INVASION OF DENTINAL TUBULES BY ORAL BACTERIA
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ABSTRACT: Bacterial invasion of dentinal tubules commonly occurs when dentin is exposed following a breach in the integrity of the overlying enamel or cementum. Bacterial products diffuse through the dentinal tube toward the pulp and evoke inflammatory changes in the pulpo-dentin complex. These may eliminate the bacterial insult and block the route of infection. Unchecked, invasion results in pulpitis and pulp necrosis, infection of the root canal system, and periapical disease. While several hundred bacterial species are known to inhabit the oral cavity, a relatively small and select group of bacteria is involved in the invasion of dentinal tubules and subsequent infection of the root canal space. Gram-positive organisms dominate the tubule microflora in both carious and non-carious dentin. The relatively high numbers of obligate anaerobes present-such as *Eubacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Peptostreptococcus micros*, and *Veillonella* spp.-suggest that the environment favors growth of these bacteria. Gram-negative obligate anaerobic rods, e.g., *Porphyromonas* spp., are less frequently recovered. Streptococci are among the most commonly identified bacteria that invade dentin. Recent evidence suggests that streptococci may recognize components present within dentinal tubules, such as collagen type I, which stimulate bacterial adhesion and intra-tubular growth. Specific interactions of other oral bacteria with invading streptococci may then facilitate the invasion of dentin by select bacterial groupings. An understanding the mechanisms involved in dentinal tubule invasion by bacteria should allow for the development of new control strategies, such as inhibitory compounds incorporated into oral health care products or dental materials, which would assist in the practice of endodontics.

**Key words.** Dentinal tubule, endodontic infections, oral bacterial adhesion, caries, invasion of dentin.

**I) Introduction**

Endodontics is the clinical discipline that deals with the prevention and management of diseases of the pulp and periapical tissues. Normally, the dental pulp (Fig. 1) is sterile and is primarily involved in the production of dentin and in tooth sensitivity. The pulp and dentin form a functional complex that is protected from exogenous substances in the oral cavity by the overlying enamel or cementum. When the pulpo-dentin complex becomes infected (Fig. 1A), the tissues react to the invading bacteria in an attempt to eradicate them. The ability of the complex to perform this function should not be underestimated, since the tissues are richly endowed with immunocompetent processes. However, in clinical terms, if the route of infection is not eradicated by these natural processes, or by operative procedures, then the burden of bacteria invading the complex overcomes the defenses and causes pulp disease, e.g., pulpitis, necrosis, and infection of the pulp chamber and root canal.

The root canal space is in open communication with the periapical tissues (periodontal ligament, cementum, and alveolar bone) via the apical foramen (Fig. 1). Bacterial metabolites and toxic products arising from bacteria present within the root canal diffuse into the periapical tissues and evoke inflammatory disease, e.g., apical periodontitis, which is characterized by resorption of alveolar bone (Fig. 1B), while localized areas of root resorption may also occur. In situations where the periodontal ligament has been damaged, e.g., after dental trauma, an infected root canal can induce extensive and rapid inflammatory root resorption. Bacterially induced periapical disease usually begins as a chronic inflammation and manifests histologically as a periapical granuloma. An acute apical periodontitis of endodontic origin indicates that the host defenses are unable to control the bacterial insult. This may be due to bacteria becoming established within the periapical tissues, with subsequent abscess formation, or due to the presence of specific bacteria within the root canal that are able to induce tissue destruction. The bacterial toxins and acute inflammatory response characteristically cause swelling and pain. The main goal of endodontic treatment is to eliminate bacteria from the root canal system and to prevent them from infecting or re-infecting the pulp, root canal, or periapical tissues. Successful treatment depends upon a sound understanding of the causative factors of the disease process.

Miller (1890) first demonstrated the bacterial invasion of dentinal tubules of both carious and non-carious dentin and reported that the tubule microflora consisted of cocci and rods. It was not until the late 1950s that experimental evidence clearly established the fundamental role of bacteria in dental caries and in pulp and periapical disease. Keyes (1960) was able to show that dental caries did not develop in germ-free animals fed a range of diets. Later, Kakehashi et al. (1965) demonstrated that pulp and periapical disease occurred in surgically exposed rat molar pulp only when bacteria were present in the oral cavity. In gnotobiotic (germ-free) rats, exposed pulps remained healthy and initiated repair by way of dentin bridging of the exposure.
Invasion of dentinal tubules by bacteria from supra- or subgingival plaque occurs whenever dentin is exposed in the oral cavity. This can be through caries lesions, restorative or periodontal procedures, tooth wear, enamel or dentin cracks, or dental trauma (Tronstad and Langeland, 1971; Peters et al., 1995; Love, 1996a). Bacteria present within coronal dentinal tubules may be responsible for pulp and periapical disease (Brännström and Nyborg, 1971) (Fig. 1A), while those within radicular dentinal tubules may be responsible for continued root canal infection (Haapasalo and Ørstavik, 1987) (Fig. 1C).

Dental caries involving the crown of the tooth can affect people at any age from when the crown erupts into the mouth. By contrast, root-surface caries occurs only when there has been loss of periodontal attachment and exposure of cementum or radicular dentin; hence it affects mainly adults. Unchecked, the advancing bacterial front of the carious process will result in infection of the dental pulp and root canal system, which will lead to periapical disease. However, bacteria that are associated with an infected root canal differ from those primarily associated with dental caries. Thus, although streptococci and Actinomyces are major components of dental plaque (Jenkinson and Lamont, 1997) and may initiate tubule and pulpal infection, obligately anaerobic bacteria are commonly present in large numbers in the infected root canal.

Streptococci are the primary bacterial colonizers of the oral cavity, and adhesion of streptococci to the acquired pellicle is an essential first step in colonization of the tooth (Gibbons, 1989; Kolenbrander and London, 1993; Jenkinson and Lamont, 1997). Streptococci express multiple surface protein adhesins (Hasty et al., 1992) that allow cells to bind to a wide range of substrates found in the oral cavity, including other microbial cells, salivary components, host cells, or extracellular matrix or serum components (Jenkinson and Lamont, 1997). However, while there are considerable data on the mechanisms involved in the formation and development of dental plaque (Kolenbrander, 2000), relatively little is known about the mechanisms by which oral bacteria penetrate or invade dentin, and cause pulpitis, root canal infection, and periapical diseases. Advances in microbial sampling methods, and in growth and identification techniques, have provided much new information on the microbial components and complexes that are associated with endodontic and periodontal infections (Sundqvist, 1994; Socransky et al., 1998).

