Ecological Hypothesis of Dentin and Root Caries

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Key Words
Bacteria · Cathepsin · Dental caries · Dentin · Ecological hypothesis · Matrix metalloproteinases · Proteolysis · Root caries

Abstract
Recent advances regarding the caries process indicate that ecological phenomena induced by bacterial acid production tilt the de- and remineralization balance of the dental hard tissues towards demineralization through bacterial acid-induced adaptation and selection within the microbiota – from the dynamic stability stage to the aciduric stage via the acidogenic stage [Takahashi and Nyvad, 2008]. Dentin and root caries can also be partly explained by this hypothesis; however, the fact that these tissues contain a considerable amount of organic material suggests that protein degradation is involved in caries formation. In this review, we compiled relevant histological, biochemical, and microbiological information about dentin/root caries and refined the hypothesis by adding degradation of the organic matrix (the proteolytic stage) to the abovementioned stages. Bacterial acidification not only induces demineralization and exposure of the organic matrix in dentin/root surfaces but also activation of dentin-embedded and salivary matrix metalloproteinases and cathepsins. These phenomena initiate degradation of the demineralized organic matrix in dentin/root surfaces. While a bacterial involvement has never been confirmed in the initial degradation of organic material, the detection of proteolytic/aminopeptidase-digesting bacteria and bacterial metabolites in dentin and root caries suggests a bacterial digestion and metabolism of partly degraded matrix. Moreover, bacterial metabolites might induce pulpitis as an inflammatory/immunomodulatory factor. Root and dentin surfaces are always at risk of becoming demineralized in the oral cavity, and exposed organic materials can be degraded by host-derived proteases contained in saliva and dentin itself. New approaches to the prevention and treatment of root/dentin caries are required.

Recent advances in our knowledge about the caries process indicate that ecological phenomena, e.g. bacterial adaptation to acidic environments (increases in bacterial acidogenicity and acidurance) and bacterial shifts to a more acidogenic and aciduric microbiota (increases in the proportion of acidogenic and aciduric bacteria), are induced by frequent and prolonged acidification. The resultant environmental acidification can tilt the balance between demineralization and remineralization toward demineralization, resulting in the initiation/progression of caries.

This paper is based on a presentation held at the 10th European Oral Microbiology Workshop, Aarhus, Denmark, in May 2014.
of caries. The ecological concept of caries was first proposed in the ‘ecological plaque hypothesis’ by Marsh [1994] and was subsequently extended by Takahashi and Nyvad [2008, 2011] based on detailed information regarding the biochemical responses to environmental acidification, i.e. the induction of bacterial acidogenicity/acidurance and the selection of acidogenic/aciduric bacteria. This ecological hypothesis forms the basis of our current understanding of caries, but mainly focuses on phenomena occurring in enamel caries. Ecological factors in dentin and root caries have only been scarcely addressed, possibly because of the complex structural composition of these tissues.

The etiology of caries has been debated over centuries, and three key hypotheses have been proposed: the chemico-parasitic, the chelation, and the proteolysis-chelation theories. There is no doubt that the etiology of enamel caries can be mainly explained by the chemico-parasitic theory, in which the dental biofilm (bacteria) produces acid from sugar, and the resultant acidic pH promotes demineralization of the enamel. Dentin and root caries can also be explained by this theory; however, the fact that dentin and root surfaces contain a considerable amount of organic material, such as collagen, has led to the notion that protein degradation (the proteolysis theory) might also play a role in caries development in these tissues. Indeed, a series of publications [Dayan et al., 1983; Tjäderhane et al., 1994; Chaussain-Miller et al., 2006] have supported this possibility.

In this review, we attempt to compile relevant histological, biochemical, and microbiological information about dentin and root caries and propose a possible mechanism for the development of dentin and root caries by including degradation of the organic matrix with the cyclic de- and remineralization processes in caries.

