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

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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. We have identified features of lipopolysaccharide structure, specifically lipid A, that influence OMV biogenesis. 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. Gingipains aid in evading the immune system by downgrading host immune responses, leading to a reduction in inflammation. We tested our hypothesis by growing *Streptococcus gordonii* alongside isolated OMVs from WT *P .gingivalis* and found that *S. gordonii* was unable to form a biofilm. We also observed dispersal of a fully formed *S. gordonii* biofilm after the addition of either WT OMVs or WT whole cell cultures. These preliminary results suggest that lipid A effects packaging of OMV cargo, a novel finding related to OMV biogenesis. We are currently working on quantifying relative amounts of gingipains for each lipid A mutant strain to determine if OMV levels correlate with relative levels of gingipains, further characterizing the role of gingipains in biofilm dispersal of *S. gordonii* and understanding how lipid A modifications effect the loading of OMV cargo.