PROCEEDINGS
Clinical

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What Constitutes Dental Caries? Histopathology of Carious Enamel and Dentin Related to the Action of Cariogenic Biofilms

ABSTRACT

Substantial pH fluctuations within the biofilm on the tooth surface are a ubiquitous and natural phenomenon, taking place at any time during the day and night. The result may be recordable in the dental tissues at only a chemical and/or ultrastructural level (subclinical level). Alternatively, a net loss of mineral leading to dissolution of dental hard tissues may result in a caries lesion that can be seen clinically. Thus, the appearance of the lesion may vary from an initial loss of mineral, seen only in the very surface layers at the ultrastructural level, to total tooth destruction. Regular removal of the biofilm, preferably with a toothpaste containing fluoride, delays or even arrests lesion progression. This can occur at any stage of lesion progression, because it is the biofilm at the tooth or cavity surface that drives the caries process. Active enamel lesions involve surface erosion and subsurface porosity. Inactive or arrested lesions have an abraded surface, but subsurface mineral loss remains, and a true subsurface remineralization is rarely achievable, because the surface zone acts as a diffusion barrier. The dentin reacts to the stimulus in the biofilm by tubular sclerosis and reactionary dentin.

KEY WORDS: caries process, caries lesion, active lesions, arrested lesions.

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INTRODUCTION

One of the objectives of the International Consensus Workshop on Caries Clinical Trials was to review modern caries definitions critically. Thus, the speaker was asked to consider what constitutes dental caries, describe the histopathology, and relate it to the action of the cariogenic biofilm. The authors have approached the subject from a clinical standpoint.

WHAT CONSITUTUTES DENTAL CARIES?

It is perhaps unfortunate that the term "caries" can be used to refer to both the caries process and the caries lesion that forms as a result of that process. The caries process is initiated in the biofilm or dental plaque (Fejerskov and Manji, 1990; Manji *et al.*, 1991; Fejerskov and Thylstrup, 1994). Biofilms form on any solid surface exposed to appropriate amounts of water and nutrient (Wimpenny, 1994). The dental tissues—enamel, dentin, and cementum—are the relevant oral solid surfaces, and these surfaces are coated by a pellicle to which the microbial cells attach. The primary colonizers and secondary organisms generate a matrix of exopolymer within which cells grow. A community of organisms is formed rather than a haphazard collection of bacteria. The community has a collective physiology which can solve the specific physicochemical problems posed by the environment at the site.

The bacteria in the biofilm are always metabolically active, causing fluctuations in pH. These fluctuations may cause a loss of mineral from the tooth when the pH is dropping or a gain of mineral when the pH is increasing (Manji *et al.*, 1991). The cumulative result of these de- and re-mineralization processes may be a net loss of mineral, leading to dissolution of the dental hard tissues and the formation of a caries lesion.

The biofilm tends to form and mature in certain locations on the tooth, notably the occlusal surface, especially during eruption, the approximal surface cervical to the contact point, and along the gingival margin. These areas are relatively protected from mechanical wear by tongue, cheeks, abrasive food, and toothbrushing. Thus, these are the sites where caries lesions may become visible. It should be noted, however, that there is nothing special chemically about these particular areas of the tooth surface—they are susceptible to lesion development only because the biofilm tends to stagnate there and remain "undisturbed" for prolonged periods of time.

ACTIVE AND ARRESTED LESIONS

The role of the biofilm in driving the caries process has some important clinical implications. If the biofilm is removed, partially or totally, mineral loss may be stopped or reversed toward mineral gain. In other words, the lesion may be arrested, and this can occur at any stage of lesion formation.

However, since the biofilm is always forming, always present, and always metabolically active, it has been suggested that the caries process might be regarded as a ubiquitous and natural phenomenon (Manji *et al.*, 1991). Strictly speaking, this process cannot be prevented, but it can be controlled to the extent that a clinically visible caries lesion never develops.

Several factors other than the composition and thickness of the biofilm will influence the magnitude of the pH fluctuations. These factors will also determine the likelihood of mineral loss and the rate at which this occurs. These determinants include the diet, the fluoride ion concentration, and salivary secretion rate, but reflect a much more complex environment than just these gross components (Fejerskov, 1997).

