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Defensins in Periodontics: A Comprehensive Review

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Abstract:- Periodontal diseases, such as gingivitis and periodontitis, are inflammatory disorders that impact the tissues supporting the teeth. These diseases are initiated by bacterial plaque biofilms that form on the tooth surface. The host's immune response to this bacterial challenge plays a crucial role in disease progression. Among the various components of the immune system, antimicrobial peptides (AMPs) such as defensins have gained significant attention for their role in maintaining oral health and their potential therapeutic applications in periodontics. This review article explores the biology, function, and therapeutic potential of defensins in periodontal health and disease.

I. INTRODUCTION

Antimicrobial peptides are polypeptides with fewer than 100 amino acids found in host defense settings, exhibiting antimicrobial activity at physiological concentrations in their tissues of their origin. In humans and other mammals, the primary antimicrobial peptide families are defensins and cathelicidins. Members of these families play a role in the antimicrobial activity of phagocytes, inflammatory body fluids, and epithelial secretions. Defensins are widely present in mammalian epithelial cells and phagocytes and often occur at high concentrations, up to millimolar levels.

➢ *Structure:*

Defensins are found in vertebrates, characterized by a β-sheet rich structure and a framework supported by six cysteines linked by disulfide bonds. These small peptides, ranging from 2 to 5 kDa in size, are cationic and feature a β-sheet core structure that is stabilized by three consistent intramolecular disulfide bonds. Classical defensin molecules can be compared to a bent paperclip, consisting of a triple-stranded β-sheet structure with a loop connecting the strands, forming the base. From this base, a β-hairpin hydrophobic finger extends nearly perpendicular. In crystalline form, human defensins create dimeric structures where the two hairpins align in a four-stranded hydrophobic β-sheet, forming the narrow section of a funnel. The broader, polar, and cationic upper part of the funnel is made up of a six-stranded β-sheet with two connecting loops facing outward. In solution and within the lipid bilayer of target cells, higher-order multimers are likely to form. The arrangement of the six cysteines has been confirmed through direct chemical analysis, as well as crystallographic and two-dimensional nuclear magnetic resonance data, making defensins effectively cyclic peptides.[7]

II. TYPES

Defensins are small, positively charged peptides that are integral to the innate immune system, serving as a crucial first line of defense against microbial infections. Mammalian defensins are categorized into α-, β-, and θ-subfamilies based on the arrangement of their six conserved cysteine residues and the patterns of intramolecular disulfide bonds with humans mainly expressing alpha-defensins and beta-defensins.[1]

Both α-defensins and β-defensins share a triple-stranded β-sheet structure with a characteristic "defensin" fold. Recently, a different subfamily known as θ-defensins has been discovered in the leukocytes of rhesus macaque monkeys. These mature θ-defensin peptides are produced through an as-yet-unknown process that splices and cyclizes two nine-amino-acid segments from α-defensin-like precursor peptides, resulting in a cyclic peptide. Although θ-defensins appear to have evolved in primates, they are inactivated in humans due to mutations that introduce premature stop codons.

In α-defensins, the disulfide bonds connect Cys1 to Cys6, Cys2 to Cys4, and Cys3 to Cys5. In β-defensins, the connections are between Cys1 and Cys5, Cys2 and Cys4, and Cys3 and Cys6. In contrast, θ-defensins have a circular structure with disulfide bonds linking Cys1 to Cys6, Cys2 to Cys5, and Cys3 to Cys4.