Structural Composition of Root and Dentin Surfaces

Dentin and root cementum surfaces are less mineralized than enamel. About 30% of dentin and root cementum is composed of organic materials, mainly collagen, while enamel is almost entirely composed of hydroxyapatite with trace amounts of organic materials [Furseth and Mjör, 1979]. These biochemical differences are reflected in the pattern of caries. While bacterial invasion of enamel occurs only after total breakdown of the tissue, root surfaces become invaded by bacteria already at an early stage of the caries process [Schüpbach et al., 1989; Nyvad and Fejerskov, 1990].

Ultrastructural studies have suggested that demineralization and breakdown of the organic matrix happens in two successive stages [Selvig, 1969; Schüpbach et al., 1989; Nyvad and Fejerskov, 1990]. In the early stages of cementum and dentin caries, minerals are dissolved by a fine gradient from the outer surface, while maintaining the characteristic cross-banding of the collagen fibers [Nyvad and Fejerskov, 1990; Deyhle et al., 2011]. The demineralized collagen serves as a scaffold for colonizing bacteria. At more advanced stages the exposed collagen is broken down by proteolytic enzymes, and collagen fibers lose their structural characteristics [Kuboki et al., 1977]. However, a recent study [Tjäderhane et al., 2015] suggests that collagen cross-banding may be lost at a relatively early stage of demineralization, in which an exposed telopeptide region of the collagen molecule can be degraded by telopeptidase activity of host-derived collagenolytic enzymes. Dentin and root surfaces are more labile than enamel in acidic conditions [Hoppenbrouwers et al., 1987; Moreno and Aoba, 1991], possibly because of the smaller hydroxyapatite crystallites in dentin [Yardeni, 1952]. Higher levels of carbonate and magnesium [Hoppenbrouwers et al., 1987] and the tubular structure of dentin [Shellis, 1996] could also add to the higher solubility.

Root and dentin surfaces are highly reactive in the oral environment. Even clinically sound root surfaces may take up minerals from the oral fluids, resulting in a higher mineral content in the surface layer compared with the normal dentin [Selvig, 1969]. Such phenomena are likely to occur as a result of cyclic demineralization and remineralization processes [Nyvad et al., 1997]. However, root and dentin surfaces are also vulnerable to physical injury. Because of the low mineral content, root surfaces are easily abraded by toothbrushing, and although cementum formation triples throughout life, reaching a thickness of about 50 μm in the coronal part of the root at the age of 20 [Furseth and Mjör, 1979], exposed cervical root cementum is often damaged and/or removed by regular scaling and root planing [Jepsen et al., 2004]. Therefore, in clinical circumstances the exposed root often presents a dentin surface. In favorable conditions a new hypermineralized surface layer may develop within a few months after over-instrumentation of root surfaces [Selvig, 1969].

Microbial Ecosystem of Dentin and Root Caries

The microbiological and structural pattern of colonization of root surfaces has been extensively reviewed [Nyvad, 1993]. A few hours after the cleaning of a root
surface the surface is colonized by pioneer species mainly belonging to Streptococcus (S. sanguinis, S. oralis, and S. mitis) and Actinomyces. In situ studies have shown that the early colonizers do not differ between enamel and root surfaces [Nyvad and Kilian, 1987], suggesting that it is the oral environment (saliva and/or gingival crevicular fluid) rather than differences in composition of the pellicle [Rüdiger et al., 2002] that determine the colonization pattern [Nyvad, 1993]. In this scenario, it is not surprising that the microbial community on clinically sound root surfaces include various acidogenic bacteria belonging to Streptococcus and Actinomyces [van Houte et al., 1994, 1996], many of which are capable of producing acid from dietary carbohydrates and lowering environmental pH to <5.5 [van Houte et al., 1994, 1996; Aamdal-Scheie et al., 1996]. In these mildly acidogenic conditions (pH 5–6), Actinomyces spp. colonizing the innermost part of the dental biofilm [Dige et al., 2009] may play an important role in controlling the mineral loss by pH-modulatory mechanisms [Takahashi and Yamada, 1999]. In addition to base production from other bacteria, such metabolic events may help to balance the demineralization and remineralization processes on root surfaces in a manner similar to that described for enamel surfaces in the dynamic stability stage [Takahashi and Nyvad, 2008].

