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Effect of photodynamic therapy on *Enterococcus faecalis* and *Candida albicans* compared to diode laser activated nanoparticle herbal irrigants

Hesham Hassan[1*], Mohamed Zaazou[2], Doaa Sadony[1], Tarek Mohamed[3]

[1]Restorative and Dental Materials Department, National Research Centre, Dokki, Giza, Egypt.

[2]Endodontic department, Faculty of dentistry, Sinai university

[3]Laser Institute for Research and Applications, Beni-Suef University, Beni-Suef, Egypt.

Abstract

Enterococcus faecalis is the most frequently found isolated bacteria after endodontic retreatment of apical periodontitis, while *Candida albicans* is more common in infected canals and peri-radicular tissues and seen in 18% of the retreatment infections. The gold standard for irrigation of root canal treatment is sodium hypochlorite. Drawbacks of NaOCl irrigant include allergic reactions, tissue toxicity, instrument corrosion, periapical tissue irritation, the limitations in removing smear layers, and an unwanted taste and smell. So alternative disinfection irrigant is required. Herbal medicine emerged with therapeutic advantages of biodegradability, nontoxicity, and cost-effectiveness.

Neem is used for root canal irrigation because of its greater efficacy. Curcumin, the major bioactive component of *Curcuma longa* (*C. longa*) with various anti-inflammatory, antibacterial, antifungal, and antiviral properties. Synthesization of curcumin herbal nanoparticles was done by application of nanotechnology. This review aims to illustrate whether herbal irrigants in the nanoform have an effect on *E. faecalis* and *Candida albicans* in comparison to standard irrigants. Another aim is to compare herbal irrigants in the nanoform with and without activation by diode laser compared to photodynamic therapy utilizing methylene blue as photosensitizer.

Keywords:

Photodynamic therapy; *Enterococcus faecalis*; *Candida albicans*; Diode laser; Herbal irrigants

*Corresponding author at: Restorative and Dental Materials Department, National Research Centre, Dokki, Giza, Egypt

E-mail addresses: heshamessameldin2020@gmail.com

1. Introduction

1.1 *Enterococcus faecalis* & *Candida albicans*

Many Studies revealed that endodontic infections have presented the presence of *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. albicans*), both of them cause root canal therapy (RCT) failure (Arslan S. 2011, Ramani N, 2012). Moreover, the exterior surface of the root can be reached by bacteria through the dentinal tubules (Love RM. 2002), causes a periradicular inflammatory lesion and could be a major factor in the inability of root canal therapy to cure the patient (Trope M. 2002). *E. faecalis* is the most frequently isolated bacteria after endodontic retreatment of apical periodontitis (Garg P. 2014). The incidence of *E. faecalis* in chronic endodontic infections ranges from 24 to 77%, which is much higher than in primary endodontic infections (40%). It can undoubtedly infiltrate the dentinal tubules and attach to dentin (Hedge V. 2009).

Extremely severe conditions, such as high concentrations of salt and an alkaline pH of 9.6, are conducive to *E. faecalis* survival (Tendolkar PM. 2003). It can even live in well-instrumented and obturated root canals on their own with minimal nutrition available (Evans M. 2002). A number of virulence factors are expressed by the bacterium (Fisher K. 2009) making it resistant to the effects of ethanol, azide, detergents, heavy metals, dehydration, and certain chemicals (Hedge V. 2009). In addition, because of E. faecalis's cell-wall structure, the bacterium has high heat resistance (Bago I. 2013).

According to Distel et al., *E. faecalis* can develop biofilms and colonize medicated root canals (Distel JW. 2002). It has the capacity to create biofilms, which are bacterial attachments embedded in a self-produced polysaccharides matrix that are affixed to a surface or interface (Costerton JW. 1999). An opportunistic dentinophillic yeast known as *C. albicans* is more common in infected canals and peri-radicular tissues. *C. albicans* seen in 18% of the retreatment infections (Ashraf H. 2007). In *C. albicans*, cells grow in unison and adher to the surface, whereas hyphal cells produce a biofilm (Mayer FL. 2013). All of the treatment approaches had the same effect on Candida. In contrast, Enterococcus generates a somewhat different and more sophisticated biofilm, *C. albicans* don't have hyphen (Meire MA. 2012).

