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# Biogenesis and function of Porphyromonas gingivalis outer membrane vesicles

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## Abstract

*Porphyromonas gingivalis* is one of the keystone pathogens associated with chronic periodontitis. All *P. gingivalis* strains examined thus far produce outer membrane vesicles. Recent studies have found that vesicles possess some well-known virulence factors of *P. gingivalis* such as adhesins, toxins and proteolytic enzymes. Carrying most of the characteristic features of their parent *P. gingivalis* cells, vesicles communicate with host cells and other members of microbial biofilms, resulting in the transmission of virulence factors into these host cells and the formation of pathogenic bacteria-dominated microbial communities. An in-depth understanding of both the nature and role of vesicles in the pathogenicity of *P. gingivalis* is both important and timely, particularly when speaking of periodontitis and its related systemic effects.

### Keywords

Gram-negative bacteria; interspecies interactions outer membrane vesicles; regulation of OMV biogenesis virulence factors

Most Gram-negative bacteria (both pathogenic and nonpathogenic) secrete outer membrane vesicles (OMV) that can be surface-bound or cell-free spherical structures. Gram-positive bacteria also produce membrane-derived vesicles, such as the oral bacterium *Streptococcus mutans* [1]. OMVs have recently gained special recognition for their role in bacterial virulence and are known to play similar roles as their parent cells with regard to mediation of adherence, biofilm formation, invasion, host cell damage and modulation of host immune responses [2]. More importantly, OMVs appear to confer distinct functional advantages over whole bacterial cells. For example, vesicles that are enriched for bacterial virulence factors are protected from dilution and proteolytic degradation, and are able to travel to distant



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targets [2]. Besides the important role of vesicles in bacterial pathogenicity, there is also an interest in bacterial vesicles for their potential as a natural adjuvant, one that contains a mixture of antigens [3]. Several recent reviews have documented the biogenesis and function of bacterial OMV [4–6].

*P. gingivalis* is a Gram-negative bacterium associated with chronic periodontitis. Vesiculation in *P. gingivalis* was first reported in the 1980s [7–9], although its central function in the physiology and pathogenicity of the organism was not immediately understood. Consistent with vesicles of other Gram-negative bacteria, *P. gingivalis* vesicles are between 50 and 250 nm in diameter but are predominantly around 50 nm (Figure 1). Recent studies using genetic, proteomic and morphologic tools have demonstrated that *P. gingivalis* vesicles may act as intermediaries that carry a wide variety of virulence factors provided by their parent cells. This particular review will focus on the characteristics of vesiculation and the virulence functions of *P. gingivalis* vesicles. Hopefully, this discussion will uncover opportunities to utilize the characteristics of *P. gingivalis* vesicles for at least two distinct purposes, namely to reduce virulence and to engineer vaccines.

## Vesiculation of *P. gingivalis* & its regulation

Biogenesis of bacterial OMV can be understood through several models (Figure 2). First, the bacterial outer membrane is pushed out through a physical force induced by accumulation of misfiled or overexpressed envelope proteins. This model is supported by a study using an *Escherichia coli* strain carrying an inducible DegP protein [10]. DegP is a member of the high-temperature requirement A (HtrA) family and functions as a chaperone at low temperatures in the periplasm of Gram-negative bacteria. McBroom and Kuehn demonstrated that the increased level of vesiculation in wildtype *E. coli* corresponded to the level of DegP overexpression [10]. Furthermore, these authors found that periplasmic protein overexpression-induced vesiculation was not specific to DegP, since there was also overexpression of periplasmic maltose-binding protein. Second, the linkage between the outer membrane and the underneath peptidoglycan layer was disrupted, which led to OMV shedding. This assumption was based on the observation that deficiencies in several proteins involved in the interconnection of the outer membrane and peptidoglycan or in lipopolysacchride formation had a dramatic impact on vesiculation [11,12]. An example is a 6.95 kDa lipoprotein (OprI) of *Pseudomonas aeruginosa* [13]. OprI is an abundant outer membrane protein and appears to covalently interact with the peptidoglycan layer [14,15]. Deletion of the *oprI* gene significantly enhances vesiculation in *P. aeruginosa*, which presumably results from loss of tethering outer membrane to peptidoglycan [14]. Finally, vesiculation may result in an increased local curvature of bacterial outer membrane. This model is supported by recent studies describing a bilayer-couple model involving a signaling molecule 2-heptyl-3-hydroxy-4-quinolone (*P. aeruginosa* quinolone signals [PQS]) of *P. aeruginosa* [16,17]. PQS appears necessary and sufficient for OMV formation through direct interaction with the bacterial membrane and expanding the outer membrane, which increases membrane curvature and leads to formation of vesicles.