However, when the mineral balance tilts toward demineralization (e.g. due to frequent sugar-induced acidification), the cariogenic processes may progress and result in the development of a clinically visible root caries lesion. Bacteria can adapt to the acidic environment and enhance their acidogenicity and acidurance, as described in the biochemical process responsible for the development of enamel caries (the acidogenic stage) [Takahashi and Nyvad, 2008]. The acidic environment promotes the introduction of more acidogenic bacteria, such as low-pH Streptococcus and Actinomyces strains, which are more capable of lowering the environmental pH than the Streptococcus and Actinomyces strains found on the surfaces of clinically sound roots [van Houte et al., 1994, 1996]. Active root caries lesions sometimes contain increased numbers of aciduric bacteria, such as mutans streptococci, Lactobacillus, or Bifidobacterium species [Nyvad and Kilian 1990; van Houte et al., 1994; 1996; Schüpbach et al., 1996; Brailsford et al., 2001; Mantzourani et al., 2009]. These observations suggest that some active lesions with an acidic environment can facilitate the establishment of aciduric bacteria, as described in enamel caries (the aciduric stage) [Takahashi and Nyvad, 2008]. However, mutans streptococci do not become dominant; even in advanced stages of active root caries, Actinomyces and non-mutans streptococci species remain dominant [Brailsford et al., 1999, 2001]. Furthermore, a molecular biology-based study [Preza et al., 2008] revealed that the microbial communities in root caries are highly complex and include not only mutans streptococci, Actinomyces, and Lactobacillus species, but also species of Atopobium, Olsenella, Pseudoramibacter, Propionibacterium, and Sele-nomonas. Yeasts, such as Candida albicans, were also detected in root caries lesions in middle-aged adults and the elderly [Shen et al., 2002; Zaremba et al., 2006], although the invasion of these species into carious dentin has been questioned [Maijala et al., 2007].

Despite similarities between the processes responsible for enamel and root caries in terms of microbial acid-induced adaptation and selection, local environmental factors on root surfaces, such as the presence of gingival crevicular fluid, thickness of bacterial deposits, and exposure to salivary flow, may modify the caries process. Most root caries lesions are shallower with a tendency to spread laterally compared to cavities in enamel, and thinner biofilms with easy access to salivary clearance of metabolites may reduce the acidity of the biofilm. Gingival crevicular fluid may also play a modulatory role in root caries because of its neutral to weakly alkaline pH [Bickel and Cimasoni, 1985]. Such weak acidic environments might facilitate colonization and invasion by Actinomyces species [Nyvad and Fejerskov, 1990], which are generally not as acidogenic or aciduric as Streptococcus species [Horiuchi et al., 2009]. Furthermore, proteolytic bacteria, such as Prevotella intermedia, Prevotella denticola, and Propionibacterium acnes, have been isolated from root surfaces [Aamdal-Scheie et al., 1996; Schüpbach et al., 1995, 1996; Hashimoto et al., 2011], suggesting that nitrogenous substrates, such as proteins, peptides, and amino acids, which can be supplied from gingival crevicular fluid, are available near the root surface.

