Anaerobic but not Aerobic Nitrogen Metabolism Drives Biofilm Calcification

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Objective: Gastronomy feeding tubes (G-tubes) are inserted into the stomach of individuals who have difficulty eating by mouth. A side-effect of extended G-tube use is the formation of extensive dental calculus, independent of oral hygiene behavior. Previously, we demonstrated that the saliva of these patients is enriched in nitrate compared to controls, and the dental calculus is enriched for bacteria associated with denitrification. Studies in environmental systems indicate that aerobic denitrification and the associated CO_2 production can result in rapid mineral deposition. In this research study, we assess the relationship between anaerobic and aerobic nitrate/nitrite reduction, biofilm formation, and biomineralization using an in vitro model system.

Methods: The bacterial strains used in this study are facultative nitrate or nitrite reducers from the oral cavity, including *E. coli*, *Rothia dentocariosa*, three species of *Neisseria*, and two species of *Actinomyces*. Single or dual species cultures were grown as anaerobic or aerobic biofilms, or aerobically with shaking for 7 days. Supernatants were assessed for nitrogen metabolism using a Griess assay, and measurement of mineral deposition by Alizarin Red staining quantification. Assays were repeated twice in duplicate, and statistical differences calculated by ANOVA with Bonferroni post-hoc tests. Correlation was assessed with Pearson's correlation.

Results: All culture conditions demonstrated nitrate/nitrite reduction, with anaerobic biofilms and aerobic planktonic metabolizing significantly more nitrogen than aerobic biofilms. In contrast, only anaerobic biofilms produced significant mineral deposits, and the amount of Greiss staining correlated with nitrogen metabolism in anaerobic conditions but not aerobically. Conclusions: In contrast to published studies on aerobic denitrification, these results indicated that denitrification under anaerobic conditions is most associated with formation of mineral deposits with oral bacteria, and that biofilms containing *Actinomyces spp* are a potential model system for future denitrification inhibition studies.

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