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The virulence system of *Porphyromonas gingivalis*: Genes, mechanism and potential role of gingipains inhibitors

Mohammad Auwal Sa'ad[1], Ramasamy Kavitha[2], Neeraj Kumar Fuloria[3], Shivkanya Fuloria[3], Manickam Ravichandran[4]* and Pattabhiraman Lalitha[1]

[1]Department of Biochemistry, Faculty of Medicine, AIMST University, Kedah 08100, Malaysia.
[2]Department of Biotechnology, Faculty of Applied Science, AIMST University, Kedah 08100, Malaysia.
[3]Pharmaceutical Chemistry Unit, Faculty of Pharmacy, AIMST University, Kedah 08100, Malaysia.
[4]Centre of Excellence for Omics-Driven Computational Biodiscovery (COMBio), Faculty of Applied Science, AIMST University, Kedah 08100, Malaysia.
Email: ravichandran@aimst.edu.my

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ABSTRACT

The oral microbiome comprises several hundreds of bacterial species that contribute to periodontitis, the most complex polymicrobial inflammatory disorder. *Porphyromonas gingivalis* is a prominent periodontitis pathogen that produces gingipains as a major virulent factor. Gingipain facilitates *P. gingivalis* survival, pathogenicity, and growth. Several genes were identified to have a role in the regulating of *P. gingivalis* pathogenesis. Studies suggest that gingipains inhibition is key for the successful treatment of periodontitis. As of now, several gingipain inhibitors have been developed, some exhibit high inhibition activity against gingipains. However, most inhibitors offer unknown toxicity and undesirable side effects. Hence, the development of highly potent and safe gingipain inhibitor is a major concern for periodontitis treatment. The present review highlights the connectivity between *P. gingivalis*, virulent factors, and its gene, periodontitis, and gingipain inhibitors. Development of gingipains inhibitors would not only treat periodontitis but would also assist in the treatment of other associated systemic diseases, for example: rheumatoid arthritis, cardiovascular diseases, diabetes, and Alzheimer's disease.

Keywords: Gingipains, *Porphyromonas gingivalis*, periodontal disease, gingipains inhibitors

INTRODUCTION

Periodontal disease (PD) is a group of complex, multifactorial, polymicrobial disease that infects tissue around the teeth (How *et al*., 2016). PD causes swelling of gums, a tendency to bleed, unpleasant breath, alveolar bone loss (that surround teeth), and can lead to teeth loss. This occurs due to the continuous growth of microorganisms over the tooth's surfaces along with the over-aggressive immune response (Olsen and Potempa, 2014). The oral cavity has several hundred bacterial species inclusive of red-complex bacteria associated with PD (Diaz *et al*., 2016). PD is a public health concern as it is prevalent in both developed and developing nations and affects 20-50% of the global population. Smoking, poor oral hygiene, and heredity are the primary risk factors associated with PD (Nazir, 2017). Globally, chronic PD is ranked as the 6th most common disease with an overall prevalence of 11.2% (Tonetti *et al*., 2017). The continuous progression of PD is associated with the biofilm in the gingival sulcus. However, the genetic variability of the host could be a factor for the maturation of PD (da Silva *et al*., 2017). Factors that lead to PD range from microbial to host to environmental to genetic level (Cavalla *et al*., 2018). Some human genetic factors have been identified and linked to PD, but their prevalence across the global population is still under investigation to explain their roles (Bonner *et al*., 2018). Apart from gene polymorphism, some cytokines like interleukins are essential for the occurrence of both chronic and aggressive PD (Li *et al*., 2018). Facts suggest oral space has around 700 bacterial species in subgingival biofilms. Some important periodontal species include *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella nigrescens*, and *Treponema denticola* (Rafiei *et al*., 2018). Most bacterial species play a role in the initiation of PD (Table 1). Among all of the bacterial species, *P. gingivalis* is reported for its strong association with initiation, maturation, and continuation of PD (Rafiei *et al*., 2018).

***Corresponding author**

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Table 1: List of microbes associated with periodontal disease. Modified from How *et al*., (2016).

Bacteria group	Genus/species
Gram-positive	
Aerobic/ facultative	*Streptococcus* (*S. gordonii, S. mitis*, *S. salivarius*)
	Staphylococcus (*S. aureus*, *S. epidermidis*)
	Enterococcus (*E. faecalis*)
	Lactobacillus (*L. casei, L. fermentum*)
	Corynebacterium (*C. matruchotii*)
	Actinomyces (*A. naeslundii, A. israelii*, *A. viscosus*)
Obligate anaerobes	*Propionibacterium* (*P. acnes*)
	Peptostreptococcus (*P. micros*, *P. anaerobius*)
	Eubacterium (*E. nodatum*)
	Parvimonas (*P. micra*)
Gram-negative	
Aerobic/ facultative	*Campylobacter* (*C. rectus*, *C. concisus, C gracilis*), *Actinobacillus* (*A. actinomycetemcomitans*)
Obligate anaerobes	*Porphyromonas* (*P. gingivalis*)
	Fusobacterium (*F. nucleatum*)
	Prevotella (*P. oralis, P. intermedia*)
	Tannerella (*T. forsythia*)
	Treponema (*T. denticola*)

Porphyromonas gingivalis

Porphyromonas gingivalis is a Gram-negative, rod-shaped, non-motile oral anaerobic bacterium that causes PD (Bostanci and Belibasakis, 2012). *Porphyromonas gingivalis* invades periodontal tissues locally, eludes the host mechanism for its survival, and avoids immune surveillance. Binding and colonization of *P. gingivalis* over tooth surface leads to periodontal pocket and biofilm formation (Hussain *et al*., 2015). Over the years *P. gingivalis* has been demonstrated as a key factor in the initiation, development, and continuation of chronic PD (Darveau *et al*., 2012). Studies suggest *P. gingivalis* as a risk factor to manifest in dementia and Alzheimer's disease (AD) (Dominy *et al*., 2019), promote oral cancer development (Wu *et al*., 2018), and develop insulin resistance and diabetes mellitus (Ishikawa *et al*., 2013). PD is also known to mediate several systemic diseases, for example: diabetes, cardiovascular disorders, osteoporosis, and respiratory infections (Figure 1), (Padmalatha *et al*., 2016).