*P. gingivalis* has been shown to express OMVs on its cell surface and release them into the environment [7–9]. Since the size of *P. gingivalis* vesicles ranges from 50 to 250 nm in

diameter (predominately 50 nm), it was suggested that similar structures, previously reported in dental plaque samples [18], were likely vesicles derived from *P. gingivalis* or other Gram-negative oral bacteria [8]. Although all *P. gingivalis* strains tested (fimbriated or afimbriated) were observed to display small membranous vesicles budding from the outer membrane [19], the level of vesiculation varied among the strains [20].

Recent publications revealed a fimbrial type or expression-dependent vesiculation in *P. gingivalis*. *P. gingivalis* strains are classified into six types (type I and Ib–V) based on the strain-specific nucleotide sequences of the *fimA* gene that encodes a subunit of major (long) fimbriae [21,22]. When comparing vesicles expressed on the surface of *P. gingivalis* strains with a fimbrial allele type I (33277), type III (49417) and type IV (W83) using transmission electron microscopy, Kerr *et al*. found much more OMV on the surfaces of 33,277 and 49,417 than those on W83 cells [23]. Interestingly, when type IV FimA was introduced into the isogenic background of 33277, vesiculation was similar between the *fimA* parent strain and the recipient strain, suggesting that the fimbrial subtype was involved in vesicle biogenesis. Our recent studies also showed a fimbrial expression-mediated vesiculation in *P. gingivalis*. A higher number of vesicles were isolated in the growth media of *P. gingivalis* 33277 when compared with vesicles found in the media of its *fimA* mutant [20]. In addition, a reduced level of vesicle release was found in the media of W83, an afimbriated strain. The results also revealed that there was a positive correlation between the expression level of FimA and the level of vesicle production in a given *P. gingivalis* strain. Interestingly, a requirement for the synthesis of flagellar proteins for vesicle production was recently found in *E. coli* W3110 [24]. In fact, the deletion or overexpression of several membrane-associated proteins was known to affect vesiculation in many Gram-negative bacteria, likely due to an alteration in the envelope structure and/or a decrease in membrane stability [25].

Besides the expression of FimA, vesiculation of *P. gingivalis* has been linked to the expressions of an OmpA-like protein (Pgm6/7) and galactose 4-epimerase (GalE) [26,27]. GalE is known to catalyze the interconversion between uridine diphosphate (UDP)-glucose and UDP-galactose. It was reported that vesicles could not be found on either the surface of a *galE* mutant or in the growth media when monitored at different time points over 3 days in culture [27]. The authors felt that this could be due to the pleiotropic effects of the *galE* mutation on truncation of LPS O-antigen and nonglycosylated form of the outer membrane Omp85 homolog, which were also observed in the *galE* mutant. By contrast, vesicles were overproduced on the surface and in the media surrounding an *ompA* mutant of *P. gingivalis* [26]. In fact, hypervesiculation was reported in the *ompA* mutants of *Salmonella*, *Vibrio cholerae* and *E. coli* [28]. Although the mechanisms were not completely elucidated, the proteins appeared to play an important role in the maintenance of bacterial outer membrane integrity and cell wall turnover. *E. coli* OmpA, which was found to interact with peptidoglycan [29], presumably crosslinked the outer membrane with peptidoglycan to provide cell wall stability. One of the mechanisms of vesicle biogenesis proposed is the loss of a link between the outer membrane and peptidoglycan [5]. *P. gingivalis* Pgm6/7 proteins share a high degree of similarity to *E. coli* OmpA, and these *P. gingivalis* proteins may also modulate vesiculation of *P. gingivalis* through a similar mechanism as that seen in *E. coli*.