This article will review current knowledge of the microbiology of dentinal tubule infections. It will also describe how recent developments have advanced our understanding of the microbial complexity of root canal and pulpal infections, and of the mechanisms by which some species of oral bacteria are able to invade dentin.

(II) Microbiology of Infection of the Pulpo-Dentin Complex

(A) Pulpo-dentin complex

Biologically and developmentally, pulp and dentin function as a complex and may be regarded as one tissue. Dentinal fluid movement, resulting in hydrodynamic activation of pulpal A-delta nerve fibers and causing dentin sensitivity (Brännström, 1986), is a common example of functional coupling of the tis-

Figure 1. Common sites of bacterial invasion of dentin. Bacteria invading from the oral cavity (i, ii, iii, iv, v) extend toward the dental pulp space (A) and may result in inflammatory disease and infection of the pulp and periapical tissues. (B) Periapical radiograph demonstrating chronic periapical periodontitis of an upper left central incisor subsequent to infection of the root canal via an enamel-dentin crack. Bacteria invading radicular dentin (v) from an infected root canal invade outward toward the external root surface (C) and may be responsible for persistent root canal infection and inflammatory disease of the surrounding tissues. (Reprinted and modified with permission from Love, 1997.)
Dentin are similar (approximately 44,243, 42,360, and 39,010 tubules at any given age within coronal, cervical, and mid-root (Tronstad, 1973; Carrigan), the overall numbers between the ages of 20 and 80 years (Bergenholtz, 1981). The pulp responds initially by cementum. Once caries, trauma, or restorative or periodontal procedures breach the integrity of this barrier, the tubules provide diffusion channels from the surface to the pulp. Bacteria can then invade these dentinal tubules, and bacterial products can diffuse across dentin to elicit pulp reactions (Vojočević et al., 1973; Bergenholtz, 1981). The pulpal odontoblasts, which begin secreting collagen at the dentino-enamel junction and then retreat centripetally, trailing odontoblast processes around which the dentin matrix is elaborated and mineralized. This results in primary and secondary dentin having a tubular nature. Tertiary or reparative dentin, which is laid down as a consequence of noxious stimuli, does not have a regular tubular form. Since the circumference of the peripheral part of the crown or root is larger than the circumference of the final pulp chamber or root canal space, the odontoblasts are forced closer together as they continue to lay down intertubular dentin. This results in changes in the relative proportions of dentinal tubules within different areas of the dentin and a characteristic S-shape course of the dentinal tubules. The number of dentinal tubules per mm² varies from 15,000 at the dentino-enamel junction to 45,000 at the pulp (Garberoglio and Brännström, 1976). Deposition of intratubular (peritubular) dentin within the tubule results in narrowing of the tubule (Linde and Goldberg, 1993). Deposition is more advanced in superficial older dentin compared with dentin closer to the pulp, and this results in a tapered tubule with the largest dimensions at the pulp (approximately 2.5 μm in diameter) and the smallest dimensions at the dentino-enamel or dentino-cemental junction (approximately 0.9 μm in diameter) (Fig. 2). Thus, a tubule is normally larger in diameter than an average oral streptococcal cell (0.5-0.7 μm).

Intratubular dentin is highly mineralized (approximately 95 vol% mineral phase) compared with the less-mineralized collagen matrix (about 30 vol% mineral phase) of intertubular dentin (Marshall, 1993), and becomes more mineralized with increasing age. This results in a decrease in size, and ultimately obliteration, of the dentinal tubules, with about 40% decrease in the overall numbers between the ages of 20 and 80 years (Tronstad, 1973; Carrigan et al., 1984). The mean numbers of tubules at any given age within coronal, cervical, and mid-root dentin are similar (approximately 44,243, 42,360, and 39,010 mm², respectively) (Carrigan et al., 1984). However, significantly fewer dentinal tubules are found in apical dentin (approximately 8190 mm²), suggesting that the formation of intratubular dentin occurs more rapidly in the apical region of the root.

(B) Intratubular content and diffusion properties

The composition of dentinal tubule fluid in vital dentin is not fully known; however, it resembles serum with proteins such as albumin and immunoglobulin G (IgG) being present (Knutsson et al., 1994). In addition, other blood proteins, such as fibrinogen, may be found in dentinal tubules after cavity preparation (Knutsson et al., 1994; Izumi et al., 1998). Dentinal fluid within non-vital root dentin is fluid originating from alveolar bone and periodontal ligament, while dentinal fluid within non-vital coronal dentin is likely to be derived from saliva.

Dentinal tubules may contain odontoblast processes, nerve fibers, and unmineralized collagen fibrils. Dai et al. (1991) examined the contents of dentinal tubules of permanent human incisor, canine, premolar, and molar teeth from patients whose ages ranged from 18 to 54 yrs. They found that unmineralized collagen was a major component within dentinal tubules, occurring in 65% of all tubules in inner dentin (closest to the pulp). In 16% of these tubules, the collagen was aggregated into large bundles that occupied more than one-fifth of the lumen. In middle dentin, the corresponding figures were 42 and 7%, and for outer dentin, 12 and 0%. These patterns of collagen distribution were similar for all tooth families and were unrelated to age, suggesting that collagen is continually laid down within dentinal tubules throughout life.