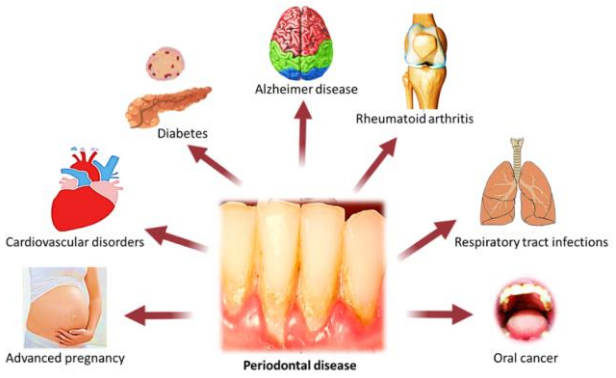

Figure 1: Systematic diseases associated with periodontal disease.

Porphyromonas gingivalis virulence factors

Porphyromonas gingivalis is unable to breakdown carbohydrates for energy. It relies on protein degradation (to generate amino acids and production of metabolic energy) and micronutrients such as vitamin K. *Porphyromonas gingivalis* is known to express a range of virulence factors (Figure 2), the molecules that contribute in the establishment and continuation of species associated with or within the surroundings of the host. Although virulence factors of *P. gingivalis* are harmful to the host, yet they assist in the development of symbiotic relations between microorganisms and hosts. Additionally, based on the environment by altering its virulence factors gene expression, the *P. gingivalis* can adjust to either more or less virulent phenotype (Khalaf *et al*., 2017).

Lipopolysaccharide (LPS)

The lipopolysaccharide (LPS) is an important virulent factor of *P. gingivalis* that is found on the outer membrane of the bacteria. LPS has the potential to cause inflammation in periodontal tissue (Nakao *et al*., 2014). *Porphyromonas gingivalis* has two distinct lipopolysaccharide macromolecules namely; O-LPS (with O-antigen tetrasaccharide repeating units) and A-LPS (with anionic polysaccharide (APS)) repeating units (Rangarajan *et al*., 2008). LPS activates host inflammation and thereby activates innate defense response. LPS initiates the expression of various gingival fibroblast intra-cellular proteins (that includes tyrosine kinase) and upregulates the monocyte chemo-attractant protein (MCP-1). These activates extracellular signal-regulated kinase (ERK-1 and 2), interleukin (IL-1) receptor-associated kinase (IRAK), nuclear factor-κB (NF-κB), and activator protein-1 (AP-1). The LPS when binds to CD-14 and toll-like receptor (TLR-4) on gingival fibroblast (GF) causes activation of the second-messenger system. LPS activates highly innate immune response receptor TLR-2 and TLR-4 on the host cell surface that leads to the secretion of IL-1, IL-6, IL-8, and

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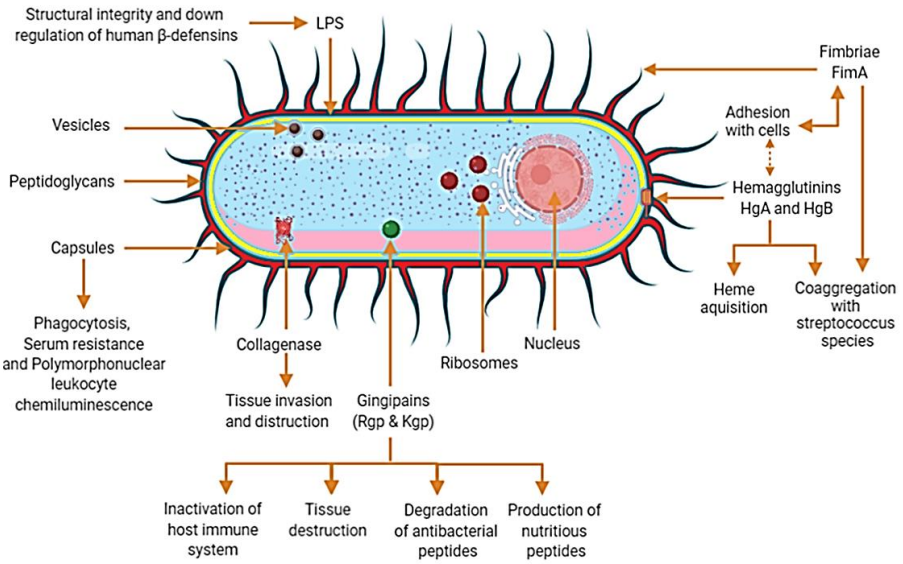

Figure 2: Mechanism of action of gingipain and other virulence factors. This figure is adapted from the research work of Kumbar *et al*.(2020).

TNF-α in host cells (Andrukhov *et al*., 2014; Nakayama and Ohara, 2017). It also has alkaline phosphatase activity, production of osteocalcin, and mineralization in periodontal ligament stem cells, that are positive for STRO-1 and stage-specific embryonic antigen-4 (SSEA-4). In terms of genes, *gtfC*, *gtfD*, *gtfE*, and *gtfF* which encodes glycosyltransferases that are involved in LPS synthesis in *P. gingivalis*, it was found that the disruption of *gtfC* and *gtfF* causes A-LPS deficiency. It has been demonstrated that for O and A-LPS production, *gtfE* is required, *gtfC* and *gtfD* may work together to synthesise two forms of LPS. Moreover, *gtfF* is essential for A-LPS production (Shoji *et al*., 2018).

