Microbiology in Endodontics

In 1894, Miller's hypotheses stated that bacteria are the causative agent of apical periodontitis. Although there are other reasons, such as chemical and physical irritation to the pulp, that can affect the pulp. This results in various degrees of inflammation.

More than 400 different microbial species have been found in infected root canals, usually in combinations. Fungi have been occasionally found in endodontic infections.

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The endodontic microbiota was clearly different in the coronal, middle, and apical parts of the root canal. Changes in the composition of the microbiota are due to changes in environmental conditions, particularly regarding oxygen tension and nutrient availability. In the very initial phases of the pulpal infectious process, facultative bacteria predominate. After a few days or weeks, oxygen decreases within the root canal as a result of pulp necrosis and consumption by facultative bacteria. Oxygen supply is affected by the loss of blood circulation in the necrotic pulp, which develops an obligate microbiota.

Root canal microbes

The most prevalent named bacterial species detected in primary infections belong to diverse genera of:

1. **Gram-negative bacteria:** Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Treponema, Campylobacter, and Veillonella.

2. **Gram-positive bacteria:** Parvimonas, Filifactor, Pseudoramibacter, Olsenella, Actinomyces, Peptostreptococcus, Streptococcus, Propionibacterium, and Eubacterium bacteria.

Other microorganisms in endodontic infections

- **Fungi** :
  Fungi are eukaryotic microorganisms that may colonize the oral cavity, especially Candida species, but they have been only occasionally detected in primary intraradicular infections. Fungi are more frequently detected in canals of teeth with post treatment disease.

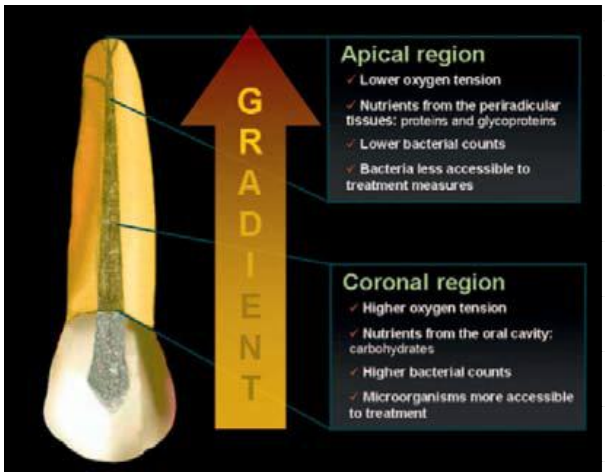
- **Archaea:**
  Archaea consist of a highly diverse group of prokaryotes distinct from bacteria. Members of this domain have been traditionally recognized as extremophiles, but some of these microorganisms have also been found to flourish in non-extreme environments, including the human body. No member of the Archaea domain has been described as a human pathogen. Several studies have failed to detect archaea in samples from primary endodontic infections. However, other studies have reported on the detection of these organisms, including a Methanobrevibacter oralis–like phylotype, in some cases. Because archaea have not been consistently detected in infected root canals, their role, if any, in the pathogenesis of apical periodontitis is questionable.

- **Viruses**
  Viruses are inanimate particles composed of a nucleic acid molecule (DNA or RNA) and a protein coat. On their own, they have no metabolism. To replicate the viral genome, they need to infect living cells and use the cell's machinery; as a consequence, they cannot prosper in the necrotic root canal. Viruses have been detected in root canals only in teeth with vital pulps. A study found the human immunodeficiency virus (HIV) in vital pulps of HIV-seropositive individuals. Another study reported the occurrence of some herpesviruses in normal and inflamed vital pulps.

Nutrition of Bacteria

The main sources of nutrients for bacteria colonizing the root canal system include:

1. Necrotic pulp tissue.
2. Proteins and glycoproteins from tissue fluids and exudate seep into the root canal system via apical and lateral foramina.
3. Components of saliva that penetrate coronally into the root canal.
4. Products of the metabolism of other bacteria