Vesiculation was also modulated in *P. gingivalis*, grown under different conditions. When observed under a transmission electron microscope, *P. gingivalis* W50 grown under hemin limited conditions displayed significantly more vesicles on its surface and released more cell-free vesicles into the surrounding environment than the organism grown under hemin excess conditions [30]. A similar observation was reported from an independent laboratory, in that, a *P. gingivalis* mutant with a deficiency in the utilization and transport of hemin produced twice as many vesicles as its parent strain did [31]. The mutant was also found to be more infectious and invasive than the parent strain when tested in a mouse model. These data suggest that there is an inverse correlation between the extent of vesiculation of *P. gingivalis* and hemin growth concentrations.

Two questions often asked are: why does *P. gingivalis* produce vesicles, and what functional advantage does vesiculation confer upon the bacteria against harmful environmental factors? In an earlier study of *P. gingivalis* vesicles, Grenier *et al*. demonstrated that vesicles interacted with chlorhexidine and, as a result, this interaction protected the organism against antibacterial treatment by acting as a decoy [32]. These authors further identified vesicle LPS as the major component involved in the binding to chlorhexidine. It was also reported that human β-defensin-3 bound specifically to hemagglutinin B of *P. gingivalis* [33]. Human β-defensins produced by gingival epithelia are known for their activity against oral bacteria including *P. gingivalis* [34]. Since hemagglutinin B is a major protein detected in *P. gingivalis* vesicles [20], it is reasonable to speculate that secretion of vesicles may reduce sensitivity of the bacteria to human β-defensin and thus facilitate survival of *P. gingivalis* in the oral environment. In addition, *P. gingivalis* vesicles may also act as decoys to absorb specific antibodies toward the bacteria. Thus, preincubation of vesicles with serum samples from periodontitis patients was able to reduce the immune reactivity of sera against *P. gingivalis* cells [35]. Another likely advantage of vesiculation is that cell associated vesicles serve as an expanded outer membrane, which optimizes membrane functionality. Outer membrane proteins including fimbrial proteins and gingipains are necessary for controlling autoaggregation, microcolony morphology and biovolume, or those processes required for maturation of *P. gingivalis* biofilms [36–38]. Moreover, the lack of cell-associated vesicles was recently linked with a decrease in the autoaggregation of *P. gingivalis*, which provided evidence of an enhanced function of the outer membrane mediated by vesicles [23].

## *P. gingivalis* OMV contents

Bacterial vesicles originate from outer membrane blebbing and contain mostly outer membrane lipids including LPS, outer membrane proteins and some periplasmic and inner membrane components [39]. DNA and RNA are also packed within some bacterial vesicles. Biller *et al*. showed earlier that *Prochlorococcus* vesicles contain DNA fragments covering 50% of the entire chromosomal sequence and RNA from 95% of all open reading frames [40]. Recently, we observed presence of ribonucleic acids in *P. gingivalis* vesicles [41]. DNA fragments of genes encoding the major subunit of fimbriae (*fimA* and *mfa1*), superoxide dismutase (*sod*) and gingipains were detected, using PCR at relatively higher levels. Sizes of some DNA fragments in vesicles appear large enough to encode a virulence factor, such as the *fimA* gene. RNAs were also isolated and identified from purified vesicles of *P. gingivalis*. Furthermore, vesicle-mediated horizontal gene transfer was detected

between *P. gingivalis* strains with efficiency at $1.9 \times 10^{-7}$ [41], suggesting that packing DNA and RNA within vesicles could be one of the driving forces for the evolution of bacteria in the oral cavity ecosystem.