Adhesin domains

Colonization of *P. gingivalis* in the subgingival region is a multistep and multistage process involving distinct gene products. The hemagglutinins, proteinases, and fimbriae, are the collections of adhesins that facilitate bacterial growth and retention over the tooth surfaces (Lamont and Jenkinson, 2000). K1-adhesin module exhibits higher *in vitro* hemolytic potential and plays a crucial role in erythrocytes recognition that contributes to Kgp's hemolytic action. The K1 adhesin module's binding ability to haem-albumin with higher selectivity and affinity indicates involvement of such domain in the gingipain assisted haem acquiring haem-albumin (Ganuelas *et al*., 2013). Facts suggest C-terminal adhesin domain to be encoded with *Rgp* gene A, which contains 4 domains namely: Hgp-15, Hgp-17, Hgp-27, and Hgp-44 (Nakayama, 2007).

Capsule

Porphyromonas gingivalis uses its capsule to evade phagocytosis, invade keratinocytes and enable its survival. The capsule of *P. gingivalis* functions as a distinctive virulent factor in an infection assorted with other organisms like the *Fusobacterium nucleatum*. The PD assortment with *F. nucleatum* makes it more brutal attributed to the capsule dependent co-aggregation (Polak *et al*., 2017). Previous research shows that enhanced encapsulation corresponds to low polymorphonuclear leukocyte chemiluminescence induction and high serum/phagocytosis resistance (Singh *et al*., 2011). The *P. gingivalis*'s capsular polysaccharide reduces the inflammatory immune response of the host attributed to the interface between pathogen and host, which allows *P. gingivalis* to bypass the immune system (Brunner *et al*., 2010). The *P. gingivalis* capsule contributes to its survival via the reduction of defensins bactericidal activity (Igboin *et al*., 2011). Seven distinct capsular serotypes have been described (K1–K7). In terms of chemical composition, K1 (strain W50) comprises mannuronic acid (ManA), glucuronic acid (GlcA), galacturonic acid (GalA), galactose, and N-acetylglucosamine (GlcNAc). The K-antigens are likely extra-cellular polysaccharides representing the capsular structure of *P. gingivalis*. Although they do not cross-react with K1, K2, or K3 immune-sera of *P. gingivalis*, except for the K2 antiserum, which partially recognized K5- and K6-antigens. On the contrary, K5 and K6 antisera do not react with the K2-antigen. Furthermore, there is a cross-reactivity after K2 antiserum absorption with cells of HG 1690 (K5) and HG 1691 (K6) strains. Although there is a difference of virulence within a capsular serotype, nevertheless, the capsule is important in determining *P.*

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gingivalis virulence. By mutation studies, the gene *epsC* (a capsular polysaccharide biosynthesis gene), which is known to encode UDP-GlcNAc 2-epimerase has a role in capsule formation (Laine *et al*., 1996; Gibson and Genco, 2006).

Fimbriae

Porphyromonas gingivalis uses fimbriae to adhere and invade the targeted sites and mediate and interact with host tissue. The fimbriae can bind with human saliva bio-components, extracellular proteins, commensal bacteria, and cellular α5β1-integrin. *Porphyromonas gingivalis* expresses two distinguish fimbria-molecules (long and short fimbriae), on the cell surface. The two molecules are associated with the development of PD. *fim*A gene encodes fimbrial proteins. Long fimbriae can be classified into six groups based on the diversity of *fimA* genes encoding FimA. The short and long fimbriae cause expressions of several cytokines, like IL-1α, IL-β, IL-6, and TNF-α that results in resorption of alveolar bones (Enersen *et al*., 2013). The fimbriae act as an important factor in atherosclerosis progression.

Proteases

Porphyromonas gingivalis produces a proteolytic enzyme that leads to PD. It neutralizes the immune defense system by hydrolyzing a variety of tissue and serum proteins using its proteases leading to tissue destruction (Grenier and Dang, 2011). *P. gingivalis* peptidyl arginine deiminase (PPAD) is one of the protease group of virulence factors which plays a role in the pathogenesis of rheumatoid arthritis (RA) (Aliko *et al*., 2018). Recent investigation reports *P. gingivalis* protease is able to activate G-protein-coupled receptor (an inflammatory mediator), activate a protein-activated receptor, and regulate the innate immune response leading to periodontal inflammation (Zhang *et al*., 2015).

Exopeptidases

The exopeptidases is an enzymes that catalyze the removal of the amino acid from the end terminal of a polypeptide chain. On the other hand, the endopeptidases enzymes cleave a peptide bond between nonterminal amino acids (Sakamoto *et al*., 2014). Exopeptidases consists of acyl peptidyl oligopeptidase (AOP), dipeptidyl peptidases (DPPs), and tripeptidyl peptidase. They assist *P. gingivalis* in obtaining proteinaceous nutrition from the mixed-species environment of the subgingival sulcus. In contrast, endopeptidase assist *P. gingivalis* in host invasion (Nemoto and Ohara, 2016).

Dipeptidyl peptidase (DPP)

Dipeptidyl peptidases (DPPs) are exopeptidases that cleave a dipeptide from the N termini of oligo- and polypeptides. *Porphyromonas gingivalis* utilizes DPP for its carbon source which is essential for its growth and development (Hromic *et al*., 2017). In *P. gingivalis*, DPP4 is encoded by the *dpp*4 gene which is known to be involved in the regulation of blood glucose levels by cleaving incretins in humans. In general, periodontal bacteremia may aggravate diabetes mellitus due to the degradation of incretins by DPP4 (Ohara *et al*., 2017).

Collagenase

Porphyromonas gingivalis expresses proteolytic enzymes in the form of collagenase on its surface, where they can encounter host cells and tissues (Holt *et al*., 1999). Collagenase is the major enzyme involved in invasion and tissue destruction (Bedi and Williams, 1994). The previous study identified the *prtC* gene with an amino acid sequence corresponding to a protein of 37.8 kDa from ATCC 53977 strain of *P. gingivalis* to be responsible for the expression of collagenase activity (Kato *et al*., 1992).