Routes of root canal infection

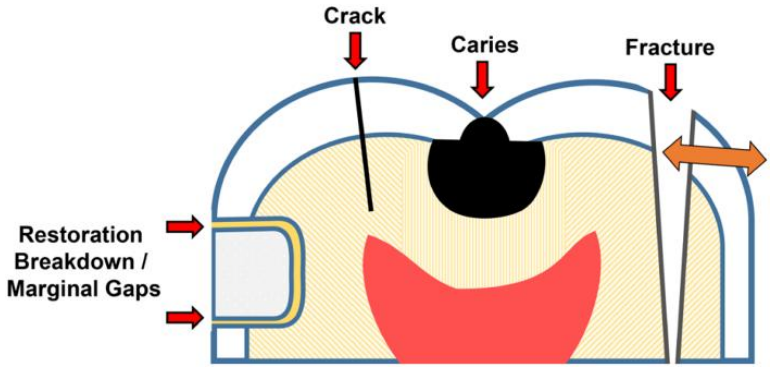
Under normal conditions, the pulpodentin complex is sterile and isolated from the oral microbiota by overlying enamel and cementum. In the event that the integrity of these natural layers is breached (e.g., as a result of caries, trauma-induced fractures and cracks, restorative procedures, scaling and root planning, attrition, abrasion) or naturally absent (e.g., because of gaps in the cementum coating at the cervical root surface), the pulpodentin complex is exposed to the oral environment.

Bacteria, usually from dental caries, are the main source of injury to the pulpal and periradicular tissues, and they enter either directly or through dentine tubules.

Modes of entry for bacteria into the pulp are as follows:

1. Through the carious cavity. Most of the bacteria in the carious process are nonmotile; they invade dentin and reach the pulp by repeated cell division. Bacterial cells may also be forced into tubules and reach the pulp by hydrostatic pressures developed on dentin during mastication.
2. Through the dentinal tubules, as in contamination during cavity preparation, through exposed root surface, and surfaces with erosion, abrasion, and attrition.
3. Periodontal bacteria can invade the root canal system via apical foramen, lateral canals, and exposed dentinal tubules, as in advanced periodontitis.

4. Through the bloodstream (anachoresis). Following trauma or inflammation to the pulp, any bacteria in the blood might be attracted to the pulp, causing pulpitis.
5. Through faulty tooth restoration.
6. Through the extension of a periapical infection from an adjacent infected tooth.



Host-parasite interaction

This interaction depends on:

1. <u>Microbial virulence factors</u>

These are microbial products, structural components, or strategies (biofilm formation) in the microorganism that give it the capability to cause tissue damage. The ability of a microorganism to cause disease is regarded as **pathogenicity**. **Virulence** indicates the degree of pathogenicity of a microorganism. Microbial secreted products, structural cellular components, and strategies that contribute to pathogenicity are regarded as virulence factors.

One example of a bacterial strategy that contributes to pathogenicity is the ability to form biofilms, which confers protection against host defenses, microbial competitors, and antimicrobial agents. Some microorganisms routinely cause disease in a given host and are called **primary pathogens**. Other microorganisms cause disease only when host defenses are impaired and are called **opportunistic pathogens**. Bacteria that make up the normal resident microbiota are usually present as harmless commensals and live in balance with the host. One of the greatest beneficial effects of human microbiota is probably the tendency to protect the host from exogenous infections by excluding other microorganisms. Nevertheless, in certain situations, the balance may be disturbed by a

decrease in the normal level of resistance, and then the commensal bacteria are usually the first to take advantage.

Most bacteria involved with endodontic infections are normal inhabitants of the oral microbiota that exploit changes in the balance of the host–bacteria relationship, becoming opportunistic pathogens.

2. <u>Host resistance factors</u>

The reaction of the host to the presence of bacteria or their products can be effective, such as:

A. Platelet factors.
B. Serum factors as antibodies (IgG, IgM)
C. Leukocytic factors as lysozymes, that hydrolyze bacterial cell walls of Gram-positive bacteria.
D. Macrophages factors.
E. Lymphocytic factors such as lymphotoxin and macrophage-activating factor.
F. Salivary factors such as lysozyme, antibodies (IgA)

Types of endodontic infections: Endodontic infections can be classified according to the anatomic location as intraradicular or extraradicular infection.

- **Intraradicular infection**: is caused by microorganisms colonizing the root canal system and can be subdivided into three categories according to the time microorganisms entered the root canal system: primary infection, caused by microorganisms that initially invade and colonize the necrotic pulp tissue (initial or "virgin" infection);
- **Secondary infection**: caused by microorganisms not present in the primary infection but introduced in the root canal at some time after professional intervention (i.e., secondary to intervention).
- **Persistent infection**: caused by microorganisms that were members of a primary or secondary infection and in some way resisted intracanal antimicrobial procedures and were able to endure periods of nutrient deprivation in treated canals.

- **Extraradicular:** infection in turn is characterized by microbial invasion of the inflamed periradicular tissues and is a sequel to the intraradicular infection.
  Extraradicular infections may be dependent on or independent of the intraradicular infection.