A. Alpha-Defensins

Alpha-defensins are mainly produced by neutrophils and the Paneth cells in the intestines. Regarding periodontal health, neutrophils are a key source of alpha-defensins, especially human neutrophil peptides (HNP) 1-4. These peptides are stored in the azurophilic granules of neutrophils and are released when bacteria invade, helping to eliminate periodontal pathogens. Alpha defensins have proven to exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses. They function by disrupting the

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microbial cell membrane through the formation of pores, leading to cell lysis. This ability to directly target pathogens makes them an essential part of the body's first line of defense. Alpha defensins are important in maintaining the balance of the gut microbiota and play a role in preventing infections in the gastrointestinal tract. They are also involved in modulating immune responses and inflammation. Dysregulation of alpha defensins has been implicated in conditions like Crohn's disease and other inflammatory disorders.[4]

According to a study by ***Gursoy et al. (2013)***, alpha-defensins contribute to oral health by being part of the antimicrobial arsenal that neutrophils deploy against bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, which are commonly implicated in periodontal diseases . This research underscores the role of alpha-defensins in controlling microbial populations in the periodontal environment. ***Sakko et al. (2016)*** explored the expression levels of alpha-defensins in gingival tissues of patients with healthy periodontium versus those with periodontal disease. Their findings revealed that alpha-defensins are expressed at high levels in healthy gingival tissues, suggesting that they play a protective role in maintaining periodontal health by targeting and neutralizing potential pathogens.[2] Research by ***Zhang et al. (2016)*** highlighted the involvement of alpha-defensins in the immune response within the periodontal tissues. Their study showed that neutrophils release alpha-defensins in response to microbial invasion, contributing to the initial defense mechanism that prevents the onset of periodontal disease. The presence of these peptides is crucial for a swift and effective immune response.[3]

Li et al. (2018) examined the potential clinical applications of alpha-defensins in periodontal therapy. Their study demonstrated that augmenting the local levels of alpha-defensins through therapeutic interventions could enhance the antimicrobial environment within periodontal pockets, potentially improving the outcomes of periodontal treatments.[2]

Eick et al. (2011) investigated the antimicrobial activity of alpha-defensins in gingival crevicular fluid (GCF). They demonstrated that alpha-defensins, particularly HNP 1-3, are effective against periodontal pathogens like *Porphyromonas gingivalis* and *Tannerella forsythia*, highlighting their role in controlling microbial infections in periodontal sites.[2]

Kawakami et al. (2017) studied the expression of alpha-defensins in periodontal tissues and found that their levels are significantly higher in healthy gingiva compared to inflamed tissues. This suggests that alpha-defensins contribute to maintaining periodontal health by being present at elevated levels in the absence of disease.[3]

Kawamoto et al. (2019) explored the role of alpha-defensins in the immune response during periodontal health. Their study showed that alpha-defensins are released by neutrophils in response to bacterial challenges, playing a crucial role in the early defense mechanism within the periodontal environment.[4]

Kumar et al. (2020) evaluated the therapeutic potential of alpha-defensins in periodontal treatment. They found that local delivery of alpha-defensins could enhance the antimicrobial efficacy of conventional periodontal therapies, suggesting their potential use in improving treatment outcomes for patients with periodontal disease.[4]

Lundmark et al. (2018) investigated the expression levels of alpha-defensins in periodontal disease and found that their levels were significantly elevated in the gingival crevicular fluid of patients with chronic periodontitis compared to healthy individuals. This suggests that the upregulation of alpha-defensins is a response to increased microbial load and inflammation in diseased periodontal tissues.[5]

van Dijk et al. (2014) observed that while alpha-defensins are generally upregulated during active periodontal disease, their expression decreases in severely inflamed tissues, possibly due to overwhelming bacterial load and immune evasion strategies by periodontal pathogens. This indicates a complex interaction between alpha-defensins and periodontal pathogens.[6]

Yoshinari et al. (2013) explored the antimicrobial properties of alpha-defensins against key periodontal pathogens. Their study demonstrated that alpha-defensins effectively inhibit the growth of bacteria such as *Porphyromonas gingivalis* and *Treponema denticola*, underscoring their importance in controlling periodontal infections.[5,6.]

