Effect of *Porphyromonas gingivalis* outer membrane vesicles on biofilm dispersal through modification of Fimbria and Lipid A phosphorylation

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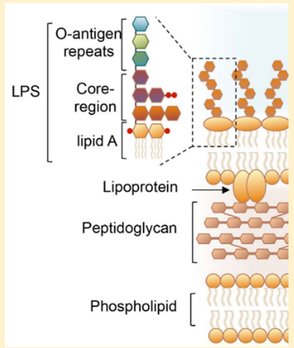
Introduction

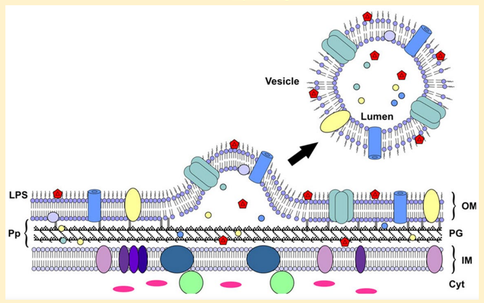
- Porphyromonas gingivalis is an anaerobic gram-negative bacteria that resides in the gum layer of the oral cavity and correlated with the progression of periodontal disease.
- The lipopolysaccharide is a conserved tripartite structure across gram-negative bacteria and serves in membrane protection and stabilization.
- Gingipains – P. gingivalis virulence factors that aid in bacterial establishment through the dispersal of non-self bacteria.
- Hypothesis #1: If Lipid A is modified via phosphorylation, then it will affect the outer membrane vesicle's ability to disperse biofilm.
- Hypothesis #2: If fimbria were knocked out from P. gingivalis, then that will reduce outer membrane vesicle-mediated biofilm dispersal.

Background

- What is a lipopolysaccharide?
- What is an outer membrane vesicle?
- How do they tie together?

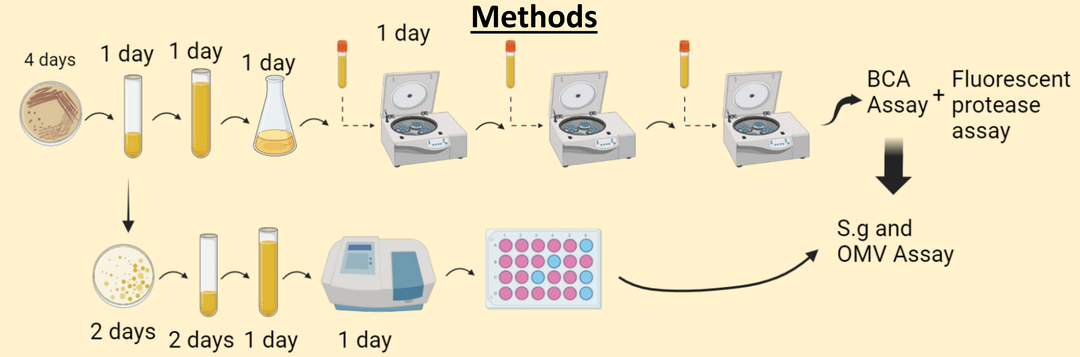


Rita F. Maldonado, Isabel Sá-Correia, Miguel A. Valvano, Lipopolysaccharide modification in Gram-negative bacteria during chronic infection, *FEMS Microbiology Reviews*, Volume 40, Issue 4, July 2016, Pages 480–493, https://doi.org/10.1093/femsre/fuw007



Gui, M.J., Dashper, S.G., Slakeski, N., Chen, Y., & Reynolds, E.C. (2016). Spheres of influence: Porphyromonas gingivalis outer membrane vesicles. *Molecular oral microbiology, 31 5*, 365-78 .

Methods



Results

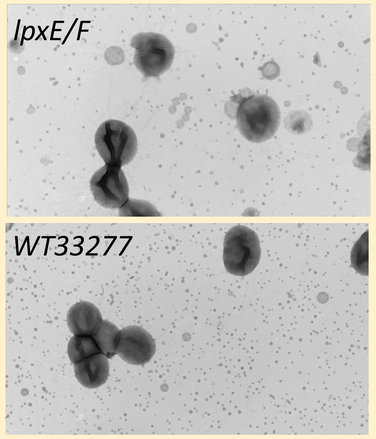


Figure 1: TEM pictures of *lpxE/F & WT33277. lpxE/F* shows a depletion of OMVs when compared to *WT33277.*

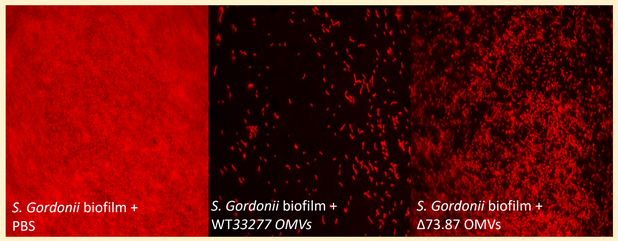


Figure 2: *Streptococcus gordonii & Porphyromonas gingivalis* OMV biofilm. The left panel shows the control. While the middle panel with *WT33277* OMVs depicts biofilm dispersal. While the panel on the right depicts less dispersal.

