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Community as the unit of pathogenicity: An emerging concept as to the microbial pathogenesis of apical periodontitis

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A current trend in the understanding of the pathogenesis of several human endogenous infections is to shift away from a reductionist paradigm to a holistic view of the microbial community as the unit of pathogenicity. Data brought about by studies on the pathogenicity of mixed infections, amalgamated with emerging information on physiological cooperative behavior of multispecies biofilms, bacterial interactions and food webs, and quorum-sensing systems reveal that the community outcome can be much more than the mere sum of its individual components. The concept of the community as pathogen is based on the widespread principle that teamwork is what eventually counts. Mounting evidence based on morphological and bacterial community profiling studies has demonstrated that apical periodontitis is a disease primarily caused by bacteria organized in biofilm communities adhered to the root canal walls. From the perspective of the single-pathogen concept, apical periodontitis can be considered as of no specific microbial etiology. However, based on the community-as-pathogen concept, it is possible to infer that, despite the high interindividual variability in endodontic microbial community composition, there are apparently some disease-related patterns. This article focuses on diverse ecological and pathogenic aspects of microbial communities, especially in relation to the pathogenesis of apical periodontitis. Therapeutic strategies based on ecological interference and other factors are also discussed. Future research should focus not only on the structure (species richness and abundance) of endodontic bacterial communities, but also on the application of methodological approaches that allow interpretation of the community behavior and function. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:870-878)**

Apical periodontitis is a bacterially induced inflammatory disease. This concept was suggested by Miller's seminal studies in 1894,[1] proven approximately 70 years later by Kakehashi et al's[2] classic study in germ-free rats, and confirmed by numerous subsequent studies.[3-5] As with any other human infectious disease, apical periodontitis has been extensively studied for the causative microbial agent(s). Studies using anaerobic culture and sophisticated molecular biology methods have demonstrated that apical periodontitis is essentially associated with a mixed infection of endogenous (oral) bacteria.[3,6-9] Therefore, unlike some classic diseases caused by exogenous pathogens, which have a "single-species etiology," apical periodontitis is similar to most of the other human endogenous infections in that no single pathogen but a set of species usually organized in biofilm communities is involved with disease causation.

This article highlights diverse ecological and pathogenic aspects of microbial communities, especially as they apply to the understanding of the pathogenesis of apical periodontitis. Insight is given on the rising concept of apical periodontitis and its different manifestations as diseases caused primarily by biofilm communities colonizing the root canal system. Implications for treatment and the ecological effects induced by intracanal procedures are also discussed in this context.

THE COMMUNITY CONCEPT

In microbial ecology hierarchy, individual microorganisms proliferate in the environment to give rise to

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populations, which in turn occur as microcolonies that interact with one another to form a *community*. Therefore, community is regarded as an integrated assemblage of populations that coexist and interact in a given environment. Following this concept, an individual root canal that becomes infected actually harbors an endodontic microbial community that is composed of several populations (microcolonies).

Individual populations may consist of a single bacterial species, but more frequently are composed of different species that collectively give rise to a mixed community.[10,11] Each population occupies a functional role (*niche*) that contributes to the overall community and maintains the ecological balance of the ecosystem. There are a limited number of niches within the community for which populations must compete. More competent populations occupy the niches and displace those less competent. As discussed in the next sections, highly structured and spatially organized microbial communities can be endowed with properties that are greater than the sum of the component populations.

REDUCTIONIST AND HOLISTIC APPROACHES

Historically, microbiologists have faced periods of "reductionism" and "holism."[12] Reductionism is based on the idea that the whole can be understood by examining smaller pieces of it, i.e., all complex systems can be completely understood in terms of their individual components. Through reductionist approaches, individual species are isolated from complex mixed communities and metabolically and genetically studied so as to allow understanding of the community by examining every single constituent. However, it has become quite apparent for the microbiota associated with many human infectious diseases that the whole may be very often greater than the simple sum of its parts. This concept has made microbiologists adopt a holistic approach to understand the community behavior associated with the pathogenesis of many infectious diseases known to have a polymicrobial etiology. Holism holds that any component cannot be thoroughly understood except in their relation to the whole. The holistic theory has been largely used in ecology, as the interplay of the different parts composing a given ecosystem will ultimately determine its properties.[12]