Vardar-Sengul et al. (2015) studied the dual role of alpha-defensins in periodontal disease, focusing on their antimicrobial and immunomodulatory effects. They found that alpha-defensins not only combat pathogens but also modulate the inflammatory response by influencing cytokine production and the recruitment of immune cells. This dual function highlights their potential as therapeutic targets for managing inflammation in periodontal disease.[6]

B. Beta-Defensins

Beta-defensins are expressed by epithelial cells lining the oral cavity. They act as natural antibiotics, helping the body to ward off infections by targeting a wide range of pathogens. Beta defensins usually resist degradation in harsh environments, such as on the skin or in the respiratory tract due to their structural stability. Their antimicrobial mechanism primarily involves disrupting the integrity of microbial membranes, leading to the formation of pores and eventual cell lysis. Human beta-defensins (hBD) 1-4 have been identified in gingival tissues and saliva. These peptides are constitutively expressed and can be upregulated in response to microbial challenge or inflammatory cytokines. Beta-defensins not only possess antimicrobial properties but also modulate the immune response by acting as chemoattractants for immune cells.[7] Beta defensins are essential in maintaining healthy mucosal barriers and

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preventing microbial overgrowth.[8] Altered expression of beta defensins has been linked to various diseases. For example, reduced levels of hBD-1 are associated with chronic inflammatory conditions like cystic fibrosis, while hBD-2 expression is often increased in inflammatory skin diseases such as psoriasis. [11]

Diamond et al. (2008) investigated the antimicrobial properties of human beta-defensins (hBD) against common periodontal pathogens. The study found that hBD-1, hBD-2, and hBD-3 exhibited strong antimicrobial activity against bacteria such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, which are known to contribute to periodontal disease. This suggests that beta-defensins are critical in controlling bacterial populations and preventing infections in the oral cavity.[8]

Dunsche et al. (2001) conducted a study examining the expression of beta-defensins in healthy and diseased gingival tissues. They found that beta-defensin expression is upregulated in response to microbial challenge and inflammation, indicating their role in the host defense mechanism against periodontal pathogens. The presence of beta-defensins in healthy gingiva highlights their preventive role in maintaining periodontal health.[8,9]

Ji et al. (2007) explored the regulation of beta-defensins in response to inflammatory stimuli and bacterial products. Their findings showed that pro-inflammatory cytokines, such as IL-1β and TNF-α, can upregulate beta-defensin expression in gingival epithelial cells, enhancing their antimicrobial defense against oral pathogens.[10]

Krisanaprakornkit et al. (2002) studied the immunomodulatory roles of beta-defensins in periodontal health. The study demonstrated that beta-defensins not only possess antimicrobial properties but also act as chemoattractants for immune cells such as dendritic cells and T lymphocytes. This recruitment helps initiate and modulate the immune response, contributing to the maintenance of periodontal health.[11]

III. AMOUNT AND DISTRIBUTION

Defensins make up about 5-7% of the total cellular protein in human neutrophils and 18% in rabbit neutrophils. Although the specific defensin proteins in murine myeloid cells have not been extensively studied, high levels of mRNA for a typical defensin, known as "cryptdin," have been found in the Paneth cells of the mouse small intestine. These cells, which also produce lysozyme, likely play a role in maintaining the gut's antimicrobial barrier. Additionally, proteins similar to defensins, referred to as "insect defensins," have been isolated from the hemolymph of infected or injured insects. This suggests that defensin-like molecules may be ancient components of host defense across the animal kingdom.[1,2]

- *Topical Applications*

The use of topical formulations containing synthetic or recombinant defensins has been investigated for the treatment of periodontal disease.[11] These formulations can be applied directly to periodontal pockets or incorporated into oral hygiene products, such as mouthwashes and toothpaste, to enhance their antimicrobial efficacy.[12] Clinical studies have demonstrated the potential of defensin-based formulations in reducing plaque accumulation and gingival inflammation. **Hancock and Sahl** (2006).[13,14]

Joly et al. (2013) investigated the application of a defensin-based gel in patients with chronic periodontitis. Their study found that the gel significantly reduced the bacterial load in periodontal pockets, particularly targeting key periodontal pathogens like *Porphyromonas gingivalis*. This suggests that topical defensins could be an effective adjunct to scaling and root planing in periodontal therapy.[14]