DIAGNOSIS

Diagnosis is a clinical judgment that precedes a treatment decision. As far as the caries lesion is concerned, diagnosis implies detecting a caries lesion, estimating its depth and degree of demineralization, and making a decision about its activity (Nyvad and Fejerskov, 1997). This information is important so that an appropriate treatment can be suggested. An active lesion requires active management. This should include preventive, non-operative treatment, but operative management may also be needed, partly to restore the integrity of the tooth surface so that the patient can clean.

The caries lesion reflects the activity of the biofilm, but, perhaps ironically, this biofilm has to be removed to allow the dentist to see the lesion. This is particularly important in the detection of the early stages of demineralization. The histologist is in a similar position, examining a reflection of the caries process in the absence of the biofilm. However, the histologist is further disadvantaged, because most histological techniques are carried out on extracted teeth, and it is often difficult to gauge lesion activity under these circumstances. Thus, dentists and histologists work on reflections of reality, and this is potentially dangerous! When one looks at a reflection, it is easy to accept the reflection as reality, perhaps forgetting where the "action" is. The "action" is initiated in the biofilm.

ULTRASTRUCTURAL CHANGES IN ENAMEL RELATED TO THE BIOFOLM

An elegant series of *in vivo* experiments (Holmen *et al.*, 1985b, 1987a,b) followed the development of the initial lesion formed under an undisturbed biofilm. The investigators created these conditions by cementing bands onto teeth which were subsequently extracted for orthodontic purposes. The bands prevented mechanical disturbance of the plaque. After various periods of time, the bands were removed and replicas of the surfaces taken for scanning electron microscopy. The teeth were then left uncovered, and mechanical plaque control was resumed, with further replicas showing how the surface reacted to resumption of biofilm removal.

After one week of undisturbed biofilm formation, no changes in the enamel were seen clinically, even after samples had been careful air-dried. However, at the ultrastructural level, there were signs of direct dissolution of the outer enamel surface. This was seen as an enlargement of the intercrystalline spaces due to partial dissolution of the individual crystal peripheries.

After two weeks with completely undisturbed plaque, the enamel changes were visible clinically after samples were air-dried. The "white spot" lesion was now visible. After three and four weeks, these changes could be seen before samples were air-dried, the lesion being opaque with a matte surface. Ultrastructurally, there was: complete dissolution of the thin perikymata overlappings; marked dissolution corresponding to developmental irregularities such as Tomes' processes, pits, and focal holes; and continued enlargement of the intercrystalline spaces.

Thus, the surface participates in the enamel reaction from the very beginning of lesion formation by direct dissolution of the outermost microsurface and enlargement of intercrystalline diffusion pathways. (Haikel *et al.*, 1983; Holmen *et al.*, 1985b; Thylstrup and Fejerskov, 1981). This direct surface erosion is most likely partly responsible for the matte surface of the active lesion.

When the orthodontic bands were removed, allowing for disturbance of the biofilm, the white appearance diminished and the surface became hard and shiny again. Ultrastructural studies showed wear of the external microsurface. This led the authors to suggest that the return to a shiny, hard surface was a result of abrasion or polishing of the partly dissolved surface of the active lesion. This important series of experiments shows the precise relationship of the lesion to the biofilm and shows that regular disturbance of the biofilm will arrest the lesion.

THE WHITE-SPOT LESION

The same series of experiments also extracted teeth at various times to allow for detailed examination of sections in polarized light (Holmen *et al.*, 1985a). After only one week of undisturbed biofilm formation, this examination showed a slight increase in enamel porosity, and the tissue beneath the porous outer microsurface was more porous than the microsurface itself. This so-called subsurface demineralization became more obvious at weeks 2, 3, and 4, and the classic histological zones of the white-spot lesion in polarized light could be identified. These zones are the surface zone and body of the lesion best seen after imbibition of sections in water and the dark zone and primary translucent zone seen after imbibition of sections in quinoline (Silverstone, 1973).

