

Effects of E-liquids on *Streptococcus gordonii* Biofilm Growth

Christina Xu*, Michelle James and Giancarlo Cuadra, PhD
Department of Biology, Muhlenberg College

Abstract

Background:

The use of electronic cigarettes, or vaping, has increased in popularity in the past decade among students in high school and college. The e-liquid contains harmful substances such as nicotine, propylene glycol, vegetable glycerol, and many flavors. The mouth is the first site of exposure to the e-cigarette aerosol, and is the home of different commensal and pathogenic bacteria. Most of these microorganisms grow as biofilms, commonly known as dental plaque. Changes in the bacterial interactions may lead to oral diseases. There are limited studies regarding the effects of vaping in the oral cavity, and even fewer studies have focused on the effects of vaping in oral bacteria. This project aims to test the effects of e-liquids with multiple flavors on the biofilm growth of oral commensal bacteria using artificial media. We hypothesize that biofilm growth may be altered as a result of the flavorings in e-cigarette liquid.

Materials and Methods:

Bacteria biofilms of *Streptococcus gordonii* were grown *in vitro*, exposing the microbes to increasing concentrations of e-liquids (1%, 3%, 5% v/v) as well as growing them over different lengths of time (1 day or 2 days). Flavors of e-liquids include blueberry, cinnamon, menthol, strawberry, and tobacco as well as flavorless. Biofilm biomass was analyzed using a crystal violet staining procedure.

Results/Conclusion:

This project shows that vaping can affect the growth of *S. gordonii*. Altering the growth of oral commensal bacteria can lead to further understanding of the effects of e-liquids on the implications of oral health. Exploring the effects of vaping in oral biology deserves more attention since oral health impacts systemic health.

Introduction

- The oral cavity is the home of many commensal bacteria, such as *Streptococcus gordonii* (Avila et al., 2009)
- Previous studies from our lab have shown that planktonic growth of bacteria is affected by e-liquid flavors (Fischman et al., 2020)
- Aim of this study: to test the effects of e-liquids with multiple flavors on the biofilm growth of oral commensal bacteria using artificial media
- Hypothesis: Biofilm growth may be altered as a result of the flavorings in e-cigarette liquid.

Materials and Methods

- *Streptococcus gordonii* (DL1) was streaked on BHI agar, and incubated at 37 °C, 5% CO₂
- Bacteria were grown and inoculated in batch cultures using artificial media (BHI).
- Bacteria concentration was adjusted to OD₅₉₅ = 1.0
- 96 well plates were treated with human saliva for 48 hours, and bacterial cultures were added to the wells to adhere to saliva-coated surfaces.
- Biofilms were grown in 50% BHI broth with increasing concentrations of e-liquids (1%, 3%, 5% v/v), as well as controls with BHI media and 5% peroxide in a 96 well plate.
- Flavors: blueberry, cinnamon, menthol, strawberry, and tobacco as well as flavorless e-liquid.
- Biofilm biomass was analyzed using a crystal violet staining procedure, and biomass was collected by observing crystal violet absorbance at 595 nm.

