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Surabaya, November 3rd – 5th, 2017

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THE ANTIBACTERIAL EFFECT OF A DIODE LASER ON
ENTEROCOCCUS FAECALIS BIOFILM

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ABSTRACT
Failure to eliminate bacteria in the root canal, especially in the form of biofilms, causes
unsuccessful endodontic treatment. Root canal preparation, both mechanically and
chemically using antibacterial agents in the form of irrigation and medication, often failing
to kill E. faecalis. This is due to the ability of E. faecalis to penetrate into dentin tubules and
form biofilms that are more resistant to antibacterial agents. Along with the development
of technology, there is a phototherapy system that brings a new breakthrough to perform
root canal disinfection through the laser application. Diode laser is one type of laser that is
widely used in dentistry. Some of the advantages of diode lasers are their small size, price
range, and their wide use of applications for dentistry.

INTRODUCTION
Endodontic treatment is divided into
three stages known as triad endodontics,
which include access opening, root canal
preparation, and complete obturation of the
canal space. Primary goal of endodontic
treatment is to eliminate polymicrobial
infection, which is the main cause of
periapical disease. Bacteria that infect
the root canal may be either planktonic or in
biofilms. Primary endodontic infections
are composed of a wide variety of bacteria
that are mostly obligate anaerobes and less
facultative anaerobes. Obligate anaerobic
bacteria are relatively easy to eliminate by
instrumentation, irrigation and medication
procedures. While facultative anaerobic
bacteria are more difficult to eliminate
because of their ability to survive from a
chemomechanical preparation.

There are about 150 species of microorganisms that can colonize in
the root canal but only 10 to 30 species
are known to the infected root canal.
These microorganisms can also be found in the dentinal tubules and may cause
root canal reinfection if not completely removed.
facalis) is a bacteria that can be found in infected root canals that have not been done for endodontic treatment and can also be found in root canals with unsuccessful endodontic treatments. In an in vitro test of 50 teeth that had undergone root canal treatment, Zoletti et al. (2006) found that 89% were infected by E. faecalis. Additionally, Wang et al. (2012) observed an E. faecalis infection in 38% of the 58 teeth with poor root canal treatments that they studied and noted a higher prevalence in teeth with poor obturation.

Enterococcus faecalis have ability to penetrate into dentine tubules, reaching 1000 µm, faster than other pathogenic bacteria and have strong adhesion to collagen. E. faecalis is able to adapt to unfavorable conditions and lack of nutrients, and can accumulate to form biofilms. Biofilm is the aggregation of various microorganisms that secrete protective and adhesive exopolysaccharide matrices, EPS (Extracellular Polymeric Substance or exopolysaccharide). In the biofilm itself there is a quantum sensing mechanism, communication between bacterial populations of the same or different species. Through this mechanism allows for the establishment of interspecies growth factors and exchange of information, causing bacteria present in the biofilm to become more resistant to phagocytosis by the body’s defense cells and also resistant to antimicrobial agents commonly used during root canal procedures.

Although there are different concepts and strategies for root canal preparation, there is mutual agreement on a chemo mechanical preparation that combines a chemical irrigant with a mechanical debridement using hand or rotary instruments. Because instrumentation alone is not enough to make root canal space free of bacteria, it is also necessary to add disinfection materials in the form of bactericidal irrigation solution. These irritant also serve to remove necrotic tissue, debris debrid and smear layer and as a lubricant.

The irrigation works in direct contact with the target and, to a limited extent, penetrates the root canal wall. Pauci (2006) reported that area remained unaffected after mechanical instrumentation using either rotary instruments or manual technique.

The irrigant was therefore unable to reach and eliminate microorganisms from the inner side of the dentine layer. This is the reason that a combination of a disinfectant solution with a supplementary irrigant is used.

Numerous antibacterial irrigants can be used in endodontic. A combination of chlorhexidine (CHX) and sodium hypochlorite (NaOCl) is the most commonly used endodontic treatment and is considered the golden standard. The first, CHX, is a wide-spectrum antibacterial agent that works on a lot of microorganisms, including E. faecalis. It is also recommended due to its substantivity, which leads to a longer therapeutic effect.
REVIEWS

Enterococci is a normal flora of the human and animal digestive system, but it is also an important pathogen that can cause infection. Enterococci comprises more than 17 species, but only few can cause clinical infections in humans. Enterococcus faecalis and Enterococcus faecium are the most common species found in humans, leading to clinical infections. Common infections are urinary tract infections, endocarditis, bacteremia, infection associated with catheter insertion, wound infection, and intra-abdominal infections. Many of the strains that cause infections come from the patient's intestinal flora, spreading and causing urinary tract infections, intra-abdominal infections, and surgical wound infections.²

