Williams Honors College
The University of Akron
Akron, OH 44325–1803

Honors Research Project Proposal

Congratulations! The inception of your Honors Research Project represents the beginning of your last phase as an undergraduate student at The University of Akron. As a member of the Williams Honors College you have maintained an outstanding academic record that has hopefully prepared you for this independent project. Your Honors Research Project Proposal is an important component of your overall project. In particular it provides a guideline and timetable for the work to be accomplished and ensures that the expectations of the project sponsor and readers are clearly defined. When preparing your written description please include the following sections:

1. **Goals and Objectives:** In the initial section of the proposal, describe the specific research questions to be addressed, as well as the goals and objectives of the project. What are the motivations for the work and the benefits if successful? This section should be written at a level that is readily accessible for non-experts in the field of study.
2. **Methodology:** Then, discuss the work to be undertaken including the methodology used. How will the goals and objectives be met and the questions described above be answered? Does this work build significantly on existing research and efforts? This section can be more in-depth than the previous section, but should still be understandable by someone in the field of study. **Please include a tentative timeline for the efforts with projected intermediate project deadlines.** While the timeline is subject to change as the research progresses, it provides an initial guide for the work to be undertaken and is an important part of the proposal. Proposals without a timeline will not be approved.
3. **Outcomes:** Describe the final output of this project, including the written report for the Honors College. If the project is to be performed or presented publicly, what will be the expected event and audience?
4. **Academic Impact:** Finally, please describe how you see your Honors Research Project building on and adding to your undergraduate experience and knowledge. How does this project serve as an enveloping experience for the undergraduate major? What is expected to be applied from the curriculum and learned from the outcomes?

Any references cited can be included in a final section when appropriate. The length of your written description is expected to be 3–5 pages. For projects involving more than one Honors student, please submit a single project description, but with a separate and completed cover sheet for each student. In addition, please include a statement regarding the expected responsibilities for each Honors student.

Provide copies of the completed proposal (cover sheet and project description) to your project sponsor, your readers, and your Honors Advisor for their approval. Once approved your proposal should be submitted online through IdeaExchange. You will be notified by email once your project proposal has been reviewed by the Williams Honors College.

Honors Research Project Proposal

Please Print

Name: Alyssa Grismer	**Student ID:** 4115139
Email (@zips.uakron.edu): aag90@zips.uakron.edu	
Title of Proposed Project: Effects of E-cigarette Vapor on Zebrafish Cell Regeneration and Wou	
Major: Biomedical Science	**Graduation (semester/year):** Spring 2021

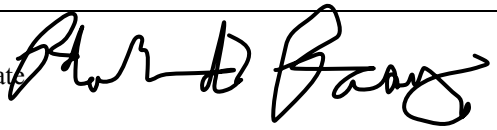
Please include a brief (maximum 200 words) summary of your proposed project

In regards to wound healing, smoking traditional tobacco cigarettes is known to have negative effects on wound healing (Akoz, 2002). An increased risk of skin necrosis has been observed in patients who smoke throughout the surgical process (Akoz, 2002). It is known that nicotine, whether introduced through inhalation or intravenously, leads to peripheral skin vasoconstriction (Akoz, 2002). The full physiological effects of e-cigarettes are not known and research is being done to improve our understanding on the effects that these products may have on our bodies. This research is intended for that purpose. Zebrafish have recently been used as a model organism for studying the effects of cutaneous wound healing (Richardson, 2013). They have an adaptive immune system as adults which gives them an advantage for research over other vertebrates for immune system and healing studies (Novoa, 2011). This research is aimed at answering the scientific question of how the chemicals in e-cigarettes may affect wound healing and regeneration of the caudal fin of adult zebrafish after prolonged use. We are interested in comparing the rate of regeneration and levels of pigmentation, in the caudal fin of the zebrafish after they undergo a minor fin clip after prolonged e-cigarette exposure.

Approval:

Honors Course No.:	**No. of Project Credits:**

Honors Project Sponsor
Signature/Date  9/30/20
Print name Brian Bagatto Email: bbagatt@uakron.edu

Reader
Signature/Date  9/29/20
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Honors Faculty Advisor
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Your approved cover sheet and proposal must be submitted to the Williams Honors College through IdeaExchange

I. Goals and Objectives

The topic that I intend to research is what effect e-cigarette smoke will have on zebrafish cell regeneration. The objectives are to quantitatively and qualitatively describe the cell regeneration in zebrafish who are exposed to the chemicals in e-cigarette vapor. Another goal is to compare the test group that is exposed to vapor to a control group of zebrafish to see if e-cigarette vapor causes any significant differences in regenerative properties, such as the rate of healing, the overall time of healing, and the pigmentation level of cells, and the level of scaring observed. If successful, this project will lead to a further understanding of the effects of e-cigarette vapor on wound healing in a model organism for humans, and open the door for further research on the mechanisms involved in wound healing that may be altered by the chemicals in e-cigarettes. A final goal for this project is to allow for undergraduate students to gain invaluable knowledge and experience from conducting educational research.

