INTERACTIONS OF HUMAN ORAL CELLS WITH ORAL BACTERIAL

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Introduction: *Streptococcus mutans* is the main etiological cause of dental caries, and it has been shown that individuals who smoke have increased dental caries. *S. mutans is* known to bind to or interact with MG63 osteoblasts. However, very little is known about the effects of tobacco directly on these bacteria on their ability to affect human pulp MG63 osteoblasts. We are hypothesizing that tobacco upregulates the expression of pro-inflammatory cytokines and MMPs to increase the pathogenic potential of *S. mutans.* The objective of this research project is to investigate the effects that nicotine, cigarette smoke condensate (CSC), and dissolvable smokeless tobacco (DST)-extract treated bacterial cells have on humanMG63 osteoblasts, in respect to their release of pro-inflammatory and anti-inflammatory cytokines, as well as MMP expression. In addition, the effects of the *S. mutans* cells will be examined for the ability to affect MG63 osteoblast growth. The long-term goal is to develop treatment modalities to reduce the effects of smoking on dental caries.

Materials and Methods: *S. mutans* UA159 was incubated in Tryptic Soy Broth (TSB), with the following concentrations: 2 mg/mL nicotine, 0.125 mg/mL CSC, 100 uL/3 mL DST-extract, and a 0 mg/mL control group. The cultures were grown in the presence of the tobacco products for 8 h at 37^{o}C in 5% CO_2, and centrifuged to isolate cells and supernatants. The cells were washed and heat-killed for 1 h at 60^{o}C. Human MG63 osteoblasts were isolated from extracted teeth, and cell passages 3-8 will be used. The tobacco-treated *S. mutans* cells and supernatants will be incubated with the osteoblasts in culture plates for 72 h and cytokine expression evaluated by reverse transcriptase polymerase chain reaction.

Results: The protein concentration of each tobacco-treated sample was found. The undiluted concentrations of the nicotine- and CSC-treated cells were slightly lower and the DST-treated cells was slightly higher than the control cells. The undiluted nicotine ($p<0.05$) and DST-treated supernatants were higher than the control, while the CSC supernatant protein concentration was lower. From our previous studies, it was found that nicotine increases bacteriocin production of *S. mutans*, so we might hypothesize that nicotine induces bacteriocin secretion, thus increasing dental caries.