In contrast to root surfaces, coronal dentin will only become colonized by bacteria after total breakdown of the enamel [Dige et al., 2014]. Dentin caries lesions possess highly diverse microbioitas [Aas et al., 2008; Obata et al., 2014; Schulze-Schweifing et al., 2014; Simón-Soro et al., 2014], which can lead to the development of strictly anaerobic conditions [Edwardsson, 1974; Hoshino, 1985]. The microbiotas in dentin caries consist of both acidogenic/aciduric bacteria, such as Streptococcus mutans, Lactobacillus, and Bifidobacterium species, and proteolytic/amino acid-degrading bacteria, such as Prevotella and Propionibacterium species [Aas et al., 2008]. Reflecting this microbiological diversity, dentin caries lesions were reported to contain a variety of organic acids, and their pH was found to vary markedly [Hojo et al., 1991, 1994].
Active dentin cavities (which were soft and yellowish-white-colored with thick carious dentin) demonstrated an acidic pH of 4.9 ± 0.2, and the dominant acid was lactic acid, while arrested dentin cavities (which were hard and darkly pigmented with thin carious dentin) had a weakly acidic pH of 5.6 ± 0.4 and exhibited mixed acid profiles, which included acetic, propionic, n/i-butyrinic, n/i-valeric, and n/i-caproic acids [Hojo et al., 1994]. The dominance of lactate and the acidic pH observed in the active cavities might have been due to carbohydrate stagnancy and carbohydrate metabolism by lactate-producing saccharolytic bacteria, including Streptococcus, Actinomyces, and Lactobacillus species. Streptococcus species are reported to mainly produce lactic acid from carbohydrates at acidic pH [Iwami et al., 1992]. Meanwhile, the mixed acid profiles seen in the arrested cavities might have been derived from the secondary metabolism of lactate to weaker acids, such as acetate and propionate, by Veillonella and other species that can neutralize acidic conditions [Takahashi, 2015]. Furthermore, these acid profiles can be created by proteolytic/ amino acid-degrading bacteria, such as Propionibacterium and Prevotella species, from nitrogenous metabolic substrates [Takahashi, 2015], which can be supplied from dentin proteins by proteolysis, as described in the next section. Such amino acid metabolism can also neutralize acidic conditions [Takahashi, 2003, 2015]. Hence, the weakly acidic conditions encountered in arrested cavities might indicate a tilt towards remineralization. However, differences in the microbiotas between active and arrested cavities have not yet been elucidated.

**Acid-Induced Denaturation of Dentin/Cementum Matrix Proteins**

As described above, dentin and root caries involves both demineralization and collagen degradation. It is important to note that protein degradation seems to occur mainly after demineralization. Proteases cannot reach proteinaceous substrates or function in mineralized tissues, i.e. their substrates have to be exposed to the aqueous phase. In addition, proteases have to be activated or detached from inhibitors since most proteases are present in their inactive forms. It is also worth bearing in mind that intact collagen molecules are resistant to several proteolytic enzymes (except for collagenases) due to their high-order conformational structure (triple helix), and thus their internal structure has to be solubilized and denatured in the aqueous phase prior to proteolytic degradation.

The conformational structure of collagen molecules remains stable under physiological conditions since the denaturation temperature of collagen (the temperature required to achieve a 5% reduction in the specific viscosity of collagen solution) is over 100°C [Cadenaro et al., 2016]. However, once collagen molecules are exposed, telopeptidic activity of host-derived collagenase can start to partly break down the telopeptide region of the molecules, as described in the previous section [Tjäderhane et al., 2015], and then the host-derived collagenase and proteases can further degrade the molecules and transform them to more water-soluble and labile components. Solubilized collagen molecules can be denatured efficiently under acidic conditions and shift their denaturation temperature toward body temperature [Hayashi and Nagai, 1973]. A study by Leikina et al. [2002] demonstrated that solubilized type I collagen can be denatured to a random coil structure even at body temperature. Collectively, these observations suggest that during acid-induced demineralization collagen molecules are exposed to the aqueous phase. Subsequently, they can be initially degraded by host-derived collagenases and acid denatured, and then the denatured collagen (gelatinized collagen) can be degraded by various proteases, such as gelatinases and peptidases, which are widely distributed in both host and bacteria.

It should be appreciated that dentin organic materials contain proteases [matrix metalloproteinases (MMPs) and cysteine cathepsins], which, unlike collagen, are resistant to the acidic environment [Tezvergil-Mutluay et al., 2013] but can be activated in the acidic environment. Details of the acid activation are described in the next section.