Biofilm and Bacterial Interactions

The community-forming ability can be regarded as essential for microbial survival in virtually all environments. Indeed, the majority of microorganisms in nature invariably grow and function as members of metabolically integrated communities, or biofilms. Biofilm can be defined as a sessile multicellular microbial community characterized by cells that are firmly attached to a surface and enmeshed in a selfproduced matrix of extracellular polymeric substance (EPS), mostly composed of polysaccharide but also proteins and nucleic acids The ability to form biofilms has been regarded as a virulence factor, and biofilm infections account for an estimated 65% to 80% of bacterial infections that affect humans in the developed world. Given its importance in varied aspects, biofilm properties have been extensively studied not only in medical microbiology but also in different sectors of industrial and environmental microbiology. As a community, biofilms are composed of populations or microcolonies (±15% by volume) that are embedded and non randomly distributed in the EPS matrix (±85% by volume) and separated by water channels. Dental biofilms can reach up to 300 or more cell layers in thickness. Individual microcolonies may consist of a single bacterial species, but more frequently are composed of several different species in a mixed community.

The EPS matrix is not only important physically as part of the scaffold that determines the biofilm structure, but it is also biologically active and can retain nutrients, water, and essential enzymes within the biofilm. The matrix also protects the biofilm community from exogenous threats and may participate in adherence to the surfaces. In most biofilms in nature, bacterial populations arise from surface colonization by planktonic (unattached) bacterial cells. Floating bacteria approach the surface to be colonized and attach to host proteins and/or coaggregate with previously

bound bacteria. This process may occur in canals that are frankly exposed to the oral cavity and filled with saliva.

Biofilm resistance to antimicrobial agents :

Bacterial cells arranged in biofilms are considered more resistant to antibiotics than the same cells grown in a planktonic state. The antibiotic concentration required to kill bacteria in the biofilm is about 100 to 1000 times higher than that needed to kill the same species in the planktonic state.

Several possible mechanisms help explain the biofilm resistance to antimicrobials:

A. The biofilm structure may restrict the penetration of antimicrobial agents
B. Starved bacteria entering the stationary phase in biofilms are much less susceptible to antimicrobials than rapidly-growing cells.
C. Presence of a subpopulation of specialized survivor cells known as persisters.

Persistent/secondary infections and treatment failure

Intraradicular infections, either persistent or secondary, can be regarded as the major causes of post treatment apical periodontitis. Other causes include extraradicular infections and non-microbial factors, for which there is only weak evidence coming from case reports and speculation. The major role of persistent/secondary infections in treatment failure is supported by two strong evidence-based arguments. First, it has been demonstrated that there is an increased risk of adverse treatment outcome when bacteria are present in the canal at the time of obturation. Second, virtually all root canal–treated teeth evincing persistent apical periodontitis lesions have been demonstrated to harbor an intraradicular infection. Based on these arguments, studies investigating bacteria remaining in the root canals at the obturation stage disclose species that have the potential to influence the treatment outcome. On the other hand, studies dealing with the microbiota of root canal–treated teeth with apical Periodontitis shows the association of species with treatment failure, because the microorganisms detected are likely to be the cause of post treatment disease.

Spread of bacteria in the body

1. Bacteremia

Bacteria, especially alpha-hemolytic streptococci, can enter the bloodstream during routine dental treatment. In a normal person, the bacteria are killed within 10 minutes by the body's defense mechanism. Infective endocarditis occurs in bacteremia to patients with a history of rheumatic fever with a cardiac murmur or mitral valve prolapse.

2. Septicemia

It is a serious, life-threatening bacterial (and their products) invasion of the bloodstream. It happens when body defense is low or when the infection is overwhelming. It is associated with severe signs and symptoms.

3. Cellulitis

It is an acute infection of the alveolar and loose connective tissue, and it is a diffuse spread of infection. Clinically, in endodontic cellulitis, it is called a flare-up, and it happens during access opening because of the environmental change of oxygen level in the root canal, which enhances the action of the facultative bacteria, and during instrumentation and obturation when debris or obturation material extrude the apical foramen.

Bacterial culturing in endodontics

There are three reasons for culturing root canal contents:

1. To determine the bacteriologic status of the root canal.
2. To assess the efficiency of the debridement procedure.
3. To isolate microbial flora for antibiotic sensitivity and resistance profiles in cases of persistent infections.

Intracanal medicaments

The main use of an intracanal medicament is to help destroy microbes.

1. **Phenol:** It is an effective medicament in the root canal.

2. **Camphorated phenol:** It is phenol liquefied in camphor. It is less toxic than the phenolic compounds.



