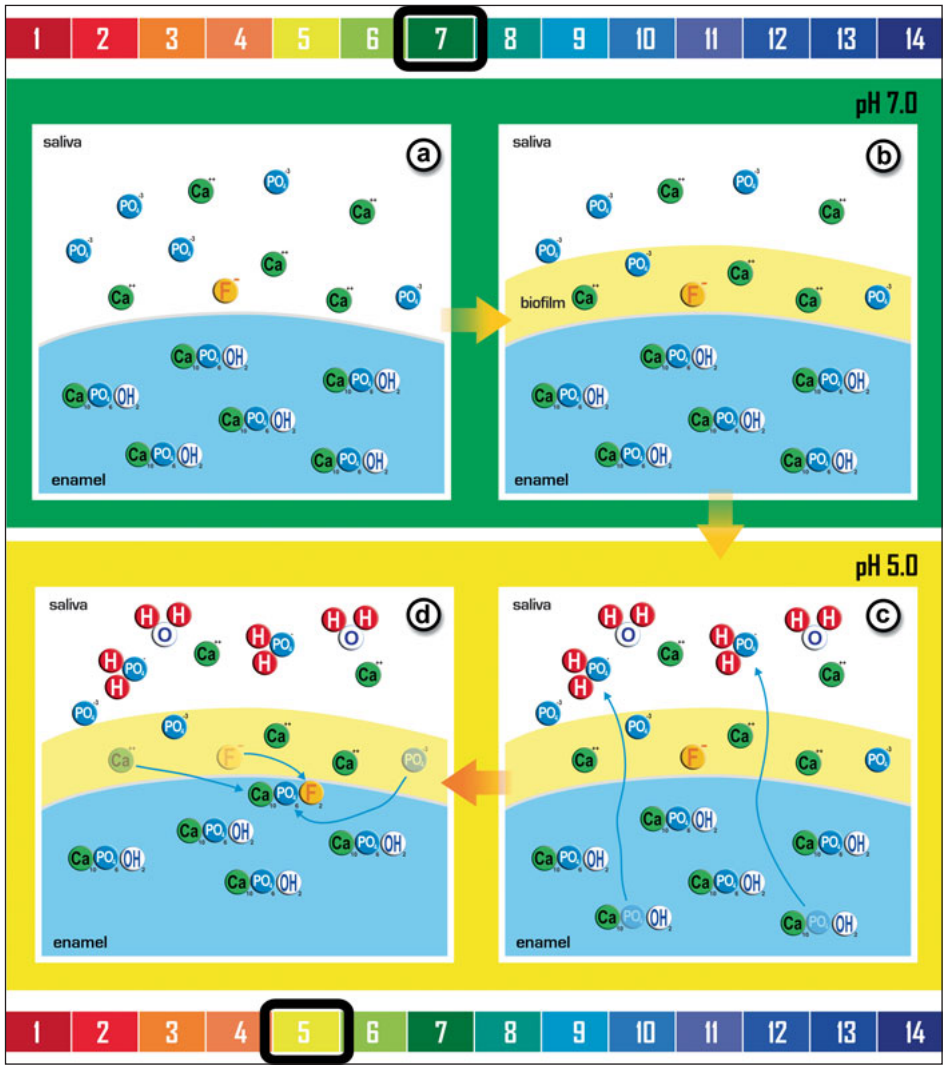

Fig. 2. Dynamics of minerals in saliva and enamel under neutral (**a**, **b**) and acidic conditions (**c**, **d**).

The pH fall has a profound effect on the solubility of hydroxyapatite and other calcium phosphates. In general, the solubility of apatite increases 10 times with a decrease of 1 pH unit. This happens because H^+ combines with PO_4^{-3} and OH^- to form $H_2PO_4^{-3}$ and H_2O (Eq. 1). As a consequence, the concentrations of free PO_4^{-3} and OH^- are reduced, thus decreasing the IAP_{HAP} and turning the solution undersaturated with respect to enamel ($IAP_{HA} < KSP_{enamel}$), promoting enamel dissolution (fig. 2c–d) [11]. The dissolution can be avoided by increasing the concentrations of Ca^{+2} and/or PO_4^{-3} in the fluid. Therefore, the lower the pH, the higher the concentrations

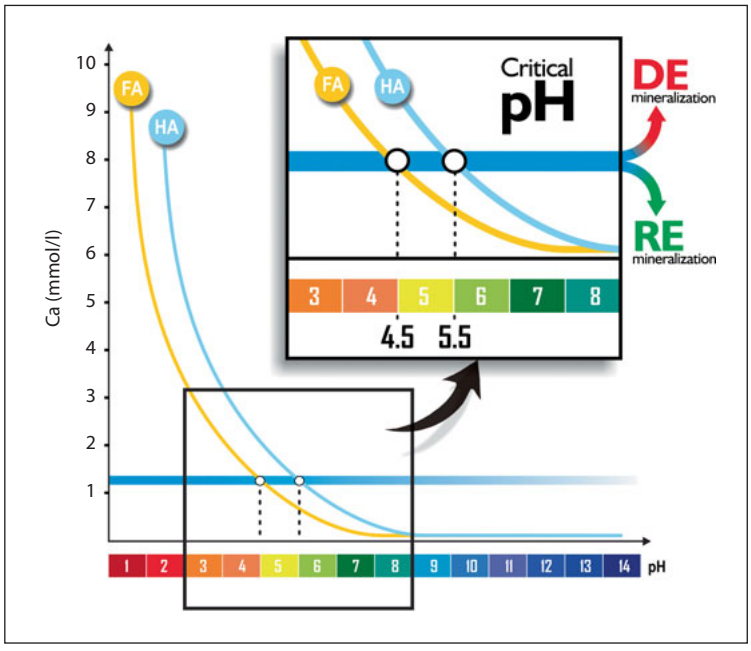

Fig. 3. Solubility of apatite as a function of pH, expressed in terms of calcium concentrations. Blue line indicates salivary calcium concentrations. The critical pH for dissolution of hydroxyapatite (HA) and fluorhydroxyapatite (FA) is 5.5 and 4.5, respectively.