1.2 Cleaning and Shaping

Cleaning and shaping have a significant impact on the endodontic treatment's success. Additionally, there are several drawbacks to root canal instrumentation, such as the creation of dentin debris and smear layers, iatrogenic mistakes, and weakened root structures (Liu R. 2013, Paque F. 2011). During cleaning and shaping, an amorphous, uneven smear layer (Torabinejad M. 2002).

Cleaning root canals require different irrigating solutions which supposed to fulfil certain requirements as to be biocompatible, nontoxic, and flavored and smelling nice (Vinothkumar TS. 2013), besides having antibacterial activity, it can have other effects such as neutralizing endotoxins, dissolving necrotic pulp tissue, and preventing the formation of the smear layer or remove it if it has already begun (Suzuki S. 2014). The quantity and flow of irrigating solutions are impacted by the canal's taper (Boutsioukis C. 2010). To enable irrigation solutions reach inaccessible parts of the root canals, such as the isthmus and fins, and to break the apical vapour lock, they require an activation mechanism. Irrigant activation enhances irrigant performance by facilitating the irrigant's direct interaction with various canal segments (Agarwal A. 2017). NaOCl can only reach 130 µm while bacteria can penetrate the dentinal tubules by 1000 µm (Rios A. 2011).

1.3 Sodium hypochlorite the gold standard for root canal irrigation

The effectiveness of NaOCl treatment may depend on the concentration of the solution, with greater concentrations producing better results (Clegg MS. 2006). On the other hand, the organic dentin matrix may dissolve at high NaOCl concentrations, such as 5% or 9% (Marending M, 2007). Among the disadvantages of NaOCl irrigant include allergic reactions, tissue toxicity, instrument corrosion, periapical tissue irritation, uneffectiveness with smear layers removal, and an unfavorable taste and smell (Garg P. 2014). It also affects the mechanical characteristics of dentine, such as the ratio of organic to inorganic matter, roughness, microhardness, elastic modulus, and flexural strength (Pascon FM. 2009). Additionally, its high surface tension shields the irrigant from making direct contact with the anatomical complexity' dentinal walls (Zehnder M. 2006).

1.4 Alternative irrigants

Chlorohexidine (CHX) is another irrigant recommended in retreatment cases (Zehnder M. 2006), (Suzuki S. 2014). In addition, The CHX molecule is hydrophobic and lipophilic in nature, with a positive charge which interacte with the microbial cell walls, lead to change in the osmotic balance of the cells due to the molecule's positive charge (Mohammadi Z. 2008). However, its inability to break down organic tissue and possible negative effects on periapical health outcomes are some of its disadvantages. That is why using it along with NaOCl is not recommended (Ng YL 2011). Furthermore, its effects seem to be restricted to planktonic cells, and their effectiveness is diminished when established biofilm structures are present (Shen Y. 2011). An alternate disinfection method is required since antibiotic-resistant bacteria are constantly growing, and synthetic drug side effects are a problem. The use of herbal irrigants as a promising alternative.

1.5 Herbal root canal irrigants

Herbal medicine has gained popularity as a therapeutic modality due to its biodegradability, nontoxicity, and low cost (Pujar m. 2011), (Pratishta J. 2014), it has improved patient acceptance and environmentally friendly characteristics can make it a more potent antibacterial endodontic irrigant that effectively eradicates E.faecalis (Afshan T. 2020).

There are many herbal irrigants available like Morinda citrifolia, Aloe Vera, Curcuma longa, Tea tree oil and Azadirachta indica (Neem) (Garg P. 2014, Ambareen Z. 2015). Numerous biological actions are present in Neem (Ghonmode WN. 2013).

1.5.1 Azadirachta indica (Neem)

Neem is an alternative to hazardous chemical irrigants like NaOCl because of its greater efficacy, which may be attributed to its various active phyto-constituents, including acid flavonoids, metabolites, alkaloids, isoprenoids, glycosides, steroids, and tannins (Biswas K.2002). Its special medical qualities are caused by its active ingredients, which include nimbinin, azadirachtin, and nimbidin. It is one of the rare herbal extracts with additional antibacterial activities that also has anti-inflammatory and anti-cariogenic qualities (Lakshmi T. 2015).