Several models have been proposed to explain the relative protection against further dissolution of the outer 10-30 microns of enamel. A physico-chemical explanation seems important. Dissolution is caused by an undersaturation with respect to enamel apatite and a formation of fluorapatite in the enamel surface caused by a supersaturation with respect to fluorapatite (Larsen, 1990). A protective role of salivary proline-rich proteins and other salivary inhibitors, such as statherin, has also been emphasized (Hay, 1984). These inhibit demineralization and prevent crystal growth. They are macromolecules and cannot penetrate the deeper parts of the enamel; thus, their stabilizing role is limited to the surface enamel. The outer enamel is special in terms of its ultrastructure and chemical composition (Weatherell *et al.*, 1984), but this is unlikely to play a significant role in caries lesion initiation.

Removal of the orthodontic bands and resumption of tooth cleaning resulted in reduced porosity of the deeper parts of lesions. The authors suggest that a gradual return of enamel fluids to supersaturation with respect to apatites causes a shift in equilibrium and reprecipitation of minerals at the sites of demineralization. Arrested lesions showed a widening of the dark zones, indicating this reprecipitation. However, although the surface of the lesion may become hard and shiny and the white spot becomes less obvious, some interior opacity remains (Årtun and Thylstrup, 1989), because some subsurface porosity is still present. It should be noted that these arrested lesions have been claimed to be more "resistant" to a subsequent acid attack than sound enamel (Koulourides *et al.*, 1980).

The shape of the white-spot lesion is determined by the distribution of the biofilm and the direction of the enamel prisms. Thus, on an approximal surface, the lesion formed beneath the biofilm is a kidney-shaped area between the contact facet and the gingival margin. Within the enamel, spread of dissolution takes place along the enamel prisms. In section, the smooth-surface lesion is conical. This conical shape is the result of systematic variations in dissolution along the enamel prisms. The oldest or most active part of the lesion is located along the central traverse. The conically shaped lesion represents a range of increasing stages of lesion progression, beginning with dissolution at the ultrastructural level at the edge of the lesion (Bjørndal and Thylstrup, 1995). This emphasizes that the lesion is driven by, and reflects, the specific environmental conditions in the overlying biofilm.

Caries on an occlusal surface is also a localized phenomenon in the deepest part of the groove-fossa system, where the bacterial accumulations receive the best protection against functional wear. The lesion forms in three dimensions, again guided by prism direction. The lesion thus assumes the shape of a cone, with its base toward the enamel-dentin junction. It appears that the active biofilm is above the entrance to the narrow fissures and grooves. Ultrastructural studies show that the deepest part of the fissure usually harbors non-vital bacteria or calculus (Theilade *et al.*, 1976; Ekstrand and Bjørndal, 1997). This has important clinical implications explaining why occlusal lesion formation can be prevented on erupting molar teeth by removal of the biofilm with a fluoride toothpaste (Carvalho *et al.*, 1992).

POROSITY HAS ITS USES

The porosity of the subsurface lesion can be turned to some advantage by the clinician. First, it explains why the white-spot lesion looks white and why a dentist, looking at a clean tooth surface, can, using vision and a three-in-one syringe, determine the depth of penetration of the lesion. The lesion that is visible only on a dry tooth surface is

probably in the outer enamel, whereas a lesion visible on a wet tooth surface has penetrated most of the way through the enamel and maybe into the dentin.

This relates to the relative refractive indices of enamel, water, and air. Enamel has a refractive index of 1.62. In the subsurface lesion, the pores are filled with a watery medium with a refractive index of 1.33. The difference in refractive index between the water and the enamel affects the light scattering and makes the lesion look opaque. If the surface is then dried, air, with a refractive index of 1.0, replaces the water. The difference in refractive index between the air and the enamel is now greater than that between the water and the enamel. This means that the lesion becomes more obvious, and an earlier lesion can be detected.

Porosity is the basis of many techniques that detect caries lesions—for instance, radiography, quantitative light-induced fluorescence (de Josselin de Jong *et al.*, 1996), electrical resistance (Ricketts, 1996). Some of these techniques allow for quantitation of the degree of porosity. Used once, they will detect demineralization, but used on a longitudinal basis, they potentially allow the dentist, or those conducting clinical trials, to follow lesion progression or lesion arrestment.

