Journal of Mind and Medical Sciences

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Primary endodontic infections - key issue in pathogenesis of chronic apical periodontitis

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ABSTRACT

Primary root canal infection is a dynamic process. All species of oral microbiota have comparable abilities to establish in the root canals of necrotic teeth. The essential ecological factors in their biological selection are nutrient availability, anaerobic environment and bacterial interactions. In chronic apical periodontitis, all selected microflora residing in the long-term infected habitat of root canals system are synergistic, and each of them can play the role of an endodontic pathogen. Microorganisms living in the root canal system of pulpless teeth progressively reach through anatomical communications to the periodontal ligament where, sooner or later, they cause the inflammatory and immunological conflict between the infection and the host. The insight into the complexity of the root canal microbiota is improved by the current pyrosequencing and next-generation sequencing diagnostic techniques, which allow the identification of multispecies of the microbiome and their targeted treatment. The insight into the complexity of root canal microbiota is improved by present diagnostic techniques of pyrosequencing and next generation sequencing, which allow the identification of multispecies of the microbiome and their targeted treatment.

Category: Review

Received: March 19, 2024
Accepted: May 23, 2024
Published: October 30, 2024

Keywords:
chronic apical periodontitis, root canal microbiome, primary endodontic infections

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Introduction

Chronic apical periodontitis is a worldwide prevalent dental disease and half of the adult population has at least one tooth affected by this condition [1]. The pathogenic key issue relies on the primary infection of root canals in pulpless teeth and on the anatomical complexity of endodontic system in humans [2,3].

The anatomy of periapex reveals a complex of three functionally integrated tissues: root cementum, periodontal ligament and alveolar bone. The local reaction of this morphological tissue unit to a range of harmful etiological agents is similar to other connective tissues of human body [3].

The root canal infections occur only in pulpless teeth, resulting either after pulp tissue necrosis or intentional therapeutic removal of the dental pulp. In necrotic endodontic systems in excess of 460 species and phylotypes have been disclosed [4].

Bacteria are the primary microorganisms described as invaders of necrotic pulp tissue and the major constituents of infected root canals. However, later on were also found fungi, viruses and even archaea [5].

The etiology of chronic apical periodontitis is explained by pivotal role of bacteria and 90% of tooth roots with associated chronic apical periodontitis, radiographic confirmed, harbored anaerobes. Without microbial infiltration from oral cavity the necrotic pulp tissue itself is not able to trigger and support the apical lesion [6,7].

The microorganisms living into the root canal system of pulpless teeth progressively reach by anatomic communications the periodontal ligament where, sooner or later, prompt the inflammatory and immunologic conflict between intruders and host.

To cite this article: Iliescu AA, Gheorghiu IM, Ciobanu S, Roman I, Dumitriu AS, Popescu GAD, Păunică S. Primary endodontic infections - key issue in pathogenesis of chronic apical periodontitis. *J Mind Med Sci*. 2024;11(2):331-336. doi:10.22543/2392-7674.1562

Because the host defense mechanisms are various, both protective and destructive, chronic apical periodontitis that involves pathologic changes of periodontal ligament, apical bone and cementum basically can be considered an immunoinflammatory response of microbial etiology following the infection originating from root canal system [8-10].

Accordingly, the pathologic features occurring in the periapex are rather the consequence of microbial toxins, their noxious metabolic byproducts and necrotic pulp remnants undergoing disintegration than of bacteria themselves [3].

Discussions

Root canal ecosystem

Commonly, all members of the oral microbiota community share comparable chances for occupying and infecting the root canals of necrotic teeth. Apparently, due to their non-shedding walls and the absence of blood flow, the microorganisms have optimal conditions to flourish [3].

However, leaving the nutrients reach milieu of oral cavity and entering the root canals they should compete to survive in this new and unique but more disadvantageous environment of endodontic system [11].

The nutrients availability, anaerobic milieu and bacterial interactions are pivotal ecological drivers that result in a conspicuous biological selection of previous 100 to 200 invaders explaining the limited number of no more than 10 to 20 bacterial species that are lastly rediscovered. Moreover, in order to survive in the specific environment of root canals the bacteria had also to bear genetic exchanges and mutations [11].

Usually in primary endodontic infections early colonizers are in position to oblige the subsequent various bacterial species to organizing them in mixed biofilm communities. The mutual close proximity of microbial cells guarantees the development of inevitable bacterial interactions that can be either positive, enhancing the survival hope by promoting a proper habitat of coexistence, or negative [3,11].

Initially the bacterial interactions are governed by nutrients availability that plays the role of the main ecological determinant. The positive interactions develop based on food chains in order to mutually use the turnover end products and to break down the various and complex nutritive substrates. Later on, by reducing the oxygen tension in the root canal, are improved the environmental conditions to developing the obligate anaerobes [3,11].