Gingipains

Porphyromonas gingivalis produces gingipains that are accountable for the regulation of its pathogenicity, growth and development, and interaction with some other species present in oral biofilm (Bao *et al*., 2014). Gingipains are a set of cysteine proteinases that are essential to adhere and colonize the epithelial cells, haem-agglutination and hemolysis, inflammatory responses manipulation, and degradation of proteins and host tissues (Li and Collyer, 2011). *P. gingivalis* manipulates innate immune responses thereby promotes chronic inflammation (Benedyk *et al*., 2016). Gingipains does not only coordinate diverse function which promotes bacterial survival, but it also activates kallikrein/kinin cascade, foster dysregulation of coagulation and complements cascade. It may also inactivate host proteinase inhibitors and degrade immunoglobulins (Singh *et al*., 2018). Gingipain activity influences the process of Th17 differentiation, however, it depends on the blocking of signaling through IL-6. It also influences the T-cells skewing towards Th17 cells via gingipains inactivation (Glowczyk *et al*., 2017).

Lys-gingipain (Kgp) and Arg-gingipain (Rgp)

Porphyromonas gingivalis is reported to produce a distinct group of gingipains, such as Lys-gingipain (Kgp) and Arg-gingipain (Rgp). *Porphyromonas gingivalis* is known to mediate host cell response and the subsequent intracellular signaling in an infected cell using these gingipains. The predominant virulence of *P. gingivalis* is attributed to its gingipains. Gingipains disrupts the host immune system, degrades the host tissues and plasma proteins, and results in an increased risk of PD (Liu *et al*., 2017). Overall, the proteases of Kgp and Rgp contribute to about 85% of the proteolytic function of *P. gingivalis*. There are two distinct but associated genes that encode Rgp proteases, namely: *rgpA*, and *rgpB*. Gingipains are grouped into Rgp (arginine-dependent gingipain R) and Kgp (lysine-dependent gingipain K). Gingipains has the

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following domains, namely: the signal peptide, the N-terminal domain, the catalytic domain (CD), the immunoglobulin superfamily-like domain (IgSF), the hemagglutinin/adhesion (HA) domain, and the C-terminal domain. The proteins RgpA (95 kDa) and RgpB (50 kDa) and Kgp (105 kDa) share almost similar catalytic domains. However, RgpB differs in that it does not contain a hemagglutinin/adhesion domain. RgpA has four HA domains (called RgpAA1 to RgpAA4) located in the middle of the IgSF and C-terminal domain. Kgp also has 3–5 such domains (called KgpAA1 to KgpAA5) in the light of different bacterial strains (Figure 3) (Jia *et al*., 2019).

These proteases functions for *P. gingivalis* infection and housekeeping, including the uptake of amino acids from host proteins and fimbriae maturation (Veillard *et al*., 2012). It is known that in PD, the *P. gingivalis* LPS is the major factor that induces osteoclasts genesis and loss of alveolar bones. Recent evidence suggests that Kgp also contributes to alveolar bone loss in periodontal disease (Yasuhara and Miyamoto, 2011). Recent studies determined three proteins, namely: Sov (*P. gingivalis* 809-810), *P. gingivalis* 534, and *P. gingivalis* 27. These proteins are important for gingipains activity and secretion. Among these proteins, Sov and *P. gingivalis* 27 protein are important factors for the growth and development of *P. gingivalis*. Therefore, besides gingipains, other factors that influence gingipain secretion are also important therapeutic targets (Saiki and Konishi, 2012). Through genome analysis, it has been determined that 11 other proteins are involved in gingipains secretion. Facts suggest that among all 11 identified proteins, the PorK, L, M, N, and W were discovered to exhibit sequence similarity when compared with gliding motility protein that belongs to *Flavobacterium johnsoniae* species (Sato *et al*., 2010). The Sov is an outer membrane protein that induces gingipain protease secretion*;* however, it is vulnerable to the extracellular environment (Saiki and Konishi, 2012).

The genome of *Porphyromonas gingivalis* and gingipains

The *Porphyromonas gingivalis* (HG66) genome is 2,441,680 bp with a GC content of 48.3% and it has a total of 2,062 genes with 1,958 predicted coding sequences (CDSs), 53 tRNAs, and 12 rRNAs (Siddiqui *et al.*, 2014). Similarly, the genome of *P. gingivalis* (W83) is 2,343,479 bp, with an average GC content of 48.3% (Nelson *et al.*, 2003). *Porphyromonas gingivalis* (W83) genome analysis uncovered a range of pathways and virulence determinants of this oral pathogen. Among these are at least six putative hemagglutinin-like genes. The genome analysis of *P. gingivalis* also reveals that it can metabolize a range of amino acids and generate many metabolic end products that are toxic to the human gingival tissue and contribute to the development of PD (Nelson *et al.*, 2003). Through comparative genome study, *P. gingivalis* genomes were sequenced from chronic periodontitis patients and healthy periodontal individuals. Results show genetic variability in the hemagglutinin genes. *Porphyromonas gingivalis* in chronic periodontitis patients were found to possess hemagglutinins genes such as *hagA* and *hagC*, whereas healthy periodontal individuals have no *hagA* and only one copy of *hagC*. Further findings revealed lower hemagglutination ability of *P. gingivalis* in healthy periodontal individuals compared to chronic periodontitis individuals. Although *P. gingivalis* in chronic periodontitis patients encodes a gene for a major fimbrium subunit FimA type 4, healthy periodontal individuals possess a FimA type 1. Results show that healthy periodontal individuals strain have lower biofilm formation and less intracellular invasion to oral epithelial cells compared to the virulent strain from chronic periodontitis patients (Mendez *et al*., 2019). Hence, it can be suggested that different strains might contain different genes and possess different virulence factors.