Dentin is very porous because of the tubular structure. However, the degree of permeability varies between different areas of a tooth and the number of patent dentinal tubules present (Pashley, 1990). The pulpo-dentine complex is normally protected from the oral cavity by the overlying enamel or cementum. Once caries, trauma, or restorative or periodontal procedures breach the integrity of this barrier, the tubules provide diffusion channels from the surface to the pulp. Bacteria can then invade these dentinal tubules, and bacterial products can diffuse across dentin to elicit pulp reactions (Vojočević et al., 1973; Bergenholtz, 1981). The pulpal responses initially by mounting an inflammatory response that increases the outward flow of dentinal fluid (Maita et al., 1991; Vongsavan and Matthews, 1994), thereby reducing diffusion of noxious stimuli through the dentinal tubules. Molecules present within dentinal tubules such as albumin, fibrinogen, and IgG have been shown to decrease fluid flow through dentin in vitro (Pashley et al., 1982; Hahn and Overton, 1997). It is therefore likely that dentinal fluid components are involved in host defenses, by both interacting directly with bacteria and products, and by reducing the permeability of dentin.

Figure 2. Transmission electron micrographs of sections of dentin colonized by S. gordonii. (A) Individual bacterial cells adhering to the wall of a dentinal tubule, with fibrillar surface material visible at the site of association between bacterial cells and tube. Bar: 0.5 μm. (B) A group of streptococcal cells in intimate contact with a tubule wall. Bar: 1.0 μm. (Reproduced with permission from Love et al., 1997.)
However, conditions that reduce the outward flow of dentinal fluid tend to increase the inward diffusion of exogenous substances. Pashley (1992) speculated that bacterial invasion of dentinal tubules would interfere more with outward fluid flow than with inward diffusion of noxious materials, due to the higher sensitivity of bulk fluid movement to changes in tubule radius, r (which varies with $r^2$), compared with diffusion (which varies with $r^4$). *In vitro* studies have demonstrated that fluid flow through dentin is indeed reduced by bacterial invasion of dentin (Michelich et al., 1980; Love et al., 1996). Reduced fluid flow might promote disease pathogenesis by allowing for an increased diffusion rate of destructive or toxic bacterial products toward the pulp. Continued stimulus results in the pulpo-dentin complex responding to the noxious challenge by activation of immunocompetent cells and inflammatory processes in the pulp and by decreasing the permeability of the dentin by the production of sclerotic or reparative dentin (for reviews, see Pashley, 1996; Jontell et al., 1998). When unchecked, bacterial invasion of dentinal tubules overcomes the pulpo-dentin defenses, resulting in infection of the pulp and root canal system.

**III. Bacterial Invasion of Coronal Dentin**

**A. Carious Dentin**

The cariogenic microflora present on the surface of fissure, smooth-surface coronal, or root-surface caries consists mainly of streptococci, lactobacilli, and *Actinomyces* spp. Members of the *mutans* group streptococci, in particular *S. mutans* and *S. sobrinus*, are considered to be the primary etiological agents in the induction of coronal and of root caries (Bowden, 1990; van Houte, 1994; van Houte et al., 1994). Samples of carious dentin from the outer surfaces of teeth contain *Streptococcus* spp., *Lactobacillus* spp., *Actinomyces* spp. and other Gram-positive rods (Loesche and Syed, 1973). Samples from the pulpal side of carious dentin lesions of extracted teeth contain larger numbers of Gram-positive anaerobic rods of *Eubacterium*, *Propionibacterium*, and *Bifidobacterium* species, with *Actinomyces* and *Lactobacillus* being the most prevalent facultative bacteria isolated (Edwardsson, 1974). In these studies, streptococci constituted only a minor group of the total isolates. Thus, different regions of carious dentin may contain quite different proportions of bacterial components in their microflora.

Greater numbers of bacteria are recovered from superficially infected dentin compared with deeper dentin (Hoshino, 1985). The application of strict anaerobic sampling and cultivation methods always gives higher recoveries of bacteria, implying that the environment of carious dentin promotes survival of obligately anaerobic bacteria. Thus, species of *Propionibacterium*, *Eubacterium*, and *Bifidobacterium* dominate the microflora of deep carious dentin, with *Actinomyces*, *Lactobacillus*, and some streptococci, but rarely *S. mutans*, being present (Table 1). Gram-negative obligate anaerobes, e.g., *Fusobacterium*, are recovered in only very low numbers, if at all (Table 1). To identify and localize bacterial species within carious dentin, Ozaki et al. (1994) detected, by immunohistochemical techniques, specific bacteria within dentin samples from fissure, smooth-surface coronal, and root-surface caries. They found that mutans group streptococci were the predominant bacteria within dentin from fissure and smooth-surface coronal caries, with higher numbers in the shallow and middle layers of dentin compared with deep dentin. Other bacteria previously identified as being dominant members of the microflora of carious human dentin—such as *Lactobacillus* spp., *Eubacterium* lactolyticum, and *F. nucleatum* (Edwardsson, 1974; Hoshino, 1985)—were frequently detected, though their relative proportions were low (Table 1). Thus, the environment within superficial carious dentin favors growth of facultative anaerobes that are associated with the carious process, e.g., mutans streptococci, while the microflora deep within the dentin is dominated by obligately anaerobic organisms.

In contrast to the microflora of fissure and smooth-surface carious dentin, *Actinomyces naeslundii* (viscosus) is the major species associated with dentin invasion in root-surface caries. *Actinomyces* species are found in shallow, middle, and deep dentin, with higher numbers of cells in deeper dentin. Mutans streptococci are frequently detected at all levels of carious root dentin, though they are mainly located in the shallow layer and do not make up a high proportion of the microflora. On the other hand, lactobacilli and Gram-negative organisms are found in low numbers, or not at all (Syed et al., 1975; Hill et al., 1977; Ozaki et al., 1994) (Table 2). Thus, the composition of the microflora associated with carious dentin differs quite considerably between coronal and root caries.