Traditionally, the study of the etiology of the main oral diseases has been based on reductionism. Under a reductionist mentality, microbiologists and infectious disease professionals are traditionally taught to look for the "causative pathogen" that is usually the single etiologic agent of a given disease.[12,13] A huge body of studies in oral microbiology has focused on certain "putative single pathogens." such as *Streptococcus mutans* for caries, and *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* for periodontal diseases, to mention only the most famous oral species. Nevertheless, recent microbiological studies have revealed that the pathogenesis of the 3 main oral bacterial diseases–caries,[14-16] periodontal diseases,[17-19] and apical periodontitis[7,8,20,21]–may be much more complex than explained by a reductionist approach. Indeed, limitations of the reductionist theory have surfaced as new concepts of biofilms, quorum-sensing systems, community behavior, species interactions, and polymicrobial/synergic infections become established. Now it is well recognized that the biofilm associated with caries or periodontal diseases represents a sophisticated community playing functions that are essential for its own architecture and physiology, with consequent pathogenetic implications.[22,23] Mounting evidence indicates that apical periodontitis can also be the result of the collaborative activities of a biofilm community.

MICROBIAL COMMUNITIES AND APICAL PERIODONTITIS

Community profiling studies[20,24,25] revealed that the bacterial composition of the endodontic microbiota differs consistently among individuals suffering from the same disease. This indicates that apical periodontitis has a heterogeneous etiology, where multiple bacterial combinations can play a role in disease causation. Moreover, community structure differs significantly between different disease forms (e.g., chronic apical periodontitis versus acute apical abscess),[8,20] suggesting the existence of a pattern associated with each one. Interindividual variability is even more pronounced when different geographic locations are studied.[24,26]

The high interindividual variability observed for samples from the same clinical disease indicates that different compositions of the bacterial communities can basically result in similar disease outcomes. Identification of the community members can reveal the presence of some species or group of species that may be important for the causation of some forms of disease. It is reasonable to realize that different magnitudes of disease, based on intensity of signs and symptoms, may be related to the species composition of the community. Indeed, it has been demonstrated that, despite individual uniqueness, bacterial communities associated with acute abscesses (severe pain and swelling) are more similar among them in comparison to bacterial communities associated with chronic apical periodontitis (no pain).[8,20] Therefore, although there may be no specific species related to some forms of disease,[27] specificity seems to be related to the community level as certain communi-

ties (species composition) are specifically associated with some forms of apical periodontitis. In other words, from the perspective of the single-pathogen concept, apical periodontitis can be considered as of no specific microbial etiology. However, based on the community-as-pathogen concept, it is possible at this point to infer that some communities are more related to certain forms of the disease.[8,20]

Even in root canal–treated teeth with posttreatment disease, recent evidence brought about by molecular methods indicates that mixed infections are found in most cases[25,28-31] and that the occurrence of mono-infection previously reported by culture studies might have been mostly an artifact derived from shortcomings of the methodology, more specifically the low sensitivity of the culture method and the occurrence of as-yet-uncultivated phylotypes.[32] Hence, mixed biofilm communities have also been implicated as the main cause of treatment failure.[33]

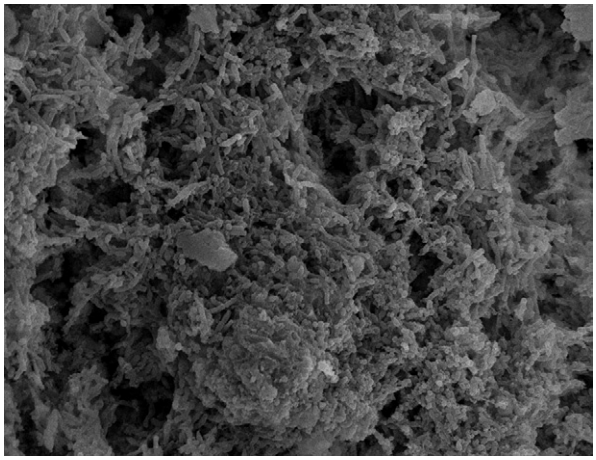

Fig. 1. One-week-old mixed bacterial biofilm formed on the root canal wall (scanning electron microscopy, original magnification ×1200). The ability to form metabolically integrated communities is arguably the most successful strategy for bacteria to survive and flourish in the root canal environment and cause apical periodontitis.