of Ca^{+2} and PO_4^{-3} required to reach saturation in respect to hydroxyapatite. This relationship is shown in figure 3.

$$Ca_5(PO_4)_3OH \rightarrow 5\,Ca^{+2} + 3\,PO_4^{-3} + OH^- \quad (1)$$

OH^- $\xrightarrow{H^+}$ H_2O

PO_4^{-3} $\xrightarrow{H^+}$ HPO_4^{-2} $\xrightarrow{H^+}$ $H_2PO_4^-$

When the pH is gradually lowered from 7.0 to 5.0, the value of pH for which the fluid becomes saturated with respect to the mineral in question (IAP = KSP) is the so-called 'critical pH'. At those conditions, equilibrium exists (no mineral dissolution and no mineral precipitation). For hydroxyapatite, the critical pH is around 5.5, while it is approximately 4.5 for fluorhydroxyapatite. When the pH is above the critical level for the formation of a respective mineral phase, precipitation of this phase occurs (remineralization). Contrarily, when the pH is below the critical level, dissolution takes place (demineralization) (fig. 3).

Carious Lesion Formation

The existence of mineral phases with different solubilities in the dental tissues explains the patterns of demineralization found in caries. Under normal conditions (pH around 7.0), the oral fluids are supersaturated with respect to both hydroxyapatite and fluorhydroxyapatite. Thus, there is a tendency towards formation of these two minerals (formation of calculus and remineralization of demineralized areas).

When bacteria metabolize sugars producing lactic acid, pH decreases in saliva and biofilm fluid (4.5<pH<5.5) rendering these fluids undersaturated with respect to hydroxyapatite while still supersaturated with respect to fluorhydroxyapatite. Consequently, hydroxyapatite dissolves from the subsurface and fluorhydroxyapatite forms in the surface layers. Saliva, in turn, has a strong buffering capacity, and this property together with

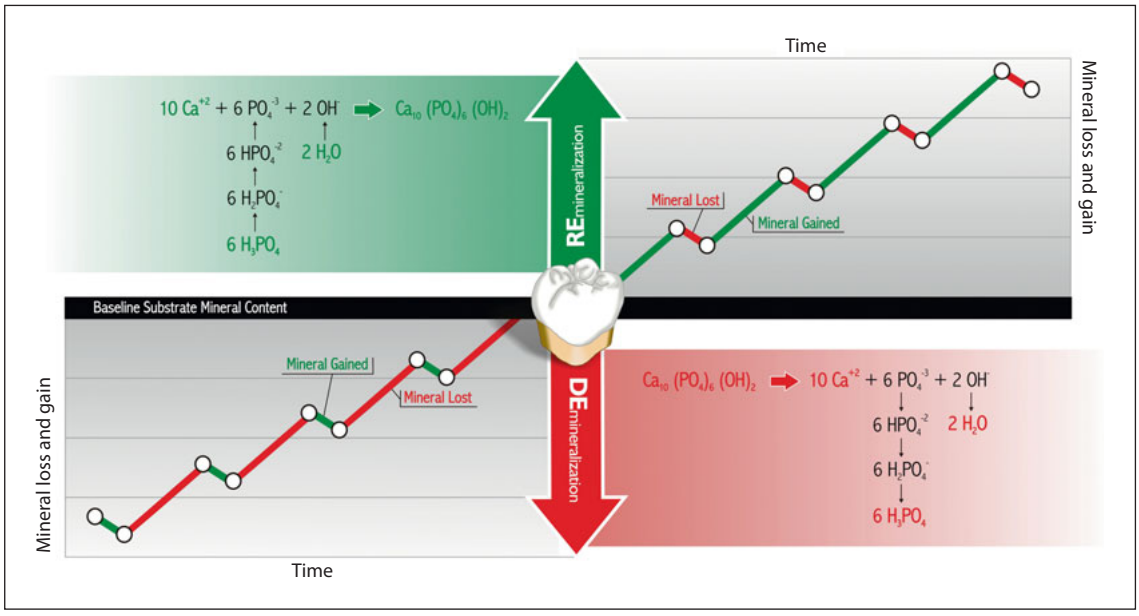

Fig. 4. Cyclic nature of de- and remineralization reactions. Source: Buzalaf et al. [68].

outward diffusion of acids makes the biofilm pH rise within a few minutes. When the pH becomes greater than 5.5, the condition of supersaturation of the oral fluids with respect to hydroxyapatite is restored; the partially demineralized crystals then undergo remineralization. The net result of successive de- and remineralization cycles with the preponderance of the former over the latter leads to caries (fig. 4).

The supersaturation of the oral fluids with respect to fluorhydroxyapatite during cariogenic challenges is responsible for the maintenance of the surface layer of carious lesions (fig. 2d). With time, formation of fluorhydroxyapatite at the expense of hydroxyapatite further increases the concentration of fluorhydroxyapatite in the surface layer. This layer has a protective role, slowing the diffusion of demineralizing agents into the lesion. On the other hand, it also renders remineralization of the lesion body more difficult [11].

Mechanisms by Which Fluoride Controls Caries

Supplementation of public water supplies with controlled levels of fluoride was the first approach involving the use of fluoride for caries control. The encouraging results coming from this measure later prompted the recommendation for the use of fluoride supplements by pregnant women in order to prevent caries in their offspring. Since the first cariostatic benefits of fluoride were observed when this element was ingested from 'systemic' sources, from the 1940s to the 1970s it was originally believed that the cariostatic mechanism of fluoride relied mainly on its uptake in the forming enamel. This would lead to the formation of fluorhydroxyapatite, a mineral phase more resistant to future dissolution. For this purpose, ingestion of fluoride was considered unavoidable and the occurrence of dental fluorosis was regarded as a necessary risk in order to achieve the cariostatic benefits of fluoride.

