Involvement of gingipains in outer membrane vesicle production and biofilm dispersal by *Porphyromonas gingivalis*

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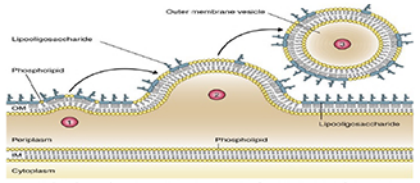
Background

Outer membrane vesicles (OMVs) are secreted by the majority of gram-negative bacteria, and serve as important virulence factors, modulating biofilm formation. *Porphyromonas gingivalis* is an anaerobic bacteria that colonizes the oral cavity and is a leading contributor to the development of periodontal disease. In *P. gingivalis* OMVs play a crucial role in evading host immune responses and the destruction of host tissues. Although their importance in pathogenesis is well characterized, OMV biogenesis is not yet fully understood.

Lipid A Modification

We have shown that lipid A structure is important for OMV biogenesis through OMV quantification of various lipid A mutant strains. We have specifically identified the phosphorylation of the C4' as crucial to inhibiting OMV biogenesis. Surprisingly, we noticed that our double mutant strain Δ73.87, which has C1' and C4' phosphatases deleted, had similar OMV biomass as our WT strain despite showing less OMVs on our TEM micrographs. This led us to believe there could be a change in OMV cargo composition based on lipid A modification, specifically of cysteine proteases secreted by *P. gingivalis* called gingipains.



Roier, S., Zingl, F., Cakar, F. et al., 2016

Figure 1. OMV biogenesis schematic

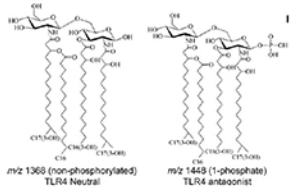
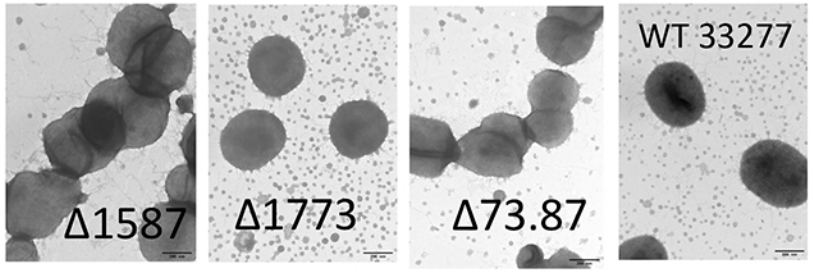


Figure 2. WT lipid A structure. Mutants shown below have modifications of the C1 and C4 phosphate groups.

Lipid A Mutants

Figure 3. TEM micrographs of *P. gingivalis* mutant strain cells and OMVs. WT reflects lipid A structure shown above, Δ1587 has C4 phosphotase deletion, Δ1773 has C1 phosphatase deleted, and Δ73.87 has both C1 and C4 photsphotases deleted.



Objectives

- Test dispersal effect of *P. gingivalis* mutant strain OMVs on *Streptococcus gordonii* biofilm
- Explain discrepancy between OMV quantification of Δ73.87 and relative amounts of OMVs on TEM images

Gingipains

- Cysteine protease packaged in *P. gingivalis* OMVs
- Gingipains aid in evading the immune system by downgrading host immune responses, leading to a reduction in inflammation.
- Facilitate sustained colonization of *P. gingivalis*

Methods

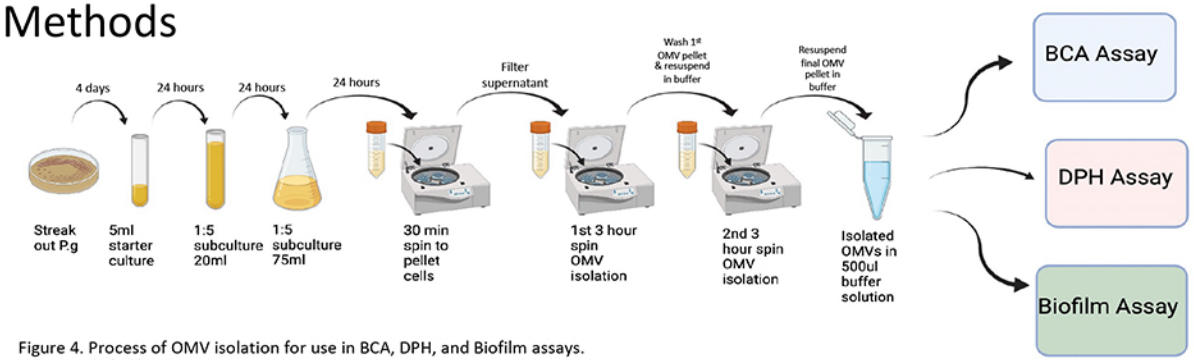


Figure 4. Process of OMV isolation for use in BCA, DPH, and Biofilm assays.
Image created using biorender.com

Conclusion & Future directions

- Despite similar DPH results WT *P.g.* OMVs and Δ73.87 OMVs have notable different effect on *S.g.* biofilm dispersal
- Lipid A modifications have possible involvement in OMV packaging
- Future experiments planned will repeat biofilm assays, quantify protein content of OMVs via BCA assay, and quantify relative amounts of gingipains

Results

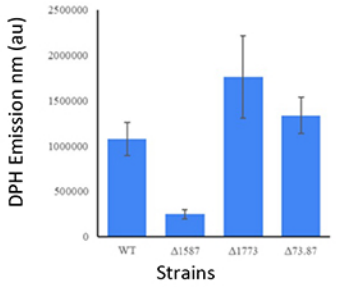
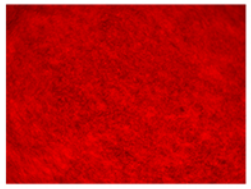
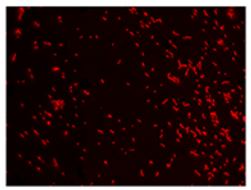


Figure 5. OMV Quantification showing relative amounts of OMVs per strain.

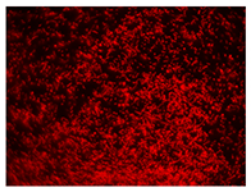
Figure 6. *Streptococcus gordonii* & *Pophyromonas gingivalis* OMV biofilm assay. When WT OMVs are added to *S.g.* biofilm there is a strong dispersal effect. A lesser dispersal effect is seen when *S.g.* biofilm is exposed to Δ73.87, indicating a difference in cargo between the two strains despite similar OMV quantification values.



S. Gordonii biofilm +PBS



S. Gordonii biofilm +WT OMVs



S. Gordonii biofilm + Δ73.87 OMVs

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