Over time the inter-bacterial positive interactions involve common protection against external menace (the release of both proteinases to inhibit the host defense mechanisms and antibiotic-inactivating enzymes), acclimatization to unfavorable changes of local milieu and, relying on individual relationships within the root canal biofilm such as quorum sensing, bacterial aggregation and coaggregation or horizontal gene transfer [3,11].

When in primary infected root canals are still pulp tissue remnants or serum-like substrates, frequently occurs bacterial association consisting of anaerobes such as Prevotella, Peptostreptococcus and Eubacteria since they are prone to enzymatic break down of peptides and amino acids [12,13].

Strong positive interactions were found between F. nucleatum while associated with P. endodontalis, P. micros and C. rectus, between P. intermedia, P. anaerobius, and P. micros or between P. intermedia, P. anaerobius, P. micros and eubacteria [14-16].

The negative bacterial interactions are focused on lowering the microbiota density by using mechanisms of competition for nutrients and niches as well as amensalism that is based on releasing of inhibitors such as enzymes, short-chain fatty acids, hydrogen peroxide or even bacteriocins [3].

The commonly used mechanisms for the survival of microorganisms into the endodontic system are biofilms and microbial aggregates generated either by autoaggregation or coaggregation, both of them being disclosed in chronic apical periodontitis [12].

Autoaggregation, which is supported by bacteria belonging to the same strain, occurs between genetically identical cells and was revealed mostly in root canal infections for genera Prevotella, Fusobacterium, Streptococcus, and Staphylococcus [12].

Coaggregation is a bacterial metabolic interaction supporting the development of biofilms and is also considered a virulence factor. In infected root canals coaggregation was noticeable in genera Prevotella, Fusobacterium, and Streptococcus [12,17].

In root canal infections Fusobacterium nucleatum holds the rank of one of the strongest bacterial participants in coaggregation and also revealed a high positive correlation with Porphyromonas endodontalis [17,18]. Similarly strong involvement of Fusobacterium nucleatum in coaggregation, this time with Porphyromonas gingivalis, was found out in extraradicular biofilms associated to refractory chronic apical periodontitis [16].

Major intergeneric pairs of coaggregated bacteria seems to be streptococci-prevotellae, streptococci-staphylococci, and streptococci-corynebacteria. Commonly fusobacteria, streptococci and prevotellae are widely accepted as coaggregation partners by many other bacterial species [19].

In endodontic biofilms associated to primary chronic apical periodontitis also occur multigeneric aggregates, which properly are amalgamated coaggregations of autonomous intergeneric coaggregation whose ecological significance had still to be elucidated [19].

Dynamics of root canal infection

Once initiated the root canal infection carries on as a dynamic process. In long-standing necrotic teeth the diminution of nutrition, fluctuating redox potential, changes in local temperature and pH or availability of receptors for

adhesins progressively modify the microflora community enabling the selection of dominant bacterial species [11].

Actually, oxygen and oxygen derivates are the main ecological determinants influencing the proportion of facultative versus obligate endodontic anaerobes. At the beginning in endodontic infection facultative anaerobe species of bacteria predominate. However, later on the cessation of blood flow and inherent subsequent oxygen supply in pulpless teeth are conducive to generating an anaerobic environment [11].

Gradually the intra-canal consumption of oxygen and production of carbon dioxide in conjunction with reduced redox potential of facultative anaerobes facilitate the shift of local microflora to obligate anaerobes. Over time, after minimum three months, the last ones outnumber the former microorganisms and become dominant [11].

Another issue of equal importance in establishing a firm microbial community in infected root canals is the nutrients delivery supporting their turnover [3,11]. The appropriate main sources are miscellaneous: saliva nutritional components, which are continuously delivered, in time exhaustible necrotic pulp tissue, organic and mineral content of dentinal tubules, proteins and glycoproteins released either from periapical tissue serum-like fluids or inflammatory exudates, and metabolic products of cohabitating bacteria from endodontic system [11].

A dichotomy occurs along the infected root canal. Facultative anaerobic bacteria involved in turnover of carbohydrates (streptococci) prevail in coronal part, opposed to anaerobic bacteria fermenting peptides and amino acids that are mostly found in the apical sector [17].

The dynamic of nutrients utilization proved to be a significant contributing issue to changes in the bacterial domination from the saccharolytic species, early disclosed in infected root canals, to asaccharolytic species that finally outnumber the initial colonists. Though facultative anaerobes grow in anaerobic setting when the direct delivery of carbohydrates from oral cavity is broken, they are severely damaged and the root canal microbiota shifts to obligate anaerobes [19,20].

