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Bacterial endotoxins in periodontal health and diseases

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Abstract: Bacterial endotoxins are a major concern in periodontal health and diseases owing to their structure and biological activity. With up-to-date knowledge of endotoxins and the recent findings about the influence of endotoxins in dental health, their probable mode of pathogenesis, and standard detection methods, this review analyzes the potential efficacy and benefits of probiotics in combination with conventional and contemporary treatment measures. In the oral cavity, Gram-negative bacteria are documented to predominate in the pulpal lesions with radiolucent areas and in the root canal with pulp necrosis, where they pose an absolute threat by promoting a series of inflammatory reactions. Endotoxin, a constituent of Gram-negative bacteria establishes a nexus between cytokine stimulation and proinflammatory reactions, therefore plays a critical role in decaying dental pulp and modulating periodontal diseases. Currently, the treatment regimen involves several biochemical preparations. In addition, probiotics have been reported to control endotoxin in gingivitis and contribute to the overall improvement of dental health. A potential benefit of a combination of probiotics as a complementary treatment along with the conventional treatment warrant more empirical evidence to elucidate its role and mechanism in resolving the clinical manifestations associated with endotoxins in the periodontal region.

Introduction

Endodontics, a recognized branch of dentistry, concerns the study of the dental pulp and surrounding tissues along with the nerve, blood, and lymphatic vessels connecting the teeth to the root. The dental pulp is secured by the barrier of enamel and cementum (Khabbaz *et al.*, 2001). Several different external factors such as caries, periodontal diseases, or trauma from post-operative procedures can destabilize this mechanical support exposing the pulp and the tissues to potential threats posed by the presence of a diverse population of microflora in the oral cavity (Khabbaz *et al.*, 2001).

An excess of 700 different microbial species have been isolated and identified using recent molecular diagnostic methods; these species belong to 185 genera and 12 phyla (Deo and Deshmukh, 2019). Both Gram-positive and Gram-negative microbes have been reported as dental colonizers and pathogens (Chenicheri *et al.*, 2017). Recent and former studies have identified several bacterial virulence factors contributing to dental plaques, caries, and periodontal diseases. In this article, we emphasize a particular pathogenic factor, which is an endotoxin produced by Gram-negative bacteria.

Endotoxins are essentially the components of the outer membrane of the Gram-negative bacteria (Kumada *et al.*, 1995) and are involved in many clinical conditions, including necrosis of the dental pulp, lesion with the radiolucent area, bone resorption, and primary and secondary root canal infection (Martinho *et al.*, 2010). When released in sufficient amounts in the dental tissues, these dormant bodies initiate a cascade of immune reactions that, in many cases, has been associated with clinical situations. This article will focus on the pathogenesis of endotoxins in the dental tissues, their association with clinical manifestations, the quantification method of endotoxins from symptomatic cases, and the probable treatment choices available.

In this current review, we have included research articles published until November 2021 that covered our topics of interest (i.e., endotoxins and periodontal diseases). We searched using the keywords 'Endotoxins,' 'Pathogenesis,' 'Periodontitis,' 'Gingivitis,' 'Endotoxin assay,' 'Oral probiotics,' and 'Clinical Studies' in the article titles from four scientific databases, including Google Scholar, Scopus, and PubMed.

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Microflora in the Exposed Dental Pulp and Tissues

The dental pulp is composed of soft connective tissues and may be exposed to the oral cavity microflora due to abrasion, caries, or trauma. Sometimes root canal treatments can also initiate such a situation. Microbiological, radiographical, and histological examinations have revealed the involvement of anaerobes in deep dentinal caries and chronic periapical inflammation (Seltzer and Farber, 1994; Chakrabarty and Karim, 2018). Typically, the bacterial load increases proportionately as the lesion size increases and vice versa (Seltzer and Farber, 1994). The presence of both Gram-positive and Gram-negative bacteria has been documented in root canals and predominantly in dentinal tubules. A study was carried out in monkeys by inoculating indigenous oral bacteria in the root canal composed of devitalized tissues, and a dynamic shift was observed in the microbiological population over the three years of the experiment (Fabricius *et al.*, 1982). Initially, a greater proportion of facultatively anaerobic bacteria was present in the samples cultured; however, over time, strict anaerobes progressively outnumbered them. Nutritional supply and interrelationship between species determine the persistence and the ultimate outcome of any oral infection (Seltzer and Farber, 1994).