Nimbidin and Nimbolide inhibit the cell membrane synthesis of the bacteria. Additionally, it has an anti-adherence action through changing the bacterial adhesion and the organism's capacity for colonization (Maragatharavlli S. 2012, Behl H. 2002). Neem's high biocompatibility makes it safer to the patients (John P. 2015). However, the main disadvantage of neem is its bitterness, which limits the patient's capacity to use. However, it can be neutralized by adding sweeteners and other flavors (Maragatharavlli, S. 2012).

1.5.2 Curcuma longa

Curcuma longa, commonly called turmeric primarily grown in India, has many biological effects, with curcumin as the main bioactive component (diferuloylmethane). It is a known cyclooxygenase-2 inhibitor and a natural poly-phenolic flavonoid (Neelakantan P. 2011). It contains certain volatile oils, including tumerone and zingiberone. (Badole GP. 2016). Curcumin (Cur) is low in cytotoxicity and has excellent antibacterial and anti-inflammatory activities (Lawande S. 2013).

Its mechanism of action suggests that it inhibits the accumulation of protein-filamenting temperature-sensitive Mutant Z (FtsZ) and increases the guanosine triphosphatase activity of FtsZ, which is fatal to bacteria (Rai D. 2008).

More recently, Curcumin was employed as an intra-canal irrigant during endodontic treatment, providing effective and promising disinfection outcomes (Devaraj S. 2016), most likely due to its permeabilization properties, which harm bacterial membranes (Tyagi P. 2015). Their extended shelf life and lack of microbial resistance have made them an effective alternative to chemical irrigants as an endodontic irrigant (Afshan T. 2020). Moreover, curcumin is photosensitive, it kills germs without adhering to them or encountering them (50. Shahzad M. 2014), this happens due to the photo-activation forming hydrogen peroxide as an intermediate molecule (Devaraj S. 2016). According to a study by da Frota 2015, found that utilizing an LED light-emitting diode (LED) unit to irrigate infected root canals while using curcumin combined with photoactivation for five minutes significantly reduced contamination (Da Frota MF. 2015). Among curcumin's many advantages are its minimal adverse effects, such as its limited oral bioavailability, which causes poor absorption, rapid metabolism as well as systemic elimination, and poor solubility in aqueous solvents (Ireson C. 2001). Furthermore, curcumin can only be dissolved in organic solvents. Therefore, because of these solvents' stated toxicity, its biological applications are limited (Jamalzadeh L. 2016).

To overcome this problem, curcumin can be synthesized as nanoparticles, which provides several benefits, including ease of entry into cells, targetability, improved bioavailability, enhanced solubility, and effective delivery (Tendolkar PM. 2003). Some studies showed that native curcumin possess lesser antifungal properties than nanocurcumin (Mima EG. 2010).

1.6 Nanocurcumin

Techniques for synthesis of curcumin nanoformulation include single emulsion, nano-precipitation microemulsion, emulsion polymerization, spray drying, solvent evaporation, thin film hydration, ultra-sonication, antisolvent precipitation, ionic gelation, solid dispersion, coacervation technique, wet milling , and Fessi method. Each one has its own pros and cons (Rai M. 2015). With Ionic gelation and the anti-solvent precipitation demonstrating better stability and solubility compared to other techniques.

Nanocurcumin has an effect on gingiva as, it has been shown to reduce vascular permeability, improve clinical symptoms such gingival bleeding, and inhibit the microsomal prostaglandin E2 synthase-1 enzyme, hence blocking prostaglandin E2 production (Naganuri D. 2016). Nanocurcumin enhanced water solubility compared to curcumin this could be attributed to nano-sized particles which lowers the chances of discoloration by increasing its removal (Areej FA. 2022), and the hydrophobic spherically shaped nanomicelles enclose all of the curcumin, have a size of about 10 nm, and make curcumin more soluble in water.

The curcumin nanoparticles have spherical shape with the particle size of 17.97 - 87.61 nm (NOP, 2010) (National organic program 2010). When compared to free curcumin, the most significant physical property of nanocurcumin that accounts for its effectiveness is thought to be its particle size. This makes it possible for nanocurcumin to enter organs that are inaccessible to curcumin (Tsai YM. 2011). Mechanical instrumentation can't prepare the entire canal surface due to the complex three-dimensional microstructure of the root canal system (Peters OA. 2001), which limit its cleaning capacities. Therefore, for effective debridement and disinfection, antimicrobial irrigant solutions must be delivered and penetrated into the three-dimensional microstructure (Paque F. 2011).

