# Biological factors in dental caries enamel structure and the caries process in the dynamic process of demineralization and remineralization (part 2)

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*Dental caries is a complex disease process that afflicts a large proportion of the world's population, regardless of gender, age and ethnicity, although it does tend to affect more indivduals with a low socioeconomic status to a greater extent. The physicochemical properties of the mineral comprising the tooth surface and subsurface modulate the development, arrestment and remineralization of dental caries. Post-eruption maturation of enamel surfaces and exposed root surfaces is important in order for more susceptible mineral phases to be modified by incorporation of soluble fluoride from the plaque into dental hydroxyapatite. The chemical reactions that occur during acidic conditions when tooth mineral dissolves (critical pH) are determined by the supersaturation of calcium and phosphate within plaque and saliva, as well as if fluoride is present.*
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## INTRODUCTION

Human dental enamel is a unique mineralized substance that is composed of hydroxyapatite (92-94%), water (2-3%), carbonate (2%), trace elements (sodium, magnesium, potassium, chloride, zinc, 1%), fluoride (0.01-0.05%) and proteins and lipids (<1%).[1-5] While hydroxyapatite forms the mineral component of bone, dentin and cementum, there is substantially more organic content (35 to 50%) in these biologic hard tissues than enamel. Also unique to enamel is lack of a resident cellular component within the tissue. Bone, dentin and cementum contain osteocytes, odontoblasts and cementocytes that maintain, repair and remodel the surrounding organic matrix and mineral. Because of the lack of cellular repair mechanisms, the events surrounding the development and reversal of caries is dependent upon physicochemical events at the tooth-pellicle/plaque interface.

The ameloblast is the cell that provides the enamel organic matrix proteins that direct mineral deposition in an orderly fashion.[1-5] Enamel is organized into rod-like structures (also referred to as enamel prisms) that begin at the dentinoenamel junction and end at the exposed enamel surface. The enamel prisms are interlocked with each other in a keyhole configuration, resulting in alteration of the prism heads and prism tails. Between each of the keyhole-shaped enamel prisms is a minute portion of organic matrix. The organic matrix determines the permeability of the enamel structure. The enamel prisms range from 4 to 6 µm in width and extend the full thickness of the enamel from the tooth surface to the dentinoenamel junction.

Each enamel prism is composed of numerous crystals of hydroxyapatite (HAP, Table) arranged in a hexagonal elongated configuration, with a basal face that is 25 nm (a-axis) by 40nm (b-axis) in width and a length of 160nm (c-axis).[1-5] A minute portion of residual organic matrix surrounds each of these HAP crystals. Within each crystal, there are unit cells that make up the entire crystal, and these are meticulously organized into the hexagonal elongated configuration of the enamel crystal. The unit cell represents the lowest denominator of HAP, $Ca_{10}(PO_4)_6(OH)_2$, composed of calcium, phosphate and hydroxyl groups. Each unit cell is composed of this

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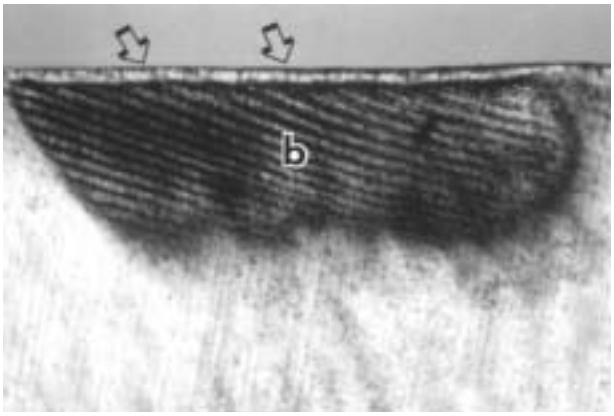
**Table.** Calcium Phosphate Compounds in Enamel Demineralization and Remineralization