II. Methodology

Background:

Since its commercial success after its release in 2003, the use of e-cigarettes have skyrocketed, not only for their use in quitting smoking, but also as a new age drug that is popular among young individuals. According to the FDA's 2018 National Youth Tobacco Survey, there has been a 78% increase in e-cigarette use in high school students from 2017 to 2018 and a 48% increase among middle school students (Tobacco, 2019). E-cigarettes are known to be less harmful than traditional tobacco cigarettes, but they are still known to have many negative health effects. In regards to wound healing, smoking traditional tobacco cigarettes is known to have negative effects on wound healing (Akoz, 2002). An increased risk of skin necrosis has been observed in patients who smoke throughout the surgical process (Akoz, 2002). It is known that nicotine, whether introduced through inhalation or intravenously, leads to peripheral skin vasoconstriction (Akoz, 2002). This leads to negative effects in wound healing. Other negative known effects on wound healing from tobacco cigarettes are from

carbon monoxide. The carbon monoxide binds more strongly to hemoglobin than oxygen making it difficult for oxygen to reach the tissue in need of repair (Akoz, 2002). The full physiological effects of e-cigarettes are not known and research is being done to improve our understanding on the effects that these products may have on our bodies. Further study is needed on e-cigarette effects on the whole body and on how prolonged exposure may affect wound healing. This research is intended for that purpose. Zebrafish have recently been used as a model organism for studying the effects of cutaneous wound healing (Richardson, 2013). They have an adaptive immune system as adults which gives them an advantage for research over other vertebrates for immune system and healing studies (Novoa, 2011).

This research is aimed at answering the scientific question of how the chemicals in e-cigarettes may affect wound healing and regeneration of the caudal fin of adult zebrafish after prolonged use of the chemicals in e-cigarettes. We are interested in comparing the rate of regeneration of the caudal, or tail, fin, of the zebrafish after they undergo a fin clip, or fin scrape. The rate of regeneration as well as the level of pigmentation in the regenerated fins, will be compared for 3 separate test groups of fish. One group will be exposed to e-cigarette vapor both before and after fin dissection, one group will be exposed to e-cigarette vapor only before the dissection, and the final group will not be exposed to any e-cigarette vapor.

We hypothesize that the rate of regeneration will be slower in the zebrafish exposed to the e-cigarette chemicals both before and after dissection, as well as for the group exposed to the chemicals only before the dissection, when compared to the rate of regeneration in the zebrafish who not exposed to the e-cigarette chemicals.

There is also a considerable teaching value to this study. It is being designed and implemented from start to finish by undergraduate students working on their honors research project. Students are learning all the aspects of designing a feasible and pertinent project, as well as how to collect, interpret, and present

the data and results. Therefore, aside from the knowledge to be gained for further study, there is enormous value to the undergraduates in performing this work.

Procedures:

Up tp 16 male and 16 female adult zebrafish will be used as a test group that is exposed to e-cigarette smoke both before and after caudal fin amputation. 16 male and 16 female adult zebrafish will be used as a test group that is exposed to e-cigarette smoke only before 50% caudal fin amputation. Up to 16 male and 16 female adult zebrafish will be used as a control group that is not exposed to e-cigarette smoke. Each group will be placed in separate tanks and acclimated for 24 hours prior to the start of the experiment. Groups will receive standard husbandry and care and will be placed in dechlorinated water that is 27-29 °C. One test group will be exposed to daily treatments of e-cigarette chemicals for a period of two weeks before the fin clip to stimulate prolonged exposure of e-cigarette vaping. One test group will be exposed to e-cigarette smoke following the amputation to represent a group with continued exposure. The control group will not be exposed to any chemicals from e-cigarette vapor during the experiment. Care will be taken to avoid exposure to e-cigarette vapors by using a suction apparatus that uses the e-cigarette to produce vapor. The device will contain the vapor and then it can be pumped into a sealed flask to be mixed with water that will be added to the tank for exposure. The risks of inhaling vapor will be minimized by the apparatus.

The groups will undergo a 50% caudal fin clip which will be performed on the adult zebrafish 2 weeks after the initial exposure period. For this procedure, the fish will be transferred from their tanks to 0.01% MS-222 will be used as an anesthetic agent. The fish may be anesthetized to loss of reflex, which will be checked with a sterilized probe. In addition gill monitoring will take place while under anesthesia. The MS-222 will be buffered with sodium bicarbonate to maintain a pH of 7. The caudal fin will be amputated using sterilized scalpels. A standard dose of Lidocaine may be used as a precautionary pain reliever. The

fish will be recovered alone in aerated tank water until swimming normally. The fish will then be returned to individual tanks so that they may be monitored post-surgical procedure. The fish require standard husbandry care after the procedure. The procedure is expected to have a minimal impact on the wellbeing of the animal, as total regeneration of the fin is expected in 1-2 weeks. The procedure is expected to take less than 20 minutes to complete.