For improving the efficacy of irrigants several techniques have been proposed including changes in temperature, concentration, addition of surfactants, and activation of irrigants (Stojicic S. 2010).

1.7 Activation of root canal irrigants

Irrigant activation seems to be a key strategy to boost the antibacterial effect of root canal irrigants (De Gregorio C. 2009). The production of hydrodynamic phenomena in well-formed canals loaded with an irrigant is the foundation for the effectiveness of sonic and ultrasonic devices, which helps to break up biofilms and increases the permeability of bacterial cell membranes to NaOCl (Plotino G. 2007).

Since Weller's invention, passive ultrasonic irrigation (PUI) has been extensively utilized as a more efficient means of eliminating irritants (Weller R.N. 1980). An acoustic streaming and cavitation effect is produced by the ultrasonic device's transmission of ultrasonic waves through the noncutting tip, which improves irrigant velocity, flow, and exchange. This allows for a more thorough cleansing and disinfection (Gründling GL. 2011). The ultrasonic device transmits ultrasonic waves through the noncutting tip, improving irrigant velocity, flow and exchange, also producing an auditory streaming and cavitation effect. (Levy G. 1996). In this way, the irrigant may be able to reach unreachable areas (Lee SJ. 2004).

Lasers have been considered as a promising alternative to the conventional approach for cleaning, disinfecting, and even shaping the root canal (Stabholz A. 2004). Laser irradiation acts primarily by photo-thermal action (Camargo SCC. 2012). Laser-induced bacterial death results in targeted heating within the bacterium as well as thermal heating of the surrounding environment exceeding lethal thresholds (Meire MA.2009, Gutknecht N. 2000) but, when laser tips come into contact with the root canal, it caused carbonize dentin and create fractures (Matsuoka E. 2005). Laser-activated irrigation (LAI) has been introduces for root canal irrigants activation (Stojicic S. 2010).

1.8 Laser activation of irrigants

Using specific wavelengths that react with water molecules to create the cavitation effect, this results in turbulent movement that aids in dispersing the irrigants into anatomical complexities by causing bubble expansion, implosions, and shock waves (Matsumoto H. 2011). The laser energy and the fiber's location determined how deep this bubble could go. It imploded at a speed of approximately one millisecond, producing a shockwave (Holzfuss J. 1998). Whose negative-pressure failure resulted in subsequent cavitation within the root canal, accompanied by a sizable bubble close to the point of collapse, typically the apex. After that, the cavitation bubble ruptured once more, and the cycle was repeated several times.

Additional factors that affect the impact of LAI include laser properties like pulse energy, length, frequency, and design and placement of the laser tip. The water in the endodontic irrigants solutions reduces the heat interaction of the laser beam on the dentinal wall, but when combined with a mid-infrared laser, they can function in concert to clear the canal (Haapasalo M. 2005). Several wavelengths are associated with bactericidal effects (Schoop U. 2004, Gurbuz T. 2008). Since the hydroxyapatite and water of hard dental tissues have little in common, near-infrared lasers (which range in wavelength from 810 to 1340 nm) can reach the pigments of bacteria (George R. 2008). As a result, deeper dentin layers can benefit from a bactericidal effect (Schoop U. 2004). However, by closing dentinal tubules they cause morphological changes of the dentinal wall while the smear layer is only partially eliminated (Kaitsas V. 2001).

Diode laser with certain wavelengths such as 940- and 980-nm are of particular interest because they are more strongly absorbed and nearly harmonics for water absorption (Gutknecht N. 2004) 940 nm and frequency system were 50–60 Hz is appropriate for endodontic applications. The irrigants can be activated by these parameters to remove debris and spread the layer (Hmud R. 2010).

Research indicates that diode lasers (DLs) have the ability to penetrate 500 μm into the surface and reduce the intra-canal bacterial count. It operated in pulsed or continuous-wave (CW) modes when releasing energy. Better control over thermal emission and damage is made possible in the latter case by the possibility of a mechanical interruption of the energy emission (appropriately referred to as "gated" or "chopped"). Milliseconds or microseconds are used to measure the pulse's duration and intervals (time on/off) (DiVito E. 2012).