Niyonsaba et al. (2007) explored the role of defensins in wound healing and tissue regeneration. Their study demonstrated that defensins not only possess antimicrobial properties but also promote epithelial cell migration and proliferation, which are essential for wound healing. The application of defensins to periodontal wounds could therefore enhance healing and reduce recovery time.

van der Weerden et al. (2012) studied the effects of beta-defensins on the healing of periodontal tissues. They found that beta-defensins, when applied topically, accelerated the closure of periodontal wounds and reduced inflammation, making them promising agents for enhancing periodontal regeneration.[15]

Schaefer et al. (2016) reviewed the clinical potential of defensins as topical agents in periodontal therapy. The study highlighted that defensins could be formulated into various delivery systems, such as gels, mouthwashes, or controlled-release devices, to enhance their application in periodontal pockets. However, challenges such as peptide stability and potential for resistance were noted, suggesting the need for further research and development.[15]

- *Gene Therapy*

Gene therapy approaches aim to enhance the expression of endogenous defensins in periodontal tissues. This can be achieved through the delivery of genetic material encoding defensins to gingival cells using viral or non-viral vectors. Preclinical studies have shown promising results in enhancing the antimicrobial and anti-inflammatory response in periodontal tissues, although further research is needed to evaluate the safety and efficacy of this approach in humans.[16]

According to **Schroeder et al. (2010)** who conducted pioneering research on the delivery of defensin genes to periodontal tissues using viral vectors. The study demonstrated that introducing the beta-defensin 3 gene into periodontal tissues significantly enhanced antimicrobial activity against key periodontal pathogens, such as *Porphyromonas gingivalis* and *Treponema denticola*. This approach showed promise in reducing bacterial colonization

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and preventing the progression of periodontal disease. **Zhang et al. (2012)** explored the use of gene therapy to overexpress human beta-defensin 2 (hBD-2) in periodontal tissues. Their study found that hBD-2 not only exhibited strong antimicrobial properties but also promoted the proliferation and differentiation of periodontal ligament cells, which are essential for tissue regeneration. This dual function suggests that defensin gene therapy could be beneficial for both controlling infection and enhancing the regeneration of periodontal tissues.[16,17.]

➢ *Combination Therapies*

Combining defensins with other therapeutic agents, such as antibiotics or anti-inflammatory drugs, may enhance their efficacy in treating periodontal disease. Defensins can synergize with antibiotics to enhance their antimicrobial activity and reduce the risk of antibiotic resistance. Additionally, defensins may complement anti-inflammatory therapies by modulating the immune response and promoting the resolution of inflammation.[16,18]

IV. CHALLENGES AND FUTURE DIRECTIONS

Despite the promising potential of defensins in periodontics, several challenges need to be addressed to fully realize their therapeutic applications.

A. Stability and Delivery

Defensins are susceptible to proteolytic degradation, which may limit their stability and efficacy in the oral environment. Developing delivery systems that protect defensins from degradation and ensure their sustained release at the site of infection is crucial for their successful application in periodontal therapy.[18]

B. Safety and Immunogenicity

The safety and immunogenicity of defensin-based therapies need to be thoroughly evaluated to ensure their safe use in humans. Although defensins are naturally occurring peptides, their synthetic or recombinant forms may elicit immune responses or exhibit cytotoxic effects at high concentrations.

C. Clinical Trials

Rigorous clinical trials are needed to assess the efficacy and safety of defensin-based therapies in periodontal disease. These trials should evaluate not only the antimicrobial and anti-inflammatory effects of defensins but also their impact on clinical parameters, such as pocket depth reduction and attachment gain.[19]

V. CONCLUSION

Defensins play a crucial role in maintaining periodontal health by exerting antimicrobial and immunomodulatory effects. Their potential as therapeutic agents in periodontics is supported by preclinical and clinical studies, although further research is needed to address the challenges associated with their application. Advances in delivery systems, gene therapy, and combination therapies hold promise for harnessing the full potential of defensins in the management of periodontal disease. As our understanding of defensin biology continues to evolve, these peptides may emerge as valuable tools in the fight against periodontal infections and inflammation.

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