Growth and Enzyme Inhibition of *Streptococcus mutans* Using Nanoceria

Alyssa Che, Sejal Gandhi, Adam Goodman, Emily Long, Herchel Patel, Kamal Patel

University of Georgia

Statement of Purpose: Dental plaque is one of the leading causes of periodontal diseases, resulting from the build-up of microbial growth and the formation of biofilms largely due to the bacteria *Streptococcus mutans*. Biofilms are caused by the proliferation of oral bacteria—some of which produce acidic byproducts such as lactic acid. Ongoing studies in cerium oxide nanoparticles (nanoceria, CeO_2) have revealed they possess promising antimicrobial capabilities of disrupting biofilm for dental application. We propose that synthesized nanoceria can be utilized to inhibit the growth of the oral bacteria, therefore reducing the amount of lactic acid that would likely cause premature tooth decay (caries, periodontitis). This study first investigates the growth patterns of *S. mutans* through OD and CFU measurements. Second, *S. mutans'* metabolism of sucrose is studied through conducting pH tests at varying sucrose concentrations. The antimicrobial effect of nanoceria on the pH of bacterial solutions will then be measured.

Methods: The pH of *S. mutans* liquid culture was measured over a period of 48 hrs. A single colony from a bacterial parent agar plate was inoculated in Tryptic Soy Broth (TSB) and grew for 24 hours in an incubator. The liquid culture was centrifuged and the broth was replaced with luria-bertani (LB) and vortexed. A large sample of culture was then made with LB to fill five 15 mL tubes with 7.5 mL and a sucrose solution was made using DI water. A series of liquid cultures were made by adding samples of LB culture and sucrose solution into the 5 tubes. The pH of each sample was measured at time 0, 24, and 48 hrs. OD and CFU measurements were prepared simultaneously. A single bacterial colony was placed in 5mL of TSB in a 15 mL tube and incubated for 24 hours. The liquid culture was centrifuged, the supernatant was removed, and 1 mL of PBS was added to the tube. A 50mL vial was filled with 19.8 mL of TSB and 200 uL of the liquid culture. This vial was used for both procedures. Using a 96 well-plate, the 1st column was filled with TSB and the next 6 columns were filled with 200 uL of the liquid culture. The Varioskan was then used to graph the optical density of each well and determine the phase of the bacteria's ideal growth. With the remaining culture, serial dilutions were performed for hours 0-4, with the culture incubated between platings. Three replicates of the serial dilutions were spread on agar plates and grown overnight. A cell counter was used to measure CFU.

Results: The bacterial culture present in 7.5% sucrose had a significant pH drop within 48 hours whereas the bacterial culture in 0% sucrose had a minimal drop in pH. This demonstrates a positive correlation between the *S. mutans* metabolism and sugar concentration, leading to increased lactic acid production. The minimal changes of pH between 24 and 48 hours can be attributed to either the bacteria completely metabolizing the sucrose or the bacteria being unable to withstand a lower pH. Using the OD to analyze bacterial growth over time, the log phase of the bacteria was determined to be at an average of 3.5 hrs. At 3.5 hours, plating serial dilutions of 10^{-4} produced a colony count of roughly 170 colonies.

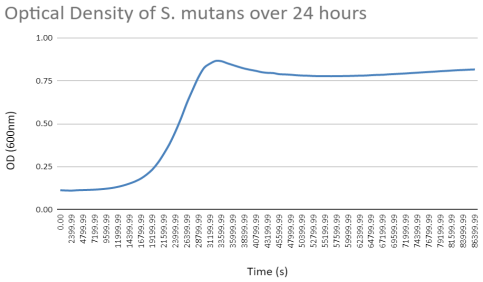

Figure 1. Optical density of *S. mutans* liquid culture measured over 24 hours.

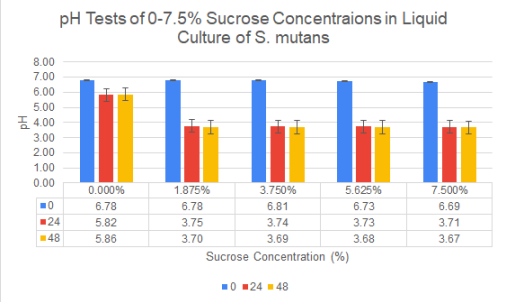

Figure 2. pH of *S. mutans* liquid cultures with varying sucrose concentrations measured over a 48-hour period.

Conclusions: This data supports the theory that higher sucrose concentrations in *S. mutans* cultures result in significant pH drops (~3) due to increased sugar concentration. Because *S. mutans* can tolerate acidic environments, it can be deduced that a pH of ~3 is the maximum acidity for the bacteria to survive. This study provides a foundation for further research into the antimicrobial properties of nanoceria, which could one day be applied to dental treatments.

References:

(Alpaslan E., et.al Sci. Rep. 2017; ISSN: 2045-2322)

(Djais AA., et.al Saudi Dent J. 2020;32:129-134)