The mid-infrared lasers' (Er:YAG: 2980nm–Er,Cr:YSGG: 2780nm) antibacterial effect, is more superficial because their primary absorption in the dentinal walls is by water (and, to a lesser extent, hydroxyapatite). This deeper irrigant release increases the root canal system's cleaning capacity by the photothermal effect, their affinity for water in dentin also causes some ablation of the surface dentin (81. Moritz A. 2006). In pulsed laser systems each pulse follows a Gaussian distribution with respect to its start time, rise, and end time. The tissue has thermal relaxation period in between pulses, which enables better control over thermal effects (Matsumoto H. 2011). They cause the dentin to undergo a surface heat phenomena and totally evaporate the smear layer (Yamazaki R. 2001).

This technique is known as photon induced photoacoustic streaming (PIPS) (De Groot SD. 2009). PIPS uses a stripped tip and radial firing to enable lateral laser energy emission in the liquids. When subablative energy (20–50 mJ) is used and supplied quickly (50 microsecond pulse duration) at a frequency of 10–15 Hz, a significant peak power is produced within the irrigant solution, leading to an explosion implosion phenomenon (photoacoustic shock wave) that prevents any direct laser irradiation on the dentin and the ensuing unfavorable thermal consequences while inducing irrigant streaming in three dimensions across the whole root canal system (Lin S. 2010). Similar to PIPS, DLs may cause an increase in the irrigants' kinetics. Although this might be the case for diode laser pulses (Hmud R. 2010). One of the limitations of using the ER:YAG laser for root canal disinfection is that the laser beam only emits in one direction, making it challenging to penetrate the entire root canal wall. To optimize the area exposed to the laser beam, the laser fiber must be moved around the root canal walls in a swirling motion on a regular basis (StabHolz A. 2004). Hydroxyapatite in particular has a great affinity for the carbon dioxide laser (10,600 nm). This wavelength's usefulness for intracanal applications is limited by its incapacity to employ a fiber-optic delivery method

One main risk is thermal injury to the surrounding periodontal tissue when using the direct laser for intracanal application (Gutknecht N. 2005). For this reason, the irrigating solution was warmed with the laser in the wet canal to maximize its disinfecting power. Additionally, the laser creates cavitation, which improves the removal of the smear layer (Zohair AE 2016). High power laser intra-canal use may result in root resorption, carbonization, ankyloses, and peri-radicular necrosis, among other harms to the periapical and dental tissue (Trindade AC. 2015) To resolve this issue:

Minimizing the dentin overheating in the root canal by moving a laser in a circular motion inside the canal (Camargo SE. 2005).

2740-nm Er,Cr:YSGG laser (20 Hz, 2W) with 200- or 320-mm fibers should be kept 2 and 3 mm away from the root apex to prevent distortion of the apical constriction (Matsuoka E. 2005).

Different bacteria could be killed, but eradication of some endodontic pathogens that grow as single-species biofilms are difficult (Lim Z. 2009). Direct laser irradiation, laser activated irrigation, and photodynamic therapy are the three categories of light-based or laser-based disinfection techniques (Olivi G. 2013). Research into more conventional forms of laser endodontics, such as direct laser irradiation, has declined (Ani´c I. 1996). Currently, the most common approaches for treating and eradicating oral biofilms are mechanical disruption and antimicrobial therapies; however, the efficacy of these approaches is constrained by the emergence of resistant microorganisms (Konopka K. 2007).

1.9 Photo dynamic therapy & combinations of photosensitizers

Several terms for PDT have been suggested, such as photodynamic antimicrobial chemotherapy (PACT), antimicrobial photodynamic therapy (APD), and photodynamic disinfection (PD) (Bergmans L. 2008).

Photodynamic therapy (PDT) was introduced for elimination and treatment of cancer (Soukos NS. 2006), and lately as an innovative alternative method for disinfecting root canals (Fonseca MB. 2008). It operates by using light at the proper wavelength to activate a photosensitizing molecule that is linked to a bacterial or fungal membrane. As a result, its electrical layers are excited to a state known as the triplet. Additionally, studies are concentrating on enhancing PDT's antibiofilm effectiveness by fusing photodynamic effects with bioactive micro- and nanoparticles (Pagonis TC. 2010).