Before leaving the subject of subsurface porosity, we add a word of caution. The dentist must be careful when using a sharp probe. It is very useful to draw the point gently across the lesion to detect a matte surface indicating an active lesion. It is most unwise, however, to jab the sharp probe into the lesion to see whether it is "sticky". The probe is likely to cause a cavity, and this will encourage biofilm stagnation and lesion progression (Ekstrand *et al.*, 1987).

DENTIN DEFENSE REACTIONS

Dentin is a vital tissue containing the odontoblast processes, and this tissue will react to transmission of stimuli from the oral cavity through the microporous enamel. The most common defense reaction by the pulp-dentin complex is the deposition of mineral within the dentinal tubules (Massler, 1967; Levine, 1974; Johnson *et al.*, 1969; Stanley *et al.*, 1983). This is called 'tubular sclerosis' or 'translucent dentin' because, due to reduced light scattering, the tissue appears translucent histologically when examined in transmitted light. The process requires a vital odontoblast.

Tubular sclerosis in dentin is visible before the advancing front of the enamel extends to the enamel-dentin junction. Once the enamel lesion comes into contact with the enamel-dentin junction, a brownish discoloration of the dentin is seen, and this is the first sign of dentin demineralization. This appearance is not lateral spread of the lesion. It has been shown never to extend beyond the limits of the enamel lesion contact area with the enamel-dentin junction. It simply represents a reaction to the biofilm at the tooth surface, transmitted in the direction of the enamel prisms (Bjørndal and Thylstrup, 1995; Bjørndal *et al.*, 1999). This is another important example of the caries lesion being driven by the acid-producing bacteria in the biofilm. This is critical to clinical management, because, once again, it implies that the lesion can be arrested by regular disturbance of the biofilm.

The other important defense reaction of the pulp-dentin complex is the formation of reactionary dentin, and this may commence before bacterial invasion of the dentin (Massler, 1967; Silverstone, 1973).

AN IMPORTANT MOMENT CLINICALLY

The formation of a cavity may be a very important moment clinically, because now the biofilm is protected within a microcavity, and unless the patient is able to clean this area, the caries process will continue. The protected environment favors an ecological shift toward anaerobic and acid-producing bacteria. Presumably, the cavity is first created by mechanical injuries during mastication, microtraumas during interdental wear, or even careless, heavy probing by dentists!

BACTERIAL INVASION: WHAT DRIVES THE CARIES PROCESS?

Bacteria are at best rarely present within the lesion before cavitation of the enamel surface, although the dentin of uncavitated lesions may be soft and lightly infected (Ratledge *et al.*, 2001). Once the cavity is directly exposed to the bacterial biomass, superficial tubular invasion occurs. Now the most superficial part of the dentin becomes decomposed due to the action of acid and proteolytic enzymes. This is called the zone of destruction. Beneath this zone, tubular invasion of bacteria is frequently seen. With rapid lesion progression, the odontoblastic processes are destroyed without having produced tubular sclerosis. These are called dead tracts in the dentin. These empty tubules are invaded by bacteria, and groups of tubules may coalesce to form liquefaction foci. This area is called the zone of bacterial penetration. In the sclerotic dentin, the translucent zone is a zone of demineralization resulting from acid demineralization (Thylstrup and Qvist, 1987).

The relevant question for the clinician now is, "What drives the caries process?" Is the process exclusively driven by the biofilm at the cavity surface, or are the bacteria within the lesion also major players? This argument is highly relevant to clinical management. The current operative paradigm is based on the removal of infected dentin before the cavity is sealed. This assumes that the bacteria within the dentin are important. However, the weight of experimental evidence (for review, see Kidd, 2000) might suggest that the bacteria in the biofilm are what matters, although we accept that this statement is controversial. It appears that lesion progression can be arrested by either simple removal of the biofilm or by the sealing of bacteria within the cavity and restoration of the tooth so that regular removal of the biofilm is possible.

