

EARLY ENAMEL LESION PART II. HISTO-MORPHOLOGY AND PREVENTION

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Summary: There has been a paradigm shift from Black's 'extension for prevention' to a minimal intervention approach in the recent time. The most important aspect in diagnosis of earliest stages of enamel demineralization involves accurate and reliable detection of such lesions. The newer diagnostic aids would enable the dentist to detect and diagnose early enamel lesions and direct appropriate preventive measures to promote remineralization and conservation of the tooth substance. A high level of caries experience necessitates preventive strategies which are more cost effective than surgical intervention and restorative procedures.

Key words: *Early enamel lesion; Demineralization; Remineralization*

Characteristics of Early Caries Lesions in Enamel

The enamel demineralization defect has a lower mineral distribution in the surface layer in comparison to the adjacent sound enamel and also a lower interprismatic mineral content. The first stage of enamel demineralization is characterized by removal of interprismatic mineral content and in the subsequent stages a well defined surface layer formation occurs which constitutes early caries lesion (4).

Although early investigators like Hollander and Saper (47) in 1935 had noticed this subsurface demineralization white opaque spot, they mistook it to be a photographic artifact. However the work of various other researchers like Applebaum, Thewlis, Besic, Coolidge et al., Gray and Francis (3, 12, 21, 41, 87), on white opaque enamel lesion and new experimental techniques like microradiography (42), polarized light experiments (23, 78), microhardness data (30), and electron microscopy shed new light in understanding the early caries lesion in enamel. These studies have demonstrated that a porous and mineral-rich surface layer covers an enamel lesion and the morphology differs a little from that of sound enamel while body of the lesion which comprises the subsurface area has low mineral content (10–70 vol %). The early caries lesion in enamel is characterized by a prominent perikymata pattern and focal holes (6, 44, 88). The main drawback of the numerous experimental techniques is that they are static measurements of caries progression at a particular time period whereas the carious process is time-dependent and is in a constant state of dynamic equilibrium wherein a balance is struck between demineralization and remineralization.

Surface layer (SL) covering early enamel lesions

The early investigators who observed the white opaque spots attributed the presence of these lesions to artifacts. They believed that the surface layer (SL) could be due to sound enamel which has a higher mineral content. These explanations were proved false by subsequent investigations by Langdon et al. (55). Their studies on pressed pellets of hydroxyapatite demonstrated that subsurface lesion could occur in an acidic gel system with 2 ppm fluoride. They also concluded that organic matrix is not important for subsurface lesion formation, and that neither a preferred crystallite orientation in the enamel prisms nor an uneven ion/mineral distribution in enamel were essential for the formation of a subsurface lesion since these are absent in pressed apatites. This is in contrast to earlier reports by Brudevold et al. (15).

Mechanism of subsurface lesion formation and progress

The bacteria in the oral plaque are acidogenic and form organic acids, including lactic, formic, acetic, and propionic acids from fermentable carbohydrates which diffuse into the enamel (30), dentin, or cementum, partially dissolving the mineral crystals (57). When this process goes unchecked minerals like calcium and phosphate diffuse out of the tooth resulting in frank cavitation. In the initial stages, demineralization can be reversed by influx of calcium, phosphate, and fluoride into the tooth and deposition on the crystal remnants in the non-cavitated lesion. This process is

termed as remineralisation. The resultant mineral crystal surface is more acid resistant than sound enamel. A shift in the balance between demineralization and remineralization frequently occurs daily and this can result in repair and reversal, maintenance of the *status quo* or cavitation (34).

Although most investigators agree that well-established caries lesion comprises of intact, mineral-rich, porous surface layer with an underlying lesion with low mineral content there is lack of agreement for a common mode of initiation of early enamel lesion. *In vitro* studies using various solutions and acid systems have been used to study lesion formation (8, 9, 20, 35, 50, 63, 65, 77). A large body of knowledge on enamel lesion has been mainly from studies on artificial caries lesions which simulate natural enamel lesions. The major advantage of these studies have been due to the ease of evaluating mechanism of caries formation and various parameters independently.