The lipids, proteins, and DNA of microorganisms can be destroyed by highly reactive oxygen species that are produced when a photosensitizer (PS) is photoactivated in the presence of oxygen (Schlafer S. 2010). In addition to being efficient against bacteria, the PDT concept also works against viruses, fungi, and protozoa (Hamblin MR. 2004). Different laser parameters affect the photodynamic effect and the degree of tissue/cell damage (Chrepa V. 2014). Lasers, light-emitting diodes (LED), and halogen lamps are the main types of light sources used in clinical PDT (Nagata JY. 2012). The destructive action of PDT is based on photochemical (Walsh LJ. 1997). The advantage of PDT is that it selectively targets the bacteria without harming the host (Shrestha A. 2010). It's possible that by strengthening the cross-links in the collagen fibers, this technique strengthens dentin (Persadmehr A. 2014). PDT is less expensive than high-power lasers and its application is easy and painless (Gursoy H. 2013) However, in contrast to other light spectra, near-IR wavelengths have a greater ability to penetrate tissues.

An ideal PS should have the following characteristics: 1. no toxicity or harmful byproducts; 2. no mutagenesis effect; 3. accumulation on specific target tissue. 4. Adaptability to topical application, 5. a triplet state of appropriate energy to allow for efficient energy transfer to ground-state oxygen, 6. Inexpensive (Allison RR. 2004), 7. high absorption coefficient in the spectral region of the excitation light, 8. long triplet state life-times, 9. high photostability (De Rosa MCCR. 2002) 10. lower surface tension of photosensitizer aqueous solutions may aid in their absorption into dentin compared to other irrigants like NaOCl (Pileggi G. 2013). The major groups of PS employed in PDT are phenothiazines, namely toluidine blue O (TBO) and methylene blue (MB) (620–700 nm), cyanine (600–805 nm), haematoporphyrin derivatives (620–650 nm), phytotherapic agents (550–700 nm), chlorines and phthalocyanines (660–700 nm) (Meisel P. 2005).

Phenothiazines are the most studied and employed dyes in PDT (synthetic nonporphyrin compounds) Toluidine blue O (TBO, tolonium chloride) and methylene blue (MB) at 650nm in several concentrations used to kill both gram-positive and gram-negative bacteria present in endodontic infections since they are amphibic (Plotino G. 2019). Compared to red lasers (620–700 nm) used for toluidine blue and methylene blue, 810 nm offers a deeper penetration depth, which is advantageous for the removal of microorganisms during root canal therapy (Boehm TK. 2011).

Nagai et al. mentioned the combination of a new PS, the azulenocyanine (Azc) with a high power laser (a GaAlAs/InGaAsP diode laser with a wavelength of 940 nm) (Nagai Y. 2018). Indocyanine green (ICG) performs its bactericidal effect through oxidative stress and affects the target cell mainly due to a photothermal effect rather than a photochemical effect (Fekrazad R. 2016).

Gram-negative bacteria's resistance to the effective destruction of antibacterial PDT arises from their distinct outer membrane architectures as well as the hydrophobic and charge-producing properties of PSs. Indeed, it seems that the charge of the sensitizer influences the photosensitivity of the bacterium (Konopka K. 2007). Both gram positive and gram-negative are inactivated by the cationic PSs, such as MB and TBO (Usacheva MN. 2001).

There have been reports of significant chromosomal DNA damage, membrane protein degradation, and functional disruption of the cell wall for *E. faecalis* cells exposed to methylene blue-mediated PDT (George S. 2008). On the same way, Fonesca et al. reported that intracanal *E. faecalis* could be treated with toluidine blue using diode laser (50-mW) causing a large reduction rate (99.9%), by increase in cell surface roughness to deform cell shape, bacterial wall disintegration, and cellular content leakage (Fonesca MB. 2008).

The biofilms subjected to MB and diode laser suffered the most damage. Photosensitizers may disrupt the integrity of the membrane and harm cytoplasmic elements in irradiated cells (Bhatti M. 1998).