In dentistry, E. faecalis is an opportunistic pathogen commonly found in secondary and persistent endodontic infections. Secondary endodontic infections occur due to bacterial contamination during the endodontic treatment process. Enterococcus faecalis can be found in the oral cavity (10%), which is derived from the digestive tract, and is commonly found in planktonic form in saliva. A non-sterile root canal procedure will cause contamination of E. faecalis from saliva into the root canal.¹⁰

Enterococcus faecalis is a Gram-positive, with a size of 0.5 - 1 μm. Enterococcus faecalis is also a facultative anaerobic bacteria that can grow in the presence of oxygen or without oxygen.
(Figure 2.1). *Enterococcus faecalis* can be found as a single, paired, or short-chain bacteria. These bacteria can use various substances; carbohydrates, glycerol, lactate, malate, and citrate; to be a source of energy.7,24

*Enterococcus faecalis* can survive in poor environments including in high pH (<11.5) and high salt concentration environments. *Enterococcus faecalis* can also grow at a temperature of 10°C to 45°C and survive at 60°C for 30 minutes.12,25. The cell wall of *E. faecalis* contains a large number of peptidoglycan and antigen in the form of teichoic acid. The peptidoglycan is a crosslinking of peptide that help to overcome the cytoplasmic pressure and provide the shape and strength of the bacterial cell wall.7,28

In the absence of nutrients, *E. faecalis* are able to maintain viability for several time and become resistant to heat, sodium hypochlorite (NaOCl), hydrogen peroxide, ethanol, and acids. In addition, *E. faecalis* can also enter a defensive state but cannot be multiplied (viable but not cultivable). This mechanism was adopted from a group of bacteria when exposed to a bad environment, and recovered when conditions improved. The ability of *E. faecalis* to adapt and adverse environmental conditions may benefit other species to settle in a biofilm.30-32

In root canal space, *E. faecalis* is able to survive even in nutritional deficiencies. This is consistent with the study of Sedgley et al. (2005) that inoculated bacterial suspensions directly into the bovine root canal, thus allowing bacterial growth into the dentin tubule through the root canal. After 48 hours of non-nutritional condition on the root surface, there was a description of the penetration of *E. faecalis* bacteria into the dentin tubule to a depth of 300 - 400 μm. In addition, *E. faecalis* is also able to maintain viability for 12 months without any additional nutrients. The ability of *E. faecalis* to survive in this bad environment, because *E. faecalis* has various virulence factors.

![Figure 1. Thin Section E. faecalis in planktonic form (TEM magnification 33000x)](image)

The various virulence factors possessed by *E. faecalis* play a role in the process of attaching bacteria to the host tissue and then invading it, the secretion of products that can form biofilms, modulate the inflammatory response and the formation of the abscess. These substances include aggregate substances (SA), surface protein chromosomes namely enterococcus surface protein (Esp), gelatinase (GelE), cytolytic toxin, lipoteichoic acids (LTA) and collagen binding protein (Ace).17,18,20,29

The aggregate substance (SA) of *E. faecalis* is an adhesin that helps contact...
between cells and facilitates the exchange of plasmids between recipients and donors. This allows the transfer of antibiotic resistance properties between *E. faecalis* and other species. Aggregate substances can also modify the phagosomal maturation of PMN and macrophages so that PMN and macrophages cannot kill bacteria. 18,29

*Lipoteichoic Acids* (LTA) in bacterial cell membranes, allow *E. faecalis* to colonize and attach to the mineral part of the tooth, triggering bacterial attachment to PMN cells and red blood cells. This LTA triggers the release of hydrolase acids, proteinases, bactericidal cationic acids and growth factors and also triggers the release of cytokines from PMN and macrophages. *Enterococcus faecalis* also has protein adhesion such as collagen binder (Ace) and enterococcus surface protein (Esp). These adhesion proteins mediate bacterial attachment to the host tissue and play a role in biofilm formation. Esp is thought to have a role in retreating the protein from the surface of the bacteria so that it is hidden from the immune system.28

Biofilms are cell populations attached to the surface and incorporated in the matrix of exopolymeric substances, polysaccharides, proteins, nucleic acid DNA. Biofilm formation is a complex process consisting of adhesion to surfaces, interactions between cells, microcolonial formation, early biofilm formation and the establishment of a three-dimensional biofilm structure (Figure 2). Bacteria in the biofilm will be more than 1000 times more resistant and phagocytic processes, the nutrients are more concentrated and facilitate communication between bacterialin the same or different popualizations. 30

![Figure 2. Biofilm Formation on Tooth Surfaces.](image)