Prevotella spp., also known as black-pigmented anaerobes, has been isolated more frequently from an acute periapical lesion and dentoalveolar abscesses compared to the asymptomatic teeth (Siqueira and Rôças, 2009). Notable among them are *Prevotella melaninogenica* and *Prevotella intermedia* (Sedghizadeh *et al.*, 2017). Other commonly isolated Gram-negative genera include *Porphyromonas* (Gomes *et al.*, 2004), *Campylobacter*, *Fusobacterium* (Shweta, 2013), *Bacteroides*, etc. (Sedghizadeh *et al.*, 2017). All these bacteria have different means of virulence and have been attributed as toxic to the apical and periapical tissues.

Less frequently isolated microorganisms include *Pseudomonas, Veillonella, Treponema, Serratia*, etc. (Piekarczyk *et al.*, 2003). Besides being Gram-negative and using endotoxin as their virulence factor, these organisms have also been reported in opportunistic oral infections (Piekarczyk *et al.*, 2003). Thorough investigations of medical history and other pre-existing conditions have demonstrated the correlation between comorbidities and periodontal infection. Lipopolysaccharide (LPS) from the periodontal pathogen can get access into the bloodstream and promote systemic disorders. Diseases involving multiorgan disturbances influence the occurrence of secondary oral infections, as is manifested in diabetic, transplant, and hemodialyzed patients (Piekarczyk *et al.*, 2003). Eighty percent of patients tested positive for human immunodeficiency virus (HIV) have been found to be associated with opportunistic oral infection. In untreated cases, an upsurge of *Prevotella*, *Veillonella*, and *Campylobacter*, among the other lingual microbiome, are correlated with elevated viremia in HIV-positive patients (Galanos, 1975). Although lacking any direct evidence, the existence of dental plaques, caries, pulp lesions, periodontitis, or any circulatory inflammatory cytokines formed thereby is suspected to be linked with the mentioned comorbidities.

Pathogenesis of Endotoxins in Periodontal Diseases

Typically, the Gram-negative bacterial population in the oral cavity may sometimes be diminished due to competition or antagonistic effects of other normal flora. However, they can leave significant virulence factors that survive longer and might induce apical and periapical lesions. Morphologically, Gram-negative bacteria possess an outer lipid membrane surrounding the peptidoglycan layer, which makes them unique from the Gram-positives. This outer membrane is composed of lipoproteins, phospholipids, and lipopolysaccharide (LPS). The lipid portion of the LPS, alternatively known as Lipid A, is generally referred to as endotoxin (Kumada *et al.*, 1995) (Fig. 1). Endotoxins are liberated as the Gram-negative bacteria die, and their cell wall undergoes lysis (Fig. 1). In addition to exerting unique pathogenicity to Gram-negative organisms, endotoxins also help in cell growth, maintaining structural integrity, and protecting the cells from external threats. Principal Gram-negative bacterial genera that inhabit the oral cavity include *Moraxella*, *Prevotella*, *Neisseria*, *Desulfobacter*, *Veillonella*, *Campylobacter*, *Desulfovibrio*, *Fusobacterium*, *Haemophilus*, *Treponema*, etc.