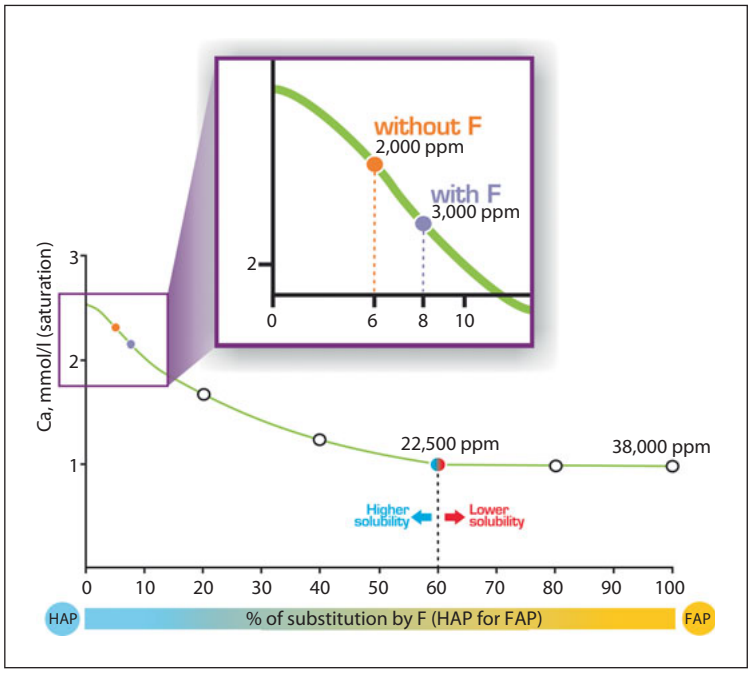

Fig. 5. Calculated solubility of fluorhydroxyapatite at 37°C in 0.1 mol/l acetate buffer at initial pH 5.0 as a function of the degree of replacement of OH^- by F^-.

However, something seemed to be missing. It was observed that fluoride concentrations typically found in enamel were unable to confer significant protection against caries. The highest fluoride concentrations in enamel are found in the surface. They are usually around 2,000 ppm (6% replacement of OH^- by F^- in hydroxyapatite) in non-fluoridated areas and 3,000 ppm (8% replacement of OH^- by F^- in hydroxyapatite) in fluoridated areas. However, these concentrations dramatically fall after the outer first 10–20 μm of enamel to around 50 ppm in non-fluoridated areas and hundreds of ppm in fluoridated areas [21]. These levels are far below those able to confer expressive reduction on the solubility of hydroxyapatite (fig. 5).

In the 1980s, the concept that fluoride controls caries lesion development primarily through its topical effect on de- and remineralization processes taking place at the interface between the tooth surface and the oral fluids was established [22, 23]. Elegant in situ studies conducted in Scandinavia greatly contributed to the consolidation of this concept. In one of the studies, the authors placed human and shark enamel slabs in removable appliances and covered them with orthodontic bands to allow plaque accumulation. Shark enamel was used because it is composed almost of pure fluorapatite (around 30,000 ppm fluoride). Microradiographic analyses revealed that carious lesions formed in both substrates, although they were less severe in shark enamel. The authors compared these data with data from previous studies with human enamel when daily mouthrinsing with 0.2% NaF was used. They observed that the mineral loss in human enamel treated with fluoride rinse was lower than that of shark enamel without any additional treatment. The lesion depths of these substrates were similar (fig. 6) [24]. These studies proved that structurally bound fluoride (shark enamel) was not very effective in inhibiting demineralization, while

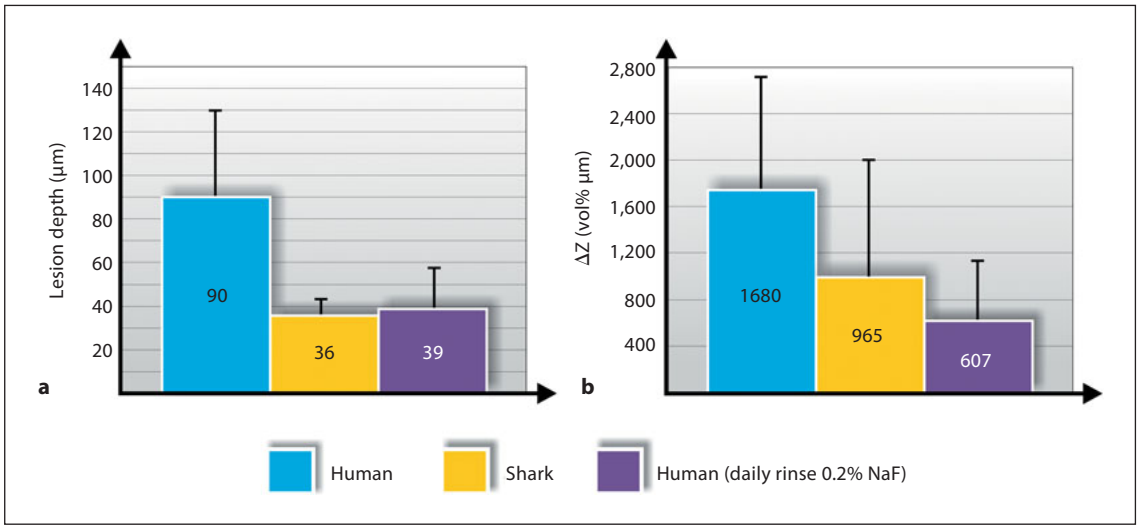

Fig. 6. Lesion depth (**a**) and mineral loss (ΔZ; **b**) in human and shark enamel after 4 weeks in situ as evaluated by microradiography. Groups human and shark refer to human and shark enamel slabs, respectively, which did not receive any additional treatment. Group human (daily rinse 0.2% NaF) refers to human enamel slabs that received daily rinses of 0.2% NaF. Bars indicate SD (n = 6). Original data from Øgaard et al. [24].

fluoride in solution (NaF rinse) led to a high degree of protection. This provided evidence that the primary action of fluoride is topical due to its presence in the fluid phases of the oral environment. It is important to stress out that the concentrations of fluoride found in shark enamel are many times higher than those typically present in human enamel, but even so they were unable to completely inhibit enamel dissolution. On the other hand, fluoride concentrations as little as 1 ppm present in an acid solution can reduce the solubility of carbonated hydroxyapatite to that equivalent to hydroxyapatite. Higher concentrations of fluoride in solution decrease the solubility following a logarithmic pattern [23].