Experimental work by Von Bartheld (92, 93) and Silverstone (77) led to the theory that acidified charged gels formed subsurface lesions. However subsequent studies (42) showed that acidified uncharged gels also formed subsurface lesions. Various other studies with acidic, unsaturated calcium phosphate solution by Larsen (56) and Moreno and Zahradnik (65) finally demonstrated gels were not needed for surface layer formation. The presence or absence of stirring on surface layer formation is irrelevant, although stirring affects the rate of surface layer formation (56). Studies on *in vitro* and *in vivo* formation of surface layer in enamel showed that pellicle is essential for formation of surface layer (60). However results from other studies revealed that pellicle did not play a crucial role in subsurface demineralization *in vitro* (56, 87, 88). Similar reports were also obtained from the *in vitro* experimental data of Ten Cate and Duysters (83, 84), Borsboom and Arends (14), and Theuns et al. (86) who found that subsurface lesion can occur even in the absence of the pellicle. A significant work by Arends et al. (7), Ogaard et al. (69), and Ogaard (68) demonstrated that pellicle does not play a major aetiologic role in subsurface demineralization *in vivo*.

Under polarized light the outer 3–9 μm *in vivo* lesions was found to be less porous in comparison with the remainder of the lesion by Thylstrup et al. (90). The investigations using microradiography and polarized light demonstrate that outer layer of early caries lesions has less mineral and greater protein content. In the literature, various researchers have reported on the surface layer thickness (d_{sL}). The relationship between d_{sL} and time varies with the observation technique and type of artificial lesion. Theuns et al. (85, 86) showed that d_{sL} increased with time in certain cases however it is largely independent of time as there was uncertainty regarding the deposition of dissolved material from the lesion body to the inner aspect of the surface layer.

The work of Gray and Francis (41) involving optical observation of microradiograms, showed an increase in d_{sL} with the demineralization. Similar conclusions were drawn

by Groeneveld and Arends (42), for enamel lesions in acidified hydroxyethylcellulose, where an increase of d_{sL} with time was demonstrated using microradiography. An increase in negatively birefringent surface zone (in water) during the demineralization period was also observed in polarized light studies by Darling (23) and Silverstone (78). Featherstone et al. (31) reported that the surface layer formation took several days and thereafter was uniformly thick. Analysis of several published works have led Theuns et al. (85, 86) to conclude that the surface layer thickness d_{sL} is not dependant on the demineralization period after it is fully developed.

Features of adsorbed material on enamel surface

1. Protection of outer enamel surface

Experiments on demineralization during caries by numerous researchers like Gray (40), Gray and Francis (41), and Francis et al. (38) showed that protective agents like fluoride, salivary proteins, polyphosphates, polyor diphosphonates, etc. conferred outer surface protection rendering it insoluble. The model suggested by them involves H^+ ions and undissociated acid molecules (HA – for example lactic acid molecules) penetrating the enamel resulting in a heterogeneous reaction and finally the outward diffusion of reaction products from the enamel.

The rate of enamel dissolution is proportional to the H^+ concentration, the content of undissociated acid (HA), and a complexation function, X, respectively. The importance of this model lies in the fact that enamel lesion is regarded as a dynamic dissolution process and it also considers undissociated acid. However its drawbacks are that it is empirical and the surface layer formation is regarded as static in nature and is formed basically by protective agents in the liquid. These agents are neither involved in the dissolution process nor in the invasion of the porous lesion. However, Weatherell et al. (94) has shown that F^- ions and proteins can invade lesions. This model also does not explain the initial surface softening which occurs *in vivo*.

2. Gradient(s) solubility product and porosity of the enamel

The thermodynamic model by Van Dijk et al. (91) is a mathematical model along the lines of original model of Zimmerman (103) for the caries formation and it describes the inward and outward diffusion of the ions Ca^{++} , phosphate, H^+ , and hydroxyapatite from the lesion and the mineral dissolution assuming that the aqueous phase ionic reactions reach equilibrium more quickly than all other processes and that mineral dissolution rate is proportional to the difference between free energy in the saturated solution and that of the solution in the enamel pores. Data from computer simulations in this study led to the conclusion that a gradient in the solubility product and porosity of the enamel, a gradient in the rate constant of the dissolution reaction were responsible for the formation of surface layer on a subsurface lesion.

The chemical gradients for ions like F^- , CO_3^{2-} , Na^+ , Mg_2^+ etc. exist in sound human enamel and these may have an effect on the rate of dissolution. The chemical gradient and gradient in the solubility product of the enamel together with the rate constant of the dissolution reaction are regarded to be closely related to each other. However it is not established whether they cause experimental lesions and also these gradients are not found in lesions occurring in pressed apatite powders (55), and on polished enamel where surface layer formation is observed. Borsboom et al. in their study found that on polished enamel where the chemical gradients existing in sound enamel are absent the formation of a subsurface lesion could be observed as it was related to the presence of fluoride in the demineralizing solution (14).