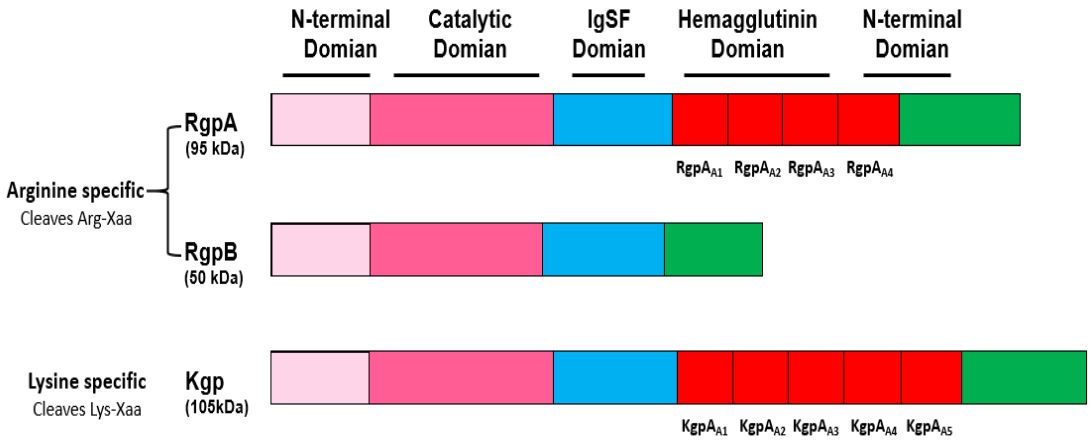

Figure 3: Structure of the gingipain proteins with various domains (Figure not to scale) (Adapted from Gibson and Genco, 2006 and Jia *et al*., 2019).

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In vivo study using mice revealed *P. gingivalis* (TDC60) to exhibit high pathogenicity to cause an abscess in comparison to strains of W83, ATCC 33277, and others. TDC60 would have attained protein-coding sequences through horizontal gene transfer from multiple periodontal pathogenic species.

A previous study determined the complete genome sequence of the TDC60 strain using pyrosequencing, and the Sanger sequencing method. The genome of TDC60 strain was found to contain a single circular chromosome (2,339,898 bp; 48.34% GC content). Further analysis of the chromosome revealed that they contain 2,220 protein-coding sequences, 4 rRNA operons, 53 tRNA sequences, and 9 noncoding RNAs (Watanabe *et al*., 2011). When the *P. gingivalis* A7A1-28 strain genome was compared with the other strains (ATCC 33277, W83, and TDC60), the protein sequences revealed that A7A1-28 possesses 119 strain-specific protein-coding sequences, of which 98 were annotated as hypothetical proteins. Genome synteny experiment revealed that gene order in A7A1-28 resembles that of A7436 and AJW4, suggesting that local mutations may produce unique phenotypes observed in A7A1-28 (Xie *et al*., 2017). A study determined 463 genes from *P. gingivalis* (ATCC 33277) that are essential for *P. gingivalis* viability. When the genes were compared to previously studied important genes (364), the 339 genes were found similar and were found shared by several species. Previously, *P. gingivalis* core genomes exhibited to encode 1476 proteins from 1909 genes. The majority of *P. gingivalis* essential genes are similar among bacterial species as a whole (Klein *et al*., 2012). The genome of *P. gingivalis* has been completely sequenced and has revealed the presence of numerous insertion sequence elements and transposons. It is expected that the understanding of the available complete sequence will explain additional virulence genes and how they are regulated (Gibson and Genco, 2006). Understanding the mechanisms involved in gene synthesis and regulation will provide valuable insight in developing therapeutic strategies.

The genes that encode gingipains that are present in the genome of *P. gingivalis* are *Kgp* and *Rgp*A. These genes encode poly-proteins which comprise pro-peptide and catalytic domain with large N- and C-terminal extensions that are required for proteolytic processing at several Rgp and Kgp residues, thereby generating mature enzymes (Hashim *et al*., 2000). It was considered that variation of DNA sequence in the 3'-coding region of the Kgp gene might determine functional biotypes, based on the variations in the non-catalytic C-terminus of the Kgp. The examination of sequential information offered three forms of Kgp gene that were corresponding to *P. gingivalis* strains (HG66, 381, and W83). Further analysis of samples revealed a fourth genotype (W83v) that showed duplication of a sequence. The N-terminal region site of Kgp from HG66 and 381 strains are identical but differ in strains of W83 and W83v. Accordingly, Kgp in W83 and W83v are different. The segment within the W83 and W83v variable region shows a 65% identity to a synthetic 20-amino-acid peptide that can inhibit hemagglutination. However, the same segment is not observed in other strains. Hence, the variation of Kgp may be relevant to the virulence of *P. gingivalis* strains (Nadkarni *et al*., 2004).

Gingipain biogenesis/activation

The gingipains activation and maturation process involves complex processes that are yet to be explained. A study suggests that *P. gingivalis* has a virulence modulation protein that is putative acetyltransferase called VimA protein that participates in gingipains biogenesis. The acetylated lysine residues have recently been identified in gingipains, indicating its role in gingipain biogenesis. Hence, this protein possibly participates in the activation/maturation mechanism of the gingipain pathway (Mishra *et al*., 2018). A previous study suggests that gingipain and some other proteins like sialidase could act with recombinant VimA of *P. gingivalis*. Although sialylation participates in the maturation of proteins, its regulation function for virulence factor in *P. gingivalis* is yet to be explored (Aruni *et al*., 2011). *Porphyromonas gingivalis* W83 strain's *PG0534* gene encodes a novel protein called PG 0534, the deletion of *PG0534* affected Rgp and Kgp activities, reducing 4–22% of its activities, while the activities of exopeptidases DPP-4, DPP-7, and PTP-A were not affected (Saiki and Konishi, 2012).