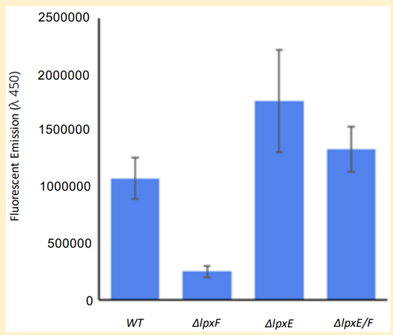


Figure 3: DPH outer membrane vesicle quantification. *WT33277 & lpxE/F* show a similar production of OMVs.

WT1 (mP)			
1:2	-60.864004245		
1:4	-56.525996063		
1:8	-53.577554051		
WT2 (mP)		lpxE/F (mP)	
1:4	-56.124665597	1:4	-54.940208247
1:16	-46.027963726	1:16	-48.352352552
1:32	-36.717798512	1:32	Uncounted
ΔGingi (mP)			
1:4	691.893240941		
1:16	442.360075812		
1:32	7.223609041		
WT1 (ΔmP)		lpxE/F (ΔmP)	
WT	-0.02477633775	Lpx E/F	-0.03130594744
1:2	-0.01572600639	1:2	-0.01322052007
1:5	-0.00527758622	1:5	-0.0106445741
1:10	-0.01503046341	1:10	-0.00369021499

Figure 4: Protease assay of outer membrane vesicles. *WT1* shows a dilution series of 1:2 – 1:8. *WT2* & *lpxE/F* show a dilution series of 1:4 – 1:32. Gingi is a control. Repeat *WT1* & *lpx*E/F shows a dilution series of 1:2 – 1:10. Dilution 1:10 shows a 5x difference in fluorescent polarization.

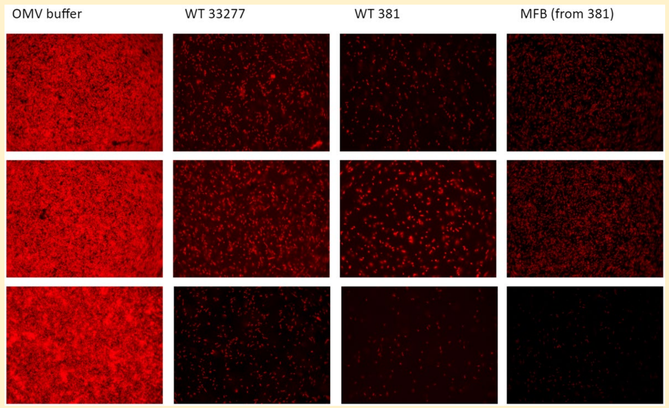


Figure 5: P. gingivalis introduction to S. gordonii biofilm. Column 1 shows S.g biofilm with 250 ul buffer as a negative control. Column 2 shows 15.09 ul of strain *33277* plus 235 ul buffer in S.g biofilm. Column 3 shows 150 ul of strain *381* plus 227 ul of buffer in S.g biofilm. Column 4 shows 23 ul of strain *MFB* plus 145 ul of buffer in S.g biofilm.

Conclusions

- Hypothesis #1: Preliminary data of the protease activity suggest that activity decreases with an increase in protein dilution. However, due to inconsistent data using the Fluorescent Protease Assay Kit, we concluded it was not an optimal measure to determine protein activity with OMVs. Future research should be directed toward optimizing an assay that adequately measures protease activity in a consistent manner.
- Hypothesis #2: If fimbriae deletion occurred then there should be little-to-no biofilm dispersal of Strep. This hypothesis proved to be inaccurate since the *MFB* strain was able to effectively disperse strep biofilm. Note: there was a miscalculation during the S.g + OMV assay as fewer *MFB* strain quantity was added into the biofilm. Since dispersal was concluded at a much smaller volume, and in conjunction with literature review; fimbria deletion consideration was no longer investigated.
- P. gingivalis morphology characterization and outer membrane vesicle production had been consistent up until Winter 2023. Upon receiving new bacterial stocks from UW Seattle, *lpxE/F* is now producing much fewer OMVs than previously characterized while *WT33277* is producing longer fimbria units.
- As of late, the lab has been focusing on characterizing the morphology of different P. gingivalis strains and will investigate genomic sequencing.
- Understanding P. gingivalis OMVs and their role in biofilm dispersal, future research could be directed toward OMV biogenesis, cessation, and progression of periodontal disease.

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