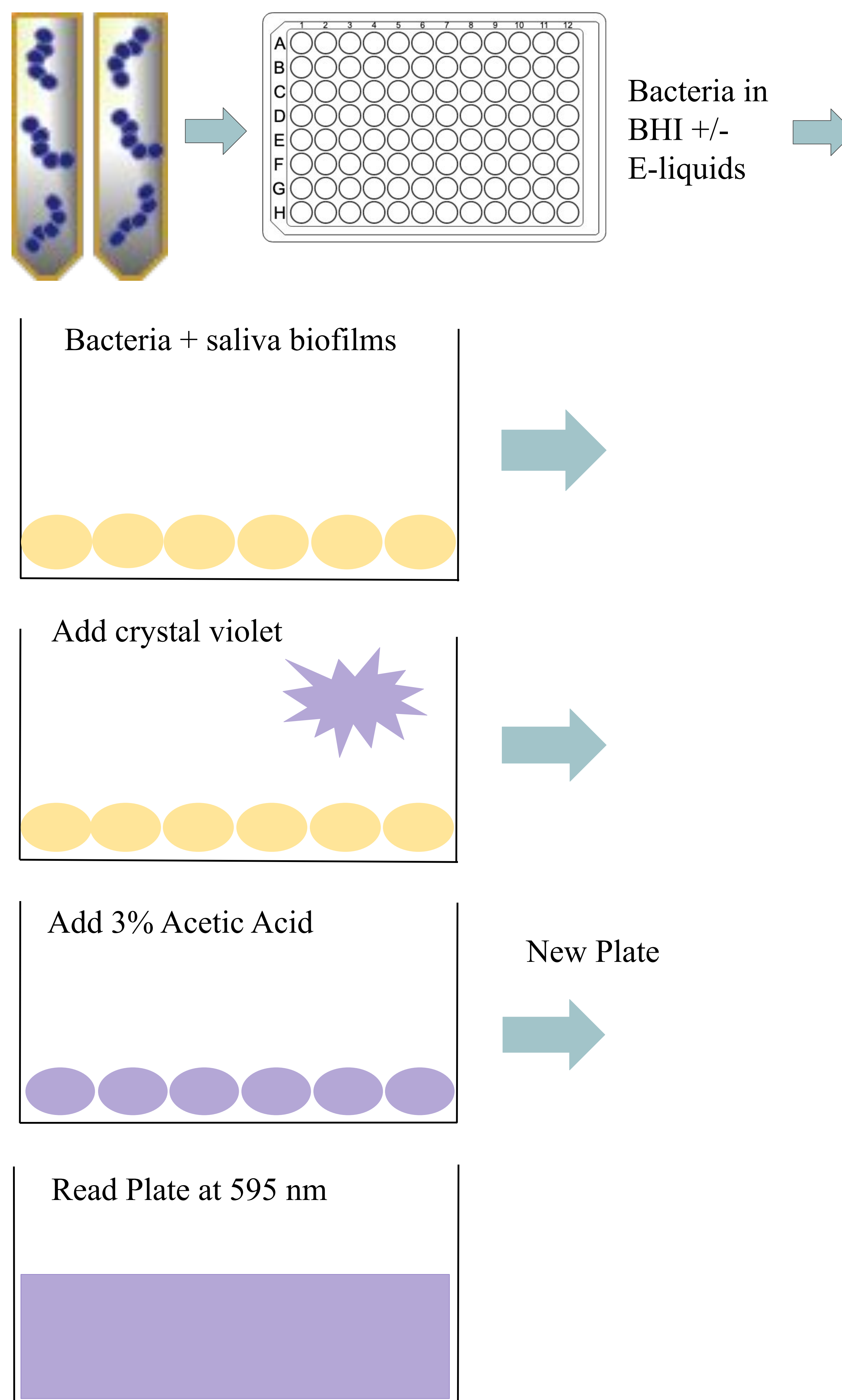


Figure 1. Crystal Violet staining procedure of biofilms in the 96 well plates

Results

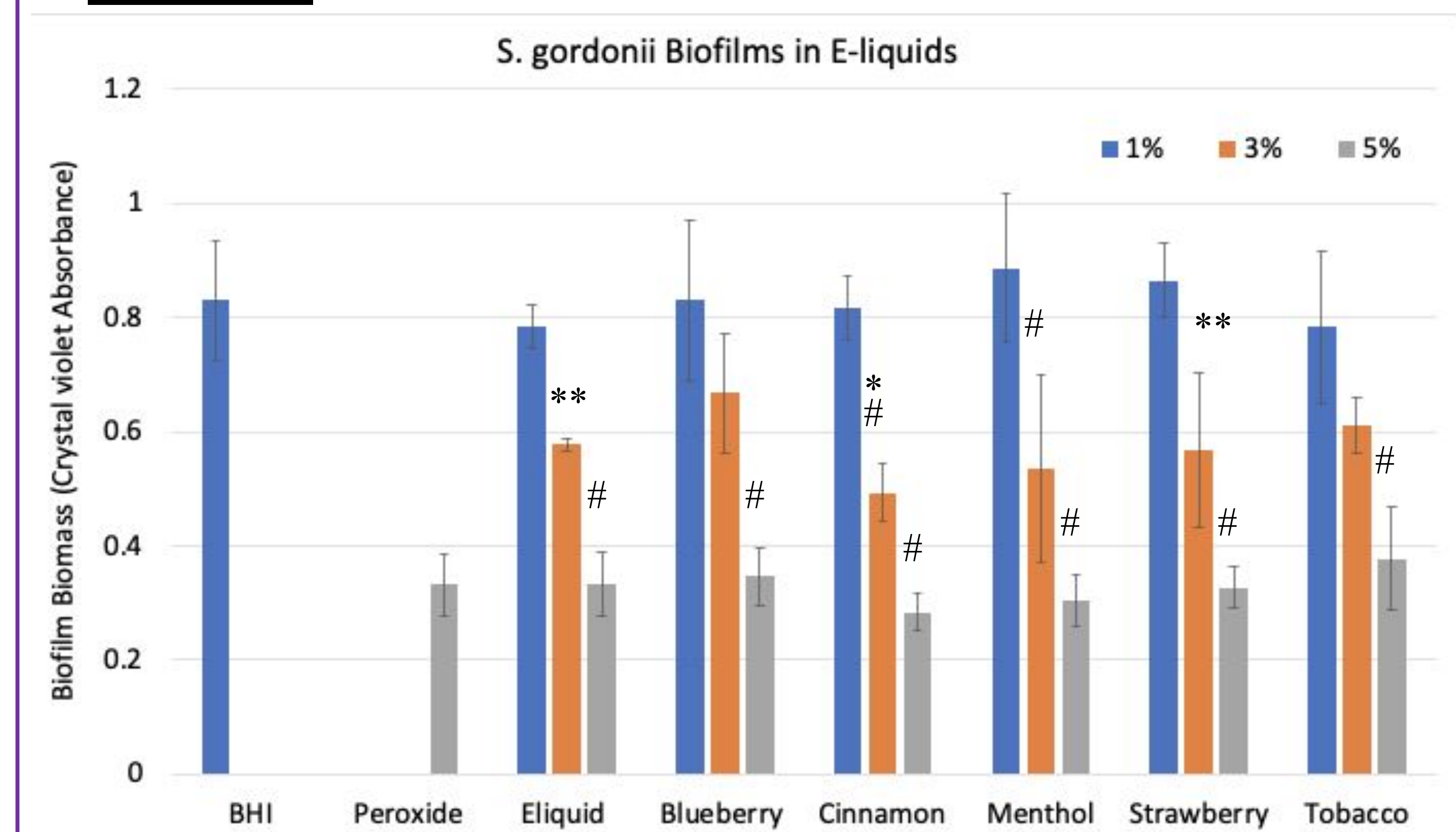


Figure 2. Biofilm Biomass. Crystal Violet Absorbance of *S. gordonii* biofilms when treated BHI with increasing concentrations of e-liquids +/- flavors in comparison to BHI and BHI + 5% peroxide controls.

- * Significant compared to flavorless e-liquid
- ** Significant compared to 1%
- # Significant compared to 1% and BHI

- Dose dependent response: as an increasing amount of e-liquids were added, the amount of biofilm biomass decreased in all conditions when compared to the BHI control or 1% e-liquids.
- 1% e-liquid growth similar to, or even greater than the BHI control.

Conclusion

- All e-liquids decrease biofilm biomass in *S. gordonii*.
- Findings with e-liquid treatments are similar to Fischman et al. findings with planktonic bacteria (Fischman et al., 2020)
- Exposure to vaping products may alter microbial microenvironments in the oral cavity, and may result in oral diseases.

Bibliography

- Avila, M., Ojcius, D. M., & Yilmaz, Ö. (2009). The Oral Microbiota: Living with a Permanent Guest. *DNA and Cell Biology*, 28(8), 405–411.
<https://doi.org/10.1089/dna.2009.0874>
- Fischman, J. S., Sista, S., Lee, D., Cuadra, G. A., & Palazzolo, D. L. (2020). Flavorless vs. Flavored Electronic Cigarette-Generated Aerosol and E-Liquid on the Growth of Common Oral Commensal Streptococci. *Frontiers in Physiology*, 11.
<https://doi.org/10.3389/fphys.2020.585416>