Thus, to interfere in the dynamics of dental caries formation, fluoride must be constantly present in the oral environment at low concentrations. In order that the mechanisms involved in this process can be more easily understood it is helpful initially to consider the different 'pools' of fluoride that can be found in the oral environment. These pools can be didactically divided into 5 categories [25] (fig. 7):

1 F_O: outer fluoride, present outside enamel (in the biofilm or saliva);
2 F_S: fluoride present in the solid phase, incorporated in the structure of the crystals, also known as fluorhydroxyapatite;
3 F_L: fluoride present at the enamel fluid;
4 F_A: fluoride adsorbed to the crystal surface, also known as loosely-bound;
5 CaF_2: 'CaF_2-like' material; globules deposited on enamel and biofilm after application of highly concentrated fluoride products; acts as a pH-controlled fluoride and calcium reservoir.

Fluoride Mechanisms of Action

Inhibition of Demineralization

If fluoride is present in plaque fluid (F_L) when bacteria produce acids, it will penetrate along with the acids at the subsurface, adsorb to the crystal surface (F_A) and protect crystals from dissolution [26]. When the entire crystal surface is covered

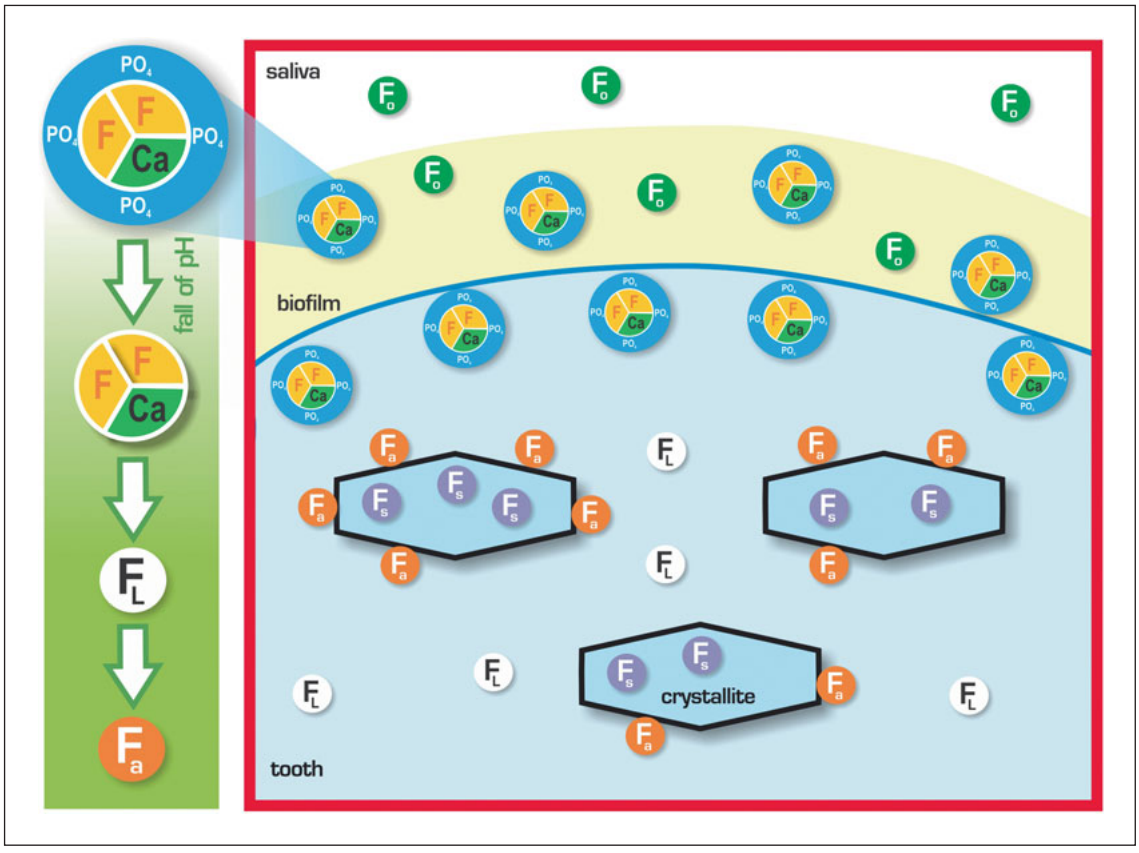

Fig. 7. Schematic representation of the different 'pools' of fluoride in the oral environment. Modified from Arends and Christoffersen [25].

by F_A (100% coverage), it will not dissolve upon a pH fall caused by bacterial-derived acids, since this type of coating makes the characteristics of the crystal similar to those of fluorapatite. On the other hand, when the coating of F_A is partial, the uncoated parts of the crystal will undergo dissolution (fig. 8) [25].

While F_A is the 'pool' of fluoride that effectively protects the crystals from dissolution, the role of fluoride present in solution (F_L) is equally important, since the higher the concentration of F_L, the higher the probability that it adsorbs (F_A) and protects the crystals. However, very low fluoride concentrations (sub-ppm range) in solution are already able to substantially inhibit acid dissolution of tooth minerals [23, 27].