With regard to microbiological and immunological significance, endotoxins are one of the major virulence factors of the Gram-negative bacteria known to deluge a number of inflammatory reactions. Moreover, being poorly antigenic, endotoxins usually go unnoticed by neutralizing antibodies. These heat-stable dormant bodies do not cause any direct cell or tissue damage but rather stimulate the immune system to release chemical mediators that, in turn, become auto-destructive for the host tissues (Martinho *et al.*, 2010; Chen *et al.*, 2022). The primary targets of these toxins are macrophages and monocytes (Munford and Hall, 1986), which are stimulated to produce cytokines. The emergence and establishment of a cytokine network due to bacterial infection generate a disturbance in the dental tissues, ultimately giving rise to periodontal lesions. Apart from macrophages, LPS has also been documented to inhibit apoptosis of neutrophils leading to excessive inflammation (Uriarte *et al.*, 2016) and induce mast cells, fibroblasts (Martinho *et al.*, 2012) to produce various proinflammatory cytokines such as Interleukin 6 (IL-6), IL-8, IL-1β, tumor necrosis factor -α (TNF-α), chemokines, and anti-inflammatory cytokines (IL-10, IL-4) (Chen *et al.*, 1992). LPS also stimulates macrophages to produce other factors such as reactive oxygen, nitrogen intermediates (nitric oxides), platelet-activating factors, kinins, bradykinins, prostaglandins, Hageman factor, etc. (Chen *et al.*, 1992). All these factors have individual or shared biological activities in endodontic diseases. TNF-α has been reported to produce bone resorption factors in addition to other chemokines and cellular adhesion molecules (Huang *et al.*, 2001). Another notable proinflammatory cytokine, IL-6, plays a role in osteoclastic differentiation and bone resorption in chronic periodontitis (Tinsley *et al.*, 2010). On the contrary, the destructive effects of IL-6 have been counteracted by the anti-inflammatory cytokine, IL-10 (Morrison and Ryan, 1987). However, LPS has also been implicated in mastocyte degranulation (Seltzer and Farber, 1994), macrophage and B-lymphocyte proliferation

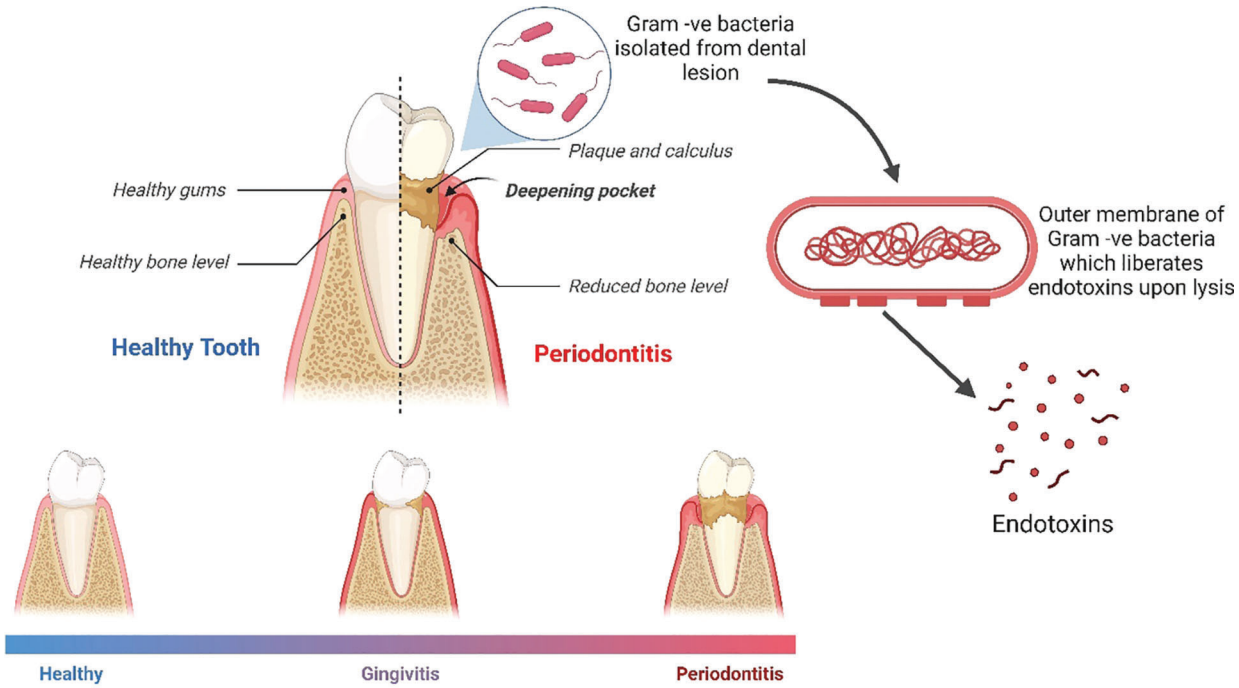

FIGURE 1. Release of endotoxin from Gram-negative organisms recovered from teeth. Top, a healthy and an infected condition of a tooth are compared. The infected portion has an inflamed gum and reduced bone level compared to the healthy one. Upon lysis, Gram-negative microbe isolated from plaque releases the outer membrane lipopolysaccharide, entitled 'endotoxins.' The bottom panel shows the progression of plaque formation, which gives rise to the characteristic features of gingivitis and eventually periodontitis. The figure was created with BioRender.com and exported under the terms of free subscription.