Gingipain glycosylation

The *Porphyromonas gingivalis* VimF mediates gingipain maturation via the transfer of galactose. Furthermore, the galactosyltransferase that is specific for gingipain glycosylation may be VimF glycoprotein (Muthiah *et al*., 2013). As RgpB mediates glycosylation processes, so RgPB inactivation leads to loss of RgpB, mt-RgpB, and mt-RgpA. Besides this, RgpB is needed for normal post-translational glycosylation of Rgp that are derived from RgpA. Also, these processes are required for enzyme stabilization (Rangarajan *et al*., 2005). The C-terminal domains of CPG70, Peptidylarginine deiminase, P27, and RgpB participate in glycosylation and por-secretion system-dependent translocation (Shoji *et al*., 2011).

Pathophysiological functions of *Porphyromonas gingivalis* proteases

The *Porphyromonas gingivalis* proteases breakdowns various serum and tissue proteins, that contribute to the suppression of the immune system making them important virulence factors in PD development (Grenier and Dang, 2011). The proteases produced by this bacterium is hypothesized to be associated with systemic pathological conditions due to kallikrein (KLK) like proteinase deregulation. This protease can inactivate SPINK6, disturb the KLKs controlling system, providing a link between PD and tumour development (Plaza *et al*., 2016). *Porphyromonas gingivalis* can modify the immune system in a way that it will be favourable for the microbial community to reside. It can also erode innate immunity to

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assist in the survival of other microbes of the periodontal biofilm community (Hajishengallis, 2011). *P. gingivalis* is known to contribute to the initiation/worsening of rheumatoid arthritis (RA) attributed to its ability to cause citrullination (Perricone *et al*., 2019). A recent study detects citrullination in collagen-induced arthritis mice when infected with *P. gingivalis*, but not in control mice. There is greater citrullination in *P. gingivalis* infected collagen-induced arthritis mice as compared to the non-infected mice. The study demonstrated that *P. gingivalis* worsens autoimmune arthritis and increased the expression of citrullinated antigens, although this depends upon the *P. gingivalis* strain. The ability of *P. gingivalis* to mediated citrullination may explain the possible link between PD and RA (Jung *et al*., 2017). Individuals with PD possess a high risk for cardiovascular disease (Liccardo *et al*., 2019). PD might initiate a pathophysiological changes in blood vessel walls and act as a precursor of atherosclerosis in susceptible hosts (Shrihari, 2012). Evidence shows that persistence exposure to oral pathogens toxins induces immune responses that facilitate coronary atherosclerosis and concurrently with other risk factors, it may lead to myocardial infarction and coronary heart disease (Shrihari, 2012). Periodontitis is considered a risk factor for diabetes causing severe complications. However, the role of the *P. gingivalis* in doing so is not fully understood (Wang *et al*., 2016). Besides, *P. gingivalis* causes an increase in blood glucose levels (Ohtsu *et al*., 2019). Recent studies revealed that *P. gingivalis* and its protease gingipains are found in Alzheimer's disease (AD) patient's brains. Infecting mice with *P. gingivalis* resulted in colonization and enhanced production of $A\beta_{1-42}$ in their brains. *In-vitro/ in-vivo* study reveals that gingipains are neurotoxic agents that exert detrimental action over tau proteins (that are required for normal neuronal functioning). Hence, developing a gingipain inhibitor will be the key step to inhibit *P. gingivalis* colonization in the brain and neurodegeneration (Dominy *et al*., 2019). Moreover, the chronic PD may lead to the development of neuro pathogenicity that is consistent with that of AD (Ilievski *et al*., 2018). Evidence suggests a high risk for an individual to develop a sporadic form of AD if the individual is suffering from chronic PD for more than 10 years. Furthermore, if chronic PD is not treated in AD patients, it may result in cognitive decline (Singhrao and Olsen, 2018).

Complement system alteration by Porphyromonas gingivalis

The "keystone pathogen" hypothesis postulates that a low-abundance microbial pathogen can orchestrate inflammatory disease by remodeling a normally benign microbiota into a dysbiotic one (Hajishengallis *et al.*, 2012). *Porphyromonas gingivalis* has the potential to alter complement-Toll-like receptor (TLRs) crosstalk in a process that aids dysbiosis and PD. *Porphyromonas gingivalis,* as a "keystone pathogen", is known to change the count and constituents of oral commensals/microbiota via its interference with innate immunity. These changes in microbiota can cause dysbiosis of the microbial population, which may result in disturbance of host-microbial homeostasis and may cause inflammatory bone loss (Figure 4) (Olsen *et al*., 2017). A noticeable attribute of gingipains is their ability to degrade the multiple complement components. At a low level, gingipains activate the C1-complex which triggers classical pathways. It also releases proteases that activate the complex, contributing to the deposition of C1q over bacterial surface resulting in a local inflammatory reaction and providing the bacteria with a colonization opportunity (Popadiak *et al*., 2007). Increased evidence shows that few bacteria developed resistance against complement systems attributed to the enzyme digestion of complement components. For instance, *P. gingivalis* produces protease that can break down C3 and C5 complement and prevent C3b deposition on the bacterial cell surface (Slaney and Curtis, 2008). Although numerous strategies have been developed by innate immune systems for control of bacterial colonization. Still, bacteria have found their ways to take the advantage of this many approaches to manipulate the immunological strengths into their advantage and thus weakening the immune system and gaining themselves an advantage (Potempa and Pike, 2009). With continuous evidence suggesting how *P. gingivalis* alters the complement system (Figure 4), it's safe to say that there is a need for effective therapeutic intervention that targets the

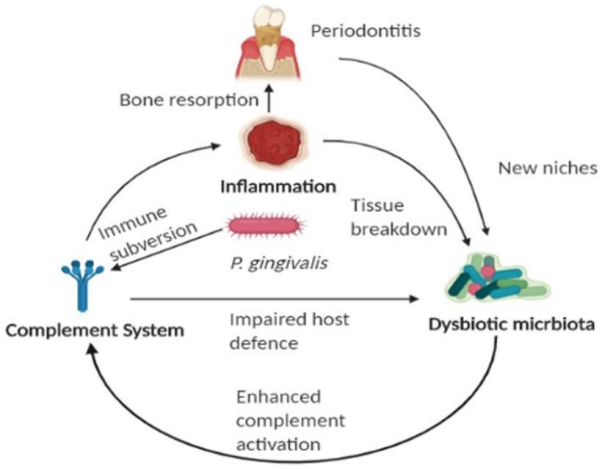

Figure 4: Dysbiosis induction by *P. gingivalis* that leads to periodontal disease. *P. gingivalis* destabilizes complement system, impairs host defense mechanism, causing an overgrowth of oral commensal bacteria. This trigger complement-dependent inflammation, leading to inflammatory tissue destruction providing it with a favorable environment to further grow and enrich with nutrient. Thus, *P. gingivalis* is providing with the perfect condition to flourish and cause bone reabsorption through the dysbiosis of microbiota with new niches for colonization. This figure is adapted from the research work of Hajishengallis *et al*.(2012).

