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

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Received 30 July 2020; Received in revised form 18 September 2020; Accepted 4 November 2020

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).

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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-kB (NF-kB), 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

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

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

**Figure 1:** Systematic diseases associated with periodontal disease.
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 mannnuronic acid (ManA), glucuronic acid (GicA), 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.
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 a5β1-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. fimA 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 peptideases (DPPs), and tripeptidyl peptidease. 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 dpp4 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 kalikrein/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
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 Porphyromonas 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).
**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).

**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).

The 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 per-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 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).
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 Aβ1-42 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

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**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).
complement system in periodontal disease (Hajishengallis, 2010).

Porphyromonas gingivalis effect on neutrophils

Porphyromonas gingivalis possesses 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 systemic 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, polymorphonuclear leukocytes bacterial 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). Chlorhexidine 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
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. acerosa* 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. acerosa* 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 (Patei 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 rRgpA DNA results in resistance development towards invasive *P. gingivalis* (W50) strain. Experimental results revealed that specific antibodies were induced by rRgpA 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 rRgpA 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* (ATCC9585) 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
a due, nevertheless, synthetic inhibitors exhibit great potential for the future.

ACKNOWLEDGEMENTS

Authors of the present study acknowledge the fund support provided by Fundamental Research Grant Scheme (FRGS) research grant no: FRGS/1/2018/SKK14/AIMST/01/1, under the Ministry of Higher Education, Malaysia.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this project.

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