"Pre-irradiation time" is the amount of time that passes between the PS's distribution and the actual photo-activation (Trindade AC. 2015). It helps in maintaining the PS inside the bacteria with greater light absorption (Usacheva MN 2001). The two most crucial elements in PDT microorganism death are the energy dose and the irradiation time (Pourhajibagher M. 2018). Curcumin was selected as the photosensitizer due to its broad absorption band spanning from 400 to 500 nm, peaking at 430 nm. This naturally occurring yellowish-orange powder is part of the Curcuma longa rhizome and possesses significant antibacterial properties against bacteria, viruses, and fungi (Moghadamtousi SZ. 2014) Unlike, Gomes-Filho et al who reported that PDT with curcumin did not inhibit fibroblast viability and was not cytotoxic (Gomes-Filho JE. 2016).

Research showed that the efficient bacterial inactivation achieved by the combination of curcumin and the blue LED light was comparable to the usual photodynamic therapy (PDT) method using a diode laser and Methylene blue (Moghadamtousi SZ. 2014). PDT investigations have revealed varying disinfection efficiency depending on the type of laser and dyes utilize (Soukos NS. 2006). Nevertheless, the treatment has never completely eliminated microbiological cells, leaving room for the possibility of recurrent infections (Fonseca MB. 2008). PUI has been presented as an option to improve root canal disinfection in the treatment of post-treatment infections (Gründling GL. 2011). PDT's antibacterial depended on the types of bacteria in the root canal system how they grow affect, how susceptible they are to PDT in a dose-dependent way (Upadya MH. 2010). Moreover, the antibacterial activity of PDT was reduced by dentin, dentin matrix, pulp tissue, bacterial lipopolysaccharides, and bovine serum albumin (Shrestha A. 2012) It seems promising to try to improve the photodynamic impact by delivering and encasing MB in polymeric nanoparticles. Additional tactics encompass the application of a solvent photosensitizer and efflux pump inhibitors (Upadya MH. 2010).

1.10 Nanoparticle irrigants' solution

The use of PDT in root canal therapy needs quickly removing by excessive saline irrigation after complete tretment because, it caused tooth discolouration and staining when methylene blue (MB) was utilized as the photosensitizer (PS) (Carvalho Edos S. 2011). The endodontic research community has been quite enthusiastic about nanoparticle-based disinfection therapy. Nanoparticles measure between 1 and 100 nm. When functionalized with other chemicals, such as photosensitizers, bioactive molecules, and medicines, they demonstrate enhanced antibacterial activity due to their small size and huge surface area to volume ratio, which results in synergistic effects (Shrestha A 2016). Moreover this types of the nanoparticles allow for greater solubility and increased reactivity (Jeevanandam J. 2018, Khan I. 2019, Khan I. 2019)

When antimicrobial nanoparticles come into contact with bacterial cell walls, they can interact electrostatically, causing damage to the cell membrane, increased permeability, the generation of reactive oxygen species, disruption of cellular processes, protein degradation, damage to DNA, and, eventually, and cell death (Beyth N. 2015).

Increased reactivity, enhanced antibacterial activity, and the ability to functionalize with other reactive compounds are just a few of the qualities that nanoparticles have that may improve the treatment of endodontic infections. This results in the creation of numerous experimental sealants and obturation materials with nanoparticle incorporation that have a variety of advantageous physicochemical characteristics, such as increased bioactivity and antibacterial efficacy.

Antimicrobial metallic nanoparticles as silver nanoparticles, zinc oxide, magnesium oxide, titanium dioxide and iron oxide have been studied as root canal irrigants (Beyth N. 2015). High surface area, positive charge density, and polycationic/polyanionic characteristics of nanoparticles increase their antibacterial activity. Combining the antimicrobial nanoparticles with additional antibacterial materials, such as calcium hydroxide, could increase their high surface area and positively charged density, which could promote synergistic effects (Akbari T. 2017, Du T. 2014)

Chitosan, an organic polysaccharide derived from a natural component found in crab shells and shrimps called chitin. Excellent biocompatibility and promising anti-biofilm and antibacterial activities are displayed by chitosan nanoparticles (Kishen A. 2008). Planktonic and biofilm *E. faecalis* cells were reported to be effectively killed by chitosan nanoparticles (Shrestha A. 2010). Research has indicated that it has the ability to eradicate the smear layer and stabilize dentine by preventing the degradation of collagenase (Kishen A. 2016).

