2020 Cover Sheet for Faculty Summer Grant Proposals

This is the first page of the application. The complete application should be sent to sherriyoung@muhlenberg.edu and danalohman@muhlenberg.edu by **noon, January 24, 2020**.

Name: Giancarlo Cuadra Date January 24, 2020

Department Biology

Project Title (25 words maximum):

Effects of Electronic Cigarettes on the Growth of Oral Bacteria

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Type of grant (place an 'X' next to all that apply):

X Research or study leading to publication, exhibition, or performance (4 or 8 wk)

___Professional growth, such as self- directed or formal skill development (4 or 8 wk)

___Crossette Family Faculty Fellowship for International Research

___Direct expenses, e.g., books, travel, equipment (not to exceed $1500, receipts needed)

Duration of summer study (available stipend):

X Eight weeks ($4000)____Four weeks ($2000)

Will you be teaching a summer course during the proposed award period? NO

If Yes, then you are not eligible for a Summer Research / Professional Development stipend. If you are awarded the Crossette fellowship, you will not be awarded an eight-week stipend (although you may be awarded a four-week stipend).

Effects of Electronic Cigarettes on the Growth of Oral Bacteria

Abstract:

The use of electronic cigarettes (ECIG) has increased over the past decade. The oral cavity is the home of hundreds of species of oral bacteria, including commensal (harmless) as well as pathogenic (harmful) organisms. The aim of this project is to test the effects of ECIG liquid without and with flavors on the growth of oral bacteria. To test this, we will grow each bacteria species while exposed to different ECIG liquids and measure growth rates, comparing to untreated controls. These results will improve our understanding of the effects of ECIG as they relate to oral disease.

Project Statement:

Electronic cigarettes (ECIG) were first developed with the intention to help with cessation of cigarette smoking. These devices consist of a battery, a tank for a liquid and a heating element that vaporizes said liquid prior to inhaling (14). There are hundreds of different brands of ECIG liquids (e-liquids) with thousands of different flavors (10). Alarmingly, children in middle school and high school are a significant population of ECIG consumption and are the target of ECIG marketing (11).

There are over one hundred published studies describing the effects of e-liquid or ECIG vapor in the airway and lung tissue. The major trends in this field is that the flavors are the most harmful ingredient of e-liquid or its vapor. These effects lead to tissue irritation, strong inflammation and injury, sometimes causing loss of function in air exchange (8). Such effects have resulted in several cases (including death) of what is now known as ECIG or vaping related acute lung injury (7).

The oral cavity houses hundreds of bacterial species, which live with each other and with the human host. In health, the interactions between bacteria and the host remain at homeostasis (1). Oral bacteria live on all surfaces of the mouth. On the teeth, these bacteria form communities referred to as dental plaque, which is an example of bacterial biofilms. Under the microscope, these biofilms include several different types of species attached to the dental surface (9). The numbers of oral pathogenic bacteria in dental plaque biofilms stay low, preventing the human host from developing any diseases. However, interference of the interactions between bacteria, or interactions between the host and bacteria, could result in a severe change that may lead to oral anomalies such periodontal disease (2) and even diseases beyond the oral cavity including diabetes, cardiovascular disease, pregnancy complications and Alzheimer's disease (3,5,6,13).

To date, little information is available on the effect of e-liquid or ECIG vapor on the oral environment. Only five articles directly test the effects of e-liquids or ECIG vapors on oral microbes, and four of these studies focus on oral bacteria. Two of the four published studies in oral bacteria were generated by our laboratory here at Muhlenberg College in collaboration with Dr. Palazzolo's laboratory at Lincoln Memorial University in Harrogate, TN. In our studies, oral commensal bacteria were exposed to flavorless e-liquid or traditional cigarette smoke and the results show little to no effect by the flavorless e-liquid to very harmful effects by traditional cigarette smoke (4,12). There are no studies to date that address the effects of e-liquids or vapors containing flavors on the growth of oral pathogenic bacteria. Therefore, the aim of this study is to investigate the effects of e-liquids with different flavors on the growth of oral bacteria. The results of this work will help us better understand the changes in bacteria-bacteria interactions and the potential gain in growth of pathogenic bacteria over commensal species.

Materials and Methods:

Four species of commensal bacteria including *Streptococcus gordonii, Streptococcus oralis, Streptococcus mitis* and *Streptococcus intermedius* as well as three species of pathogenic bacteria including *Porphyromonas gingivalis, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* will be employed in this project. Bacteria will be grown in broths with nutrients appropriate for each strain at 37° Celsius (human body temperature). Each broth will contain a final volume of 1% to 5% of e-liquid with five different flavors: blueberry, cinnamon, menthol, strawberry and tobacco. In addition, in separate test tubes no e-liquid will be added to broths, which will serve as controls. As bacteria grow in their broths, absorbance of the cultures will be read using a spectrophotometer at 595 nm wavelength. Absorbance will be measured at 0, 2, 4, 6, 8 and 24 hours of growth.