(Torabinejad *et al.*, 1985), and arachidonic acid metabolism. The products of this active metabolism have been further associated with a periapical lesion in human teeth by means of bone resorption (Torabinejad *et al.*, 1985).

LPS has also been reported to activate the complement cascade utilizing either the classical or the alternative pathway. The lipid A portion is responsible for the activation of the classical pathway, while the polysaccharide portion activates the alternative or properdin pathway (Horiba *et al.*, 1992). Because of this activation, anaphylatoxins (C3a and C5a) are produced. In general, these components are known to act as smooth muscle contractors as well as chemotactic factors for monocytes, neutrophils, and mast cells and contribute to kinin formation, all of which eventually lead to inflammation. C5a has been particularly documented to stimulate oxidative metabolism of monocytes and neutrophils and enhance the immune response by inducing the secretion of IL-6, IL-8, and TNF-α (Liu *et al.*, 2019). About 1 μg/mL of LPS along with lipoteichoic acid (LTA) has been found to activate C5a expression, which is further positively correlated to the secretion of IL-6 and the duration of inflammation in dental pulp (Gewurz *et al.*, 1968). The possible mode of pathogenesis is illustrated in Fig. 2.

A more recent study (Aziz *et al.*, 2021) hypothesized the association of metallothionein and zinc in the development and progression of periodontitis. LPS from *Prevotella gingivalis* initiates acute phase response and thus upregulates metallothionein, which consequently leads to a zinc-deficient condition in the patient, a hallmark of periodontitis. The involvement of LPS in a zinc deficient condition can give rise to chronic periodontitis by initiating dendritic cell maturation or inhibiting the anti-inflammatory actions of regulatory T-cells (Aziz *et al.*, 2021).

Association of Lipopolysaccharides with Clinical Manifestation

The clinical signs and symptoms in endodontics are more frequent due to the presence of anaerobic Gram-negative bacteria and their diverse pathogenic factors. One such example is *Porphyromonas gingivalis*, a representative of periodontopathogens. Endotoxins from this organism are highly toxic to fibroblasts and spread infection rapidly. However, controversies cloud the involvement of endotoxin or any other bacterial components responsible for this manifestation (Torabinejad *et al.*, 1985).

In root canal infections, endotoxin is found in significantly high amounts, which is responsible for inflammation of periapical tissues. A clinical study of 27 root canal patients established a significant correlation between the presence of endotoxin and pulpal lesion with radiolucent areas (Horiba *et al.*, 1991); similarly elevated endotoxin level was evident in root canals with pulp-less teeth as compared to individuals with vital pulp (Schein and Schilder, 2006). Teeth with radiolucent areas harbor five times more endotoxin content compared to teeth without them (Martinho *et al.*, 2010). Spontaneous pain, swelling, and purulent exudates have been documented as the general symptoms, among many more. Even a minute amount of endotoxin is capable of causing periapical lesions, and its concentration increases proportionately with time (Martinho *et al.*, 2017).