Assay of LPS staining patterns demonstrated that LPS profiles in the envelope fraction were almost identical in both *P. gingivalis* parent cells and their corresponding vesicles [26]. This was not the case when protein profiles of *P. gingivalis* cells and the vesicles were compared. Thus, accumulating evidence indicates that a select protein cargo exists within bacterial vesicles. In general, some outer membrane-associated virulence factors are enriched in vesicles [2]. Gingipains, a group of proteinases produced by *P. gingivalis*, were found preferentially packed into vesicles [42,43]. Therefore, a three- to fivefold enrichment of gingipains was observed in vesicles derived from *P. gingivalis* 33277 and W83, respectively, as compared with levels in their parent bacterial strains [20]. The mechanism(s) responsible for this selective assembly of protein cargos in *P. gingivalis* strains has not been identified. A study by Haurat *et al*. reported that the major outer membrane proteins RagA/B were excluded from vesicles of *P. gingivalis* W50, however, these proteins were detected in the vesicles of a *porS*$^{-}$ mutant with a deficiency in LPS biosynthesis [43]. RagA/B was also undetectable in the vesicles of the *porS*$^{+}$ complement strain. Therefore, the authors suggested that *P. gingivalis*' LPS plays an important role in the selective exclusion of RagA/B from vesicles. It is proposed that LPS may be responsible for compartmentalization of the bacterial surface based on polysaccharide composition or length [43]. Some microdomains that are considered 'hot spots' for vesicle budding selectively contain or have an enriched subset of proteins. A selective protein cargo was also recently identified in *P. gingivalis* vesicles. When comparing protein ratios of vesicles/cell outer membrane using proteomic analyses, Veith *et al*. demonstrated that all proteins with a C-terminal secretion signal (CTD), including gingipains, exhibited mid-to-high ratios in vesicles, indicating an enrichment of these proteins in *P. gingivalis* vesicles, whereas some outer membrane proteins with a peptidoglycan-binding domain such as Omp40 and 41 and RagA/B showed lower ratios and had reduced levels in vesicles [42]. It was proposed that enriched CTD proteins promote formation of an electron dense surface layer via their connection with anionic LPS (A-LPS), and that this electron dense surface layer provides vesicles with a smooth surface layer [44].

Strain variations were found when the protein contents of vesicles derived from *P. gingivalis* 33277, W50 and W83 were analyzed. Not surprisingly, fimbrial proteins (FimA, C, D, E and Mfa1) were exclusively detected in 33277 vesicles, which is consistent with expression of these proteins in *P. gingivalis* 33277 cells [20,35,42]. A most striking finding was that tetratricopeptide repeat (TPR) domain proteins were not detectable in 33277 vesicles (Supplementary Table 1), while four or five TPR domain proteins were found in the vesicles from W50 and W83, even though there are eight genes encoding TPR proteins in the *P. gingivais* genome. Proteins with a TPR domain are found in both eukaryotic and prokaryotic cells and are involved in diverse cellular processes including transcriptional regulation, mRNA processing and protein folding and translocation [45]. Previous studies, using a mouse subcutaneous infection model, showed that expression of TprA protein (PG1385 identified in W50 and W83 vesicles) was upregulated in *P. gingivalis* W83 grown in a

mouse subcutaneous chamber, and that the survival rates of mice infected with the *tprA* mutant were significantly higher than those of mice infected with the parent strain W83, suggesting a role of TprA in *P. gingivalis*' virulence [46]. However, the virulence mechanism of TprA remains to be clarified.

A recent study, using LC–MS/MS, identified 151 proteins from *P. gingivalis* W50, and all but one likely originated from the bacterial outer membrane or the periplasm [42]. This study further classified the proteins based on their known localization; 79/151 proteins were membrane proteins and the rest were assigned either as extracellular, luminal or undetermined. The importance of these findings is that the identification and the understanding of the distinct features of *P. gingivalis* vesicles may provide a molecular basis for the development of a safer vaccine using vesicles, rather than their parental bacterial cells. One of the most successful OMV-based vaccines is the one against *Neisseria meningitides*, which has been clinically used in several countries outside of the USA [47]. There is concern, however, that the complex contents of OMVs could contain risk factors leading to a clinical complication. For example, LPS, especially lipid A (also known as endotoxin), can induce a strong innate immune response in humans and cause a severe complication called endotoxic or septic shock. In the initial development of OMV-based vaccines, efforts have been focused on a reduction in LPS content by detergent extractions, and more recently, OMV vaccines have been developed from bacterial mutants producing an attenuated nontoxic LPS [47]. It is worthy to note that *P. gingivalis* LPS elicits a much weaker immune response compared with *E. coli* LPS and can be agonistic or antagonistic with respect to Toll-like receptor 4 activation based on the lipid A structure in the bacterial subpopulations [48]. Therefore, it is promising to know that investigators may take advantage of some unique features of *P. gingivalis*' LPS to identify and engineer safer liposome-based vaccine.

