Investigation of *Porphyromonas gingivalis* Outer Membrane Vesicle Role in Biofilm Dispersal

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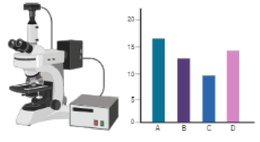
Abstract

Porphyromonas gingivalis is a keystone pathogen in the pathogenesis of periodontal disease. It produces several major virulence factors - such as lipopolysaccharides, proteins, and outer membrane vesicles (OMVs) - that can evade host immune defenses. These virulence factors assist *P. gingivalis* in the colonization of the host at a cellular level – such as biofilm dispersal - and are involved in the alteration of oral microbial community structure and destructive inflammation in the periodontal tissue.

Previous studies show that *P. gingivalis WT 33277* and *WT 381* strains are nearly genetically identical, however, they have significant differences in host immunological responses. These differences are thought to be correlated with strain-specific differences in virulence factors such as OMV abundance – in which *WT 381* secretes more than *WT 33277*.

We hypothesized that a strain with higher OMV production (*WT 381*) would have greater biofilm dispersal.

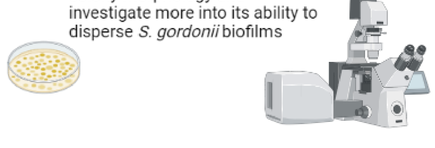


Quantify the results from isolated OMV and supernatant experiments to determine if there is a significant difference between wild types.

Investigate protease activity in outer membrane vesicles to determine their correlation to biofilm dispersal

Ongoing Studies

Characterize *381 WT* strain colony morphology and investigate more into its ability to disperse *S. gordonii* biofilms



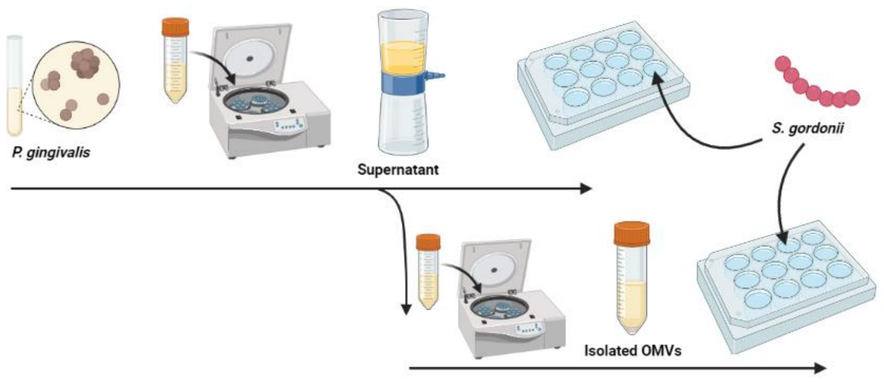


Figure 1. Methodology for experiments

Isolation of OMVs from bacterial culture undergoes centrifugation and Polyether sulfone (PES) filtration of bacterial culture from whole cells. The supernatant contains OMVs as well as other secretions by *P. gingivalis*. Isolated OMVs continue to undergo high-speed centrifugation and suspensions. Supernatant and isolated OMVs are added to coverslips in a 12-well plate already inoculated with *S. gordonii.*

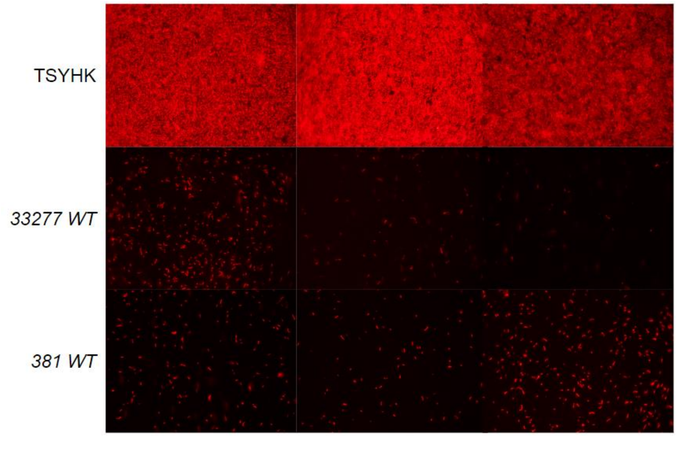


Figure 2. The dispersal of *S. gordonii* biofilms is influenced by major virulence factors in the supernatant of *P. gingivalis*

Across shows 4 technical replicates from the same 12-well plate. TSYHK is the growth media for *P. gingivalis*, used as the control with no addition of *P. gingivalis.* The addition of *33277 WT* and *381 WT* strain supernatants is also shown. Whole-cell concentrations were normalized via optical density before being filtered.

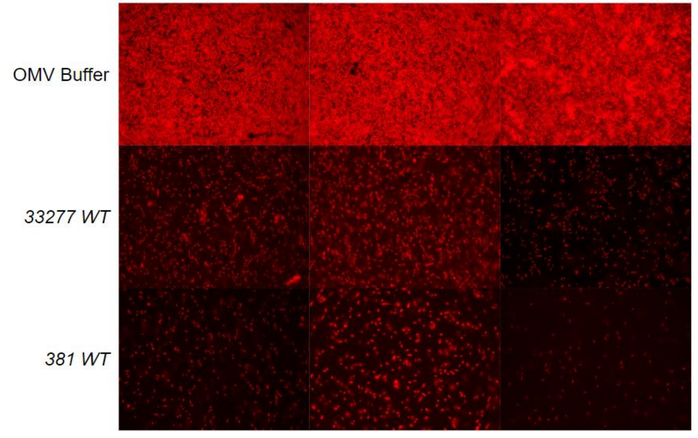


Figure 3. The dispersal of *S. gordonii* biofilms is influenced by isolated OMVs of *P. gingivalis*

The addition of *P. gingivalis* isolated outer membrane vesicles (OMV) to mature *S. gordonii* biofilms. Across shows 3 technical replicates. OMV buffer is the solution that OMVs are resuspended and stored in after being centrifuged and used as the control. *P. gingivalis* was not added to OMV buffer biofilms. The addition of *33277 WT* and *381 WT* strains' isolated OMVs is also shown. OMV concentrations were normalized via optical density.

CONCLUSIONS

- **When OMV concentration is normalized between wild type strains, they have same dispersal patterns qualitatively – indicating that OMVs have an influence in dispersal patterns**
- **The dispersal of *S. gordonii* biofilms with *P. gingivalis* supernatant shows slight qualitative differences among strains – indicating a different level of OMV production among strains.**

Works Cited

Coats, S. R., Kantrong, N., To, T. T., Jain, S., Genco, C. A., McLean, J. S., & Darveau, R. P. (2019). The Distinct Immune-Stimulatory Capacities of Porphyromonas gingivalis Strains 381 and ATCC 33277 Are Determined by the fimB Allele and Gingipain Activity. Infection and Immunity, 87(12). https://doi.org/10.1128/IAI.00319

Zhang Z, Liu D, Liu S, Zhang S, Pan Y. 2020. The Role of Porphyromonas gingivalis Outer Membrane Vesicles in Periodontal Disease and Related Systemic Diseases. Frontiers in cellular and infection microbiology. 10:585917–585917. doi:10.3389/fcimb.2020.585917.

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