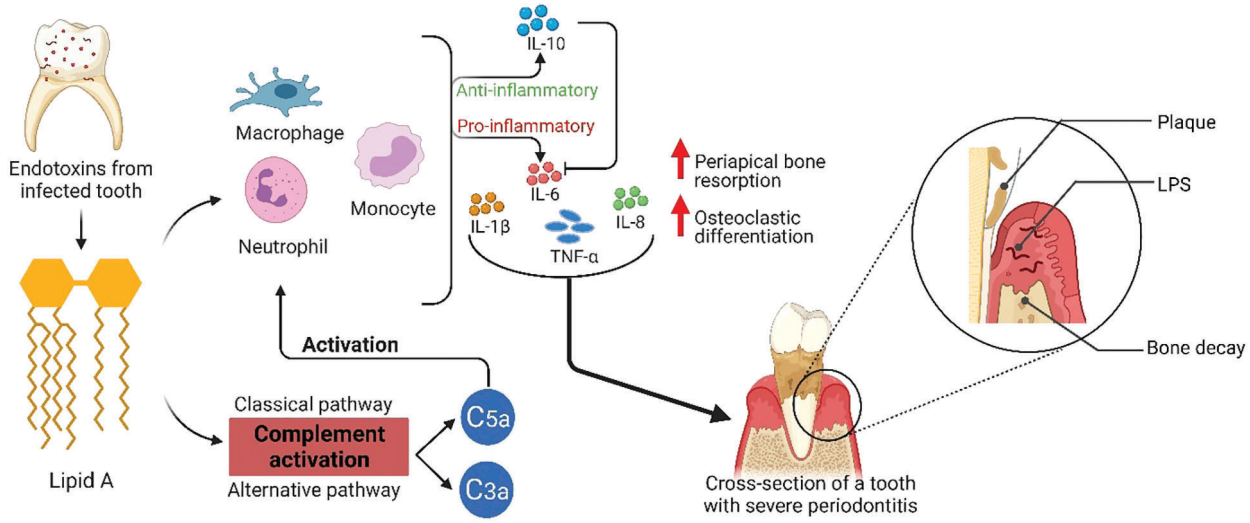

FIGURE 2. A Possible mode of pathogenesis exerted by endotoxins in periodontitis. Lipid A, the most active component of endotoxin, stimulates the phagocytic cells and both the classical and alternative complement pathways, releasing anaphylatoxins, i.e., C3a and C5a, that in turn act as the chemotactic factors for the phagocytic cells. Phagocytic cells, including macrophages, monocytes, and neutrophils, enhance the immune response by producing various proinflammatory cytokines such as IL-6, IL-8, TNF-a, IL-1b, etc. An influx of these cytokines is responsible for inflammation, enhanced periapical bone resorption, and osteoclastic differentiation, ultimately leading to periodontitis. Conversely, anti-inflammatory cytokines such as IL-10 are formed occasionally, which resists the destructive actions of proinflammatory cytokines. The figure was created with BioRender.com and exported under the terms of free subscription.

A systematic review and meta-analysis carried out by Martinho and colleagues (Martinho *et al.*, 2017) evaluated the association of endotoxins with clinical symptoms in patients with endodontic infections. This comprehensive analysis revealed that individuals with previous episodes of pain, having teeth with tenderness to percussion, and radiographic lesions of >2 mm had higher levels of endotoxins than their counterparts. Root canal exudation has also been associated with higher levels of endotoxins (Martinho *et al.*, 2011a; Parolia *et al.*, 2014).

The activation of macrophages or other mononuclear cells by LPS results in a rapid influx of cytokines in the infected dental tissues. This phenomenon makes LPS one of the major modulators of inflammation and the consequences thereafter. Several studies conducted on primary root canal infections have revealed a positive correlation between the number of anaerobic Gram-negative bacteria, endotoxin contents released from them, and the levels of cytokines and chemokines, in particular TNF-α, IL-1β, and Prostaglandin E (Martinho *et al.*, 2011b; Parolia *et al.*, 2014; Martinho *et al.*, 2015). The expression of cytokines has also been coupled to the appearance of clinical symptoms. Elevated levels of TNF-α and Interferon-γ have been positively correlated with previous episodes of pain, tenderness to percussion, and bone destruction, while several other cytokines, including IL-4, IL-5, and IL-13, have been found to exhibit protection from such manifestations (Miller *et al.*, 1975).

Patients with endodontic infections experience several episodes of pain at the onset of inflammation, which is again linked with the endotoxin contents. Endotoxin activates the Hageman factor, which in turn drives the production of bradykinin, a potent mediator of pain. Exposure of leukocytes to endotoxins has also been reported to produce bradykinin (Torabinejad *et al.*, 1985; Schein and Schilder, 2006). Investigations of infected root canals and periapical lesions have revealed the presence of more endotoxins in the periapical region in painful teeth compared to those with no symptoms (Munford and Hall, 1986).