**B. Non-Carious Dentin**

*In vivo* studies show that bacteria are able to penetrate the tubules of non-carious coronal dentin exposed to the oral environment. Invasion of tubules occurs readily and is evident within a week of exposure (Lundy and Stanley, 1969; Olgart et al., 1974). With time, the numbers of tubules infected and the depth of infection increase (Lundy and Stanley, 1969). The pattern of invasion is characterized by variable numbers of tubules penetrated and variable depths of penetration between different areas of dentin (Fig. 3A) (Tronstad and Langeland, 1971; Olgart et al., 1974). Inflammatory changes within the pulp are commonly observed and can be seen within a week of exposure (Olgart et al., 1974). Other studies have demonstrated that microleakage of oral bacteria around restorations allows for bacterial invasion of exposed dentinal tubules at the base of the cavity (Brännström and Nyborg, 1971; Vojinovic et al., 1973), resulting in pulp inflammation (Vojinovic et al., 1973) or periapical disease (Ray and Trope, 1996). Likewise, microleakage through enamel cracks and fractures as a result of trauma may lead to bacterial invasion of...
### TABLE 1
Bacterial Species Identified in Carious Coronal Dentin

<table>
<thead>
<tr>
<th>Bacterial Genus or Species</th>
<th>Isolation Frequency in Carious Dentin</th>
<th>Bacterial Genus or Species</th>
<th>Isolation Frequency in Carious Dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial</td>
<td>Deep</td>
<td>Superficial</td>
</tr>
<tr>
<td><strong>Streptococcus</strong></td>
<td>High</td>
<td>Low-moderate</td>
<td>Propionibacterium</td>
</tr>
<tr>
<td>S. mutans</td>
<td></td>
<td></td>
<td>P. acnes</td>
</tr>
<tr>
<td>S. sobrinus</td>
<td></td>
<td></td>
<td>P. avidum</td>
</tr>
<tr>
<td>S. intermedius</td>
<td></td>
<td></td>
<td>P. lymphophilum</td>
</tr>
<tr>
<td>S. morbillorum</td>
<td></td>
<td></td>
<td>P. propionicum</td>
</tr>
<tr>
<td>S. sanguinis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>Low</td>
<td>Low</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>P. anaerobius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. parvulus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. micros</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomyces</td>
<td>High</td>
<td>Moderate</td>
<td>Fusobacterium nucleatum</td>
</tr>
<tr>
<td>A. israelii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. naeslundii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. odontolyticus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sanguinis e.g., P. acnes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mutans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sobrinus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptococcus spp.</td>
<td>Low</td>
<td>Low</td>
<td>Peptococcius</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>High</td>
<td>High</td>
<td>Clostridium spp.</td>
</tr>
<tr>
<td>E. alactolyticum AQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. aerofaciens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. saburreum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veillonella spp.</td>
<td>Moderate</td>
<td>Low</td>
<td></td>
</tr>
</tbody>
</table>

Modified from Edwardsson, 1987; Ozaki et al., 1994.

### TABLE 2
Bacterial Species Identified in Carious Root Dentin

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Isolation Frequency</th>
<th>Bacterial Species</th>
<th>Isolation Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>Low-high</td>
<td>Propionibacterium</td>
<td>Low-moderate</td>
</tr>
<tr>
<td>S. sanguinis</td>
<td></td>
<td>e.g., P. acnes</td>
<td></td>
</tr>
<tr>
<td>S. mitis</td>
<td></td>
<td>Lactobacillus</td>
<td>Low</td>
</tr>
<tr>
<td>S. mutans</td>
<td></td>
<td>L. casei</td>
<td></td>
</tr>
<tr>
<td>S. sobrinus</td>
<td></td>
<td>L. plantarum</td>
<td></td>
</tr>
<tr>
<td>Actinomyces</td>
<td>High</td>
<td>Peptostreptococcus</td>
<td>Low</td>
</tr>
<tr>
<td>A. naeslundii</td>
<td></td>
<td>micros</td>
<td></td>
</tr>
<tr>
<td>A. odontolyticus</td>
<td></td>
<td>F. nucleatum</td>
<td>Low</td>
</tr>
<tr>
<td>A. viscosus</td>
<td></td>
<td>P. endodontalis</td>
<td></td>
</tr>
<tr>
<td>Eubacterium spp.</td>
<td>Low-moderate</td>
<td>Veillonella spp.</td>
<td>Low</td>
</tr>
<tr>
<td>e.g., E. alactolyticum</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Modified from Edwardsson, 1987; Ozaki et al., 1994.
the pulpo-dentin complex and act as a cause of pulpal disease (Love, 1996a). Hence, sealing of dentin from exogenous substances and bacteria in the oral cavity, in both vital and non-vital teeth, is a critical step in tooth restoration.

The composition of the microflora invading exposed non-carious dentin has not been fully elucidated but is dominated by Gram-positive cells (Lundy and Stanley, 1969; Brännström and Nyborg, 1971; Tronstad and Langeland, 1971; Vojinovic et al., 1973; Olgart et al., 1974) and probably resembles the composition of the biofilm infiltrating the tooth-restoration interface (Edwardsson, 1987). This biofilm resembles mature plaque and is composed mainly of streptococci and Actinomyces spp. Anaerobic Gram-positive cocci, e.g., Peptostreptococcus micros, and Gram-negative organisms tend to be present in only low numbers (Mejàre et al., 1979, 1987).

(IV) Microflora of the Infected Root Canal

Bacteria may enter the root canal system directly via caries lesions or via pulp exposure following trauma. However, many infections of the pulp occur as a result of supra- or subgingival bacteria penetrating exposed dentin, enamel-dentin cracks, and around restorations (Pashley, 1990; Peters et al., 1995; Love, 1996a) and then invading dental tubules. Almost all bacteria recovered from the root canal systems of teeth with intact crowns belong to the oral microflora (Wittgog and Sabiston, 1975; Sundqvist, 1976; Le Goff et al., 1997). The concept that bacteria can gain access to the pulp system via the blood stream has not been proven. In fact, Delivanis and Fan (1984) were unable to demonstrate the presence of bacteria in unfilled cat root canals after repeated intravenous injections of *S. sanguis* (*sanguinis*). More than 300 bacterial species are recognized as components of the oral microflora (Moore and Moore, 1994). However, only relatively few species appear to be able to invade the root canal space and infect the root canal (Kantz and Henry, 1974; Sundqvist, 1976; Dahlén and Bergenholtz, 1980). This suggests that many species of oral bacteria do not have the properties necessary to invade tubules and survive within the intratubular environment.

