The Oral Microbiome in Periodontal Disease: Interactions Between *Porphyromonas gingivalis* and Commensal Bacteria

Sirith Bahia and Sarah Alaei

Department of Science and Mathematics, University of Washington, Tacoma, WA

Introduction

- Porphyromonas gingivalis (Pg) is the keystone oral pathogen implicated in periodontal disease that has been hypothesized to interact with different commensal bacterial species, including *Bacteroides thetaiotaomicron and Streptococcus gordonii*
- Periodontitis is an inflammatory disease initiated by dysbiosis of bacteria in the oral microbiome.
- Within the oral microbiome, bacteria interact with each other through the secretion of outer membrane vesicles (OMVs) and proteins. These secretions can alter microbial behavior and the host's immune response, contributing to increased disease progression.
- Whether interactions between Pg and commensal bacteria influence MV secretion and pro-inflammatory cytokine release by innate immune cells remains to be determined.

Hypothesis

We hypothesized that when Pg is co-cultured with *Bacteroides thetaiotaomicron and Streptococcus gordonii* there will be an increase in OMV production and IL-6 secretion.

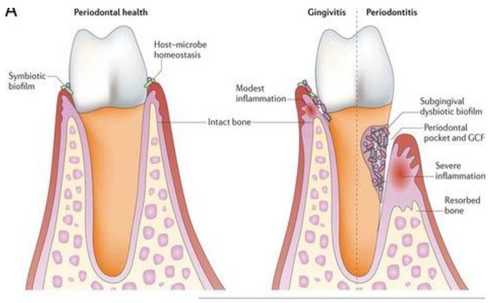

Figure 1.Initiation of Periodontitis. Dysbiosis of oral bacteria triggers an overactive and destructive immune response leading to the breakdown of tissue and bone.

Figure adapted from Olsen, I., Lambris, J. D., & Hajishengallis, G. (2017). *Porphyromonas gingivalis* disturbs host-commensal homeostasis by changing complement function. *Journal of oral microbiology*, *9*(1), 1340085. https://doi.org/10.1080/20002297.2017.1340085

Methods

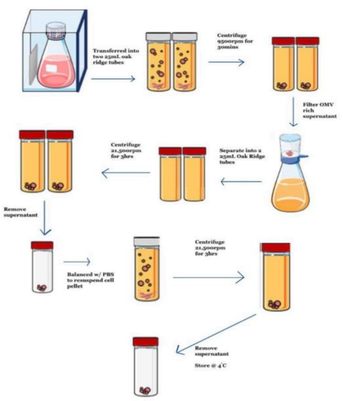

Figure 2. OMV Isolation. OMVs were collected from both bacterial mono- and co-cultures. OMVs were then stored and analyzed for subsequent analysis.

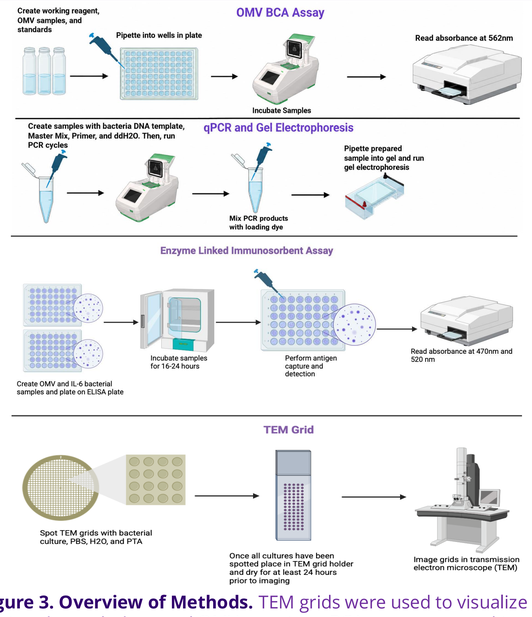

Figure 3. Overview of Methods. TEM grids were used to visualize bacterial morphology and OMV secretion. BCA assay was used to quantify OMVs present in bacterial cultures. PCR and gel electrophoresis was used to determine abundance of bacterial DNA in cultures and quality control. Enzyme-linked immunosorbent assay was used to test immune response triggered by bacterial culture exposure.

Results

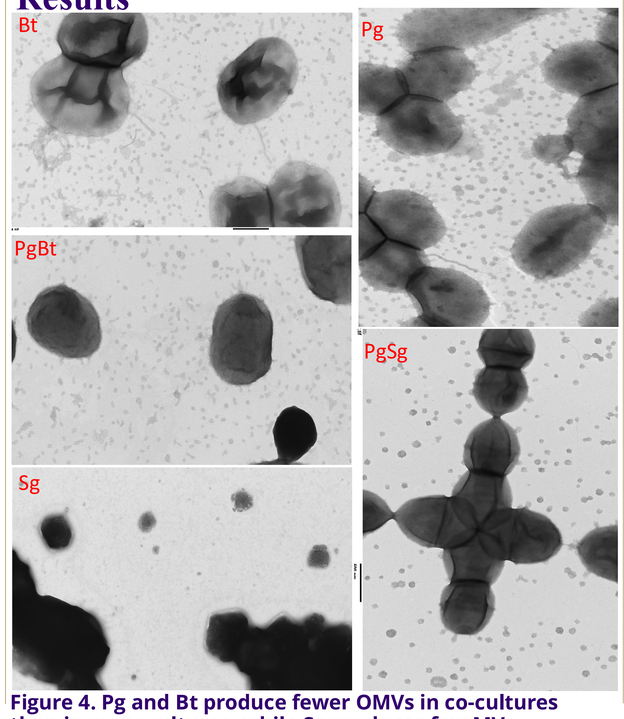

Figure 4. Pg and Bt produce fewer OMVs in co-cultures than in monocultures, while Sg produces few MVs. Transmission electron microscopy was used to view bacterial cultures. Differences in OMV abundance in mono- and co-cultures in these images can indicate that bacterial interactions influence OMV production.