Investigators, however, have different perceptions on the involvement of endotoxin alone in the clinical signs and symptoms of endodontics. In some cases, invasion of dental pulp with Gram-negative bacteria followed by the release of different enzymes such as proteases and acyloxyacyl hydrolases have contributed to inflammation by their own mode of pathogenesis, or may have even reduced the detrimental effects of LPS (Torabinejad *et al.*, 1985). Unlike the Gram-negative organisms, Gram-positives have a peptidoglycan layer which accounts for the induction of acute inflammation and sometimes its succession into a chronic one. They do so in a manner similar to endotoxins by stimulating the macrophage, granulocytes, and lymphocytes to secrete IL-1 and TNF-α, and producing oxygen radicals, nitric acids, etc. (Torabinejad *et al.*, 1985).

Detection of Endotoxins

Endotoxin testing has provided strong evidence to associate the presence of LPS with different endodontic signs and symptoms. The classic detection method, known as the Limulus amoebocyte lysate (LAL) assay, was developed in the 1950s. LAL is essentially an extract of amoebocyte cells found in the Atlantic horseshoe crab, *Limulus polyphemus*. On exposure to bacterial endotoxins, these cells release a clotting factor in blood as a defense and end up coagulating the blood cells. Based on this principle, the LAL assay was developed, which uses a serine protease to activate a coagulation cascade in the presence of Gram-negative bacterial endotoxins. The formation of gel-clot, as a result, is the basis of interpreting the result (Martinho *et al.*, 2011a).

Given its high sensitivity, the LAL assay is the most widely used method by endodontic researchers.

Endodontic Treatment for Endotoxin Reduction

The high toxicity of endotoxin is responsible for root canal infection, bone resorption, periapical inflammation, and cytokine influx. Therefore, curative measures can be directed to either the removal of endotoxin-producing bacteria or inactivating and eliminating endotoxin and other substances from infected teeth. To ensure infection control, some measures are to be taken before starting the endodontic treatment, such as using sterile equipment to operate the root canal, antisepsis of the operative region, and using chemical irrigants to minimize the microbial count and their virulence factors. While bacterial cells cannot be eliminated, endotoxins pose an extra threat, being highly heat-resistant dormant entities, which essentially makes their removal challenging. However, several chemicals have been utilized to inactivate endotoxins in the treatment procedures. Such chemicals include calcium hydroxide [$Ca(OH)_2$], sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), etc. Apart from using these chemicals, recent studies have focused on employing probiotics in endodontic treatments to improve oral health.

Calcium hydroxide [$Ca(OH)_2$]

The introduction of $Ca(OH)_2$ in dentistry dates back to 1920 and has received immense attention since Hermann utilized it as a pulp-capping agent. It is dissociated into calcium (Ca^{2+}) and hydroxyl (OH^-) ions once solubilized in water and is slowly absorbed in the fluid of vital tissues (Safavi and Nichols, 1993). The generation of (OH^-) ions creates an alkaline environment that causes the denaturation of bacterial proteins resulting in cell lysis (Safavi and Nichols, 1993). In addition to antimicrobial activity, $Ca(OH)_2$ mediates the breakdown of intracanal soft tissue remnants, reduction of periapical exudates, and induction of mineralization and periapical healing (Ba-Hattab *et al.*, 2016).

In vitro, $Ca(OH)_2$ has been found to inactivate LPS by hydrolyzing the potent toxic component, lipid A, and transforming them into nontoxic fatty acid and amino sugars (Oliveira *et al.*, 2005). Another *in vitro* study (Buck *et al.*, 2001) evaluated intracanal medicament, focusing on polymyxin B, the antibiotic against Gram-negative organisms. While the antibiotic in combination with steroid was unable to act on endotoxins, both polymyxin B and $Ca(OH)_2$ were found to detoxify endotoxins in root canals and furthermore stimulated the production of B-lymphocytes (Oliveira *et al.*, 2005). Long-term use of $Ca(OH)_2$ as root canal medication has been reported beneficial when endotoxin is suspected. Application of alkaline $Ca(OH)_2$ for 1, 2, and 5 days detoxified more LPS compared to other irrigants (Tanomaru *et al.*, 2003). Moreover, when applied for 14 and 30 days, multiple-session treatment using $Ca(OH)_2$ was more effective in reducing endotoxin from root canal infection as compared to single-session (Nascimento *et al.*, 2021).

