Tobacco and cigarette smoke increase the risk of periodontal disease, one of the most widespread human diseases. It has been established that Porphyromonas gingivalis, a gram negative anaerobic bacterium, is one of the main causative agents of periodontal disease. Prior research indicates that P. gingivalis binds to Fusobacterium nucleatum in oral biofilms. It is not yet understood if nicotine, a major component of cigarette smoke, affects the growth of bacteria differently if added in the planktonic phase, defined as the primary subculture from agar to broth before the start of a biofilm formation experiment, or the biofilm phase, defined as the secondary subculture from broth culture to a microtiter plate. Therefore, the main objective of this study is to understand this methodological difference.

F. nucleatum and P. gingivalis were both grown in anaerobic GasPak containers on blood agar plates. The media for primary subculture consisted of a Brain Heart Infusion (BBL) broth supplemented with 5 g/L yeast extract and 5% vitamin K & hemin serum at 37°C. F. nucleatum was subcultured in the absence of nicotine and plated on a 96 well plate to establish biofilm. P. gingivalis was subcultured in varying concentrations of nicotine and subcultured on top of the F. nucleatum biofilm. Biofilm mass was analyzed using the crystal violet technique and samples were measured in a spectrophotometer at 490 nm. The results demonstrated a statistically significant increase in biofilm formation when P. gingivalis was subjected to a higher nicotine concentration in the planktonic phase in comparison to a lower nicotine concentration in the biofilm phase. This data suggests a nicotine assisted activation of receptors on the surface of P. gingivalis specific for binding to F. nucleatum. Further testing on the receptors through a biotinylation assay will confirm the results.

Advisor: Richard L. Gregory, Department of Oral Biology, Indiana University School of Dentistry, Indianapolis, IN 46202