For improved anti-biofilm effectiveness by photodynamic activation, chitosan nanoparticles have also been functionalized with photosensitizers including Rose Bengal and methylene blue (Shrestha A. 2012), resulting in improving collagen cross-linking while also making it easier for chitosan nanoparticles to bind to collagenase, which reduced collagenolytic activity. Its use has been shown to improve dentine's resistance to fatigue (Persadmehr A.2014).

1.11 Alternatives nanoparticle irrigants

Chitosan nanoparticles is a promising contender for novel irrigants (Del Carpio-Perochena A. 2015), with a broad-spectrum antibacterial activity (Kong M. 2010). Electrophoresis, diode laser application, high-intensity focused ultrasound and manual dynamic activation have been introduced in order to increase the distribution and the effects of chitosan nanoparticles (Ionescu A. 2020).

Since they work on multiple levels, silver nanoparticles (AgNPs) have antifungal effects and antibacterial (Bapat RA. 2018). Poly (vinyl alcohol) (PVA) coated AgNPs were effective against *C. albicans*, E. faecalis, and Pseudomonas aeruginosa, when used as an irrigating solution (Chávez-Andrade GM. 2019). Ultrasonic activation and Nd:YAG laser irradiation were observed to enhance the antibiofilm and antibacterial capabilities, respectively (Kushwaha V. 2018, Abbaszadegan A. 2015). When an AgNPs-based irrigant was employed as a last rinse instead of NaOCl, the fracture resistance of teeth that had endodontic treatment was nearly doubled (Jowkar Z. 2020). AgNPs inhibit the bacterial cell's DNA replication as well as the production of ribosomes and other cell proteins. Additionally, it disrupts the bacteria's cycles of energy transmission (Bragg PD. 1974).

With a mode of action close to AgNPs, zinc oxide nanoparticles, or ZnONPs, have been recognized for their bactericidal capabilities (beyth N. 2015). It was discovered that a ZnONPs-based irrigant maintained its antibacterial effectiveness while eliminating planktonic *E. faecalis* and disturbing the biofilm matrix (Shrestha A 2010). Furthermore, teeth with root canal therapy had a mean fracture resistance that was around 400N higher when ZnONPs-based solution was used as a final irrigant than when NaOCl was utilized (Jowkar Z 2020).

In vitro and ex vivo studies have demonstrated the long-term antibacterial efficacy of a nano-magnesium oxide solution against *E. faecalis* (Monzavi A. 2015). When hydrogen peroxide was added to an irrigating solution, iron oxide nanoparticles had peroxidase-like activity, which led to antibiofilm and bactericidal action against *E. faecalis* (Bukhari S. 2018)

Overall, Research on irrigants based on nanoparticles could lead to the development of creative and original endodontic disinfection techniques. To completely understand the potential of different nanomaterials to be used as endodontic irrigants, more research is required. Furthermore, investigations should look into the ways to include nanoparticles into irrigation solutions, their long-term antibacterial action, and their in vivo efficacy. While limiting any potential negative or detrimental consequences (Bapat RA. 2018).

Nanotoxicology emerged as a specialized field dedicated to investigating the potential risks linked to exposure to nanomaterials (Oberdörster G.). The very characteristics that make nanoparticles unique also contribute to their potential toxicity, posing risks to oral tissues, overall health, and the environment (Dreher KL, 2004).

Due to their size being comparable to biological molecules, nanoparticles can be readily absorbed by various organs and tissues. They can enter the human body via multiple pathways, including inhalation, skin contact, and ingestion. Once inside, they may circulate systemically and have been found to accumulate in organs such as the lungs, liver, and components of the reticuloendothelial system.

When present at toxic levels, nanoparticles can cause harm through both oxidative stress-dependent and independent mechanisms. Oxidative stress, in particular, can lead to cellular damage by triggering DNA mutations, the release of inflammatory cytokines, protein degradation, lipid peroxidation, and programmed cell death (Oberdörster G.).

Furthermore, nanoparticles may act as environmental contaminants. Since their toxic effects often depend on concentration, their accumulation in ecosystems could potentially lead to long-term toxicity in living organisms (Dreher KL, 2004).

Conclusion:

Given these concerns, the possible negative consequences of using nanoparticles in endodontics should not be underestimated. Their impact could affect treatment outcomes, patient well-being, and ecological health. Therefore, it is essential to carefully assess their safety and develop application methods that reduce biological and environmental risks while enhancing therapeutic effectiveness.

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