Calcium fluoride (CaF_2) is an important source of fluoride to the oral fluids (F_L). It is known as pH-controlled fluoride and calcium reservoir. This compound forms when the fluoride concentrations in the solution bathing enamel are higher than 100 ppm. The formation of CaF_2 is a two-stage reaction. Initially, a slight dissolution of the enamel surface must occur to release Ca^{+2} that in a second stage will react with fluoride that is applied, thereby forming CaF_2 globules. These globules precipitate not only on sound enamel surfaces but also and more importantly on biofilm,

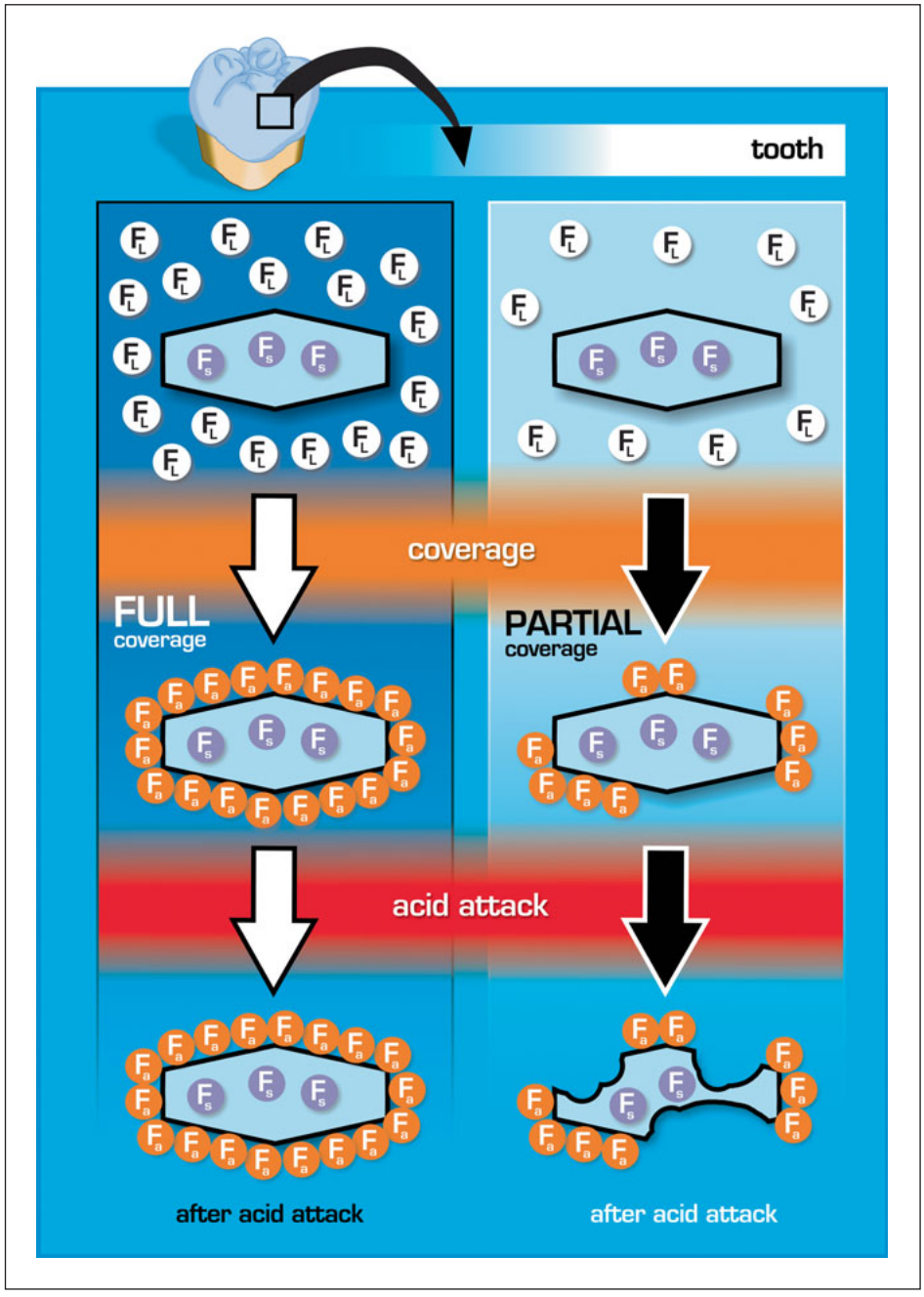

Fig. 8. Events taking place at the subsurface of enamel upon a cariogenic acidic challenge. Fluoride (F_L) penetrates at the subsurface along with the acids, adsorbs to the surface of the crystal and protects it from dissolution (left chart). When coverage is partial, uncovered portions of the crystal will dissolve (right chart). Modified from Arends and Christoffersen [25].

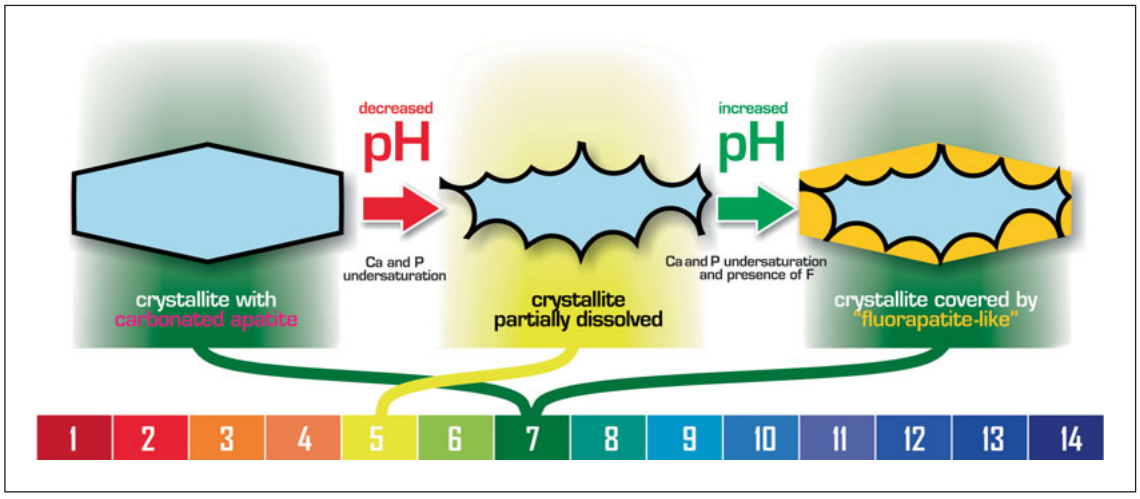

Fig. 9. Schematic representation of remineralization occurring in the presence of fluoride. Fluoride speeds up the process of remineralization and leads to the precipitation of a coat poor in carbonate and rich in fluoride on the partially demineralized original crystallite. This renders the tooth structure more resistant to subsequent acidic challenges. Modified from Featherstone [26].

pellicle and enamel porosities. The dissolution rate of CaF_2 globules is limited by the adsorption of HPO_4^{-2} that is lost under acidic pH, thus allowing CaF_2 to dissolve and fluoride and calcium to be released. This fluoride will add to the 'pool' of F_L [11, 28].

