Investigation of Co-Aggregation and RNA Expression in Single and Multispecies Periodontal Biofilms

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Objectives: The oral cavity is home to over 700 bacterial species, and the success of their colonization is linked to their ability to adhere to surfaces and co-exist in biofilms. It was previously investigated in a periodontal biofilm model that Fusobacterium nucleatum polymorphum (strain 1-5) was present in strong coaggregation pairs with Parvimonas micra (1-4), Actinomyces oris (1-8), and Veillonella dispar (1-3). A. oris was the strongest driver of co-aggregation with Porphyromonas gingivalis (1-1) and the best binder to surfaces. In this study we will investigate RNA expression and changes in gene expression during co-aggregation and biofilm formation between 1-1, 1-5, and 1-8.

Experimental Methods: The strains investigated for gene expression in communities were Porphyromonas gingivalis (1-1), Fusobacterium nucleatum polymorphum (1-5), and Actinomyces oris (1-8). Bacterial species were grown anaerobically and in single and multi-species combinations for planktonic and biofilm gene expression. Overnight cultures were extracted for total RNA using the Direct-zol with Triazol method (Zymo Research) and RNAseq was performed by Azenta Biosciences. Total amounts of RNA were assessed with Nanodrop. Total amounts of RNA extracted from duplicates of each condition were compared with Students T-Test.

Results: Single species planktonic growth on average, produced slightly more RNA than biofilm growth (2967 ng vs 1838 ng) for single species biofilms, however the differences between planktonic and biofilm values were not significant (P=0.28). In contrast, three species grown together planktonically produce significantly more RNA (P= 0.02) than three species grown in a biofilm (11406 ng to 3955 ng).

Conclusions: The significant increase in RNA expression levels during planktonic interaction of three species implies that bacteria are able to detect the presence of co-aggregation partners and adjust levels of gene expression to form community biofilms. Future studies will identify the specific changes in gene expression induced by mixed-species coaggregation.