Bacteria will also be grown in biofilms. To achieve this, sterile plastic plates will be treated with sterile human saliva, following the protocol of our IRB (IRB Cuadra_S19_18). Briefly, saliva will be collected from healthy non-smokers and non-vapers that meet the donation criteria in the IRB. Human saliva from at least five donors will be pooled and processed to remove any microbial contaminants. Sterile human saliva will be used to coat plastic surfaces of plates, simulating saliva-coated teeth. The seven aforementioned strains of bacteria will be added to these plates separately or together and allowed to adhere, similar to dental plaque on real teeth. Once adhered, bacteria will be incubated at 37 °C with broths containing 1% to 5% e-liquids with the five different flavors or control as described above. After 24 hours of biofilm growth, biomass will be measured and biofilms images will be acquired using the scanning electron microscope in the biology department. Moreover, level toxicity of e-liquids with flavors against oral bacteria will be assessed using the fluorescent microscope in the department. Furthermore, overall amounts of specific species will be determined with molecular probes and equipment in the department.

Preliminary Data:

To ensure meaningful results and productive protocols, my laboratory has already started pilot experiments of this project. As seen in Figure 1, *S. intermedius* was grown in BHI broth without and with 1% e-liquids containing strawberry, menthol and cinnamon flavors. Based on the growth rates, all three e-liquids with flavors significantly

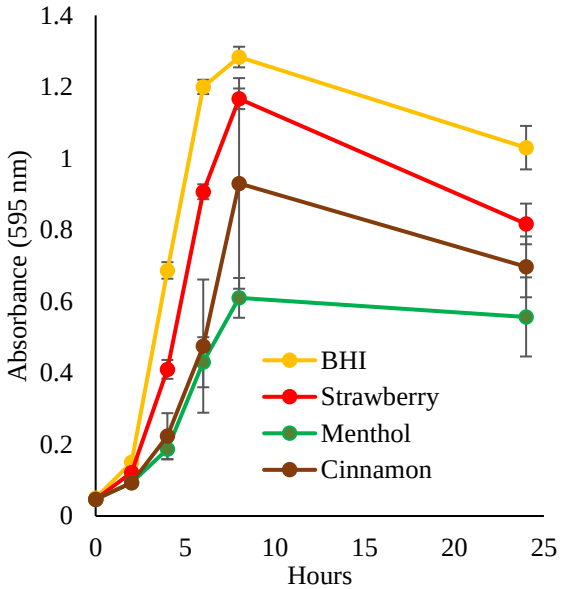

Fig 1. *Streptococcus intermedius* growth after exposure to 1% e-liquids containing three different flavors. Averages and SD (error bars) of three cultures for each condition.

reduce the growth of the strain compared to BHI control. The data indicate that these e-liquids with flavors at only 1% in broth (low dose) do affect the growth of this commensal species. Based on this, e-liquids with flavors will be used to test the growth of all seven species separately as well as altogether in biofilms, mimicking their normal communal biology in the oral cavity.

Expected Outcome and Professional Value:

Based on the preliminary data, we expect significant differences in the growth of these bacteria when comparing treatments to controls. It will be interesting to observe the multi-species biofilm formation and architecture after e-liquid treatments. All these results will add to the current limited knowledge of the effects of ECIG use within the oral cavity, as this may relate to oral diseases. After publishing the first two studies on the effects of flavorless e-liquid on oral bacteria, we have created a preamble to the studies on e-liquid flavors on the same microorganisms. Therefore, we expect to present this study at the American Society of Microbiology (ASM) meeting in Chicago IL on June of this year. The ASM is the biggest organization in microbiology in the country and it is the most appropriate platform to present this work and showcase our students as co-authors. In addition, we also expect to prepare a manuscript summarizing the resutls of all above experiments and we are confident that this study will be accepted for publication in the same journal where our previous two studies have been published.

This study, its presentation and publication will give rise to several more in-depth studies for each of the species of bacteria mentioned above. For example, in the future we can study the ability of any of these species to tolerate or even become resistant to e-liquid and flavors. We can also investigate the interactions of these resistant bacteria on the host, using oral epithelial cells,

which we routinely grow in our laboratory. I am very confident that these e-liquid-related projects will attract several students to work in my laboratory, especially those that are thinking of a healthcare career such as medicine or dentistry.

Project Requirements:

All facilities and resources to complete this project already exist in the biology department.

Project Schedule and Expenses:

Bacterial growth experiments with e-liquids will be performed in the second half of May of 2020. Biofilm experiments and microscopy will be done in June and July of 2020. The ASM presentation will be in late June. Manuscript preparation will start in early August. Except for molecular probes and fluorescent stains, most materials and supplies for this project are already in my laboratory. Travel expenses to the ASM will be paid for out-of-pocket. Publication fees will be covered by the Biology department. I have not applied to any other funding for this project. I do not have any other professional commitment during the time covered by this grant. In case the project is not completed by August of 2020, I expect to complete the remaining experiments by December of 2020.

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