## Virulence of *P. gingivalis* vesicles

In addition to carrying the major outer membrane contents of *P. gingivalis*, vesicles have shown the primary characteristic features of this organism, such as induction of inflammatory responses, impairment of host cells and transmission of virulence factors into host cells [27,35]. A recent study showed that after treatment with *P. gingivalis* vesicles (5–10 μg $ml^{-1}$) for 1 h, oral epithelial cells in a confluent monolayer were detached from the wells of culture plates [35]. In agreement with these results, we found detachment of human epithelial cells or gingival fibroblasts from wells that were exposed to vesicles (>3 μg $ml^{-1}$) [20]. However, the detachment of the cells was not observed with a lower concentration of vesicles (0.5 μg $ml^{-1}$). The detachment effect was abolished when the vesicles were treated at 100°C for 30 min or in the presence of antigingipain antibodies suggesting a gingipain-dependent host cell impairment [35].

One of important features of *P. gingivalis* is its ability to invade and survive within host cells including fibroblasts, epithelial and endothelial cells [49]. Several well-known membrane proteins involved in *P. gingivalis* attachment to, and invasion of, host cells have been identified within vesicles, including FimA, hemagglutinin A and heat-stress protein (HtrA) [50 –53]. Not surprisingly, therefore, vesicles derived from *P. gingivalis* have been

found to invade human primary oral epithelial cells, gingival fibroblasts and human umbilical vein endothelial cells [20,54–56]. Consistent with the invasive activity of *P. gingivalis* cells, 33277 vesicles, a strain with relatively high invasive activity was found to enter gingival epithelial cells and fibroblasts more efficiently than W83 vesicles. Notably, the effect of vesicle invasion on host cells has not as yet been fully established. However, a depletion of the intracellular transferrin was observed in epithelial cells invaded by *P. gingivalis* vesicles, which resulted from degradation of the cellular transferrin receptor within 1 h after vesicle invasion [54]. Another important question is, if internalization of both *P. gingivalis* cells and their vesicles into human epithelial cells are established using a similar mechanism. It can be said that at least both of them depend upon endocytic pathways for entry [55,57]. One key step of *P. gingivalis* host cell entry appears to involve secretion of the serine phosphatase SerB, which dephosphorylates the Ser 3 residue of the actin-depolymerizing molecule cofilin [58]. However, SerB was not found in *P. gingivalis* vesicles [42]. Thus, it is likely that *P. gingivalis* vesicles utilize a different mechanism for epithelial cell internalization. Another important feature of *P. gingivalis* invasion is the ability of the internalized cells to disseminate from one cell to another through actin-mediated membrane protrusions or an endocytic recycling pathway [57,59]. It will be interesting to determine, if some of the intracellular vesicles can also exit and re-enter other host cells. In opposition to this suggestion, Furuta *et al*. showed that *P. gingivalis* vesicles were sorted to lysosomes after being internalized via an endocytic pathway [55].

Chronic periodontitis is known to be the result of a breakdown of periodontal tissue-microbe homeostasis, which then leads to uncontrolled inflammation. A study using a mouse model demonstrated that after using an intranasal immunization method, vesicles effectively elicited *P. gingivalis*-specific serum IgG and IgA as well as salivary IgA 5 weeks after the initial immunization, whereas whole cells did not [27]. It was suggested that the increased antigenicity found in the vesicles might result from the more concentrated immune-dominant determinants on the vesicles compared with *P. gingivalis* cell surfaces. *P. gingivalis* vesicles are also known to play a role in the induction of inflammatory responses. Kou *et al*. found that stimulation of human gingival epithelial cells with vesicles led to an increased expression of cyclooxygenase-2, IL-6, IL-8 and matrix metalloproteinases (MMP-1 and MMP-3) [60]. Production of IL-8 is well known for its role in innate immune responses in periodontal tissues since this chemokine is involved in the recruitment of neutrophils from the vascularized gingival tissue to the gingival crevice [61]. By contrast, *P. gingivalis* vesicles appeared to repress immune responses induced by IFN-γ [62]. Expression of several genes involved in IFN-γ signal transduction, including genes encoding class II transactivator, Janus kinases (Jak1 and Jak2), were downregulated in vascular endothelial cells in the presence of *P. gingivalis* vesicles [62]. Since major histocompatibility complex class II molecules are essential for antigen presentation, it is likely that inhibition of their expression facilitates *P. gingivalis* escape from immune surveillance. Overall, these studies suggest that the effect of *P. gingivalis* vesicles on the human immune response system is a complicated matter, which may depend on both their strain-specific origins and the growth conditions of the parent cells.

