

Effect of Nicotine on Planktonic and Biofilm Growth Phases of an Experiment

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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. nucleate* 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.

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