**Protein Degradation by Host- and Bacteria-Derived Proteases**

Recent studies have suggested that host-derived MMPs and cysteine cathepsins are directly involved in dentin matrix degradation during caries formation. Several types of MMP, such as MMP-2 (a 72-kDa gelatinase), MMP-8 (collagenase-2), and MMP-9 (a 92-kDa gelatinase) [Tjäderhane et al., 1998], as well as cysteine cathepsins B and K [Nascimento et al., 2011; Vidal et al., 2014], have been found in dentin caries lesions. The dentin matrix mainly consists of type I collagen, and MMPs and cathepsins can degrade such collagen after bacterial acid-induced demineralization, as stated above. These host proteases are also contained in saliva [Tjäderhane et al., 1998; Nasci-
mento et al., 2011; Hedenbjörk-Lager et al., 2015] and usually secreted in their inactive forms; however, they are automatically activated under acidic conditions (around pH 4.5). Cysteine cathepsins can function actively under acidic conditions, while MMPs have an optimal pH around neutral. In addition, Miyoshi et al. [2010] reported that the proteolytic activity of these proteases was gradually activated in saliva even at neutral pH and 37°C, although the mechanism responsible for this activation has not been elucidated.

Dentin itself is also known to contain proteases, such as MMP-2, 3, 8, 9, and 20, and cysteine cathepsins B and K [Sulkala et al., 2002; Mazzoni et al., 2009; Shimada et al., 2009; Toledano et al., 2010; Boushshet et al., 2011; Buzalaf et al., 2015; Tjäderhane et al., 2015]. These proteases are produced during dentin formation and then become embedded in the dentin matrix (in their inactive forms) together with protease inhibitors, such as tissue inhibitor of metalloproteinases (TIMP)-1 and -2 [Ishiguro et al., 1994; Niu et al., 2011]. However, once dentin is demineralized or exposed to the aqueous phase, these proteases are activated and start to degrade the dentin organic matrix. Cysteine cathepsin B is reported to inactivate TIMP-1 and -2 [Nagase, 1997].

On the other hand, some oral bacteria possess their own proteases, including collagenases, gelatinases, and peptidases. Proteolytic bacteria, such as Prevotella and Propionibacterium species, have been isolated from dentin carious lesions [Aas et al., 2008] and root surfaces [Preza et al., 2008; Hashimoto et al., 2011], suggesting that bacteria may also contribute to dentin matrix degradation. In particular, Prevotella intermedia is known to exhibit protease activity at a wide range of pH (from neutral to acidic) as well as acid-producing activity [Takahashi and Schachtele, 1990]. No studies have identified a bacterial involvement in the initial degradation of dentin matrix, but the detection of bacterial metabolites in carious dentin cavities [Hojo et al., 1991, 1994] suggests that bacteria digest partly degraded dentin matrix and metabolize it into organic acids. Nevertheless, bacterial acidification is essential for the acid-induced demineralization of tooth minerals, the acid-induced denaturation of exposed and partly degraded proteins, and the acid-induced activation of host-derived proteases. Furthermore, proteolytic/amino acid-degrading bacteria can utilize denatured and/or partly digested host proteins as metabolic substrates [Takahashi and Yamada, 2000; Takahashi et al., 2000; Takahashi and Sato, 2001, 2002; Takahashi, 2015].

Ecological Hypothesis for Dentin and Root Caries

Based on the findings described above, the extended ecological caries hypothesis [Takahashi and Nyvad, 2008, 2011] can be refined to include the etiology of dentin/root caries (fig. 1), in which a proteolytic stage has been added to the previous hypothesis.

In the previous hypothesis, bacteria-induced acidification was considered to be the major ecological driver of caries formation, i.e. acid formation was considered to promote bacterial acid-induced adaptation and selection within the microbiota and to tilt the balance of demineralization and remineralization towards demineralization via a process involving dynamic stability and acidogenic and aciduric stages [Takahashi and Nyvad, 2008, 2011]. In the refined hypothesis, bacterial acidification also induces the exposure of organic matrix and the activation of dentin-embedded and salivary MMPs/cathepsins. These proteases may initiate a partial degradation of the acid-exposed tooth organic materials (mainly collagen), which can then be further denatured and broken down by bacterial acids and proteolysis.