ROOT-SURFACE CARIES

Root caries is similar to enamel caries in being a subsurface demineralization, but, unlike enamel caries, the surface may appear softened at an early stage of lesion development. Bacteria penetrate at an earlier stage than in coronal caries (for review, see Fejerskov and Nyvad, 1996). Even when microcavities are observed, the demineralization is deep to a relatively well-mineralized surface layer. Interestingly, although these lesions may appear rather extensive, they are seldom more than 0.5 to 1 mm deep. This slow rate of bacterial invasion and tissue degradation gives the opportunity for these lesions to be arrested by plaque control with fluoride toothpaste (Nyvad and Fejerskov, 1986; Nyvad *et al.*, 1997). Once again, it is the biofilm at the surface of the lesion that is driving the process.

SECONDARY CARIES

Secondary or recurrent caries is primary caries at the margin of a restoration (for review, see Mjör and Toffenetti, 2000). The histological picture will show primary caries next to the restoration margin, and there may be lines of demineralization, called wall lesions, running along the cavity wall. These are a consequence of microleakage, but clinical and microbiological studies appear to indicate that this leakage does not lead to active demineralization beneath the restoration.

SUMMARY

The caries process is initiated by activity within the biofilm and manifested in the underlying enamel or dentin. The caries lesion may be active or arrested, and reflects the activity in the biofilm. It should be considered the sign or symptom of the disease.

REFERENCES

Årtun J, Thylstrup A (1989). A three-year clinical and SEM study of surface changes of carious enamel lesions after inactivation. *Am J*

Dentofac Orthop 95:27-33.

Bjørndal L, Thylstrup A (1995). A structural analysis of approximal enamel caries lesions and subjacent dentin reactions. *Eur J Oral Sci* 103:25-31.

Bjørndal L, Darvann T, Lussi A (1999). A computerized analysis of the relation between the occlusal enamel caries lesion and the demineralised dentine. *Eur J Oral Sci* 107:176-182.

Carvalho JC, Ekstrand KR, Thylstrup A (1992). Results of 3 years of non-operative occlusal caries treatment of erupting permanent first molars. *Community Dent Oral Epidemiol* 20:187-192.

de Josselin de Jong E, Hall AF, van der Veen MH (1996). Quantitative light-induced fluorescence detection method: a Monte Carlo simulation model. In: Early detection of dental caries. Stookey GK, editor. Indianapolis: Indiana University, pp. 91-102.

Ekstrand KR, Bjørndal L (1997). Structural analysis of plaque and caries in relation to the morphology of the groove-fossa system on erupting mandibular third molars. *Caries Res* 31:336-348.

Ekstrand KR, Qvist V, Thylstrup A (1987). Light microscope study of the effect of probing in occlusal surfaces. *Caries Res* 21:368-374.

Fejerskov O (1997). Concepts of dental caries and their consequences for understanding the disease. *Community Dent Oral Epidemiol* 25:5-12.

Fejerskov O, Manji F (1990). Risk assessment in dental caries. In: Risk assessment in dentistry. Bader JD, editor. Chapel Hill: University of North Carolina Dental Ecology, pp. 215-217.

Fejerskov O, Nyvad B (1996). Dental caries in the ageing individual. In: Textbook of geriatric dentistry. Holm-Pedersen P, Löe H, editors. Copenhagen: Munksgaard, pp. 338-372.

Fejerskov O, Thylstrup A (1994). Different concepts of dental caries and their implications. In: Textbook of clinical cariology. 2nd ed. Thylstrup A, Fejerskov O, editors. Copenhagen: Munksgaard, pp. 259-283.

Haikel Y, Frank RM, Voegel JC (1983). Scanning electron microscopy of human enamel surface layers of incipient carious lesions. *Caries Res* 17:1-13.

Hay DI (1984). Specific functional salivary protein. In: Cariology today. Guggenheim B, editor. Basel: Karger, pp. 98-108.

Holmen L, Thylstrup A, Øgaard B, Kragh FA (1985a). A polarised light microscopic study of progressive stages of enamel caries in vivo. *Caries Res* 19:348-354.