| | |
|---|---|
| **Dental Hydroxyapatite** (Enamel, Dentin, Cementum; Ca/P = 1.61 to 1.64) | $(Ca,Mg,Na)_{10}(PO_4, HPO_4, CO_3)_6\ OH)_2$ |
| **Hydroxyapatite** (HAP; Ca/P = 1.67) | $(Ca)_{10}(PO_4)_6(OH)_2$ |
| **Fluorhydroxyapatite** (FHAP; Ca/P = 1.67) | $(Ca)_{10}(PO_4)_6(F,OH)_2$ |
| **Fluorapatite** (FAP; Ca/P = 1.67, Shark Teeth) | $(Ca)_{10}(PO_4)_6F_2$ |
| **Carbonateapatite** (CAP; Ca/P = 1.67) | $(Ca)_{10}(PO_4)_6\ CO_3$ |
| **Carbonatehydroxyapatite** (CHAP; Ca/P = 1.7 to 2.4) | $(Ca,X)_{10}(PO_4,CO_3)_6(OH)_2$ |
| **Calcium-Deficient Hydroxyapatite** (CaDHAP; Ca/P >1.67) | $(Ca,X)_{10}(PO_4HPO_4)_6(OH)_2$ |
| **Dicalcium Phosphate Dihydrate** (DCPD; Ca/P = 1.1) | $CaHPO_4 2H_2O$ |
| **Tricalcium Phosphate** (TCMP; Ca/P = 1.5) | $(CaMg)_3(PO_4)_2$ |
| **Octacalcium Phosphate** (OCP; Ca/P = 1.33) | $Ca_8H_2(PO_4)_6 5H_2O$ |
| **Amorphous Calcium \| Phosphate** (ACP; Ca/P = 1.1 to 3) | $(CaC)_x(PO_4,Y)_y$ |

X = calcium substituted by Mg, Na, Zn, Sr, and other)
Y = substitute for $PO_4$

Compiled from references: 1-45,26




Histopathologic appearance of enamel caries lesion viewed with polarized light microscopy.

An intact negatively birefringent surface zone (arrow) is present overlying the positively birefringent body of the lesion (L). The negative birefringence of the intact surface zone (arrow) is due to a pore volume (mineral loss) of less than 5%; whereas, the positive birefringence of the body of the lesion (L) is due to a pore volume (mineral loss) of >5%. The adjacent sound enamel is negatively birefringent and typically has a pore volume of 0.1% as determined by quantitative polarized light microscopic means. (polarized light, water imbibition, refractive index of water = 1.33, refractive index of sound enamel = 1.62).

The translucent zone (arrow) represents the advancing front of the enamel caries lesion and is a zone of demineralized enamel that is negatively birefringent when imbibed with quinoline (refractive index of quinoline = 1.62, identical to refractive index of sound enamel = 1.62). The pore volume (mineral loss) of the translucent zone is approximately 1%, approximately 10 times that for the adjacent sound enamel (pore volume of 0.1%). The dark zone 9 (d) is a zone of remineralization that is adjacent to the translucent zone and may have a secondary dark zone adjacent to the overlying surface zone. This zone has a pore volume (mineral loss) of 2 to 4%, in contrast to a minimum pore volume of 5% for the body of the lesion. The positive birefringence of the dark zone, when imbibed with quinoline, is due to a microsieve network of porosities in this zone that preferentially excludes quinoline due to its molecular size and retains air within the smaller porosites (refractive index of air = 1.0; refractive index of quinoline). The differences in the refractive indices of pores filled with air compared with those filled with quinoline allows for detection of the dark zone by polarized light microscopy, when quinoline imbibition is used.

(Figure reprinted with permission: Hicks MJ, Flaitz CM, Silverstone LM: Fluoride uptake in vitro of sound enamel and caries-like lesions of enamel from fluoride solutions of relatively low concentrations. J Pedod 1986;11:47-61.)

structure and represents the individual building block that when combined with numerous other unit cells form the enamel crystal. In turn, the enamel crystal is the building block for the enamel prism when it joins with numerous other HAP crystals to form the keyhole-shaped enamel prism. Finally, numerous interlocking keyhole-shaped enamel prisms form what we visualize as tooth enamel. With dentin, bone and cementum, the formation of HAP crystals is organized about more abundant organic material and is more intimately associated with collagen interaction. The crystals of these less mineralized tissues are formed by HAP with crystal dimensions of 3 nm (a-axis and b-axis) in width by 60 nm (c-axis) in length.