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complement system in periodontal disease (Hajishengallis, 2010).

Porphyromonas gingivalis effect on neutrophils

Porphyromonas gingivalis possess the capacity to destabilize this defense system and turn it to advantage through neutrophils degradation and innate immunity evasion (Olsen and Hajishengallis, 2016). In periodontitis, generally, neutrophils cannot phagocyte the *P. gingivalis* available in the biofilm. While frustrated phagocytosis, the neutrophils induce reactive oxygen species and produce enzymes to destruct pathogens. Inflammatory disruption of gingival tissue and alveolar bone is attributed to these secreted molecules (Sochalska and Potempa, 2017).

Gingipain inhibitors

Gingipain inhibitor is any substance or molecule that can prevent the production of gingipains, thereby slow its progression or prevent it from causing periodontitis and other associated diseases. These substances/molecules can be either synthetic or natural compounds, antibiotics, antibodies, or antiseptics (Table 2). Although a potent and safer gingipain inhibitor is yet to be developed. The gingipains inhibition represents a newer strategy for the treatment and prevention of periodontitis and other systematic diseases associated with it (Olsen and Potempa, 2014). A previous study showed that benzamidine derivatives possess the potential to inhibit the activity of gingipains and also impede the growth of *P. gingivalis* with results supporting the use of benzamidine as a periodontitis treatment (Frohlich *et al*., 2013). Besides, another study revealed that prenylated flavonoids have inhibitory activity against *P. gingivalis* (Kariu *et al*., 2017). The use of add-on systemic antibiotic treatment remains the alternative therapy for periodontitis. The disadvantages of such treatment include the inability of drugs to achieve high gingival crevicular fluid concentration, unknown toxicological effects and it may yield undesirable side effects. Moreover, recent evidence shows that bacteria are developing antibiotic resistance, which is a major drawback (Barca *et al*., 2015).

Protease

Proteins found in rice such as RA17, seed allergens, and alpha-amylases are found to possess inhibition activity against both the Rgp and Kgp. The study suggests rice protein as a useful nutraceutical for the prevention, protection, and management of PD (Taiyoji *et al*., 2009). It does that by inhibiting bacterial proteinase activities. Hence protein extracted from rice could be a potent Rgp inhibitor to prevent periodontitis (Taiyoji *et al*., 2013). Besides, evidence suggests that non-dialyzable material extracted from cranberry juice can also reduce the progression of *P. gingivalis* (Bodet *et al*., 2006). Other protease inhibitors such as leupeptin also assist in inhibition and attenuation of some destructive task of Rgp, which includes inhibition of platelet aggregation and prevention of degradation of LL-37 (Jain, 2017). A mammalian pancreatic secretory Kazal-type trypsin inhibitor can alter and block Kgp activity, as compared to bovine inhibitor, which specifically blocks the activity of Rgp (Bania *et al*., 2008).

Proteins-derived peptides and peptide analogues

Histatin-5 (salivary protein) is known to possess inhibitory activity against several proteinases, among which include cysteine-proteinases gingipains. Histatin-5 shows that it has the potential to treat diseases associated with cysteine-proteases such as cardiometabolic diseases (Gusman *et al*., 2001). Kappa casein (κ-casein (109-137) has been shown to have an inhibitory activity of *P. gingivalis* peptide proteinases. Results show that there's a substantial reduction in the development of lesion in an infected murine model when incubated with κ-casein (Toh *et al*., 2011). Lactoferrin the 80-kDa iron-binding glycoprotein is prevalent in gingival crevicular fluid, tears, and saliva, exhibit high inhibitory activity against gingipains by inhibiting the specific activity of purified RgpB. It also inhibits RgpA/Kgp activity (Dashper *et al*., 2012). Facts suggest that some peptide derivatives synthesized from human saliva histatins exhibit their inhibitory potential against Rgp and Kgp. These peptide analogues exhibit strong inhibition against virulence due to host proteins (immunoglobulin, fibronectin, type-I collagen, and fibrinogen) degradation, poly-morphonuclear leukocytes bactericidal action disruption, and vascular permeability enhancement. Their exceptional inhibition activity presents a wide application to develop new PD treatment (Kadowaki *et al*., 2004). The incubation of *P. gingivalis* with DX-9065a has inhibited its growth activity. The DX-9065a can inhibit RgpA and RgpB amidolytic activity (Matsushita *et al*., 2006). Azapeptides Michael acceptor derivatives also possess high potent inhibition activity against Kgp (Ekici *et al*., 2004).