LOW-ABUNDANT MEMBERS–ARE THEY REALLY IMPORTANT?

The need for a holistic view must not be interpreted as a superficial approach to the problem. Quite conversely, the problem must be seen as a much more complex question that needs an integrated approach to be fully understood. In this context, every single part of the community should be studied and assessed, not individually, but as an important integrant of the whole system. What niche a single species occupies and how this species interacts with other members and the habitat represent an essential piece of knowledge for understanding the community behavior and its relationship with the host. The fact that the community as a whole is what matters does not imply that every single member of the community and its respective function does not need to be fully identified and appreciated. For instance, for a thorough understanding of a whole community, there is a need to know the members and their "professions" (niches), as well as their relative proportion to the overall community. Thus, one can predict the final outcome and understand how this information can interfere with the global effect of the community as a whole, including how profitable (or virulent!) it will be. In other words, it is important to identify who is present, at what proportion, and which niche it is occupying.

The first step in community analyses is to produce detailed inventories of the constituent members, from high- to very low abundant taxa.[34,35] In this regard, it may be important to know even rare dwellers, which can provide the community with uncommon and unique but potentially advantageous properties. It has been widely accepted in microbial ecology that even low-abundant members might serve as keystone species within complex communities.[34,35] Low-abundant members might simply be the products of historical ecological change with the potential to become dominant in response to shifts in environmental conditions (e.g., when local environmental changes favor their growth). Therefore, low-abundant populations at a sample site might eventually become dominant in response to environmental changes.[35] It is worth pointing out that dominant populations in one individual can correspond to low-abundant populations in another one.[36] From this discussion, it becomes quite apparent that a consistent understanding of the ecology and pathogenicity of a microbial community depends on the knowledge of every component involved, including identification of species present at low levels in the environment. Minimizing or disregarding their occurrence may be interpreted as oversimplification.[37]

THE BIOFILM LIFESTYLE

The community-forming ability can be regarded as essential for microbial survival in virtually all environments. Indeed, the vast majority of microorganisms in nature invariably grow and function as members of metabolically integrated communities–or biofilms (Fig. 1).[13,23] Biofilm can be defined as a sessile multicellular microbial community characterized by cells that are firmly attached to a surface and emmeshed in a self-produced matrix of extracellular polymeric substance (EPS, usually polysaccharide).[13,38] The ability to form

biofilms has been regarded as a virulence factor.[39] It has been estimated that biofilm infections comprise 65% to 80% of the bacterial infections that affect humans in the developed world.[40]

Biofilms are not merely passive assemblages of bacterial cells that are stuck to surfaces, but are structurally and dynamically organized complex biological systems. Bacterial cells in biofilms form microcolonies (about 15% by volume) that are embedded and nonrandomly distributed in the EPS matrix (about 85% by volume) and separated by water channels.[10,38,41,42] Dental biofilms can reach up to 300 or more cell layers in thickness.[10]

Many naturally occurring biofilms have a highly diverse microbiota. As a community of bacteria, biofilms have a collective physiology, responding in concert to environmental challenges. The component species form populations that are not randomly distributed but are spatially and functionally organized throughout the mixed community. Indeed, populations are strategically positioned for optimal metabolic interaction and the resultant architecture favors the ecological role of the community. The properties displayed by a mixed biofilm community are mostly dictated by the interactions between populations, which create novel physiological functions that cannot be observed with individual components.

