Redefining the Persistent Infection in Root Canals: Possible Role of Biofilm Communities

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Abstract

Current concepts suggest that persisting infections subsequent to endodontic therapy are caused by one or two bacterial species that are "too robust" to be eliminated by conventional treatment measures. As a consequence, numerous studies are exploring the characteristics of these "most" resistant organisms to define an effective treatment strategy to eradicate them from root canals. By taking an ecological perspective, the main objective of this review is to present evidence that the nature of persisting endodontic infections depends not on the robustness of the organisms in the infected site, but on their capability of adapting their physiology to the new environmental conditions set by the treatment. Changes in the environment, such as an increase in pH by calcium hydroxide or the effect of antimicrobials, are capable of triggering genetic cascades that modify the physiological characteristics of bacterial cells. Surface adherence by bacteria to form biofilms is a good example of bacterial adaptation and one that is pertinent to endodontic infections. Increasing information is now available on the existence of polymicrobial biofilm communities on root canal walls, coupled with new data showing that the adaptive mechanisms of bacteria in these biofilms are significantly augmented for increased survival. This ecological view on the persisting infection problem in endodontics suggests that the action of individual species in persisting endodontic infections is secondary when compared to the adaptive changes of a polymicrobial biofilm community undergoing physiological and genetic changes in response to changes in the root canal environment. *(J Endod 2007; 33:652–662)*

Key Words

Bacterial adaptation, *Enterococcus faecalis*, microbial ecology, pathogens, physiological changes

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A traditional concept that explains infectious processes occurring in humans suggests that diseases are produced as the result of the aggressive invasion of harmful microorganisms, which battle with the human host's defenses, triggering mechanisms that release antibodies and immune cells. The impact of such an approach generates a predisposition to search for those "most dangerous" microorganisms that can cause/trigger the most severe damage to the host. In line with this view, infectious processes of the oral cavity were proposed to be caused by a relatively small number of organisms from the diverse collection of species found in the human mouth (1). In caries, for example, the frequent isolation of *Streptococcus mutans* from carious lesions (2–4) generated a considerable number of studies to explore the ex vivo features of this bacterium. Research findings showing the significant acid-tolerant capabilities of *S. mutans* defined this organism as "the" agent responsible for initial enamel and dentine demineralization. Similarly, in periodontal disease, the frequent recovery of proteolytic microorganisms from deep periodontal pockets, such as *Porphyromonas gingivalis*, increased the attention of periodontists to these bacteria because they were considered key etiological agents of the disease (5, 6). The main disadvantage with this traditional view of the infectious process, especially in oral infections, is that the determination of true cause-and-effect relationships is not always possible. Consequently, the predominance of certain microorganisms at a given site may be the result of the disease itself rather than that of the initiating agent (7). Recently, the "ecological plaque hypothesis" (8–13) has improved on these classic infectious concepts to explain the etiology of caries and periodontal disease. This hypothesis suggests that the organisms associated with the disease may also be present at sound sites, but at levels too low to represent a clinical threat. In other words, disease is produced as the result of changes in the local environmental conditions that will shift the balance of the resident flora.

Root canal infections have a different nature than that of caries or periodontitis because they become established in originally sterile compartments of the oral cavity. In many cases, this led to the concept that the etiology of root canal infections involves only a single pathogen. For example, the predominance of certain proteolytic black-pigmented anaerobic organisms in cultures from infected root canals associated with acute symptoms suggested that these organisms are foremost etiological agents in such cases (14, 15). Recently, the frequent recovery of *Enterococcus faecalis* in root canals associated with persistent infections brought about an intense research interest in this bacterium. *E. faecalis* has become the ideal organism to test different irrigants, medicaments, and antiseptic solutions used in endodontics ex vivo, with findings that revealed its innate resistance capacity (16–18). This extensive interest in *E. faecalis*, perhaps driven by its ability to grow under almost any laboratory condition (19), resulted in the concept that the organism is the sole etiological agent for chronic endodontic infections. Consequently, the focus on *E. faecalis* resulted in much less information on the existence of other organisms in such infections that may possess similar tolerating characteristics to *E. faecalis* and that would shed light on the existence of a polymicrobial persisting community. Thus, it is not surprising that ecological parameters in root canal infections are not often discussed.

From an ecological perspective, the root canal can be considered a highly controlled environment with a limited number of niches. Although niches are composed by a variety of environmental factors that limit the growth of one species relative to others (20), the main limiting factors in root canal niches that influence bacterial colonization are, for instance, oxygen and nutrient availability (21). After root canal treatment, other

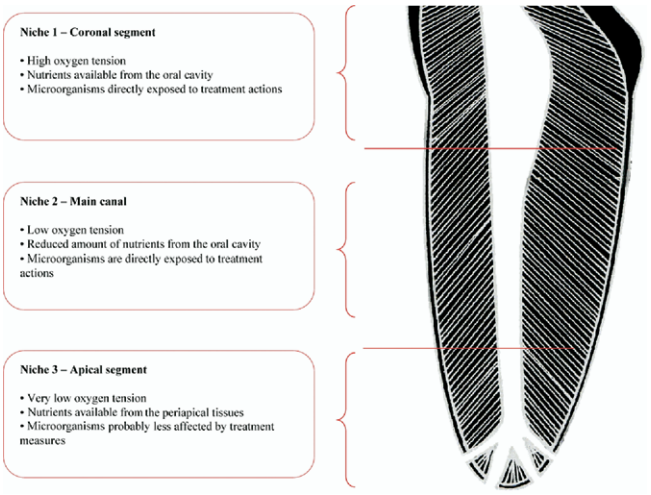

Figure 1. Different niches and limiting factors in the root canal environment.

limiting factors become involved, such as pH and the short-/long-term effects of the antibacterial medicaments applied. The limiting factors for three different niches in root canals are depicted in Fig. 1, from which it can be reasonably assumed that bacterial survival in such controlled environments, especially after root canal treatment, is based on the capacity of organisms to adapt to the existing conditions.

