

Lipid A Phosphorylation Modulates *Porphyromonas gingivalis* Outer Membrane Vesicle Function in Biofilm Dispersal

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Porphyromonas gingivalis is a Gram-negative bacterium that secretes small membrane-enclosed packages known as outer membrane vesicles, or OMVs. Previous research has shown that *P. gingivalis* uses OMVs as virulence factors to shape host-pathogen interactions, leading to dysbiosis and the progression of periodontal disease. However, less is known about how *P. gingivalis* uses these virulence factors to shape interactions between bacteria within biofilms. We hypothesized that lipid A structure could influence OMV mediated biofilm dispersal, possibly by altering OMV cargo. Lipid A structure can be modified by enzymes, such as the phosphatases LpxE and LpxF, after being synthesized in its bis-phosphorylated form. OMVs were isolated from wild type ATCC33277, Δ *lpxF*, and Δ *lpxF*/ Δ *lpxE* strains of *P. gingivalis*, normalized based on total protein concentration and then added to pre-formed biofilms of *Streptococcus gordonii*, which is another common bacterium found within the oral microbiome. After 24 hours, the ability to disperse the biofilm was determined using fluorescence microscopy. The Δ *lpxF* strain showed decreased OMV production compared to the wild type and Δ *lpxF*/ Δ *lpxE* strains, indicating that lipid A phosphorylation is an important modulator of OMV biogenesis. The biofilm dispersal ability of the Δ *lpxF*/ Δ *lpxE* strain was greatly diminished compared to the wild type, indicating that changes to lipid A structure affect packaging of OMVs, despite the strains sharing similar OMV biomasses. This is a novel finding that helps increase our understanding of how *P. gingivalis* shapes bacterial communities within the oral mucosa.