Antibiotics and antiseptics

Chlorhexidine is the most common and widely used antigingivitis and antiplaque agent. Chlorhexidine acts as an antiseptic because of its anti-bacterial property that may increase permeability (through the bacteria cell membrane) resulting in the disruption of cell lysis (Balagopal and Arjunkumar, 2013). Chlorohexidine has been proven as a productive way of reducing bacteria colonization (Paolantonio *et al*., 2008). Over the years, antibiotics have been integral in treating bacterial infection including *P. gingivalis.* Antibiotics azithromycin and erythromycin are suggested to be effective against *P. gingivalis* attributed to their ability to reduce biofilms formation (Yamamoto *et al*., 2015). Evidence suggests that periodontal pathogens found in chronic PD are susceptible to antimicrobial compounds such as amoxicillin, clindamycin, and metronidazole. However, recent shreds of evidence show that they are developing sensitivity which may suggest their resistant development to the previously known active antibiotic in some

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population (Ardila *et al*., 2010). Despite the challenges and the ability of *P. gingivalis* to develop resistance against other antimicrobial agents, yet some antibiotics like levofloxacin have a significant activity when used (Pradeep *et al*., 2015). Furthermore, moxifloxacin yields a very effective and significant improvement when used in adjunct therapy together with root-planing in treating aggressive periodontitis as compared to the use of only mechanical treatment (Ardila *et al*., 2015).

Plant extracts

Sword bean extract and canavanine inhibit gingipains produced by *P. gingivalis* with an efficacy comparable to that of leupeptin and with lower toxicity compared to chlorhexidine gluconate on KB cells (Nakatsuka *et al*., 2014). *Rumex acetosa* L. extracts are rich in proanthocyanidins and could reduce *P. gingivalis* adherence in a dose-dependent way. The previous study reported that galloylated proanthocyanidins, *R*. *acetosa* and procyanidin B2-di-gallate have the potential to inhibit *P. gingivalis* adherence with the host cell, which protects cells against bacterial infection. Although procyanidin B2-di-gallate and *R*. *acetosa* seem to be a potential prospect for upcoming oral mouth care products, at the moment it appears difficult from the toxicological side to determine what ramification it could have. However, reports indicate no toxicity in the *in-vitro* assay at lower concentrations (Schmuch *et al*., 2015). *Dodonaea viscosa Angustifolia* plant extract is reported to control oral infections and PD. *Dodonaea viscosa Angustifolia* significantly reduced proteinase produced by this bacterium (Rgp (24%) and Kgp (53%). Despite its ability to impair periodontal pathogens, further study needs to be done to find out the substances accountable for its beneficial effects (Patel *et al*., 2013). Catechins from green tea plant extracts also can lessen periodontal break down caused by gingipains. Catechin derivatives are reported to inhibit Kgp activity, but to a lesser inhibition compare to that of Rgp (Okamoto *et al*., 2004). Garlic has a long history to possess high antifungal and antibacterial activity. However, there is a little known on its activities when it comes to oral bacterial species particularly periodontal pathogens or their enzymes. Nevertheless, evidence reveals that garlic extract could inhibit the range of oral microbes and also *P. gingivalis* protease activity (Bakri and Douglas, 2005). The biomolecules like quercetin, resveratrol, catechin, epicatechin, orcinol demonstrated to inhibit *P. gingivalis* fimbriae. However, additional research over various strains of *P. gingivalis* needs to be done to support the aforementioned results (Murakami *et al*., 2015).

Antibodies and vaccines

A study revealed that infecting mice with *P. gingivalis* causes stimulation of a protective immune responsive antibody that is directed to the amino-terminal region of the Rgp catalytic domain (Genco *et al*., 1998). Immunization of egg yolk antibodies isolated from hens yolks show a decrease in hydrolysis activity. Furthermore, there was a dose-dependent adhesion loss by gingipains incubated cells. Gingipains pretreatment with egg yolk antibodies can induce cell detachment in gingipains and can be effective immunotherapeutic agents to treat PD in humans (Yokoyama *et al*., 2007). The *P. gingivalis* vaccination by recombinant RgpA (rRgpA) revealed that peptide can impede the loss of bone and prevent PD induced by *P. gingivalis* (Wilensky *et al*., 2017). Hemagglutinin domain-specific antibodies are actively produced when mice are immunized subcutaneously with RgpA, thereby contributing to the inhibition of PD. Furthermore, immunizing mice using a vaccine of *rgpA* DNA results in resistance development towards invasive *P. gingivalis* (W50) strain. Experimental results revealed that specific antibodies were induced by *rgpA* DNA vaccines against enzymes, and it can offer protection immunity against *P. gingivalis* infection. Moreover, the alveolar bone loss sustained due to *P. gingivalis* infection can be prevented by the *rgpA* DNA vaccine immunization (Miyachi *et al*., 2007).

Other bacteria

Bacteria found in human subgingival may interact with *P. gingivalis,* leading to a reduction of its cytotoxicity to oral epithelial cells, hindering its growth, and altering gingipain activity (Tenorio *et al*., 2011). A recent study reveals that probiotic is a promising treatment approach towards provoked inflammatory disease with a complex polymicrobial aetiology. Although *P. gingivalis* is known to have a different mode of interaction with innate immune responses of the host when compared to other pathogens. Nevertheless, *Lactobacillus rhamnosus* (ATCC9595) could modulate the inflammation signal and introduce *P. gingivalis* to the immune system via induction of CXCL8 secretion (Mendi *et al*., 2016).

CONCLUSION

The oral cavity encompasses around 700 bacterial species inclusive of red-complex bacteria associated with PD. The present review describes the function of *P. gingivalis* and its proteases (gingipains) in the pathogenesis of PD. Although many essential genes that contribute to *P. gingivalis* virulence factors were identified, yet there is a need to understand the mechanisms involved in their biogenesis and regulation. Such understandings will assist in the development of new strategies to treat PD. Evidence suggests several highly potent gingipains inhibitors from synthetic/natural sources, bacteria, antibodies, antibiotics, and antiseptics. Development of gingipains inhibitors not only treats periodontitis but will also aid in the prevention of other associated systemic diseases contributed by PD. However, the majority of gingipain inhibitors are unsuitable for *in-vivo* studies due to the high/unknown toxicity level. Few antibiotics exhibit strong inhibitory activity against *P. gingivalis*, but they offer antibiotic resistance by *P. gingivalis*. At the moment, a safer and highly potent gingipain inhibitor for the *in vivo* study is yet

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a due, nevertheless, synthetic inhibitors exhibit great potential for the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this project.

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