Although traditional views suggest that the organisms surviving root canal treatment are a selected group of the "most robust" organisms, the application of ecological parameters indicates that bacterial survival after root canal treatment will depend not on the robustness of the organisms, but on how good an adaptor the organism is to the new limiting factors in their corresponding niches. Furthermore, as in every natural microenvironment, the adaptive capabilities of individual organisms are exponentially augmented when growing in biofilm communities. As proposed in this review, data now exist (Fig. 2) that provide an argument for the inclusion of the biofilm concept in the etiology of persisting endodontic infections. The foundation for this ecological approach to endodontic infections suggests that the most dangerous "pathogen" is not an individual species, but a polymicrobial entity that undergoes physiological and genetic changes triggered by changes in the root canal environment.

Pathogens and Virulence

The accepted definitions of microbial pathogen and microbial virulence were formulated largely from the study of infections caused by a single etiological agent, although both represent combinations of highly complex parameters. As a result, classifications of microbial pathogens place the primary responsibility for causing a determined disease at all times on the microorganism [for a review see Casadevall and Pirofski (22)]. Likewise, the virulence of a pathogen is generally defined as the degree of pathogenicity or ability of the organism to cause disease measured by an experimental procedure (23).

The theory of the single pathogen as the etiological agent of a specific disease is derived from Robert Koch's Postulates, which are based on the work accomplished by Koch and his coworkers on diseases, such as, tuberculosis, diphtheria, anthrax, and cholera, that were determined to be caused by specific microbial entities at a time when the most prominent medical and scientific communities denied their importance [see Kaufmann and Schaible (24)]. By current standards, however, there are very few microorganisms to which the term *pathogen* can be applied invariably (25). A common example is group A streptococci, which are etiological agents of acute rheumatic fever, rheumatic heart disease, post-streptococcal glomerulonephritis, and invasive infections causing at least 517,000 deaths each year (26). Not included in such a traditional "pathogenic" categorization are the various microorganisms that we encounter on a daily basis coexisting peacefully with humans and from which a small number of them may be capable of a pathogenic phase. For example, *Escherichia coli* is ubiquitous, asymptomatically colonizing the human intestines and is widely distributed in the environment, yet, after experiencing specific genetic variations, this organism can cause epidemic dysentery and neonatal meningitis, among other diseases (27). Another example is *Listeria monocytogenes*, which is well adapted as a saprophyte for peaceful survival in soil and decaying vegetation, but it has also another phase where it acts as an intracellular invader capable of causing serious infections in humans (28–30). Current research suggests that the ability of these opportunistic organisms to switch from the harmless to the pathogenic state appears to occur in response to environmental changes that are mediated through complex regulatory pathways, which reversibly modulate the expression of virulence factors. The advent of bacterial genomic studies has significantly increased our understanding of the pathogenic state of many microbes with the on/off virulence switch, in fact, constituting a valuable marker of individual microbial pathogens (31–33).

Oral Pathogens

Historically, much of the earlier research into dental caries and the various periodontal diseases was focused on correlating a single specific organism with the disease to satisfy Koch's Postulates. We now know that not all diseases are the result of the action of a single organism and this is particularly true of the oral cavity where all of the microbial diseases associated with tissue destruction involve more than one type of organism and are, therefore, "mixed" infections (34). This polymicrobial nature of oral disease has its basis in the characterization of the organisms present in dental plaque and their potential roles in dental caries, gingivitis, and periodontitis (35–37). Early data (38) recognized the association of mutans streptococci, including *Streptococcus mutans* and *S. sobrinus*, with the initial phase of human dental caries because their acidogenic and aciduric properties permitted them to create a low-pH environment in dental plaque after the ingestion of sugars. In addition, lactobacilli and certain acid tolerant non-mutans streptococci can now be considered virulent with respect to dental caries (39, 40). In periodontal disease, the use of animal models suggested that *Actinomyces naeslundii* was involved in the destructive alveolar bone loss characteristic of advanced periodontitis (41), whereas evidence was also presented in the 1970s that black-pigmented organisms, such as *Porphyromonas gingivalis*, were directly involved in periodontitis (36).

Marsh (7) proposed the "ecological plaque hypothesis" to explain changes in the ecology of dental plaque that lead to the development of caries or periodontal disease. This hypothesis constitutes a dynamic model in which plaque-mediated diseases are the consequence of imbalances in the resident microflora resulting from an enrichment within the microbial community of the above mentioned "oral pathogens" (9–11, 13). In caries, for example, potentially cariogenic bacteria may be found naturally in dental plaque, but at neutral pH and with a conventional low-sugar diet, the levels of such potentially cariogenic bacteria are clinically insignificant. If the intake of fermentable carbohydrates increases, the low pH provoked in plaque favors the proliferation of acidogenic and aciduric bacteria, such as mutans streptococci and lactobacilli, which promote enamel demineralization.