In studies where strict avoidance of contamination is attempted, sampling has been done of teeth with intact pulp chamber walls (Sundqvist, 1976). Consequently, the bacteria that are detected in the root canal must have gained entry by invading dentinal tubules. Sundqvist (1976) studied the microflora of human teeth that had become non-vital as a result of trauma, but which otherwise were intact and caries-free. Utilizing strictly anaerobic sampling techniques, he demonstrated that bacteria could not be isolated from teeth with normal periapical tissue, while bacteria were regularly isolated from teeth from patients who had apical periodontitis. Likewise, Möller et al. (1981) showed that only devitalized and infected pulps of monkey teeth showed signs of apical periodontitis, whereas devitalized and uninfected pulps did not develop periapical bone destruction. The pioneering studies by Sundqvist (1976) and later by Möller et al. (1981) demonstrated that, in addition to streptococci, lactobacilli, and *Actinomyces*, obligately anaerobic species of *Fusobacterium*, *Peptostreptococcus*, *Eubacterium*, *Propionibacterium*, *Veillonella*, *Prevotella*, and *Porphyromonas* dominated the root canal microflora (Table 3). Other micro-organisms such as yeasts, *e.g.*, Candida and Saccharomyces (Lana et al., 2001), and spirochetes, *e.g.*, Treponema (Jung et al., 2000; Röças et al., 2001), have been occasionally recovered from an infected root canal. Most of the oxygen-sensitive members of the root canal microflora are not readily cultivable without the strict application of anaerobic methods (Carlsson et al., 1977), and this may explain why, in earlier studies, many teeth with apical periodontitis did not appear to harbor bacteria in the root canal.

Obligately anaerobic bacteria dominate the microflora of established asymptomatic infected root canals, with streptococci making up a significant proportion of the facultative species. Commonly, between 2 and 8 bacterial species are recovered from infected root canals, with *F. nucleatum, P. intermedia*, and streptococci being often present (Sundqvist, 1994; Le Goff et al., 1997) (Table 3). A series of studies on the dynamics of experimental root canal infections of monkey teeth has shown that facultative anaerobic bacteria, mainly streptococci, are the first colonizers of the root canal, but that by 6 months, obligate anaerobes dominate the microflora. When combinations of bacterial strains, isolated originally from an endogenously infected root canal, were re-inoculated into further canals with devitalized tissue, the dominance of anaerobic bacteria was again established. Furthermore, the original proportions of the bacterial strains were re-established, despite equal numbers of the different strains being inoculated into the canals (Fabricius et al., 1982a,b). These observations support the notion that associations between specific bacteria enable the root canal microflora to grow and survive in a highly specialized and selective environment (Sundqvist, 1992a).

### TABLE 3

**Bacterial Species Commonly Found in Asymptomatic Infected Root Canals**

<table>
<thead>
<tr>
<th>Gram-positive Cocci</th>
<th>Gram-positive Rods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus anginosus</em></td>
<td><em>Actinomyces israeli</em></td>
</tr>
<tr>
<td><em>S. sanguinis</em></td>
<td><em>A. naeslundii</em></td>
</tr>
<tr>
<td><em>S. mitis</em></td>
<td><em>Eubacterium alactolyticum</em></td>
</tr>
<tr>
<td><em>S. mutans</em></td>
<td><em>E. lentum</em></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td><em>E. nodatum</em></td>
</tr>
<tr>
<td><em>P. micros</em></td>
<td><em>E. timidum</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gram-negative Cocci</th>
<th>Gram-negative Rods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Capnocytophaga ochracea</em></td>
<td><em>Fusobacterium nucleatum</em></td>
</tr>
<tr>
<td><em>C. sputigena</em></td>
<td><em>Prevotella intermedia</em></td>
</tr>
<tr>
<td><em>C. curvus</em></td>
<td><em>P. melaninogenica</em></td>
</tr>
<tr>
<td><em>C. buccae</em></td>
<td><em>P. denticola</em></td>
</tr>
<tr>
<td><em>P. buccalis</em></td>
<td><em>P. granulosum</em></td>
</tr>
<tr>
<td><em>Campylobacter rectus</em></td>
<td><em>Lactobacillus</em></td>
</tr>
<tr>
<td><em>C. curvus</em></td>
<td><em>Porphyromonas gingivalis</em></td>
</tr>
<tr>
<td><em>C. endodontalis</em></td>
<td><em>Bacteroides gracilis</em></td>
</tr>
</tbody>
</table>

Adapted from Sundqvist, 1992a,b, 1994; Le Goff et al., 1997.
Mixed root canal infections result in larger periapical lesions than do mono-infections (Fabricius et al., 1982a,b). However, while the components of the root canal microflora are well-established, it is interesting that no single bacterial species has been indicted as the major pulp and periapical pathogen in chronic asymptomatic conditions. *P. gingivalis*, which is strongly implicated in destructive adult periodontal disease (Socransky and Haffajee, 1992; Lamont and Jenkinson, 1998), is recovered in low numbers from asymptomatic chronic root canal infections (Sundqvist, 1994; Le Goff et al., 1997). However, numbers of *Porphyromonas* and *Prevotella* species increase dramatically when there are signs and symptoms of acute periapical infection (Haapasalo, 1989; Sundqvist et al., 1989; Hashioka et al., 1992). The dominance of Gram-negative species in the latter stages of root canal infection supports the evidence that a highly selective environment continues to develop within the root canal system. Moreover, mechanisms may exist that allow these Gram-negative obligate anaerobes, e.g., *Porphyromonas* and *Prevotella* species, to penetrate dentin, even though the bacteria are not routinely isolated from the tubule microflora.

The microflora of carious and cavitated dentin of teeth with pulpitis is similar to that previously reported for intact carious dentin (Hahn et al., 1990) (Table 1). Gram-positive organisms predominate, especially *Lactobacillus* spp. and streptococci. Gram-negative bacteria, e.g., *P. intermedia*, are found in lower numbers in superficial to deep dentin, but are more prevalent within dentin at the pulpal wall. Investigating the degree of cellular infiltrate and degenerative changes in the pulp of teeth with cavitated carious dentin, Massey et al. (1993) reported no association between the microbial load within the dentin and histopathology of the pulp. However, there was a positive correlation between the presence of *P. intermedia* and *P. melaninogenica* and extensive inflammation of the pulp.