Several factors affecting bacterial attachment to the substrate are the surface energy of the substrate, temperature, pH, fluid flow rate passing through the substrate, the length of bacteria in contact with the substrate, surface hydrophobicity and nutrient availability. The surface structure of bacteria that plays an important role in bacterial attachment is pili, flagella and EPS. Adhesin or ligand in bacteria will bind to receptors on the substrate. In the third phase occurs the multiplication and metabolism of bacteria inherent and form a complex community. In this phase there is biofilm maturation. 29,30,31

Biofilm formation is regulated by quorum sensing system by some pathogenic bacteria. The formation of biofilms is influenced by physicochemical factors, which consist of layers of substrate and planktonic bacteria that will be attached to the substrate layer. A nutrient-
rich environment, with both aerobic and anaerobic conditions, produces a biofilm structure with bacterial aggregates located on the surface with a water channel. While in minimal nutritional conditions, biofilms that form, like irregular clumps of cells.

Factors affecting biofilm formation are nutrient content in growth medium, serum, iron, carbon dioxide, pH and temperature. Changes in osmotic pressure also affect the formation of *E. faecalis* biofilms. Isolate *E. faecalis* that produces extracellular polysaccharides so as to produce biofilms is more susceptible to macrophages than isolates that do not produce biofilms. In anaerobic and nutrient-rich conditions, *E. faecalis* showed a mature biofilm with water channels on the root canal wall.

LASER is an acronym of light amplification by stimulated emission of radiation. The laser is a form of energy in the form of particles called photons and moves in waves. Each wave of photons has three basic properties: velocity (light velocity), amplitude (energy of wave) and wavelength (horizontal wave measurement). Lasers are generated by the medium present in the tube. The medium may be gas, crystal or liquid. After the energy is given, the photons will move and are reflected by two mirrors present at each end of the tube. This reaction continues to move forward and backward. In one mirror there is a small hole so that the laser light can come out. The outgoing laser light is then delivered through optical fiber (fibre optic).

Laser Diodes are one of the most widely developed types of lasers in dentistry (Figure 3). Some of the advantages of diode lasers are their small size, price range, and wide use of dental treatment applications. The relatively small size of the diode laser provides benefits for the relatively small area of the workspace, easy to carry and move, and relatively light. In addition, the diode laser has a more affordable price range than any other laser system. Another advantage of diode lasers is its short application time, requiring only a few seconds for the laser beam to be emitted after the laser system is turned on. In general, other laser systems require several minutes to reach the ready condition to emit light. Electricity consumption of diode lasers is also small compared to other laser systems, it can be said to be more efficient and play a good role against environmental protection.

![Figure 3. Diode Laser](image-url)

The diode laser is a type of laser with an active medium of semiconductor, having four different wavelengths, 810nm, 940nm, 980nm and 1064nm. The laser light of the diode is channeled through the plastic fibers and concentrated at the ends.
of the fibers causing a heat effect. Laser diodes can be used in various dentistry procedures. Generally used in soft-tissue therapy procedures such as surgery and periodontal pocket therapy, but also can be applied to procedures involving hard tissues such as teeth. One is in endodontic treatment such as root canal disinfection.\textsuperscript{37}

Laser diodes can convert electrical energy into light using the same principle as other types of lasers, but with internal reflection capabilities that give rise to resonators, the stimulated light can be reflected back to front and backwards so that only certain wavelengths can be generated.\textsuperscript{7,34,39}\textsuperscript{\textsuperscript{\textdagger}}The active medium used in diode lasers is solid, such as GaAlAs (Gallium Aluminum arsenate). Due to the crystalline nature of the active medium, the crystal tip can be selectively polished for the internal refractive index resulting in a total or partial reflection surface. This provides the same functionality as an optical resonator in a larger laser system (figure 4).\textsuperscript{39}

\textbf{Figure 4} Semiconductor Component In Diode Laser\textsuperscript{39}

Laser diodes use flexible glass fibers to channel energy to the desired target tissue area. Generally, these flexible glass fibers are incorporated into the handpiece. Some things to consider when using glass fiber, such as the selection of fiber diameter to be used. The flexible glass fiber in the diode laser is available in diameters of 200-320 μm. The diameter affects the light energy emitted by the diode laser system. In addition to diameter, which is worth noting is the speed of movement of the fiber end during treatment. Burned networks are an unwanted side effect due to excessive force or movement of the fiber ends too slowly. Glass fibers can be driven 1-2 mm with a light vibration such as using a brush and move quickly when working on soft tissue.\textsuperscript{31,38}

Debris can accumulate on the glass fiber end during treatment and this causes the tip of the fiber to become very hot and act as a cutting iron. This can cause unwanted tissue heating and lead to additional damage. Therefore, the flexible glass fiber condition should be routinely checked, if the fiber end becomes blacker then a 2-4 mm cut from the fiber ends.\textsuperscript{33,38}