Holmen L, Thylstrup A, Øgaard B, Kragh FA (1985b). Scanning electron microscopy study of progressive stages of enamel caries in vivo. *Caries Res* 19:355-367.

Holmen L, Thylstrup A, Årtun J (1987a). Clinical and histological features observed during arrestment of active enamel carious lesions in vivo. *Caries Res* 21:546-554.

Holmen L, Thylstrup A, Årtun J (1987b). Surface changes during the arrest of active enamel carious lesions in vivo. A scanning electron microscope study. *Acta Odontol Scand* 45:383-390.

Johnson NW, Taylor BR, Berman DS (1969). The response of deciduous dentine to caries studied by correlated light and electron microscopy. *Caries Res* 3:348-368.

Kidd EAM (2000). Caries removal and the pulpodentinal complex. *Dental Update* 27:476-482.

Koulourides T, Keller SE, Manson-Hing L, Lilley V (1980). Enhancement of fluoride effectiveness by experimental cariogenic priming of human enamel. *Caries Res* 14:32-39.

Larsen MJ (1990). Chemical events during tooth dissolution. *J Dent Res* 69(Spec Iss):575-580.

Levine RS (1974). The microradiographic features of dentine caries. *Br Dent J* 137:301-306.

Manji F, Fejerskov O, Nagelkerke NJD, Baelum V (1991). A random effects model for some epidemiological features of dental caries. *Community Dent Oral Epidemiol* 19:324-328.

Massler M (1967). Pulpal reactions to dental caries. *Int Dent J* 17:441-460.

Mjör IA, Toffenetti F (2000). Secondary caries: a literature review with case reports. *Quintessence Int* 31:165-179.

Nyvad B, Fejerskov O (1986). Active root surface caries converted into inactive caries as a response to oral hygiene. *Scand J Dent Res* 94:281-284.

Nyvad B, Fejerskov O (1997). Assessing the stage of caries lesion activity on the basis of clinical and microbiological examination. *Community Dent Oral Epidemiol* 25:69-75.

Nyvad B, ten Cate JM, Fejerskov O (1997). Arrest of root surface caries *in situ*. *J Dent Res* 76:1845-1853.

Ratledge DK, Kidd EAM, Beighton D (2001). A clinical and microbiological study of approximal carious lesions. Part 2: Efficacy of caries removal following tunnel and Class II cavity preparations. *Caries Res* 35:8-11.

Ricketts DNJ (1996). Electrical conduction detection methods. In: Early detection of dental caries. Stookey GK, editor. Indianapolis: Indiana University, pp. 67-80.

Silverstone LM (1973). Structure of carious enamel including the early lesion. In: Oral sciences reviews. No. 3. Dental enamel. Melcher AH, Zarb GA, editors. Copenhagen: Munksgaard, pp. 100-160.

Stanley HR, Pereira JC, Spiegel EH, Broom C, Schultz M (1983). The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin, and dead tracts beneath various types of dental lesions according to tooth surface and age. *J Oral Pathol* 12:257-289.

Theilade E, Fejerskov O, Hørsted M (1976). A transmission electron microscope study of 7-day old bacterial plaque in human tooth fissures. *Arch Oral Biol* 21:587-588.

Thylstrup A, Fejerskov O (1981). Surface features of early carious enamel at various stages of activity. In: Proceedings of a workshop on tooth surface interactions and preventive dentistry. Rølla G, Sønju T, Embery G, editors. London: IRL Press, pp. 193-205.

Thylstrup A, Qvist V (1987). Principal enamel and dentine reactions during caries progression. In: Dentine and dentine reactions in the oral cavity. Thylstrup A, Leach SA, Qvist V, editors. Oxford: IRL Press, pp. 3-16.

Weatherell JA, Robinson C, Hallsworth AS (1984). The concept of enamel resistance—a critical review. In: Cariology today. Guggenheim B, editor. Basel: Karger, pp. 223-230.

Wimpenny JWT (1994). The spatial organisation of biofilm. In: Bacterial biofilms and their control in medicine and industry. Wimpenny J, Nichols W, Stickler D, Lappin-Scott H, editors. Bioline, pp 1-5.