The organization of the HAP crystal is important to understand because certain factors intrinsic to the enamel unit cell may lead to its instability in acidic conditions during a cariogenic challenge.[1-5] The c-axis with hydroxyl groups present is less stable than when fluorine replaces hydroxyl groups. If systemic fluoride during amelogenesis and/or topical fluoride following eruption of immature enamel into the oral cavity are available, it is possible to substitute fluorine for hydroxyl groups resulting in fluorapatite (FAP), $Ca_{10}(PO_4)_6\ F_2$ when all hydroxyl groups are substituted. However, in nature, partial substitution of fluorine for hydroxyl groups occurs with fluorhydroxyapatite (FHAP), $Ca_{10}\ (PO_4)_6(F,OH)_2$, being formed. Both FAP and FHAP formation results in less internal

crystalline stress and strain, a decreased c-axis, an increase in crystal size and thickness, and a decrease in calcium-deficiency. These physicochemical alterations in HAP by partial or complete substitution result in increased crystalline stability and decreased susceptibility to acid dissolution during cariogenic challenges.

Although in theory enamel, dentin, bone and cementum are composed of HAP, the actual circumstance is that most mineral crystals in these hard tissues have a certain proportion of substitutions for calcium, phosphate and hydroxyl groups $[(Ca,Mg,NA,ST,SE,ZN)_{10}(PO_4,CO_3,HPO_4)_6(OH,F)_2]$.[1-5] Calcium may be replaced with magnesium, sodium, zinc, selenium and strontium. Phosphate may be substituted by carbonate and acid phosphate. As discussed previously, fluorine may be found in place of hydroxyl groups. While fluoride stabilizes mineral crystals, some of these other substitutions (magnesium, sodium, selenium, carbonate, acid phosphate) destabilize the crystals and lead to increased caries susceptibility. The incorporation of other trace elements in the HAP mineral is dependent upon the trace elements in the water, soil and foods from a specific geographic area. More than 40 different trace elements have been identified in enamel HAP crystals, with the vast majority of only minor importance.

**Post-eruption maturation of enamel**

With eruption of a tooth into the oral cavity, the enamel surface is immediately coated by a salivary protein pellicle and plaque begins to accumulate.[1,6,7] At the time of eruption, the caries susceptibility of enamel is at its greatest level. The enamel surface undergoes maturation by exchanging more soluble mineral components for less soluble mineral. As described previously, plaque is supersaturated with respect to calcium and phosphate and contains increased fluoride in comparison to saliva. During episodes of acidogenic challenge, more soluble carbonate-rich HAP is replaced by more acid-resistant HAP or FHAP. In the presence of fluoride ion availability, it is possible for fluoride to be acquired by: 1) adsorption of fluoride onto HAP crystals; 2) exchange of fluoride for hydroxyl groups in HAP; 3) dissolution and reprecipitation of HAP to form FHAP; 4) and precipitation of mineral phases with FHAP crystal growth.[1-5] It is also thought that the salivary proteins making up the pellicle may also provide a certain degree of protection by binding to HAP and protecting it from organic acids generated in plaque by mutans streptococci and lactobacilli.

It is particularly advantageous to expose recently erupted teeth to topical fluoride.[1,6-8] Caries reduction in newly erupted teeth exposed to sodium fluoride (43%), acidulated phosphate fluoride (36-63%) and stannous fluoride (61-84%) treatment is substantial. When compared with newly erupted teeth, the reduction in caries for previously erupted teeth is approximately 50% less with subsequent topical sodium fluoride (19%), acidulated phosphate fluoride (21-31%, and stannous fluoride (21-44%) treatment.

Similar findings regarding maturation of exposed root surfaces has been suggested.[1,9-13] Root surfaces that have been gradually exposed to the oral environment over time due to ongoing periodontal disease have a lower caries prevalence than root surfaces exposed rapidly to the oral cavity following periodontal surgery. Perhaps, immediate topical fluoride treatment with subsequent frequent periodic exposures may help alleviate the high prevalence of root surface caries in teeth undergoing periodontal surgery.

In addition, permeability of recently erupted enamel is quite high.[1-5] The organic material and water content allow soluble fluids from the plaque to penetrate the enamel up to a depth of 200 µm. With post-eruption maturation, access to the underlying enamel is restricted to the outer most 20 µm of the enamel. This may reflect preferential dissolution of the more soluble mineral phases and replacement with less soluble mineral phases of larger cross-sectional crystal diameter than the original crystals, effectively reducing the organic matrix space and decreasing permeability.