As with other types of lasers, in laser diodes, the effects of rays on a target cell or network rely on the formation of molecules that then react with tissue molecules.\textsuperscript{73} There are 4 types of laser light interaction with target tissue: reflection, absorption, transmission and scattering as shown in Figure 5.\textsuperscript{38} These four interactions depend on the optical properties of a network
such as water content, presence or absence of pigmentation and wavelength of the laser light used. In the reflection properties, the laser beam is reflected by collimated and diffuse light properties without having any effect on the target tissue. In some cases, the nature of this reflection may be dangerous because the laser energy may change direction of the eye or another area of tissue.

The second interaction of laser-tissue is absorption. The laser energy absorbed by the target tissue is a less favorable laser trait. The nature of this absorption results in weakening of the strength of light and the reaction between the photon with the material that passes. Transparent water cannot absorb the energy emitted by diode type lasers and Nd:YAG. Therefore this interaction becomes the basis of consideration of the selection of a type of laser to the target network. The third effect is transmission. The transmission effect is strongly influenced by the wavelength of the laser light used. And the last interaction is scattering or spreading laser energy. This interaction is advantageous because it can diffuse the radiated power emitted. The direction of the photon can be spread so that heat transfer to the target tissue through the medium passes through the ray, as shown in illustration of figure 5 and figure 6. Through the reflection, transmission and dissemination, allowing photons to pass through a material without damaging the tissue. However, when the photon is absorbed, it allows the physical or chemical reaction between the laser and the tissue.

**Figure 5** Potential Laser-tissue Interactions

In endodontics, several studies have suggested that the effectiveness of laser diodes against root canal disinfection is not much different from the solid-based laser system Nd: YAG. It is found by testing the bactericidal effect of diode laser and Nd: YAG against *E. faecalis*. The glass fiber diameter used in laser diodes is capable of effectively channeling laser light on the root canal system to reduce bacterial contamination. Antibacterial effects are seen to reach 1 mm into the dentine, far exceeding the ability of chemical disinfectants such as NaOCl irrigation materials. This shows the effectiveness of antibacterial effect of *E. faecalis* even to the deeper layers of dentine.

**Figure 6** Scattering effect on Dentine.
The laser directly acts as an effective adjunct irrigant in the process of root canal disinfection. The laser beam penetrates into the canal space which irrigation solutions cannot reach such as the accessory root canal and deep dentin tubules. The antibacterial effect of diode laser is obtained by ionic reactions and the resulting heat (photothermal). Although laser light is weakened, the bactericidal effect can be maintained because enamel or dentin tubules act as light conduectors. Several studies of diode lasers with different parameters show that the diode laser can effectively reduce intracanal bacteria and penetrate to a depth of 500 μm into the dentin.

The diode laser application is by gently moved of the fiber, in a circular direction with a spiral-shaping motion from the apical direction to the coronal root canal during laser operation. The procedure is repeated four times for five seconds. Always pay attention to moving the glass fibers that deliver the light at the tip of the fiber. This is to prevent the increase in surface temperature of teeth that can damage the tissues around the teeth. If required, treatment with the laser beam of diode laser may be repeated after three to seven days, but not more than twice overall. Power strength should be set at 1-1.5 W.8,9

CONCLUSION

The main goal of endodontic treatment is to remove all infected or necrotic pulp tissue, bacteria and endotoxins from the root canal system. Root canal treatment consists of three stages known as endodontic trials ie access opening, root canal preparation and obturation. The root canals have complex anatomy and can complicate mechanical cleaning with endodontic instruments, consequently root canal preparation with mechanical instrumentation alone will only partially reduce bacteria from the root canal.

Failure to eliminate bacteria in the root canal, especially in the form of biofilms, causes the failure of endodontic treatment. The most common bacteria found in cases of unsuccessful endodontic treatment is E. faecalis. Root canal preparation mechanically and chemically using antibacterial ingredients in the form of irrigation and medication, often failing to kill E. faecalis. This is due to the ability of E. faecalis penetrate into dentinal tubules and form biofilms that are more resistant to antibacterial agents. Much research has been done to find the most effective way of removing E. faecalis from root canals, one of which is by irrigation of root canals.

Along with the development of technology, there is a phototherapy system that brings a new breakthrough to perform root canal disinfection through laser applications. Endodontic laser applications have been introduced since 1980 and the number of users has continued to increase to date. Some studies say that the laser is able to lift the smear layer from the root canal space and has a bactericidal effect...
on pathogenic bacteria grown in the root canal. Nevertheless, the effectiveness of laser use in clinical reporting is still.

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