Activated MMPs do not function efficiently under acidic conditions as their optimal pH is usually around neutral, while activated cathepsins are active and stable under acidic conditions around pH 5. However, when the acidic environment is neutralized by oral clearance, dissolved tooth mineral, and/or bacterial alkali production/acid neutralization through amino acid degradation [Takahashi, 2015], MMPs start to function efficiently and degrade acid-exposed and acid-denatured proteins. At the same time, the de- and remineralization balance may begin to tilt towards remineralization; however, once organic matrices are partly degraded or lost, complete remineralization may no longer be possible [Kuboki et al., 1977]. In addition, bacteria can metabolize partly degraded proteins via their proteolytic activity, although it is still questionable whether bacteria play a role in the initial stages of the degradation of the organic components of teeth. The highly diverse anaerobic microorganisms found in dentin and root caries lesions, which include both acidogenic/aciduric bacteria, such as mutans streptococci, Lactobacillus, and Bifidobacterium species, and proteolytic/acid-degrading bacteria, such as Prevotella, Propionibacterium, and Fusobacterium species [Aas et al., 2008], seem to thrive on their nutritionally rich environments, in which both exogenous metabolic substrates (dietary carbohydrates, saliva, gingival crevicular fluid, etc.) and endogenous metabolic substrates (degraded collagen, etc.) are available.
Recently, Simón-Soro et al. [2013] proposed a tissue-dependent hypothesis of dental caries, which states that acid-producing bacteria act as a vehicle for penetrating enamel and allow dentin-degrading microorganisms to expand cavities. However, as described above, the contribution made by bacteria to the initial degradation of tooth organic materials remains questionable, and host-derived proteases seem to be the main contributors to this process. Nevertheless, proteolytic/amino acid-degrading bacteria can metabolize proteins, peptides, and amino acids [Takahashi and Yamada, 2000; Takahashi et al., 2000; Takahashi and Sato, 2001, 2002; Takahashi, 2015], which are made available via an initial degradation step involving host-derived proteases. This modification of the nutritional conditions might be an ecological determinant in the establishment of the microbial communities observed in dentin and root caries, i.e. highly diverse anaerobic microbiotas including both saccharolytic and proteolytic/amino acid-degrading bacteria. In this context, current thinking regarding the tissue dependency of caries formation might need to be revised.

We hypothesize that bacterial acids demineralize enamel/dentin, expose the dentin matrix, and activate host-derived proteases to promote the initial degradation of the dentin matrix concurrently with its acid denaturation (fig. 1). Subsequently, proteolytic/amino acid-degrading bacteria can settle in the modified tissue together with acid-producing bacteria. These phenomena happen in parallel with a continuous degradation of dentin matrix by bacterial and host-derived proteases. In vitro de-mineralized dentin matrix can be self-degraded by endogenous matrix-embedded proteases without the presence of bacteria [Brackett et al., 2015; Seseogullari-Dirihan et al., 2015; Altinci et al., 2016]; however, in in vivo dentin caries, bacteria are always present, at least in the infected layer of the carious dentin, and inevitably contribute to further degradation of matrix through their proteolytic/peptidic and metabolic activity. In both enamel and dentin caries bacterial acid production initiates the processes resulting in caries. Furthermore, in dentin caries, host-derived proteases are deeply involved in the processes.