Besides the role of *P. gingivalis* in periodontitis, an association between *P. gingivalis* and atherosclerosis has been extensively investigated *in vitro*, *ex vivo* and in animal models [63–67], which has made this bacterium a model of atherosclerosis pathogenesis initiated by microorganisms [68]. Previous studies have focused on intact *P. gingivalis* cells, based on the discovery of proteins and DNA of this bacterium in *ex vivo* samples. It was speculated that *P. gingivalis* may enter microvasculature following tooth brush or other dental procedures, which may lead to a transient bacteremia [69,70]. However, it has not been confirmed if live cells of *P. gingivalis* cause low-grade inflammation in the walls of arterial vessels. With recent findings, including the efficient invasive activity of vesicles and the presence of vesicle-associated major outer membrane proteins, DNA and RNA in *P. gingivalis* vesicles [20,41,42], it is likely that vesicles serve a significant role in atherosclerosis and represent a 'Trojan horse' to induce infections at secondary sites, such as in the walls of vessels than intact *P. gingivalis* cells. This concept is supported by *in vitro* studies that *P. gingivalis* vesicles, like their originating cells, were able to induce macrophages to form foam cells [71] and to serve as an activator of platelet aggregation [72].

## Interspecies interaction mediated by *Porphyromonas gingivalis* vesicles

Dental plaque is a multispecies microbial biofilm. Interactions between/among different species are established by the specific recognition between each adhesin and its receptor. *P. gingivalis* expresses multiple adhesins that mediate coaggregation with many other oral bacteria [73–75]. All of these well-known adhesive molecules including fimbrial proteins, gingipains and hemagglutinin are found in *P. gingivalis* vesicles. Therefore, once released, vesicles can act as representatives of *P. gingivalis* to communicate with other oral bacteria. Studies of interspecies bacterial interactions revealed that *P. gingivalis* vesicles play a central role in introducing some bacteria to dental plaque (biofilm) and in promoting the microbial diversity of multibacterial structures. One example is that *Staphylococcus aureus*, frequently found in the nasal cavity and pharynx, was able to coaggregate with predominant oral microbes such as *Streptococcus, Actinomyces* and the mycelium-type *Candida albicans*, only after they were treated with *P. gingivalis* vesicles [76]. In another instance, *P. gingivalis* vesicles mediated coaggregation of *Treponema denticola* and *Lachnoanaerobaculum saburreum* [77], a process that did not occur in the absence of *P. gingivalis* vesicles or in the presence of heat-treated vesicles. Thus, it is conceivable that vesicles serve as a tether between these two bacteria. Binding of *P. gingivalis* to *T. denticola* is known to involve *P. gingivalis* fimbriae and *T. denticola* dentilisin [78], however, it is not clear what specific molecular mechanism is involved in the interaction between *P. gingivalis* and *L. saburreum*. Moreover, *P. gingivalis* vesicles were also able to enhance the attachment and invasion of *Tannerella forsythia* to epithelial cells, and showed a greater ability to do this than the originating cells [79]. Although this effect is not fully understood, bacterial molecules enriched in these vesicles are likely involved.

## Conclusion & future perspective

Vesicles carrying most of the bacterial virulence factors are probably the best weapon of *P. gingivalis* for its survival in the oral cavity (Figure 3). As evidenced above, they have

diverse abilities, including the mediation of biofilm formation, organism–host interactions and immune responses. Characteristic features of vesicles, such as concentrated proteinases, virulence factors and the ability to travel to distant sites, may make them more important not only for the initiation and progression of periodontitis but also for the secondary pathophysiological effect of periodontitis-associated systemic disorders. Therefore, manipulating and/or blocking vesiculation of *P. gingivalis* and other periodontitis-associated oral pathogens may be one of therapeutic targets for virulence reduction. Future studies of vesicles may also focus on taking advantage of this acellular organelle to develop vaccines against *P. gingivalis* infection, since virulence mechanisms among different *P. gingivalis* strains vary and are strain specific. A comparison of functionally different vesicles derived from different *P. gingivalis* strains may provide an opportunity to select distinct vesicles, which can be modulated and utilized as a safer vaccine than their parental cells.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Executive Summary