A similar kind of comparison between the biomechanical preparation of different irrigating solutions and $Ca(OH)_2$-based root canal dressing was performed *in vivo*. The trial was done in dog's teeth containing endotoxin. While the biomechanical preparation was ineffective, the dressing was found to resolve the effects of endotoxins (Nelson-Filho *et al.*, 2002).

Auxiliary irrigating solution

Biomechanical preparations of several chemicals, such as NaOCl and/or CHX, are most frequently used and are regarded as auxiliary chemical substances (Nelson-Filho *et al.*, 2002). In addition to antimicrobial activities, hypochlorite has a unique capacity to degrade the components of the smear layer and necrotic tissues (Spratt *et al.*, 2001). When used in disinfection, it achieves its efficacy comparable with chlorhexidine or iodine at varying concentrations. LPS inactivation using NaOCl has been documented; however, the outcomes are insignificant as compared to $Ca(OH)_2$ (Nelson-Filho *et al.*, 2002).

CHX, on the other hand, is a strong base and deploys its bactericidal effects by binding to negatively charged microbial cell walls and altering their osmotic equilibrium. In terms of antimicrobial activity, it is effective against both Gram-positive and Gram-negative organisms, facultative and strict anaerobes, yeast, and fungi (Greenstein *et al.*, 1986). However, the use of CHX as the principal irrigant is not encouraged for its less efficacy on Gram-negative organisms. Interestingly, when applied in combination with $Ca(OH)_2$, a significant reduction in endotoxin content was evident (Nelson-Filho *et al.*, 2002). A clinical study on 54 primarily infected root canals compared the efficacy of 2.5% NaOCl and 2% CHX gel on the reduction of LPS. A higher percentage of endotoxin was reduced when 2.5% NaOCl was used compared to CHX. However, neither could completely eliminate bacterial LPS (Gomes *et al.*, 2009). In secondarily infected root canals, chemo-mechanical preparation of 2% CHX gel and 17% EDTA was shown to have more efficacy in reducing the bacterial count (99.61%) than endotoxin (60.6%) (Endo *et al.*, 2012).

The chemical auxiliary substances are good for use as irrigating solutions during root canal procedures for several reasons, including their high antimicrobial activity, reducing cytotoxicity and cytokine influx, degrading necrotic tissues, etc. Nevertheless, these can be employed along with $Ca(OH)_2$ to attain maximum endotoxin reduction in infected teeth.

Probiotics

'Probiotics' has revolutionized the arena of therapeutics by shifting the conventional outlook of considering bacteria as harmful to rather integrating bacteria for health benefits. These are, in other words, called 'the good bacteria'. Recent studies on probiotics have reported their capacity to function as antimicrobial, anti-cancer, and anti-toxin agents (Gulzar *et al.*, 2019). Owing to such properties, probiotics have been evaluated for their potential in reducing dental caries, plaque, or other periodontal diseases.

Probiotics have potential applications in preventing alcohol-induced liver disorders and inflammatory bowel diseases (Allaker and Stephen, 2017). *Bifidobacterium* and *Lactobacillus* spp. are among those probiotics that also contribute to controlling oral microbial growth (Krasse *et al.*, 2006). When present, they interfere with plaque-forming

biofilm by initiating a competition for binding sites on the host tissues and nutrients. Such a demonstration has been observed by applying *Lactobacillus reuteri* in individuals with moderate to severe gingivitis. A modification in the cariogenic microbiota has resulted in the reduction of inflammation and plaque formation (Krasse *et al.*, 2006). Furthermore, lactic acid bacteria produce a number of antimicrobial agents, including hydrogen peroxides, bacteriocins, and other low molecular weight antimicrobial peptides. These properties have been utilized to reduce periodontal pathogens in plaque, control the level of cariogenic bacteria in saliva and remodel the oral microflora (Allaker and Stephen, 2017).