The community lifestyle affords a number of advantages to biofilm bacteria, including (1) creation of a broader habitat range for growth of a more diverse microbiota; (2) increased metabolic diversity and efficiency due to food webs; (3) protection from competing microorganisms, host defenses, antimicrobial agents, and environmental stress; (4) facilitated genetic exchanges; and (5) enhanced pathogenicity.[10,22,38,39,41,43-45] As for the latter, it is highly likely that the steps involved in pathogenesis of apical periodontitis require the concerted action of bacteria in a community. It is possible that certain species have more than one role in disease, whereas different species can perform similar functions. This helps explain why communities with different bacterial composition can be found in different individuals with similar disease.[8,20,24-26] In mixed communities, a broad spectrum of relationships may arise between the component species, ranging from no effect (rare) or reduced pathogenicity to additive or synergistic pathogenic effects. Endodontic abscesses are examples of polymicrobial infections whereby bacterial species that individually may have low virulence and are unable to cause disease can do so when in association with others as part of a mixed consortium (pathogenic synergism).[46,47]

COMMUNITY-BASED MICROBIAL PATHOGENESIS

A current trend in medical microbiology when it comes to endogenous infections is to move away from the dominant paradigm that a single species causes a disease to the understanding of the microbial community as a pathogen. This has become mostly apparent from studies revealing that virtually all human endogenous anaerobic infections are mixed infections.[48] A critical appraisal of data brought about by studies on the pathogenicity of mixed infections,[46,49] combined with emerging information on physiological behavior of multispecies biofilms,[13,22] bacterial interactions,[12,50] and quorum-sensing systems,[51,52] reveals that the community is in many diseases the unit not only of survival but also of pathogenicity. In this regard, multiple microorganisms forming an organized community are necessary to produce disease.

Mechanisms of survival and induction of tissue damage by bacteria forming biofilm communities are a result of collective and cooperative activities and quite differ from mechanisms used by planktonic cells of specialized pathogens.[13] Consequently, the outcome of biofilm community infections usually differs when compared to infections caused by pathogens occurring in a planktonic state.

ACUTE (PLANKTONIC) AND CHRONIC (BIOFILM) INFECTIONS

Depending on several factors, apical periodontitis can be chronic or acute. The following discussion is based on some emerging concepts in medical microbiology as to the pathogenesis of acute and chronic infections that have the potential to be applied to apical periodontitis and help fill the current gap in knowledge. An acute infection is usually caused by a highly virulent bacterial community. Such high virulence may be because of the presence of virulent species or strains and/or the occurrence of synergism between species. Acute infections are usually related to bacterial cells in a planktonic state, at high counts, and with some tissue invasion ability, counteracted by diminished host resistance.[53] It has been demonstrated for some pathogens that genes coding for many virulence factors are much more expressed in planktonic cells than in sessile (biofilm) cells, suggesting that planktonic cells are more likely to participate in acute infections.[54] The phenotype of cells in a planktonic state is fundamentally different from the much more diverse biofilm phenotype.[55-57] The shift in gene expression toward a planktonic phenotype is usually conducive to rapid growth and mobility. Many enzyme and toxin genes are turned off when bacteria grow in the biofilm phenotype, but production of these factors can be reinitiated and am-

plified when individual cells are released from biofilms and assume the planktonic phenotype[13]; however, it must be recalled that transition to a planktonic state renders cells more susceptible to antimicrobial agents and phagocytosis.[39,58,59]

A chronic disease is in turn usually associated with low virulence of the bacterial community involved, which however generally represents a persistent source of aggression to the tissues. Persistence in chronic infections is usually related to community organization in biofilms and the inaccessibility to host defenses because of the anatomic location of the infection.[42,59,60] The juxtaposition of bacterial biofilms to tissues not accustomed and adapted to their presence triggers destructive inflammatory responses. In chronic infections, bacteria basically maintain their presence next to susceptible tissues that respond in a detrimental way.[13] This is clearly observed in marginal periodontitis.[10,11] Likewise, in chronic apical periodontitis, bacteria in the necrotic root canal cause chronic infection by forming protected biofilms on the canal walls, maintaining a close contact with the apical periodontal ligament, which reacts by persistent inflammation.