(V) Bacterial Invasion of Radicular Dentin from the Root Canal

Once bacteria gain access to the root canal system, they invade root canal dentinal tubules (Fig. 1C) and may be responsible for persistent root canal infection (Haapasalo and Ørstavik, 1987; Ørstavik and Haapasalo, 1990). Shovelton (1964) examined histologically 97 extracted, clinically non-vital teeth and found that 61 of the teeth showed bacterial penetration of the radicular dentinal tubules. The numbers of tubules containing bacteria were highly variable from tooth to tooth and among sections of an individual tooth. The depth of penetration by bacteria into the tubules was also found to be variable. It was noted that the presence of bacteria within the tubules was related to the clinical history of the tooth, such that chronic infections had more bacterial invasion and that tubule invasion did not occur immediately after the bacteria appeared in the root canal. These observations were similar to those reported in later histological studies on the invasion of non-carious coronal dentin (Lundy and Stanley, 1969; Brännström and Nyborg, 1971; Tronstad and Langeland, 1971; Vojinovic et al., 1973; Ölgart et al., 1974).

The microflora within radicular dentinal tubules of teeth with infected root canals (Ando and Hoshino, 1990) resembles that of deep layers of carious coronal dentin (Edwardsson, 1974; Hoshino, 1985) (Table 1). Lactobacilli, streptococci, and *Propionibacterium* spp. are predominant, with other bacteria such as Gram-positive anaerobic cocci, *Eubacterium* spp., and *Veillonella* spp. being present in low numbers. Obligately anaerobic Gram-negative bacteria were recovered in very low numbers or not at all (Edwardsson, 1974; Hoshino, 1985; Ando and Hoshino, 1990), but are known to be present in infected root canals, as previously discussed. The inability to detect fastidious anaerobes within invaded coronal or radicular dentin may have been due simply to difficulties in cultivating these bacteria. By utilizing specific antisera, Ozaki et al. (1994) demonstrated that *P. endodontalis* was present, albeit in low numbers, within dentinal tubules of carious dentin. Recently, Peters et al. (2001) demonstrated that the flora recovered from mid-root radicular dentin of teeth with apical periodontitis of endodontic origin was similar to that reported in previous studies (Ando and Hoshino, 1990), while Gram-negative bacteria including *F. nucleatum*, *P. gingivalis*, and *P. intermedia* were commonly recovered. Clearly, Gram-negative obligate anaerobic bacteria are more frequently found, and in higher cell numbers, in infected root canals than in carious and non-carious infected dentin. Undoubtedly, the application of novel molecular techniques that detect bacteria in samples without the necessity for laboratory cultivation (Dymock et al., 1996), or the presence of bacteria in situ, will assist greatly in future analyses of infected dentin, root canals, and pulpal tissues.

(VI) Bacterial Invasion of Radicular Dentin from a Periodontal Pocket

Bacterial invasion of radicular dentin of periodontally diseased teeth has been demonstrated by light microscopy (Kopczyk and Conroy, 1968; Langeland et al., 1974; Adriaens et al., 1987b) and by microbiological studies (Adriaens et al., 1987a; Giuliana et al., 1997). It has been suggested that the dentinal tubule microflora associated with a periodontal pocket could act as a reservoir for re-colonization of the pocket after debridement (Adriaens et al., 1987a; Giuliana et al., 1997). The majority of species recovered from radicular dentin are Gram-positive bacteria (*P. micros*, *S. intermedius*, *A. naeslundii*), with lower numbers of Gram-negative organisms (*P. gingivalis*, *P. intermedia*, *Bacteroides forsythus*, *F. nucleatum*, *V. parvula* (Giuliana et al., 1997).

While it is clear that bacteria are able to invade radicular dentin from the periodontal pocket, a contentious issue is whether bacteria invade healthy cementum prior to dentin penetration, or if bacteria gain access to dentin only via breaches in the cementum layer. Several studies have described invasion of the cementum of periodontally diseased teeth (Hartzell, 1911; Daly et al., 1982; Adriaens et al., 1987a,b; Giuliana et al., 1997). However, it was not evident from any of these studies if the invaded cementum was intact, healthy, or diseased. Exposed cementum is a thin, often discontinuous layer (Moskow, 1969), and commonly shows surface defects, e.g., at sites where Sharpey’s fibers attach to the cementum matrix (Adriaens et al., 1987b). Exposure of cementum to crevicular fluid, bacterial enzymes, or acidic metabolites may induce physicochemical and structural alterations, such as localized resorptive lacunae or demineralization (Daly et al., 1982; Eide et al., 1984; Adriaens et al., 1987b). It seems likely, therefore, that bacterial invasion of exposed cementum associated with periodontal disease occurs after the cementum has been altered by physiological, bacterial, or environmental factors.
TABLE 4
Bacteria Associated with in vivo Dentin Caries and Root Canal Infection that can Invade Root Dentinal Tubules in vitro

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Streptococcus sanguinis</td>
<td>Akpata and Blechman, 1982</td>
</tr>
<tr>
<td></td>
<td>Ørstavik and Haapasalo, 1990</td>
</tr>
<tr>
<td></td>
<td>Perez et al., 1993</td>
</tr>
<tr>
<td>Streptococcus gordonii</td>
<td>Love, 1996b</td>
</tr>
<tr>
<td></td>
<td>Love et al., 1996</td>
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<tr>
<td></td>
<td>Love et al., 1997</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Akpata and Blechman, 1982</td>
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<tr>
<td></td>
<td>Haapasalo and Ørstavik, 1987</td>
</tr>
<tr>
<td></td>
<td>Ørstavik and Haapasalo, 1990</td>
</tr>
<tr>
<td>Streptococcus sobrinus</td>
<td>Nagaoka et al., 1995</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>Nagaoka et al., 1995</td>
</tr>
<tr>
<td>Actinomyces viscosus (naeslundii)</td>
<td>Nagaoka et al., 1995</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>Love et al., 1997</td>
</tr>
</tbody>
</table>

(VII) Bacterial Invasion in vitro

In vitro studies have examined penetration of coronal or root dentin by a limited number of oral bacteria that are associated with carious or non-carious dentin. Cells of S. mutans, S. sanguinis, and A. naeslundii have all been shown to penetrate dentin discs in vitro (Michelich et al., 1980; Meryon et al., 1986; Meryon and Brook, 1990). Invasion of root dentinal tubules by pure cultures of streptococci or enterococci associated with root canal infections in vivo, or with dentinal caries, has been demonstrated histologically (see Fig. 3A) (Table 4). In contrast, invasion of dentin by mono-cultures of Gram-negative anaerobic bacteria is less clear, but invasion has not been generally recognized. Neither Bacteroides melaninogenicus ss. melaninogenicus nor P. intermedia (Akpata and Blechman, 1982; Perez et al., 1993) invaded root dentin after 21-28 days' incubation. On the other hand, limited invasion by P. intermedia has been reported (Berkiten et al., 2000), while P. endodontalis and P. gingivalis both showed low-level penetration of dentinal tubules of bovine roots that had the cementum removed (Siqueira et al., 1996).