Enhancement of Remineralization

After an acidic challenge, salivary flow buffers the acids produced by the bacteria. When the pH is higher than 5.5, remineralization will naturally occur (fig. 3) since saliva is supersaturated with respect to the dental mineral. Traces of fluoride in solution during dissolution of hydroxyapatite will make the solution highly supersaturated with respect to fluorhydroxyapatite. This will speed up the process of remineralization. Fluoride will adsorb to the surface of the partially demineralized crystals and attract calcium ions. Since carbonate-free or low-carbonate apatite is less soluble, these phases will tend to form preferentially instead of the original mineral, under the nucleating action of the partially dissolved minerals. This new coating will be less soluble due to the exclusion of carbonate and incorporation of fluoride, rendering the enamel more resistant to future acidic challenges (fig. 9). After repeated cycles of dissolution and reprecipitation, enamel crystals may be completely different from their original state [11, 26].

Role of 'Systemic' Fluoride

As mentioned above, the main mechanisms of action of fluoride rely on its topical use since low, sustained levels of fluoride in the oral fluids can significantly control caries progression and reversal. However, this concept does not invalidate the use of 'systemic' methods such as fluoridated water. More than 60 years of intensive research attest to the safety and effectiveness of this measure to control caries [4]. In this case, however, it should be emphasized that despite being classified as a 'systemic' method of fluoride delivery (as it involves ingestion of fluoride), the mechanism of action of fluoridated water to control caries is mainly through its topical contact with

the teeth while in the oral cavity or when redistributed to the oral environment by means of saliva. Since fluoridated water is consumed many times a day, the high frequency of contact of fluoride present in the water with the tooth structure or intraoral fluoride reservoirs helps to explain why water fluoridation is so effective in controlling caries, despite having fluoride concentrations much lower than fluoride toothpastes, for example [29]. This general concept can be applied to all methods of fluoride use traditionally classified as 'systemic'. In the light of the current knowledge regarding the mechanisms by which fluoride control caries, this system of classification is in fact misleading.

One point that deserves attention regarding the mechanism by which fluoridated water leads to caries control is that even recent studies have shown a beneficial pre-eruptive effect of water fluoride on caries control. Well-designed cohort studies have reported that pre-eruption exposure to fluoride is important for caries prevention, especially in pit and fissure surfaces of permanent first molars. This could be due to the difficult access of topical fluoride to these areas. The anticaries protection may occur due to pre-eruption fluoride uptake in the crystalline structure (F_S) of the developing enamel, its adsorption on the crystal surface (F_A) or its presence in the enamel fluid (F_L). Upon post-eruption acidic challenge, F_S would be released to the fluid phase (F_L), thus inhibiting demineralization and enhancing remineralization [30, 31].

Effects in Oral Bacteria

Although the main action of fluoride on the dynamics of dental caries is on de- and remineralization processes that occur on dental hard tissues, it has also been proposed that the fluoride ion can affect the physiology of microbial cells, including cariogenic streptococci, which can thus indirectly affect demineralization [32, 33]. The inhibitory effect of fluoride in pure cultures of oral streptococci was described over 70 years ago, and since then many reports have been published on direct and indirect effects of fluoride on the energy and biosynthesis of streptococci [34]. Bacterial metabolism can be affected by fluoride through several complex mechanisms that are beyond the scope of the present chapter and therefore will be presented only briefly.

Fluoride exerts its effects on oral bacteria by a direct inhibition of cellular enzymes (directly or in combination with metals) or enhancing proton permeability of cell membranes in the form of hydrogen fluoride (HF) [33, 35]. The biological effects and mechanisms of action of fluoride on oral bacteria are summarized in table 1.

According to the reaction $H^+ + F^- \rightleftharpoons HF$, HF is formed more easily under acidic conditions ($pK_a = 3.15$) and enters the cell due to a higher permeability of HF to bacterial cell membranes. HF then dissociates in H^+ and F^- in the cytoplasm, which is more alkaline than the exterior environment [34]. This intracellular F^- inhibits glycolytic enzymes, resulting in a decrease in acid production from glycolysis. F^- in the cytoplasm also lowers cytoplasmatic pH (which decreases the entire glycolytic activity), affecting both the acid production and acid-tolerance of *S. mutans* [33]. Cell membrane-associated H^+-ATPases are also inhibited by F^- because excreted protons are brought back into the cell, therefore decreasing excretion of H^+ from the cell (fig. 10) [35, 36].

It is known that fluoride concentrations in plaque can be increased for several hours after exposure to a fluoridated dentifrice [37–40]. Lynch et al. [41] concluded that low levels of plaque and salivary fluoride resulting from the use of 1,500 ppm fluoride toothpastes are insufficient to have a significant antimicrobial effect on plaque bacteria. A recent review, however, concluded that fluoride concentrations as found in dental plaque have biological activity on critical virulence factors of *S. mutans* in vitro, such as acid production and glucan synthesis, but the in vivo implications are still not clear [33].