Biofilms are by and large better tolerated in sites where in equivalent counts planktonic cells would be otherwise significantly damaging. This can be explained by the fact that many enzymes and toxins that significantly contribute to the virulence of the planktonic phenotype are not produced by their counterparts in the biofilm state.[13] Consequently, bacteria in biofilms comprise a persistent low-grade aggressive behavior that evokes a counterattack by host defenses. However, bacterial biofilms in the avascular root canal (resultant of necrosis or treatment) are by and large protected from host defense cells and molecules. Even in the situation where the biofilm extends beyond the apical foramen to cover the external root surface adjacent to the foramen, sessile bacteria are more resistant to phagocytic challenges, irrespective of the presence of opsonins, such as antibodies and complement molecules.[59] One can then assume that organization in biofilm communities is the most successful strategy for bacteria to survive and thrive in virtually every environment. Biofilm bacteria benefit from the environment for long periods without the need to mount a sophisticated apparatus of virulence. Disease is usually mediated by host-derived factors in an attempt to eliminate these sessile communities.

To sum up, biofilms are generally less aggressive in causing immediate tissue damage but potentially dangerous because they can stimulate persistent inflammation associated with collateral tissue damage. Also, biofilms serve as potential foci for acute exacerbations by releasing sufficient planktonic cells to initiate an acute infection. Tissue damage and resultant inflammation are generally proportional to the cellular density and species composition of the biofilm.

METHODS TO STUDY MICROBIAL COMMUNITIES

More than 400 species-level taxa have been found in the different types of endodontic infections, of which about 20 to 40 have been more frequently detected and then regarded as candidate endodontic pathogens. The fact that every molecular or culture study of the endodontic microbiota shows different species composition and different most prevalent species associated with the same clinical disease lends strong credence to the community-as-pathogen concept.

The recognition of the importance of the community as pathogen makes it important to develop research approaches that allow identification of the structure and physiology of the whole microbial community. Many molecular biology methods have been applied to the investigation of bacterial communities associated with endodontic infections. They include methods for microbial identification, such as broad-range polymerase chain reaction (PCR) followed by clone library analysis,[7,8,21,28] checkerboard,[29,61-64] and DNA microarray approaches.[65] Microbial community profiling techniques, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), and ribosomal intergenic spacer analysis (RISA), are mostly used if a fingerprint of the whole community is desired, although information on species identification can also be obtained.[26,32,36,66] Current methods that can provide invaluable information on the community physiology and function, such as metagenomics (genome-wide analysis of DNA obtained directly from the environment), transcriptomics (RNA expression analysis), proteomics (large-scale protein expression analysis), and metabolomics (metabolite profile analysis), have still to be applied in endodontic research. In addition to allowing prediction of function, these methods have the potential to disclose patterns of molecules associated with clinical conditions, which may serve a role as outcome predictors.

TREATMENT CONSIDERATIONS–ECOLOGICAL INTERFERENCE

Consciously or unconsciously, endodontic treatment has been fundamentally based on nonspecific elimination of intraradicular microorganisms. In fact, the current concept of endodontic infections as mixed communities that exhibit a very high interindividual variability lends support to the application of broad-spectrum antimicrobial approaches to treat endodontic

infections. Consequently, no specific species are targeted, but the community should be.

In this regard, in addition to chemical and mechanical direct effects promoted by preparation and interappointment medication, ecological effects may also be important. By ecological interference, one should understand as any event that causes significant disturbances in the ecosystem, affecting communities and compromising in some way their survival. For instance, eradication of key elements in a community may lead to an ecological disaster for the community, which would ultimately succumb by death of the remaining members. Examples of key components include species involved with cross-feeding and acquisition of essential nutrients for less competent species, species that modify the environment and favor the establishment of others, species producing protective enzymes that degrade antibiotics and host defense molecules, and so forth. Another positive ecological effect induced by treatment refers to influence on nutrient availability by eliminating existing nutrient sources (necrotic pulp tissue and bacterial partners) through chemomechanical preparation and medication and preventing further nutrient supply (by sealing the canal and precluding protein-rich tissue fluids and/or saliva seepage into the canal) through obturation.