***Porphyromonas gingivalis***

- Porphyromonas gingivalis, a Gram-negative bacterium, is one of the keystone pathogens associated with chronic periodontitis and several systemic diseases.
- All *P. gingivalis* strains examined thus far produce outer membrane vesicles with size ranging from 50–250 nm.

***Porphyromonas gingivalis* outer membrane vesicles contents**

- LPS profiles in the envelope fraction were almost identical in both *Porphyromonas gingivalis* parent cells and their corresponding vesicles.
- DNA and RNA are packed within *P. gingivalis* vesicles, including DNA fragments of genes encoding *fimA, mfa1, sod, rgp* and *kgp*. Sizes of some DNA fragments in vesicles are large enough to encode a virulence factor.
- Major outer membrane-associated virulence factors of *P. gingivalis* are exported through vesicles. Gingipains, a group of proteinases, are found preferentially packed into vesicles.

**Virulence of *Porphyromonas gingivalis* vesicles**

- Vesicles derived from *Porphyromonas gingivalis* are able to invade human primary oral epithelial cells, gingival fibroblasts and human umbilical vein endothelial cells.
- The increased antigenicity found in the vesicles might result from the more concentrated immune-dominant determinants on the vesicles compared with *P. gingivalis* cell surfaces.
- Vesicles serve a significant role in atherosclerosis and represent a ‘Trojan horse’ to induce infections at secondary sites, such as in the walls of vessels than intact *P. gingivalis* cells.
- *P. gingivalis* vesicles were also able to enhance the attachment and invasion of *Tannerella forsythia* to epithelial cells, and showed a greater ability to do this than the originating cells.

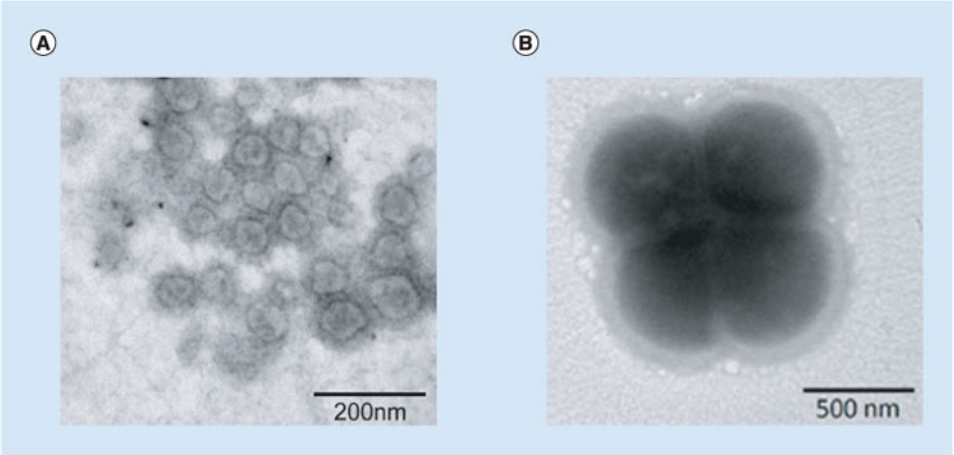


**Figure 1. *Porphyromonas gingivalis* cells and vesicles shown by negative-stain transmission electron microscopy**

**(A)** The purified vesicles from *P. gingivalis* 33277 growth medium, showing the single membrane surrounding spherical structures. **(B)** Outer membrane blebbing on the surface of *P. gingivalis* 33277 cells.