Beyond Deep Dentin Caries

The polymicrobial invasion of dentin and its subsequent breakdown are considered to trigger inflammatory responses within the dental pulp, resulting in (dental)...
pulpitis (fig. 1). Both physical stress (attributed to the short distance to the pulp) and chemical stress (caused by the presence of bacterial components and metabolites) induce pulp inflammation. Bacterial components such as lipopolysaccharides (from Gram-negative bacteria), muramyl dipeptide (from both Gram-positive and Gram-negative bacteria), and lipoteichoic acid (from Gram-positive bacteria) can be sensed by Toll-like receptors (TLRs) [Kawai and Akira, 2010] and hence activate the host defense inflammatory cascade of TLR2 and TLR4, which are involved in Gram-positive and Gram-negative bacterial sensing, respectively. These TLRs have been detected in the odontoblast cell membrane [Jiang et al., 2006; Veerayutthwilai et al., 2007] and are considered to be involved in sensing microbial invasion and subsequent odontoblastic defensive responses, such as the production of antibacterials (defensins and nitric oxide) and molecular messaging molecules (chemokines and cytokines) [for review, see Farges et al., 2015]. On the other hand, bacterial metabolites, including short-chain fatty acids (butyrate, etc.), ammonia, sulfur compounds (hydrogen sulfide and methyl mercaptan), and amines (histamine, etc.) are known to be cytotoxic and immunomodulatory [Niederman et al., 1997; Kurita-Ochiai et al., 2008], and thus probably initiate and promote pulp inflammatory responses. These substances are derived from bacterial peptide/amino acid metabolism [Takahashi, 2015], i.e. the bacterial metabolic processes of the partly degraded dentin organic matrix. The observation that proteolytic/amino acid-degrading anaerobes, such as Prevotella, Fusobacterium, and Porphyromonas species, were frequently detected in advanced dentin caries lesions with pulpitis [Martin et al., 2002; Chhour et al., 2005; Rôças et al., 2015] may support this notion. Furthermore, dental fluid contains gelatinolytic enzymes [Mazzoni et al., 2012; Pessoa, 2013] which increase in pulpal inflammation [Zehnder et al., 2011] and MMP-20 [Sulkala et al., 2002]. It is possible that these host- derived enzymes, together with microbial proteases, promote the degradation of dentin matrix in deep caries lesions.

In deep progressive dentin caries, polymicrobial infections occur in the dental pulp. These infections involve a subset of the genera encountered in carious dentin including Actinobacteria (Actinomyces, Bifidobacterium, Parascaradia, and Propionibacterium) and Firmicutes (Lactobacillus and Streptococcus) [Nadkarni et al., 2010; Chalmers et al., 2015], taxa that have also been recovered from the advancing front of carious dentin [Edwardsson, 1974]. Among them, Lactobacillus species were reported to play a prominent role in the initial stages of pulp infection [Nadkarni et al., 2010], although their specific pathogenic mechanisms are unclear. As pulp infections progress, the microbiota within the lesion is reported to shift to an anaerobe-dominant polymicrobial composition (as represented by Coriobacteriaceae and Lachnospiraceae species) [Nadkarni et al., 2010], which resembles the microbiota seen in infected necrotic root canals, indicating that the environment is suitable for promoting the growth of these bacteria rather than pathogenic activity.

The caries process, from the formation of dentin/root caries to infection of the root canal via pulpitis, involves various physiological phenomena associated with ecological polymicrobial transition. These processes seem to be continuous; however, it is still unclear which mechanisms are responsible for the microbial transition and the concomitant transition from a reversible to an irreversible pulpitis. Importantly, as the dentin is broken down during caries, bioactive molecules released from the dentin matrix itself have the capacity to stimulate regeneration of the dentin-pulp complex [for review, see Smith et al., 2012] (fig. 1). If these modulatory responses are within the capacity of the pulp to recover (or heal) pulpitis would be abrogated.