On the other hand, elimination of some community members may leave some niches available to be occupied not necessarily by the same species but even by more virulent or resistant species that were previously absent or in low abundance in the environment. Depending on innumerous factors, this change in species composition and/or proportion might lead to a community with altered overall properties, including dominance by more robust species and/or with increased virulence. This may predispose to unfavorable treatment outcome or flare-ups, respectively.

Therefore, treatment procedures can promote total or partial elimination of the endodontic bacterial communities. Although ideal, total elimination is allegedly a difficult or even impossible goal to achieve with the methods and substances currently available.[67] Partial elimination is what can be achieved. However, one must be aware that partial elimination of the community represents disturbances that can lead to death of the residual community or reorganization in different proportion of species, which can even jeopardize treatment outcome. To avoid the latter, i.e., community reorganization, the clinician should direct his or her efforts to maximal and broad-range elimination of the endodontic bacterial community through chemomechanical preparation supplemented by an intracanal antimicrobial medication.[68-72] The role of the obturation would be to eliminate or reduce space for reorganization of the community by surviving bacteria and prevent further nutrient supply. As there is a threshold of residual bacteria beyond which obturation cannot successfully cope (by entombment),[67,73-77] the effects on the community have to be maximized so as to reduce bacterial populations significantly before a root canal filling is placed.[67]

It is reasonable to assume that ecological interference may be much more complex and insightful than the current treatment paradigm, which stands essentially on trying to eliminate all community members or denying substrate for remaining species. Research has been linearly and tirelessly looking for protocols and techniques that promote total bacterial elimination or a perfect apical or coronal antibacterial seal and no plausible conceptual alternative has been devised or at least proposed. Although the success rate of the endodontic treatment under controlled conditions is satisfactorily high following the existing paradigm,[78-81] the overall success rate when performed by the general population is astonishingly low.[82-87] This may be mostly a result of technical difficulties, because the high failure rates are related to treatment performed under substandard conditions.[85,88,89] Interference strategies focusing on the community ecology and virulence, on host responses, or even on possible modifying (genetic?) factors of the disease may be potential therapeutic targets for future research, especially if associated with less complex treatment technologies.

CONCLUDING REMARKS

Multiple microbial species forming a biofilm community are usually necessary to produce apical periodontitis, and monoinfections rarely, if ever, occur. In this setting, attempts to link a single species to the disease have proven fruitless. Historically, most endogenous infections (including apical periodontitis) were studied on the single-pathogen basis in spite of many of them having long been recognized as mixed infections. With the results from pathogenicity studies in animal models demonstrating the occurrence of synergism between several oral species, bacterial pairs started to arouse the interest of researchers involved with anaerobic infections. More recently, with molecular methods exhibiting a higher resolution to disclose the diversity and complexity of most endogenous infections, more complex bacterial interactions have surfaced and started to be investigated under the perspective of species clusters, which paved the way for a still more holistic view of the overall community as the unit of pathogenicity.

Study of species richness and abundance in endodontic communities continues to be of utmost importance, as community behavior is obviously dependent on its

diversity. However, the focus also needs to be on the collective cooperative behavior of potentially pathogenic bacteria and how the myriad of associations that can occur within the community affect and modulate virulence of species. The virulence ability of a given species is allegedly different when it is in pure culture, in pairs, or as part of a large bacterial "society" (community). The concept of the community as pathogen is based on the principle that teamwork is what eventually counts. For a holistic understanding of endodontic communities and their role in the pathogenesis of apical periodontitis as well as how they respond to treatment, there is a need for introduction of sophisticated methodological approaches that allow not only the cataloguing of bacterial species present but also the interpretation of the community behavior and function as it can be inferred by global gene expression (transcriptomics) and comprehensive inventories of released proteins (proteomics) and metabolites (metabolomics). All this information integrated in a dynamic network has the potential to provide a comprehensive view of the disease process, which will certainly increment the current conceptual model of apical periodontitis pathogenesis. This knowledge may serve as the foundation for a shift in the current therapeutic paradigms.

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