**Demineralization of dental hydroxyapatite**

Partial dissolution of dental hydroxyapatite (enamel, dentin, cementum) occurs with exposure to organic acids derived from acidogenic bacteria in the dental plaque or ingestion of acidic beverages and foods.[1-5,14] This exposure of HAP to acid leads to an increase in calcium, magnesium, phosphate, acid phosphate, carbonate and bicarbonate in the microcosm of the incipient carious lesion. When acid is present, both DCPD and OCP (Table) may form directly from the ions released from the dissolved HAP. When the acidogenic attack has resolved and a neutral pH is reached, DCPD can hydrolyze to OCP in the presence of calcium, bicarbonate and carbonate ions derived from either dissolved HAP, plaque or saliva. Both DCPD and OCP under these conditions may form apatite or carbonatehydroxyapatite. When magnesium ions from dissolved HAP, plaque, saliva, or ingested food and drink are present, magnesium-substituted tricalcium phosphate may arise directly following HAP dissolution or from DCPD. This inhibits the formation of OCP, and does not allow DCPD or OCP to form HAP.

When low levels of fluoride ions derived from mouthrinses or dentifrices are present, FHAP or FAP (Table) are created from the dissolution products of HAP.[1-5] The creation of FHAP or FAP inhibits DCPD and OCP formation. With high levels of fluoride ions acquired from topical fluoride gels, calcium fluoride ($CaF_2$) will be formed in deference to HAP or FHAP. If acid phosphate or phosphate ions are present, the resulting calcium fluoride will hydrolyze to FHAP. Calcium fluoride can act as a reservoir for both calcium

and fluoride and release these ions during acidic challenges and inhibit further HAP dissolution, and encourage FHAP formation. In general, the presence of fluoride ions restricts the formation of acidic, more soluble calcium phosphates (DCPD, OCP) and facilitates creation of more acid-resistant FHAP. Of interest is the fact that fluoride is most effective in inhibiting HAP dissolution when calcium and phosphate ions are also present in solution (plaque/pellicle/saliva). In addition, both plaque and the carious lesion tend to be supersaturated with DCPD and OCP. This allows for conversion of the DCPD and OCP into HAP or FHAP under resting conditions when fluoride and other mineral elements are available.

The process of acid dissolution of a HAP crystal begins with dissolution of the central core and progresses to dissolution of the remaining peripheral "shell" of the crystal.[2-5,15] The central core is preferentially attacked due to its higher carbonate concentration and crystalline imperfections. The initial sign of dissolution is the detection of a centrally placed etch pit, demonstrated by the presence of a small indentation at the terminal end of the HAP crystal. The dissolution continues down the center core, resulting in a hollowed out HAP crystal. In laboratory studies, lactic acid rapidly dissolves the core (few minutes). The remaining peripheral "shell" of the crystal requires a considerably longer time period to dissolve in lactic acid (hours).

**Subsurface white spot lesion formation**

The caries process is a gradual one that requires repeated episodes of prolonged exposure to acidic conditions consistently below the critical pH for enamel dissolution (pH 5.5, demineralization) with intervening periods of return to the resting pH of plaque (pH 7.0, remineralization period).[1,9-13,15-22] In the face of failure to remove plaque from retentive tooth areas, a diet high in refined carbohydrates, and frequent carbohydrate ingestion, the dynamic equilibrium between demineralization and remineralization will be tipped towards demineralization with the development of clinically detectable white spot lesions. The early enamel lesion is characterized by four distinct histopathologic zones, as described previously (Figure).[23-25] Two zones of demineralization are present: 1) the translucent zone (1% pore volume) along the advancing front of the lesion; and 2) the body of the lesion (>5 to 25% pore volume) representing the majority of the lesion and situated approximately 15 to 30 um beneath the overlying intact enamel surface. Two zones of remineralization are also present: 1) the dark zone (2-4% pore volume) situated near the advancing front just superficial to the translucent zone; and 2) the surface zone (1 to <5% pore volume) forming the intact surface overlying the lesion.

The initial formation of the lesion is due to the dissolution of HAP from the enamel prisms forming the enamel surface.[1-5,23-26] The initial dissolution results in loss of a small amount of mineral within the enamel and would have a similar appearance to the translucent zone (negatively birefringent in quinoline). With continuing demineralization without the benefit of remineralization of this initial lesion, a surface zone (Figure) that resembles the surrounding sound enamel, with respect to its negative birefringence (water imbibition) is formed. With ongoing removal of mineral from the underlying enamel (Figure), a positively birefringent body of the lesion (water imbibition) develops and separates the overlying surface zone from the translucent zone at the advancing front. If lesion development occurs over a relatively long period of time, a zone of remineralization (the dark zone, positive birefringence in quinoline) with reprecipitation of mineral phases from the translucent zone will occur (Figure). If lesion formation is over a short period of time, the dark zone will not form and there will be rapid advancement of the front with a large, heavily demineralized body of the lesion and a surface zone of minimal thickness. Once a certain degree of demineralization has occurred the lesion will take on a white spot appearance and become clinically detectable.