3. **<u>Camphorated monochlorophenol (CMCP):</u>** It is more toxic than phenol, but it is also a more active antiseptic, but does not last for more than 3 days. It is less irritating than tricresol formalin.



4. **<u>Tri cresol formalin:</u>** The compound is a mixture of three isomers. It has a powerful antibacterial action that lasts for up to 7 days.

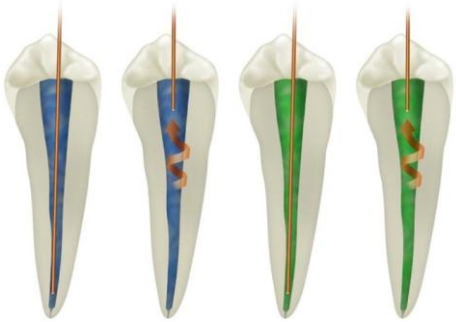


5. **<u>Calcium hydroxide:</u>** This is biocompatible and can be used to disinfect the root canal for more than one week. The antimicrobial activity of calcium hydroxide is related to (OH-) in an aqueous environment. Hydroxyl ions(OH-) are highly oxidant free radicals that show extreme reactivity, reacting with several biomolecules.



6. <u>Photoactivated disinfection (PAD):</u>

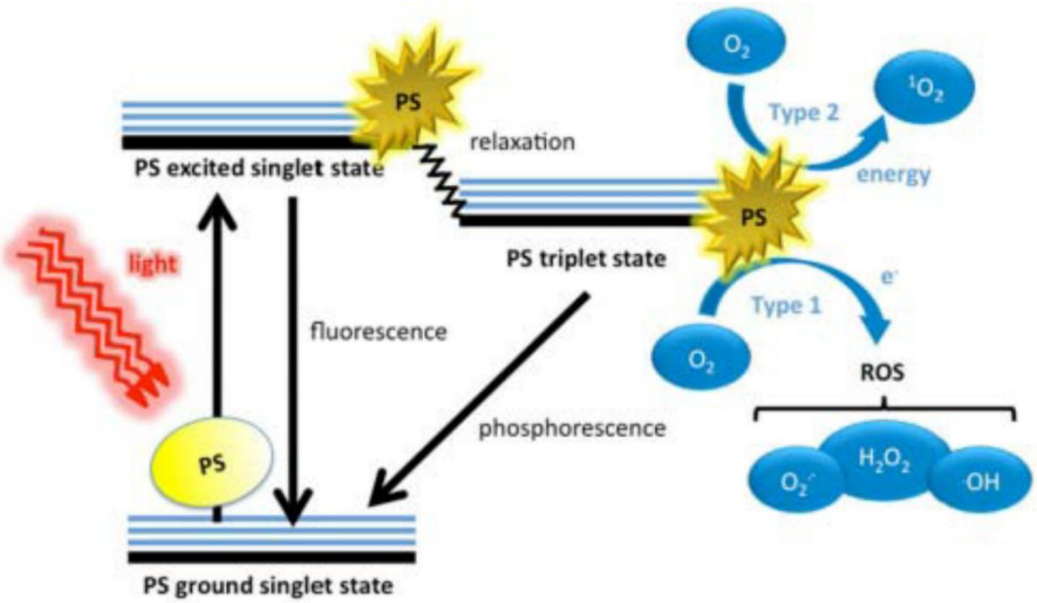
Is a medical treatment that utilizes light to activate a photosensitizing agent (photosensitizer) in the presence of oxygen. The exposure of the photosensitizer (PS) to light results in the formation of oxygen species, such as singlet oxygen and free radicals, causing localized photodamage and cell death.





Mechanism

PAD involves three components: light, a photosensitizer, and oxygen. A PS is administered to the root canal. Upon irradiation with light of a specific wavelength, PS undergoes a transition from a low-energy ground state to an excited singlet state. Then the PS may decay back to its ground state, with emission of fluorescence, or may undergo a transition to a higher-energy triplet state. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing rapid and selective destruction of the target tissue.



Antibiotics in endodontics

Antibiotics are used when infection spreads to the alveolar bone, with swelling of the area above the affected tooth, and drainage does not relieve the swelling.

Most of the bacterial species involved with endodontic infections are susceptible to penicillins, which makes them the first-line drugs of choice. In more serious cases, including life-threatening conditions, combining amoxicillin with clavulanic acid or metronidazole can achieve optimum antimicrobial effects as a result of the extended spectrum of action to include penicillin-resistant strains. Erythromycin is used in cases of penicillin allergy.

In conclusion, unnecessary use of antibiotics increases the risk of developing resistant species of bacteria.