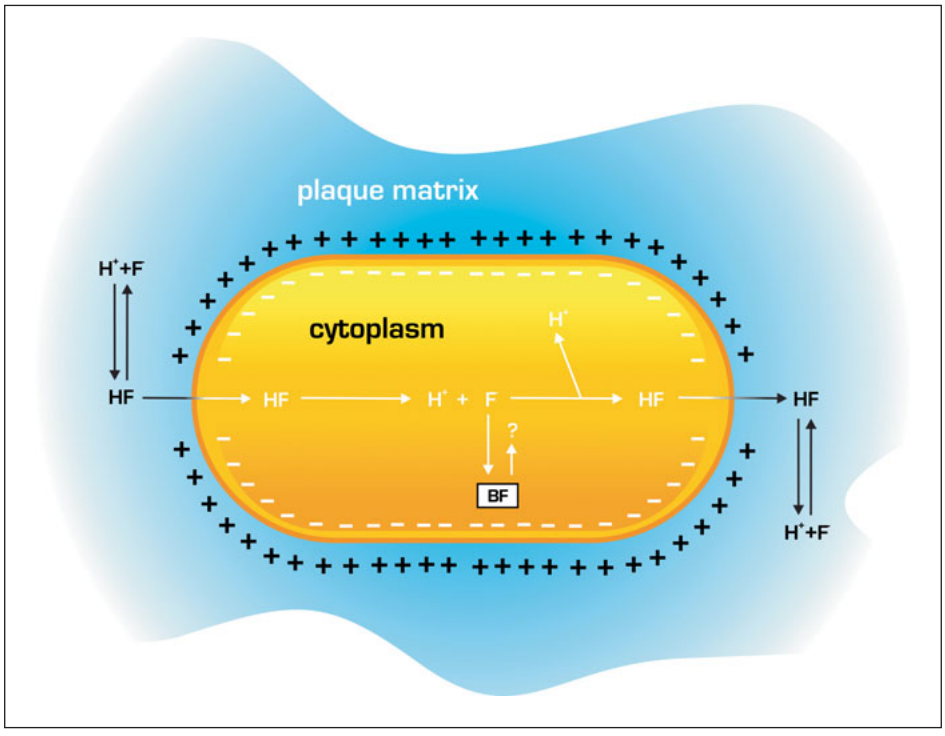

Fig. 10. Fluoride accumulation, distribution and efflux from bacterial cells. BF=Bound fluoride. Modified from Hamilton and Bowden [69].

Table 1. Biological effects and mechanisms of action of fluoride on oral bacteria

Biological activity	Examples	Mechanism
Enzyme inhibition (at sub-millimolar levels of fluoride)	enolase, urease, P-ATPase, phosphatases, heme catalase, heme peroxidase	direct binding of F^- or HF
	F-ATPase, nitrogenase , RecA, CheY	binding of metal-F complex
Dissipation of proton gradient/ motive force (at micromolar levels of fluoride)	acidification of cytoplasm (inhibition of glycolysis, PTS system, and IPS formation)	action as transmembrane proton carrier
	inhibition of macromolecular synthesis and export	

PTS system = Phosphotransferase sugar transport system; IPS formation = intracellular polysaccharide formation.
Source: Koo [33].

As most of the evidence of antimicrobial effects of fluoride on oral bacteria comes from in vitro studies, caution must be taken when interpreting these results. Clinical studies addressing the subject, however, seem to indicate that fluoride does have an antimicrobial effect, and that this effect is dependent on factors such as the fluoride concentration applied and associated antibacterial components. With regard to fluoride concentration, studies with different research protocols have shown significantly lower plaque scores in subjects using a 5,000 ppm fluoride toothpaste, in comparison with formulations containing 500, 1,100 and 1,500 ppm fluoride [42, 43]. Concerning other components with inhibitory effects on plaque growth, it was also demonstrated that the combination of high levels of fluoride (5,000 ppm) and sodium lauryl sulphate reduces de novo plaque formation in subjects using slurries of dentifrices with different fluoride concentrations [43]. Also, the association of fluoride with other ions in formulations containing stannous fluoride or amine fluoride has been shown to be effective in promoting lower plaque formation and acid production, either alone or in combination [44–46]. The use of a stabilized stannous fluoride/sodium hexametaphosphate dentifrice [47, 48] as well as a stannous-containing sodium fluoride dentifrice [49] have also proven to be effective in reducing plaque formation.

Fluoride-releasing materials have also been shown to provide antimicrobial effects. Results from in vitro and in situ studies indicate that fluoride released from glass ionomer cements has an inhibitory effect on the pH fall and the acid production rate of *S. mutans* and *S. sanguinis* [36]. Reduced *S. mutans* growth and lower pH fall on plaque formed on glass ionomer cements has also been shown to occur when compared with composite resin [50–52].

Fluoride in Intraoral Reservoirs

Besides interfering in de- and remineralization processes, along with effects in oral bacteria, fluoride retained in intraoral reservoirs plays an important role on the mechanism of action of the ion. It is known that plaque and salivary fluoride levels decrease rapidly after the application of a fluoride vehicle, following a bi-phasic exponential pattern [53]. These levels, however, are significantly elevated for many hours after the exposure to the fluoridated agent when compared to baseline levels, indicating that fluoride is bound to intraoral reservoirs and subsequently released to saliva over time [29, 37–40].

Fluoride can be deposited on dental hard tissues as CaF_2 (as discussed above), bound to the oral mucosa and retained by dental plaque components. Oral mucosa has been shown to be an important fluoride reservoir, mainly due to its large surface area, releasing fluoride to saliva over time [54]. Although all fluoride reservoirs contribute to the maintenance of the ion in the oral cavity, fluoride retained in dental plaque is likely more relevant from a clinical perspective [for details, see Vogel, this vol., pp. 146–157], as it is the site where de- and remineralization processes take place. Considering that most subjects do not completely remove dental plaque after toothbrushing, the amount of fluoride retained in plaque can help determine the fate of the enamel underneath it [37–39].