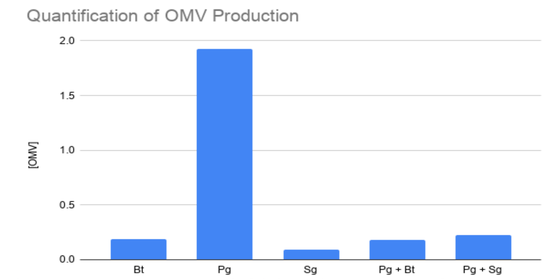

Figure 5. Protein content is highest in Pg monoculture OMVs. Bicinchoninic acid assay (BCA) was used to quantify protein amounts in isolated OMVs. Triplicates of each sample were used in the assay (n = 2 replicate cultures) and the mean was calculated for each sample. We can see that Pg mono-culture produces the most OMVs and the Sg mono-cultures produces the least. Furthermore, we can see when Pg is co-cultured the OMV concentration changes, leading to the conclusion that bacterial interactions alter OMV production.

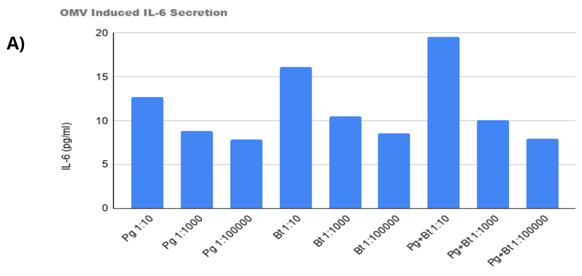

Figure 6. Cultures with higher [Bt] induce higher IL-6 secretion by MM6 cells. ELISA was conducted with MM6 cultures tested in triplicates (n=3) from 5/21 to 5/26 and absorbance was measured at both 450nm and 570 nm. A trned can be observed where samples containing higher [Bt] induce more IL-6 secretion. **(A)** IL-6 secretion was tested with exposure to bacterial OMVs. **(B)** IL-6 secretion was tested with exposure to live bacterial cell cultures.

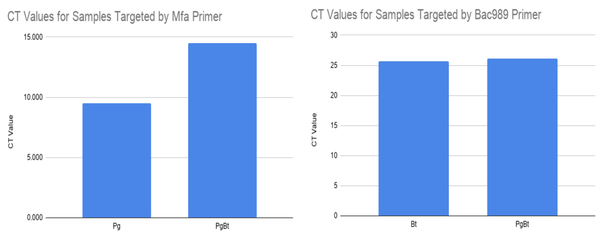

Figure 7. qPCR CT values showing lower Pg abundance in PgBt culture than in monoculture. CT values indicate amount of genetic material present. Low CT values equate to higher amounts of genetic material. Two replicate cultures were used to generate the data shown (n=2). The difference in abundance of Pg and Bt in PgBt culture can support that a certain abundance of bacterial species in a culture can alter OMV production.

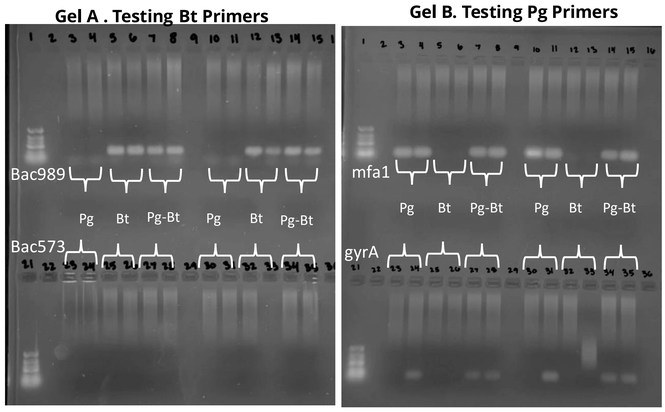

Figure 7. Gel eletrophoresis quality control test for primers used in taxon specific qPCR. The gels were ran with 2 pairs of duplicates. Samples and layout is indicated above and is the same for both gels. Gel A tested for Bt using Bac989 and Bac573 primers. Gel B tested for Pg using mfa1 and gyrA primers. Only the bacteria specific primers showed bands for each gel indicating that specific bacteria is present.

Findings and Future Steps

- Cultures containing Bt cause a higher immune response, releasing more IL-6 and leading to chronic inflammation and progression of periodontitis.
- Polymicrobial interactions altered OMV production of Pg-Bt and Pg-Sg resulting in lower OMV abundance. This may be essential in understanding which bacteria are responsible for OMV production that prolongs periodontitis.
- CT values indicate lower quantity of Bt in PgBt culture which can indicate that a certain abundance of bacteria is required to promote OMV production.
- Further understanding of the mechanism/pathways which are used to secrete and change the amount of IL-6 released is needed. This may allow better treatment of periodontitis by blocking pro-inflammatory cytokines that cause further degradation of bone and gum.
- Investigating the different types of cytokines released by immune cells can help determine the effect they have on initiating periodontitis.
- Studying the exact signal causing the degradation of the gums and teeth can help by creating treatment to block that signal so further damage does not occur.

Contact

Sirith Bahia,
sbahia06@gmail.com

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