3. Model of dissolution-precipitation mechanisms

Another model based on a dissolution-precipitation mechanism has been proposed by Margolis and Moreno (58) and Margolis et al. (59) to explain the formation of subsurface lesion. They suggested that there is a flow of H^+ (or undissociated acid HA) from the inner enamel to the surface layer resulting in phase transitions and DCPD (dicalcium phosphate dihydrate) and FAP (fluorapatite) in the surface layer of an enamel lesion. Thus the surface layer consists hydroxyapatite, dicalcium phosphate dihydrate and fluorapatite. The chemical potential of $Ca(OH)_2$ is greater in the deeper portion of the enamel in comparison to the surface layer and vice versa for the chemical potentials of H_3PO_4 and HA. The advantage of this model is that it is able to reasonably explain the intact nature of the surface layer overlying the area of demineralization in an enamel lesion. The two basic assumptions in this model were that the rate of transport of dissolved enamel does not occur at a faster rate such that reprecipitation occurs with surface layer formation and the second assumption was that favourable conditions already exist in the enamel for the reprecipitation reaction.

The inherent drawback of this model is that the chemical potentials of H_3PO_4 and $Ca(OH)_2$ are considered which is more suitable for thermodynamic process, however similar expressions cannot be used for the rates of crystal growth and dissolution. Besides this model excludes the specific effects of ions (19). According to Nielsen and Toft (66) and Nielsen (67) in systems with non-equivalent concentrations of the constituent ions and diffusion-controlled crystal growth the rate limiting factor is the deficient component and this rate is likely to become independent of the concentration of the excess component when there is large supersaturations or large deviation from equivalence (66, 67).

4. Model of outer surface protection combined with a dissolution-precipitation mechanism

An outer surface protection combined with a dissolution - precipitation mechanism model has been suggested

to explain the formation of subsurface lesion by Featherstone (31) and Featherstone et al. (35) which takes into account Gray's theory (40) of adsorption of protective agents and precipitation-dissolution model put forth by Moreno (64). F^- ions are not considered to be essential in this model. The outer surface of enamel adsorbs acquired pellicle and this confers protection. Later there is diffusion of undissociated acid (HA) into the enamel and through the surface layer and reacts with the enamel after dissociating into H^+ and A^- . This is followed by an outward diffusion of CaA_2 , $Ca(H_2PO_4)_2$, and $CaHPO_4$ (reaction products) in ionized and un-ionized forms and the precipitation at certain positions of $CaHPO_4$ (monetite). Thus an equilibrium between rate of loss to the exterior solution and surface layer deposition results in an apparently intact surface layer (70 % vol mineral and up to 70 μ m thick). The disadvantages of this model are identical to those of the earlier models.

5. Kinetic model

Kinetic model for subsurface lesion formation and progress has been proposed by Christoffersen and Arends (18) Arends et al. (5), and Christoffersen (17) wherein inhibitors of enamel dissolution such as F^- or specific proteins in the external demineralizing solution and from the enamel fluoride ions present near the outer surface of sound enamel (e.g., fluoride, ions like Mg_2^+ , CO_3^{2-} , proteins, etc. on the outer surface of sound enamel) play a crucial role in subsurface lesion formation and progression with time. In this model the dissolution rate of the crystallites varies with fraction of the surface covered by the inhibitor and on the pH, degree of saturation, and type of inhibitor in the solution.

The rate of adsorption of inhibitors onto the crystallite surface occurs at a higher rate when the external solution has a high inhibitor concentration resulting in retardation of dissolution process and the composition of the solution in the lesion and the external solution becomes identical. Here the lesion progresses slowly and this depends on the surface process occurring on the crystallite surface.

Mechanism of in vivo formation of a surface layer by inhibitors

The inhibitor fluoride (F^-) is mainly responsible for the mechanism of formation of a surface layer covering a subsurface enamel lesion as it decreases the rate of demineralization and causes increased crystallite growth. *In vivo* inhibitor e.g., a protein also act by similar mechanism. It is likely that in vivo surface layer is formed by various inhibitors.