Whether probiotics have any direct or particular effect on endotoxins is not clear yet. However, probiotic treatment significantly reduces Gram-negative periodontopathogens that can be linked to the control of endotoxin in clinical situations. Many strains of *Lactobacilli* and *Streptococci* isolated from healthy oral cavities show antibacterial activity against some of the dominant Gram-negative periodontopathogens, including *Prevotella intermedia, Porphyromonas gingivalis*, and *Fusobacterium nucleatum* (Zhu *et al.*, 2010; Terai *et al.*, 2015; Khalaf *et al.*, 2016). Since most probiotics are lactic acid bacteria, they produce lactic acid as the major metabolic end product of carbohydrate fermentation. The acidification of the environment largely prevents the growth of pathogenic microorganisms. While this is generally assumed as the antibacterial role of probiotics, they have been reported to employ several strategies to combat Gram-negative periodontopathogens. In one study, *Lactobacillus delbrueckii* isolated from commercial yogurt products significantly reduced the growth of *P. gingivalis* by producing inhibitory amounts of hydrogen peroxide to disturb the integrity of the pathogen's cell wall by generating short-lived radicals (Cornacchione *et al.*, 2019). Probiotics are also well documented to produce bacteriocins. Nisin from *Lactobacillus lactis*, reuterin from *Lactobacillus reuteri*, salivaricin from *Lactobacillus salivarius*, and plantaricin from *Lactobacillus plantarum* are amongst the significant bacteriocins associated with oral cavity (Masdea *et al.*, 2012; Baca-Castanón *et al.*, 2015; Heeney *et al.*, 2019). Nisin has demonstrated antibiofilm and antibacterial activity against many Gram-negative periodontopathogens, including *P. intermedia, P. gingivalis, F. nucleatum, and Treponema denticola*, without exhibiting any cytotoxicity to human oral cell lining (Shin *et al.*, 2015). In addition, plantaricin also inhibits the growth of *P. gingivalis (Khalaf et al., 2016)*. To evaluate the efficacy of probiotics, *Lactobacillus reuteri* lozenges were applied in subgingival plaque associated with changes in salivary and subgingival microflora. A significant reduction of *P. intermedia* and *P. gingivalis* was evident in saliva and subgingival samples, respectively. An increase in the prevalence of *P. gingivalis* was only evident four weeks post-probiotic treatment (Mayanagi *et al.*, 2009).

At least five different randomized preclinical trials have demonstrated that treatment with probiotics significantly prevents alveolar bone loss and, to some extent, contributes to an increase in bone density. The reduction in alveolar bone loss was more prominent in ligature-induced periodontal sites that received *Bacillus subtilis* treatment than that in those that did not (Messora *et al.*, 2013; Garcia *et al.*, 2016). *B. subtilis* was applied to a rat model of periodontitis, and C-terminal peptide was evaluated both in the presence and absence of probiotic therapy. C-terminal peptide is a marker of bone resorption and is decreased in the presence of probiotics, decreasing bone loss and osteoclastic activity (Foureaux *et al.*, 2014).

On the other hand, an indirect mechanism of the probiotics in the oral cavity is the modulation of both innate and specific immunity whereby their interaction with macrophages, T-cells, and other immunocompetent cells can lead to cytokine production (Allaker and Stephen, 2017) that can be a potential threat in an already existing endotoxemia in the periodontal region.

When applied, lactic acid bacteria shift oral microbiota to address periodontitis, but it might also influence the gut microbiota and be involved in initiating aggressive immunomodulation. These long-term changes in oral or gut microbiota are not well studied and warrant further investigations. Given the broad-spectrum beneficial activities of probiotics, more convincing pieces of evidence are needed to support the use of these bacteria in the clinical management of periodontal diseases.

Conclusion

Bacterial endotoxins are strongly associated with endodontic diseases. The endotoxins have a fundamental role in initiating chronic periapical lesions and bone resorption by creating a cytokine network and directing self-damage to the host. While the conventional biochemical preparations can generally act on clearing out endotoxins, the routine application can result in chronic pulpal inflammation. Considering that, the use of probiotics as a prospective alternative in the treatment has already been demonstrated by several *in vitro* and *in vivo* studies. However, further studies are recommended to evaluate the potency and long-term effect of probiotics in the oral microbiota and their overall applications in endodontics.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

Author Contribution: All authors have materially participated in the research and article preparation. MMK conceptualized and designed the study. FN reviewed the literature and prepared the graphical presentation. FN drafted the article. MMK and MTR revised the article critically for important intellectual content. All the authors approved the final article before submission.

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Conflicts of Interest: The study design, data collection, and analysis, and decision to publish or preparation of the manuscript were absolutely the authors' concerns. Therefore,

no competing or conflicting interests exist amongst the authors.

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