The formation of an intact surface overlying the carious lesion has been difficult to mimic in artificial caries models.[1,5,19,23-28] Surface softening and exposure to organic acids results in extensive surface loss and erosion without formation of an intact surface zone. Some methods result in the maintenance of a surface layer with considerable mineral loss, but not a true surface zone that histologically resembles the adjacent sound enamel on polarized light examination. In order to create lesions that mimic the natural process of white spot lesion formation, artificial caries model systems using diffusion-controlled acidified gels (continuous demineralization), demineralizing solutions containing calcium, phosphate and fluoride ions (continuous demineralization), or cyclic exposure to demineralizing and remineralizing solutions (intermittent demineralization and remineralization) had to be developed. It is now possible to create artificial or caries-like lesions in enamel that mimic naturally occurring white spot lesions (Figure).

The maintenance of an intact surface during caries formation (Figure) is quite remarkable. At first, this was considered to be unique to surface enamel.[1,5,23-28] Later, it was proven that an intact surface can be reproduced even when the surface enamel is ground away, and artificial caries is created in the remaining abraded enamel. Likewise, it was shown that compressed blocks of HAP display a surface zone when artificial caries is formed within the HAP.

The subsurface white spot lesion with an intact surface occurs due to the physicochemical parameters of demineralization of HAP.[26] The mechanistic approach to demineralization of enamel is based

upon the primary driving force being hydrogen ion transport from the dental plaque at a pH of 5.0 into the underlying enamel at a pH of 7.0. The concentration gradient for hydrogen is much less in enamel than that in dental plaque, during episodes of acidogenesis by mutans streptococci and lactobacilli. Hydrogen ions are transported to the advancing front of the lesion. Once the hydrogen ions encounter susceptible tooth mineral, dental hydroxyapatite undergoes dissolution with the resultant dissolved mineral transported from the advancing front to the dental plaque. Of interest is the fact that the fluid phase at the advancing front has a much lower calcium and phosphate concentration (0.1mmol/L) than that at the enamel surface (5 to 8 mmol/L). This implies that calcium and phosphate are being transported against their concentration gradient and this requires energy input to accomplish this. The energy for this active transport of solubilized mineral phase from the advancing caries front is supplied by the influx of the hydrogen ions (hydrogen ion diffusion) driven by a 100-fold concentration gradient in the plaque compared with enamel along the advancing front. Mineral phases may become entrapped in the zones of remineralization (dark zone and surface zone) if the caries process is a slow process. During the intervening periods of remineralization and return to a resting neutral plaque pH, partially demineralized crystals may be repaired or new crystals formed from the available dissolved mineral phases within the lesion and dental plaque. Demineralization may be markedly decreased in individuals with higher plaque levels of calcium, phosphate and fluoride. The increased calcium and phosphate in plaque would require a lower pH (a higher hydrogen ion gradient) between plaque and the advancing front to allow for active transport of calcium and phosphate from the advancing caries front into the plaque. This effectively would result in a lower critical pH in order to induce demineralization. The presence of increased levels of fluoride in plaque favors reprecipitation of dissolved mineral and also allows for incorporation of fluoride into reconstituted HAP. Likewise, increased fluoride content of native enamel in the form of FHAP would lessen the extent of demineralization and favor mineral reprecipitation.

## SUMMARY

Dental caries is a complex disease process that afflicts a large proportion of the world's population, regardless of gender, age and ethnicity, although it does tend to affect more indivduals with a low socioeconomic status to a greater extent. The physicochemical properties of the mineral comprising the tooth surface and subsurface modulate the development, arrestment and remineralization of dental caries. Post-eruption maturation of enamel surfaces and exposed root surfaces is important in order for more susceptible mineral phases to be modified by incorporation of soluble fluoride from the plaque into dental hydroxyapatite. The chemical reactions that occur during acidic conditions when tooth mineral dissolves (critical pH) are determined by the supersaturation of calcium and phosphate within plaque and saliva, as well as if fluoride is present.

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