Experiments involving *in vitro* caries systems by Ten Cate and Duisters (83,84), and later by Borsboom et al. (14), showed that surface layer is formed by F^- ions which exist in the liquid phase. Data from *in vivo* studies on early enamel lesions by Arends et al. (7), Ogaard et al. (69), proved that a surface layer is seen only after a month. The

position of outer enamel surface of the lesion is not seen to change in relation to the sound enamel surface during the formation of lesion (16). The formation of subsurface lesion is not influenced by fluoride content in the solid sound enamel as is shown by lack of *in vivo* decrease in caries in proportion to fluoride level in sound enamel as proposed by Englander et al. (27), Aasenden et al. (1), and Stem et al. (81).

The first stage in lesion formation is the softening stage where the outer surface has lesser mineral content but more in the deeper layers. During demineralization there is inward flow of fluoride and other ions into the porous enamel from the saliva or plaque. These fluoride ions exist in the liquid phase between the crystallites. When the fluoride level in the external solution of saliva reduces, the fluoride ions in the aqueous phase in the lesion also reduces from the surface along the lesion front. On the other hand in an active lesion when the level of fluoride is high, the crystallites near the enamel surface are in longer contact with fluoride which eventually get adsorbed and prevents further acid dissolution, when F^- adsorbs at vacant OH^- sites in the surface of the crystallites. This protection by the inhibitory effect of fluoride increases if the crystallites are in contact with the fluoride-containing solution for longer time period due to deeper penetration into the individual crystallites. In this case no new crystallographic phase is formed. In an *in vivo* caries process the periods of de- and remineralization are interrupted numerous times in a day and this influences the progression of the lesion. The fluoride in this case is derived from saliva, food and beverage. When subsurface lesion is formed under plaque, there is influx of fluoride (derived from excess fluoride from dentifrices, salivary proteins, etc.) into the lesion and prevention of progression of caries (4).

Prevention

The importance of exploring new strategies for prevention and therapy lies in the fact that initial caries has the potential to progress (32). Minimally invasive dentistry is a recent concept in preventive dentistry wherein the priority of the dentist is to preserve demineralized non cavitated enamel and dentin (28).

Chlorhexidine digluconate mouth rinse is a highly effective antibacterial therapy in decreasing the cariogenic bacteria (53) and the protective factors can then prevent or reverse the progression of dental caries (34). However when the lesion formation continues, the antibacterial substances in saliva like lactoferrin and the immunoglobulins are not effective. Less frequent consumption of fermentable carbohydrates is known to reduce the risk of caries. The use of sugar substitutes like xylitol instead of the fermentable carbohydrates such as glucose, sucrose, and fructose reduces the likelihood of dental caries (45, 80).

A total reduction of 30 % and 70 % caries is seen with fluoride toothpaste, mouth rinse, and office topical. Fluoridated water is also highly effective in preventing caries (33). Good plaque removal and oral hygiene techniques, placement of pit and fissure sealants, use of fluoride-releasing preventive and restorative materials, constitute other caries preventive techniques.

Approximal initial lesions have been subjected to fluoride treatment for remineralization and this prevents restorative intervention (25, 54, 76). Long - term studies have revealed that the use of fluoride has not been highly effective (61). An alternative to this is the possibility of using adhesive resin-based materials as a non-invasive treatment for early approximal enamel lesions for sealing noncavitated enamel carious lesions on premolar and molar (13, 26, 70). This treatment modality has received immense attention as certain long - term studies have shown that the caries in occlusal areas where caries was left underneath the intact sealant remained arrested until 10 years (39, 62, 79, 82).

In an *in vitro* study on argon laser (AL) irradiation and remineralizing solution (RS) treatment alone and in combination on caries like lesion formation in primary tooth enamel it was concluded that the greatest decrease in lesion depth in primary tooth enamel occurred with the use of RS (calcium, phosphate and fluoride in a carbopol base) in combination with AL irradiation (11). A lower enamel solubility and dissolution rates can be obtained with non-invasive caries preventive regimen of treating primary and permanent tooth enamel with low-fluence argon laser (AL) irradiation, either alone or in combination with topical fluoride treatment (96).

Conclusion

The progression of early enamel lesion is determined by the dynamic balance between demineralization and remineralization. The diagnostic armamentarium includes novel technologies and non-invasive techniques like fibre-optic transillumination and electrical resistance methods which are very useful in detecting posterior approximal dentinal caries and occlusal caries. Radiographs and direct digital imaging are still important tools in estimation of caries. A clear understanding of the mechanism of subsurface lesion formation and progression, possibilities and limitations of newer methods and their clinical applications need to be recognized by the dentist to direct preventive strategies to the high caries risk individuals.

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Submitted June 2008.
Accepted September 2008.

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