The ability of mixed cultures of bacteria, associated with coronal or root caries, to invade dentin was investigated by Nagaoka et al. (1995). Analysis of their data suggested that invasion of L. casei was enhanced when co-cultured with S. sobrinus or A. naeslundii. More recently, it has been shown that dentinal tubule invasion by P. gingivalis was promoted when co-cultivated with S. gordonii (Love et al., 2000). These experiments demonstrate that bacteria may compete for invasion of dentinal tubules, and also that they may co-operate in invasion. Both these interactions may be significant in determining the outcome of tubule infections.

(VIII) Factors Influencing Tubule Invasion by Bacteria

(A) Dentin Structure

Whenever dentin is cut or abraded, a smear layer of debris forms on the instrumented surface and packs into the superficial portion of the dentinal tubule. In vitro experiments suggest that the presence of a dentinal smear layer prevents the penetration of coronal or root dentinal tubules by streptococci (Michelich et al., 1980; Love et al., 1996), and this is confirmed by in vivo studies. Bacterial invasion of dentinal tubules occurs more readily when the smear layer has been removed from the dentin, compared with smeared dentin where the degree of tubule invasion is low (Vojinovic et al., 1973; Olgart et al., 1974). Additionally, the degree of pulp inflammation appears less pronounced under smeared dentin.

Depth of bacterial invasion may depend, at least in part, upon tubule diameter, since this determines the rate of solute diffusion (Pashley, 1992). Sclerotic or obliterated tubules will physically impede bacterial invasion and can result in regional differences in bacterial invasion of dentin. Invasion of coronal and mid-root dentin occurs readily by S. gordonii, while the extent and depth of invasion are significantly less in apical dentin (Love, 1996b). This is because of the lower number of patent tubules in this region due to dentinal sclerosis, which is always more advanced in the apical region compared with coronal and mid-root dentin at any age.

Intact cementum is crucial to limitation of the bacterial invasion of radicular dentinal tubules from the pulpal surface. Penetration is enhanced when the overlying cementum is resorbed (Valderhaug, 1974; Haapasalo and Ørstavik, 1987; Love, 1996b), a common occurrence in the presence of inflammatory periapical disease and after traumatic injuries that damage the periodontal ligament.

Limiting nutritional supply may influence the depth of bacterial penetration. This is partly dependent upon the potency of the tubule, since diffusion of substances into tubules from the oral cavity or pulpal fluid is proportional to tubule diameter (discussed above). This may account for the higher numbers of cariogenic bacteria present within superficial dentin (Edwardsson, 1987), where the presence of fermentable carbohydrates and oxygen from the oral cavity is likely to be higher than in deeper dentin. Also, the anaerobic environment and the possible presence of tissue components, e.g., hemin, within dentin close to the pulp is likely to favor growth and survival of organisms such as P. intermedia and P. gingivalis (Hahn et al., 1990).

(B) Bacterial adhesion

The pivotal nature of streptococcal interactions with deposited salivary proteins and glycoproteins on oral surfaces and other organisms is well-recognized in the development of the complex dental plaque biofilms (Gibbons, 1984; Malamud, 1985; Banas et al., 1990; Terpenning et al., 1993; Kolenbrander, 2000). A great many streptococcal protein adhesins have been identified that can interact with salivary molecules. These include the antigen I/II family polypeptides (Jenkins and Demuth, 1997), amylase-binding proteins (Scannapieco, 1994), surface lectins (Murray et al., 1986; Takahashi et al., 1997), fimbrial adhesins (Olino and Fives-Taylor, 1993; Wu and Fives-Taylor, 1999), EP-GP binding protein (Schenkels et al., 1993), and glucan-binding proteins GBP59 (Banas et al., 1990) and GBP59 (Smith et al., 1994). The possession of multiple salivary adhesins favors colonization by a range of mechanisms. Interbacterial co-aggregation is also an important aspect in plaque development (Kolenbrander and London, 1993). Streptococci co-adhere with other early colonizers, such as Actinomyces spp., and are also bound by later colonizers such as P. gingivalis and B. forsythus (Lamont et al., 1992; Yao et al., 1996). Later colonizers are often strict anaerobes and increase in
plaque when a more anaerobic environment develops, which may be due, in part, to the actions of earlier colonizers. Despite our extensive knowledge about adhesive interactions between bacteria and substrates in the oral cavity, the influence of bacterial adhesion and inter-bacterial binding in tubule invasion is relatively poorly understood.

Collagen type I, a major organic component of dentin, is recognized by oral streptococci, and when adsorbed onto hydroxyapatite surfaces, it serves as an adhesion substrate (Liu and Gibbons, 1990; Liu et al., 1990). Strains of S. mutans are able to bind to unmineralized collagen and to particles of root dentin (Switalski et al., 1993). The ability of oral streptococci to bind to collagen may facilitate bacterial adhesion to exposed dentin or cementum, and subsequently tissue penetration. The antigen I/II polypeptides, expressed on the surfaces of most indigenous species of oral streptococci (Jenkinson and Demuth, 1997), play a major role in mediating adhesion of streptococci to collagen (Love et al., 1997). Strains of P. gingivalis also readily bind to collagen-coated hydroxyapatite, and to bovine bone collagen (Naito and Gibbons, 1988; Naito et al., 1993). This binding is due, at least in part, to the adhesion fimbriae that bind strongly to collagen in vitro (Naito et al., 1993). Fimbriae are involved in other adhesive interactions important in host colonization by P. gingivalis, such as binding to salivary receptors, epithelial cells, fibronec tin, and other oral bacteria (Isogai et al., 1988; Goulbourne and Ellen, 1991; Li et al., 1991; Lamont and Jenkinson, 2000), and in the invasion of epithelial cells (Lamont et al., 1995; Weinberg et al., 1997).