Culturing on human serum subgingival plaque microorganisms in the first stage occur the consumption of low-level carbohydrates and in the second stage proteins hydrolyze and degradation of remaining carbohydrates originating from serum glycoproteins. The third and last stage has been characterized by final protein decompose [11,19,20].

In second stage dominated Prevotella intermedia, Fusobacterium nucleatum, Veillonella parvula and Eubacterium species. Peptostreptococcus micros, Fusobacterium nucleatum and eubacteria have been disclosed as dominant bacteria in the third stage [21-23]. The adaptation of Peptostreptococcus micros to the ecological niche of infected root canals relies on its wide range of proteolytic enzymes, mostly peptidase [24].

Considering bacterial nutrition, a further change occurs as the necrotic pulp tissue is gradually degraded and consumed by endodontic microflora. Actually, this primary source of nutrients for bacterial turnover is out and cannot be any longer renewed.

Nevertheless, a new source of bacterial nutrients is generated when the root canal infection reaches the periradicular tissues to induce a chronic apical periodontitis. Relying on exudates components, such proteins and glycoproteins, released in the apical section of the root canal by periapical granulation tissue, the proteolytic bacteria may use this nutritional protein substrate in their turnover as suitable source of carbon and nitrogen [3,11].

Bacterial interactions

Many bacterial interactions in primary endodontic infections are linked to their food chain, some of bacteria providing essential nutrients for survival of other members of microbial community in need [20].

Though well equipped with peptidases, fusobacteria, peptostreptococci and eubacteria are not able to breakdown normal, chemically intact, proteins [11]. In contrast, Prevotella intermedia, Porphyromonas gingivalis and Porphyromonas endodontalis are efficient in cleaving serum proteins up to peptides and amino acids [18,21].

In the same line, an association of Peptostreptococcus micros with Prevotella intermedia or other black-pigmented anaerobic rods such as Porphyromonas gingivalis and Porphyromonas endodontalis, having a recognized enzymatic capacity of cleaving serum proteins, are highly involved in generating acute periapical abscesses [18,21,23].

A special mutual relationship is also established between Campylobacter rectus and Porphyromonas sp. by delivering to Porphyromonas a growth factor related to hemin [25]. On its turn Campylobacter rectus get the source of energy within the local bacterial consortium they are living together since it uses a particular respiratory mechanism based on hydrogen and formate as electron donors respectively oxygen, fumarate or nitrate as electron acceptors [26,27].

The most evident prove of bacterial interactions in infected root canals is the survival of a bacterial strain such as Prevotella oralis only if inoculated with other bacteria and not alone. Moreover, experimentally inoculating in equal amount in sterilized root canals separate bacteria strains isolated from an infected root canal, over time was reestablished the same proportion between bacteria as initially recorded in former infected root canal [28].

Microbiota diversity in primary endodontic infections

Historically the microorganisms from the root canals of pulpless teeth were observed initially in the 17th century by Leeuwenhoek and described by Miller two centuries afterwards as cocci, bacilli and spirochetes [29].

In oral milieu are disclosed 13 distinct phyla out of over 700 bacterial taxa, such as Fusobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Spirochaetes, Synergistes, Proteobacteria, Cyanobacteria, TM7, Sulphur River 1, Acidobacteria, Deinococcus and Chloroflexi [30].

Revealing and recognizing the bacteria participating in primary infection of root canals, including the chronic apical periodontitis, traditionally culture-based microbiological techniques have been carried out [7]. In primary endodontic infection participates a mixed microflora that is dominated by anaerobes. Although rarely occurred fungi, archaea and viruses [31,32].

The density of bacteria per root canal of necrotic teeth is as high as 103 to 108 [6,33] counting 100 genera and 9 phyla [33,34]. Focusing on chronic apical periodontitis, the subsequent molecular techniques revealed in average 10 to 20 bacterial species or phylotypes [33,34].

Not clinically unworthy is the conspicuous direct relationship between the radiographic size of apical lesions and the number of bacteria and species found in root canals [6]. As higher is the microbial concentration and variety, the larger is the apical image of radiolucency [34].

Actually, in apical chronic periodontitis associated with radiographic image below 5 mm were disclosed approximately 12 taxa whereas in apical lesions ranging from 5 to 10 mm around 16 taxa. The extended radiographic images, over 10 mm, were observed when the infected root canals lodged 20 to 40 taxa and even more [34].

Astonishing, compared to periodontal pockets, the bacterial species found in chronic apical periodontitis revealed an unexpected reduced diversity, between minimum 4 and maximum 12 taxa for each infected root canal as compared with 20 to 30 cultivable genera commonly isolated [4,35].