Control of Dentin and Root Caries

According to our refined hypothesis for dentin and root caries the caries processes are reversible (fig. 1). Case studies have demonstrated that root and dentin caries lesions can be controlled/ arrested for years by daily mechanical cleaning and use of fluoride toothpaste [Nyvad and Fejerskov, 1986; Kidd et al., 2015], even in cases of manifest cavity formation into dentin [Nyvad and Fejerskov, 1997]. Success of the treatment requires that the lesion can be accessed for cleaning. Evidence supporting the effect of such nonoperative treatments came from experimental studies in which natural root caries lesions were arrested in situ over 3 months by daily cleaning with a fluoride toothpaste combined with two professional topical fluoride applications by 2% NaF [Nyvad et al., 1997]. The dual fluoride treatment resulted in a mineral gain in the surface layer and in a zone next to the lesion front. A recent review suggested that higher fluoride concentrations may be needed to control caries in root surfaces compared to enamel surfaces [Wierichs and Meyer-Lueckel, 2015]. Further studies should explore the use of fluoride in the arrest of root and dentin caries. Such studies might also include evaluation of the caries-controlling
effect of fluoride-releasing dental materials, although the clinical relevance of fluoride release from dental materials is still debatable [Dionysopoulous, 2014; Cury et al., 2016].

Our refined hypothesis highlights the vulnerability of exposed root surfaces. However, once the root/dentin surface has been demineralized protease inhibitors can be effective at preventing the degradation of dentin organic materials. MMP inhibitors, such as nonantimicrobial chemically modified tetracyclines and 2-(imidazolyl)-1-hydroxyethane-1,1-bisphosphonic acid (zoledronate), inhibited caries progression in rats [Tjäderhane et al., 1999; Sulkala et al., 2001]. Other reagents, including chlorhexidine [Pashley et al., 2004; Garcia et al., 2009], fluoride [Kato et al., 2014], silver diamine fluoride [Mei et al., 2012], doxycycline [Osorio et al., 2011], ethylene diamine tetraacetic acid [Thompson et al., 2012], benzalkonium chloride [Tezvergil-Mutluay et al., 2011b], quaternary ammonium compounds [Tezvergil-Mutluay et al., 2011a], various metal ions such as zinc [Toledano et al., 2012] and iron [Kato et al., 2010], and natural substances [Chaussain-Miller et al., 2006] have been reported to inhibit host proteases such as MMPs and cathepsins in vitro. Recently, hesperidin was reported to reduce the degradation of human dentin matrix (acid-demineralization) in situ [van Strijp et al., 2015]. These observations suggest that novel approaches for the protection of the organic matrix based on protease inhibitors might refine and improve therapies for root and dentin caries in the future.

The prevalence of root caries is expected to increase in the coming years because populations get older and retain an increased number of teeth with gingival recession. When root surfaces are exposed to the oral cavity, they will always run a risk of becoming demineralized, and exposed organic materials can be degraded by host-derived proteases contained in saliva and dentin itself. This process of root surface loss seems to be very slow but inevitable. New approaches to the prevention and treatment of dentin and root caries based on the etiology of the condition are required, especially for the elderly/medically compromised patient.

Acknowledgment

This study was supported in part by a KAKENHI grant (No. 60183852) from the Japan Society for the Promotion of Science.

Author Contributions

Both authors participated in the preparation of the manuscript.

Disclosure Statement

The authors declare no conflicts of interest.

References


DOI: 10.1159/000447309

ECOLOGICAL HYPOTHESIS OF DENTIN/ROOT CARIES

The authors declare no conflicts of interest.


Edwardsson S: Bacteriological studies on deep ar-

Horiuchi M, Washio J, Mayanagi H, Takahashi N: Acid profiles and pH of carious den-

Horiuchi M, Washio J, Mayanagi H, Takahashi N: Acid production by streptococci growing at low pH in a chemostat under anaerobic con-

Iwami Y, Abbe K, Takahashi-Abbe S, Yamada T: Acid production by streptococci growing at low pH in a chemostat under anaerobic con-

Jepsen A, Ayna M, Hedederich J, Eberhard J: Signif-

Kato MT, Leite AL, Zarella BL, Salo T, Tjäder-

Kato MT, Leite AL, Zarella BL, Salo T, Tjäder-

Kato MT, Leite AL, Zarella BL, Salo T, Tjäder-


Kuboki Y, Oghushi K, Fusayama T: Collagen bio-


Meiyoshi Y, Watanabe M, Takahashi N: Autoacti-