Fluoride has a strong affinity to both organic and inorganic components of plaque, and can be found as ionic, ionizable and strongly bound forms. Although the amount of fluoride in the ionizable fraction is considerably larger than in the ionic pool, it adds to the amount of ionic fluoride in plaque fluid, which is responsible for the cariostatic action of fluoride [29]. The clinical relevance of fluoride retained in plaque is that it can be released under acidic conditions during cariogenic challenges. In other words, fluoride is released when it is most needed to reduce demineralization, to enhance remineralization of early lesions, or both. Clinical studies support the concept that the amount of fluoride in oral reservoirs is of paramount importance in its cariostatic effectiveness, as caries incidence and activity have been shown to be inversely related to fluoride

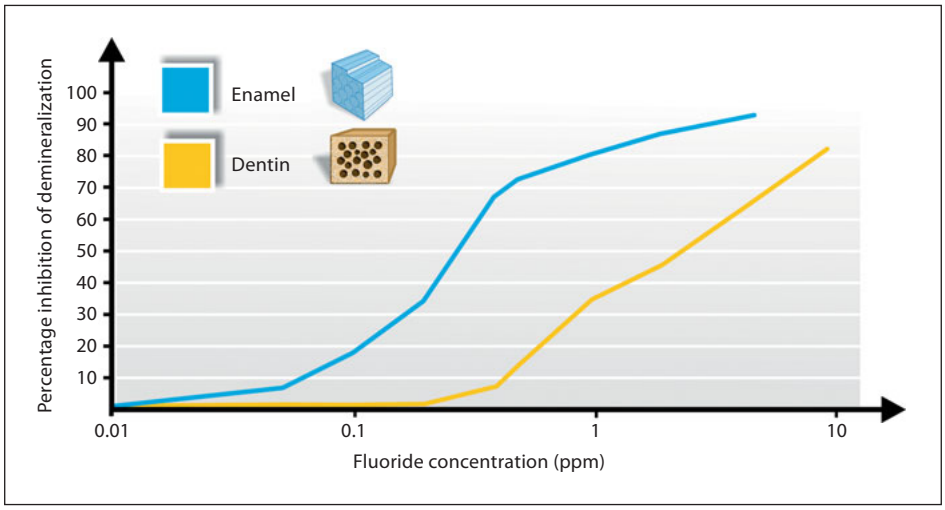

Fig. 11. Inhibition of demineralization of enamel and dentin at different concentrations of fluoride in solution. Data are expressed as percentage of demineralization at 0 ppm fluoride. Modified from ten Cate et al. [11].

concentrations in saliva and/or dental plaque [55–57].

Dentin De- and Remineralization and the Protective Effect of Fluoride

The essence of de- and remineralization processes, as well as the interactions with fluoride that were described above for enamel, also apply to dentin. The main differences of both substrates are:

(1) Dentin is more susceptible to caries attack than enamel, with a critical pH more than 1 pH unit higher than that for enamel [58].

(2) Dentin demineralizes faster and remineralizes slower than enamel under the same experimental conditions [59, 60].

(3) More concentrated fluoride is needed to inhibit demineralization [61, 62] (fig. 11) and to enhance remineralization [63] of dentin when compared with enamel. In fact, clinical trials show a beneficial effect of 5,000 ppm fluoride over 1,100 ppm fluoride dentifrices to arrest root carious lesions [42, 64].

(4) Dentin seems to benefit from a higher daily frequency of exposure to fluoride [65] and also from the combination of methods of fluoride use [66] which is not necessarily the case for enamel.

(5) Dentin contact area with cariogenic acids is larger than that of enamel. For this reason, dentin is apparently much more permeable to acids, with demineralization taking place at a relatively large depth, while mineral deposition is restricted to the outer layers. If the crystallites surrounding the diffusion channels (tubules) are coated with a fluoride-rich mineral, the acids will bypass these relatively resistant minerals, while mineral and fluoride ions will readily be deposited. Thus, the lesion front in dentin moves deeper, while the surface layer becomes broader. In enamel, on the other hand, diffusion is much slower and allows acids to 'sidestep' into smaller intraprismatic porosities and dissolve crystallites that are still unaffected by either acid or fluoride. Thus, mineral uptake and loss occur at similar depths for enamel

lesions, while for dentin lesions mineral uptake is predominant at the surface and mineral loss at the lesion front [60].

It has also been recently shown that very deep lesions extending through enamel into dentin can be remineralized. Although this process is slow, it indicates that remineralization might be used to treat deep lesions [67].

Conclusion

Knowledge of the mechanisms by which fluoride promotes caries control is essential for the achievement of the maximum benefits of this element with minimum risk of side effects. The main action of fluoride for caries control occurs through its topical effect. Fluoride present in low, sustained concentrations (sub-ppm range) in the oral fluids during an acidic challenge is able to absorb to the surface of the apatite crystals, inhibiting demineralization. When the pH is reestablished, traces of fluoride in solution will make it highly supersaturated with respect to fluorhydroxyapatite, which will speed up the process of remineralization. The mineral formed under the nucleating action of the partially dissolved minerals will then preferentially include fluoride and exclude carbonate, rendering the enamel more resistant to future acidic challenges. Topical fluoride can also present antimicrobial action. Fluoride concentrations as found in dental plaque have biological activity on critical virulence factors of *S. mutans* in vitro, such as acid production and glucan synthesis, but the in vivo implications of this are still not clear.

Evidence from cohort studies also supports fluoride's systemic mechanism of caries inhibition in pit and fissure surfaces of permanent first molars when it is incorporated into these teeth pre-eruptively. In this case, upon post-eruption acidic challenge, F_S would be released to the fluid phase (F_L), thus inhibiting demineralization and enhancing remineralization. Additionally, ingested fluoride can exert a topical mechanism of action when it recirculates in the oral environment through saliva.

The essence of de- and remineralization processes, as well as the interactions with fluoride that were described for enamel, also apply to dentin. The main differences are that dentin is more susceptible to caries attack than enamel, with a critical pH more than 1 pH unit higher. Consequently, dentin demineralizes faster and remineralizes slower, requiring higher fluoride concentrations and frequencies of application when compared with enamel.

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