From the very beginning, by using culture methods, it has been observed the frequent presence of some bacteria belonging to both gram-negative genera such as Fusobacterium, Porphyromonas, Prevotella, Dialister, Tannerella, Treponema, Campylobacter or Veillonella and gram-positive, including Streptococcus, Peptostreptococcus, Actinomyces, Parvimonas, Pseudoramibacter, Olsenella, Filifactor and Eubacterium [19,34,35].

Moreover, some other associated bacterial species such as Fusobacterium nucleatum, Porphyromonas endodontalis, Porphyromonas gingivalis, Prevotella intermedia, Pseudoramibacter alactolyticus, Parvimonas micra, Prevotella nigrescens, and to a lees extend Filifactor alocis, Tannerella Forsythia, Dialister invisus, Dialister pneumosintes, Prevotella baroniae, Olsenella uli and Treponema sp presumed to be involved in the etiology of chronic apical periodontitis in the era of culturing methods, are presently confirmed by molecular techniques as candidate pathogens [19,36].

According to clone library analyses in chronic apical periodontitis about 55% of taxa colonizing the infected root canals are still uncultivable phylotypes, namely bacterial species recognized only by 16S rRNA gene sequence waiting for to be characterized [37-39]. Spirochetes are also joining to this group. Furthermore 64% of Treponema species occurring in chronic apical periodontitis previously were uncultivable [36].

However, the optimistic perspective is supported by successful culture of some formerly uncharacterized phylotypes such as Prevotella baroniae, Dialister invisus and Jonquetella anthropi [40] and the novel molecular technologies introduced in microbiologic research [41-43].

Molecular investigation also succeeded to find some uncultivable phylotypes of genera Prevotella, Selenomonas, Fusobacterium, Eubacterium, Solobacterium, Dialister, Synergistes, Olsenella, Veillonella or of Lachnospiraceae family [44,45].

Reminding the amount of aforementioned uncultivable oral microflora [4] these bacteria should not to be underestimated because some of their yet uncharacterized phylotypes might significantly contribute in the pathogenesis of various types of chronic apical lesions, including acute painful exacerbation [14,24].

The reason might be for some of these uncultivable bacteria the sensitivity to oxygen (strict anaerobes), inadvertent laboratory media and transfer conditions or nutrients deprivation, especially of those provided by other associated taxa [46].

Another explanation relies on the state of dormant bacteria that are characterized as viable but uncultivable. Actually, the dormant state is a protection mechanism of these bacteria against the inappropriate environment [47].

Compared to bacterial identification from DNA fragments, the physiological state of microorganisms living in the infected root canals, such as viable, injured or dormant, might be rather considered the crucial marker of their involvement in chronic apical periodontitis [47,48].

Current concept of endodontic pathogen

An endodontic pathogen is considered that microbe taking in charge the development of inflammatory tissue and associated bone destruction around the tooth apex, which are defined as chronic apical periodontitis.

In order to satisfy classic Koch's Postulates, the tendency to explain an oral disease was previously focused on its correlation with a single specific microorganism. However, the proved polymicrobial nature of root canal infection rejected this opinion [47].

Presently no evidence can support that one specific component of endodontic microflora is more virulent than others [47]. Moreover, the advances in microbiologic techniques failed to identify the main culprit and paradoxically the catalog of putative candidate endodontic pathogens even expanded [19].

The contemporary revised definition reminds that commonly a pathogen is a microbe responsible to bring

about host damage but highlights an alternative harmful mechanism, either direct microbial or indirect, managed by host immune reply [2].

From ecological viewpoint the major challenges for microorganisms on their way to find survival niches once established in the root canals of necrotic teeth depend on nutrition sources, inter-microbial competition, and defensive response of host.

In chronic apical periodontitis all members of selected microflora that reside in the habitat of the long-term infected root canals system are synergistic and each of them might be considered endodontic pathogen [19,38].

Conclusions

Primary root canal infection is a dynamic process relying on selection of those members of oral microbiota capable to survive in the demanding habitat of the root canals that occurs in necrotic teeth. All members of microflora residing in the selected ecological niche that characterizes an established chronic apical periodontitis are synergistic and each of them can play the role of endodontic pathogen. The insight into the complexity of root canal microbiota is improved by present diagnostic techniques of pyrosequencing and next generation sequencing that enable to identify the microbiome multispecies.

Contributions

AAI: conceptualization, supervision, methodology, data acquisition, writing-original draft, writing-review and editing; IMG: conceptualization, literature review, formal analysis, writing. SG: literature review, formal analysis, writing-review and editing; IR: literature review, formal analysis; ASD: supervision, writing-review; SP: conceptualization, supervision, writing-review and editing. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. Informed consent was obtained from all subjects involved in the study.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

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