Recent data have provided strong evidence for bacterial adhesion specificity as playing a major role in determining the invasion of dentinal tubules. Experiments utilizing isogenic mutants of S. gordonii or S. mutans deficient in the expression of antigen I/II polypeptide surface adhesins clearly demonstrate that these polypeptides not only mediate streptococcal binding to collagen, but also are necessary for bacterial invasion of dentin (Love et al., 1997). It seems that recognition of type I collagen may facilitate bacterial adhesion to dentin (Fig. 2) as well as a morphological growth response manifested by long-chaining of streptococcal cells (Love et al., 1997). In support of this suggestion, acid-soluble type I collagen fragments completely inhibit dentinal tubule penetration by streptococci in vitro (Fig. 3B). These and subsequent experiments with mixed cultures of oral bacteria have led to the following model (Fig. 4) for dentinal tubule invasion by streptococci and P. gingivalis. It is envisaged that antigen I/II family polypeptides produced by S. gordonii, S. mutans, and other oral streptococci mediate primary binding of bacteria to intratubular collagen type I. Streptococcal growth and metabolism promotes localized demineralization together with release of collagen peptides. The presence of these peptides leads to up-regulation of antigen I/II production (A), long-chaining of cells, and colonization along the length of the tubule. In the lower diagram, P. gingivalis cells (●) and S. gordonii cells both adhere to collagen (1), but P. gingivalis is unable to penetrate the tubules further in monoculture. The presence of S. gordonii (2) provides an additional binding substrate for P. gingivalis and promotes intratubular colonization by P. gingivalis. Up-regulation of streptococcal antigen I/II adhesin production (3) provides additional binding sites for P. gingivalis. These bacteria remain in association with the streptococci (4), and the dentinal tubules become invaded by a mixed bacterial population.

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**Figure 4.** Streptococcal invasion of dentinal tubules (upper diagram) and co-invasion with P. gingivalis (lower diagram). Streptococcal cells (●) adhere to unmineralized collagen type I ( ― ― ) via antigen I/II polypeptide adhesin (A). Growth of streptococci in the presence of collagen peptides leads to up-regulation of antigen I/II production (A), long-chaining of cells, and colonization along the length of the tubule. In the lower diagram, P. gingivalis cells (●) and S. gordonii cells both adhere to collagen (1), but P. gingivalis is unable to penetrate the tubules further in monoculture. The presence of S. gordonii (2) provides an additional binding substrate for P. gingivalis and promotes intratubular colonization by P. gingivalis. Up-regulation of streptococcal antigen I/II adhesin production (3) provides additional binding sites for P. gingivalis. These bacteria remain in association with the streptococci (4), and the dentinal tubules become invaded by a mixed bacterial population.
polypeptides (Love et al., 2000). Invasion is not dependent on production of major adhesion fimbriae by P. gingivalis, that bind collagen, since isogenic P. gingivalis mutants defective in major fimbriae are still able to co-invade with S. gordonii (Love et al., 2000). On the other hand, the antigen I/II polypeptide SpaP of S. mutans binds only weakly to P. gingivalis cells, and S. mutans cells do not allow the invasion of dentinal tubules by P. gingivalis (Love et al., 2000).

It is likely that other bacterial interactions between host proteins and other bacteria may influence tubule invasion. Recently, it has been demonstrated that dentinal tubule invasion and adhesion to collagen by S. mutans or S. gordonii were inhibited by human serum, suggesting a protective mechanism of serum (Love, 2001). In contrast, cells of E. faecalis, a species commonly recovered from the root canals of failed endodontic cases, maintained their ability to invade dentin and adhere to collagen in the presence of serum (Love, 2001). It was suggested that, following root canal therapy, this ability may allow residual E. faecalis cells in radicular dentin to re-colonize the obturated root canal and participate in chronic failure of endodontically treated teeth (Love, 2001).

Analysis of these data demonstrates that specific adherent interactions between oral bacteria may facilitate tubule invasion. The observations should stimulate more detailed investigations of other bacterial interactions and their role in determining the composition of the dentinal and root canal microflora and the outcome of endodontic infections.

**IX) Summary and Future Prospects**

Bacterial invasion of dentinal tubules and the clinical consequences thereof have been recognized for over a century. However, while many components of the infected dentinal tubule microflora have been identified, it seems likely that there are etiological agents of endodontic infections that have not yet been recognized. Molecular techniques of identification and quantification will be powerful tools in future studies of endodontic infections. Bacterial invasion of dentin occurs rapidly once the dentin is exposed to the oral environment, and in the early stages of infection, Gram-positive plaque bacteria dominate the microflora. The identification of adhesins that mediate these initial interactions of bacteria with dentin is important for the design of adhesion-blocking compounds. For example, agents that block antigen I/II polypeptide recognition of collagen, or that block the co-adhesion-mediating properties of antigen I/II protein, could be effective in controlling or preventing the initial invasion of dentin via the dentinal tubules. With time, fastidious obligately anaerobic bacteria become established as principal components of the microflora and can be found within the deep dentin layers. Unchecked bacterial invasion leads to inflammatory pulp disease, root canal infection, and periapical disease. It is important, therefore, that the mechanisms of invasion and interbacterial adhesion at all stages of the process be understood if novel control strategies are to be developed. These might include compounds that are added to dentifrices or mouthwashes, or that could be incorporated into dental materials, to inhibit the bacterial invasion of dentinal tubules. With longer retention of dentition in populations in many regions of the world, and increased incidence of root exposure, it is likely that infections of the pulp and periapical disease will have wider clinical implications in the very near future.

**Acknowledgments**

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**REFERENCES**