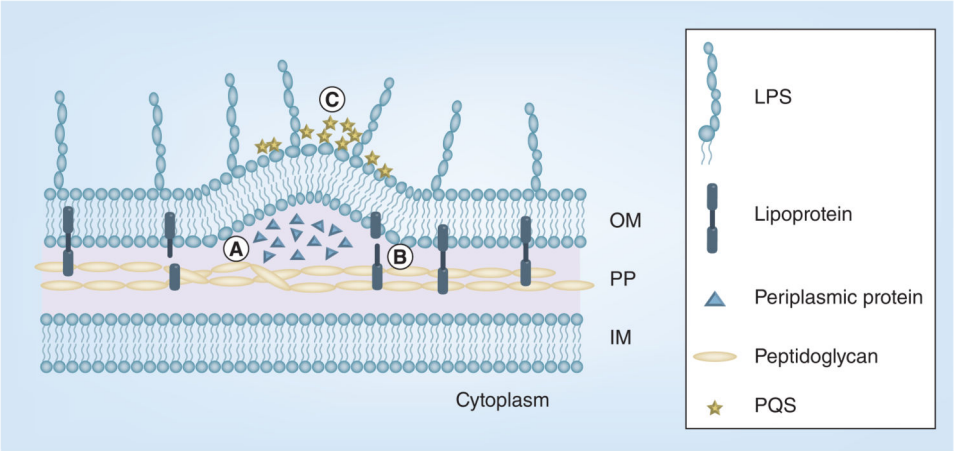


**Figure 2. Models of bacterial outer membrane vesicles biogenesis**

Three models are presented. **(A)** A physical force induced by accumulation of misfiled or overexpressed envelope proteins pushes out outer membrane vesicles. **(B)** The linkage between the outer membrane and the underneath peptidoglycan layer is disrupted. **(C)** Local curvature of bacterial outer membrane is enhanced by extracellular signals including PQS. IM: Inner membrane; LPS: Lipopolysaccharide; OM: Outer membrane; PG: Peptidoglycan; PQS: *P. aeruginosa* quinolone signal.

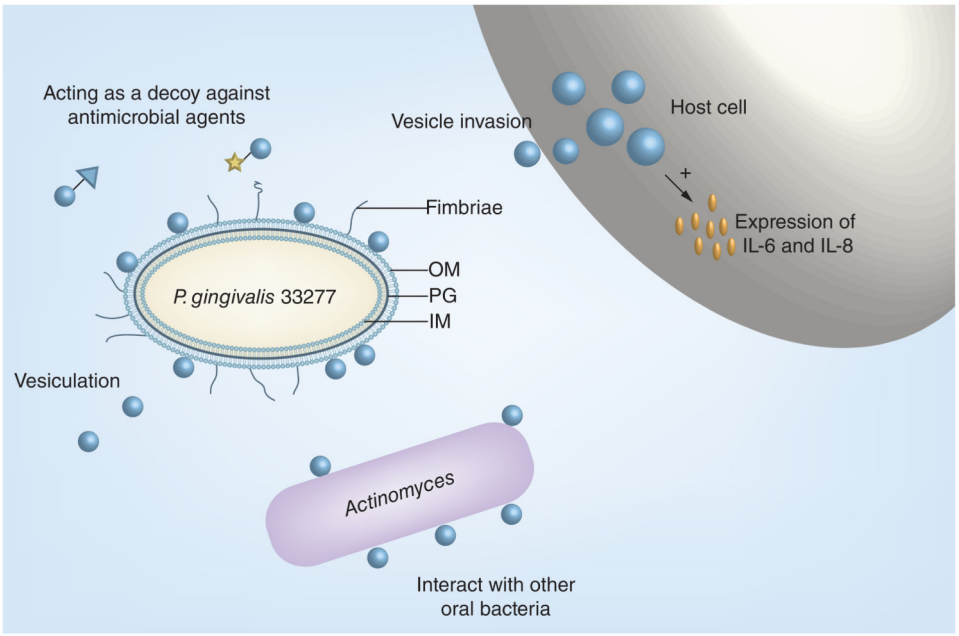


**Figure 3. *Porphyromonas gingivalis* vesicle and its functions**

The formation of outer membrane vesicless is through the blebbing and pinching-off of the OM. Arrows show OM, PG and IM, respectively. Bacterial cell-free vesicles may function as decoys neutralizing antimicrobial agents, interacting with other oral bacteria, host cells and inducing host immune responses. This is a schematic presentation and is not to scale. IM: Inner membrane; OM: Outer membrane; PG: Peptidoglycan.