During the experiment, the fish will be photographed several times for up to 2 weeks after their fin clips. Photographs will be taken so that a measurement of the rate of growth and regeneration can be recorded. All fish exposed to e-cigarette chemicals will be euthanized at the end of the experiment using an overdose of MS-222 and the remaining fish in the control group will be returned to the in house breeding stocks.

Fish will be identified by the container they are placed in. It is impractical to identify individual fish when they are in the general population. When isolated fish will be identified by the container label. Fish will be monitored once a day during the two week exposure to the e-cigarette vapor. Their water will be changed as needed and deceased or diseased fish will be removed, if necessary. Fish will be fed daily and the temperature will be maintained by an automated heater. Water changes will be done as necessary to maintain proper husbandry and care of the animals. Records will be maintained on charts corresponding to the tanks to record the water temperature, cleaning, feeding, and experimental progress.

To the best of my knowledge, the effects of e-cigarette exposure on regeneration of tissue has not been explicitly tested in zebrafish, adult or larvae; therefore it is unknown whether e-cigarettes will negatively impact the healing process of zebrafish. Some experiments done on humans have shown that nicotine exposure causes a decrease in cutaneous blood flow. (Davies, 2016). This is often cited as the reason for negative effects in healing with people who are regular smokers. It is important to study wound healing in zebrafish because of their remarkable ability to regenerate tissue with little scarring. (Poss, 2003).

This is an ability which is hard to find in many other model organisms in the lab. Zebrafish have also been used as a model organism for wound healing (Richardson, 2013). Because this type of experiment has never been accomplished, the usage of zebrafish for exposure to e-cigarette vapor is necessary and justified. The exposure of e-cigarettes is hypothesized to compromise the healing and regeneration processes in the zebrafish and therefore it is expected that the zebrafish exposed to the vapor would have a longer recovery time, a slower regeneration rate, and a higher possibility of scarring and hyperpigmentation.

Tentative Timeline:

August: IACUC Approval Received for Project

September-October: Compile Supplies and Set Up Preliminary Testing

November-December: Run Trials and Collect Data for Research Project

January-February: Complete Statistical Analysis and First Draft to be Submitted to readers

February-March: Receive Feedback from Readers and Revise Drafts as Needed

March-May: Finalize Project and Prepare to Turn In

III. Outcomes

The final output of this project is expected to be a written report that will be published on Idea Exchange, along with a presentation poster highlighting the findings of this project and areas for further research, as specified by the Biology Departments requirements. The mode of expected presentation of the project will likely be determined at a later date and time. Due to the current pandemic, it is unclear as to whether the possibility of presenting this project to an audience will be available to us. As mentioned in the previous section, it is hypothesized that the zebrafish exposed to the vapor would have a longer

recovery time, a slower regeneration rate, and a higher possibility of scarring and hyperpigmentation. These findings will be reported upon in the final project.

IV. Academic Impact

Because this research has never been performed, I see it as an opportunity to increase the wealth of knowledge in the scientific community. I believe that this research has value in that it could be used in the development for further studies involved in designing treatments to decrease the negative side effects associated with nicotine. It could potentially reveal a unique ability of zebrafish to resist scaring and slow regeneration of tissues, which would incite research into the mechanisms behind this ability, which may be utilized for human applications. This would further my undergraduate experience by allowing me the opportunity to implement and design a study from start to finish, and accomplish the task of contributing my findings to the scientific community. This project will potentially be able to further my career aspirations in medical school as I am interested in the speciality of dermatology. Having published research in a field related to dermatology could make me a more competitive applicant for certain residency programs.

V. Expectations and Responsibilities of Students:

This research project is expected to be completed by myself, Alyssa Grismer, as well as Jenna Pumneo. Both of us will be expected to share the work of carrying out the procedures of the experiment. This will include sharing the responsibilities of preliminary testing, setting up the experiment, preparing and delivering the vapor, performing the fin clip, sharing husbandry care, and collecting data. Each of us will be analyzing different components of the collected data in order to generate separate results. Jenna will be responsible for collecting data on the rate of regeneration as well as the differences in regeneration times. I will be responsible for collecting data related to scarring and hyperpigmentation. We will have separate conclusions from our observations but will share much of the responsibility of performing the experiment itself.

VI. Citations

Aköz, T., Akan, M., & Yıldırım, S. (2002). If You Continue To Smoke, We May Have a Problem: Smoking's Effects on Plastic Surgery. Aesthetic Plastic Surgery, 26(6), 477-482. doi:10.1007/s00266- 002-2045-3

Center for Tobacco Products. (2019, February 11). Youth & Tobacco - Youth Tobacco Use: Results from the National Youth Tobacco Survey. Retrieved from https://www.fda.gov/TobaccoProducts/PublicHealthEducation/ProtectingKidsfrom Tobacco/ucm4051 73.htm

Davies, C. S., & Ismail, A. (2016). Nicotine has deleterious effects on wound healing through increased vasoconstriction. Bmj, I2709. doi:10.1136/bmj.i2709

Richardson, R., Slanchev, K., Kraus, C., Knyphausen, P., Eming, S., & Hammerschmidt, M. (2013). Adult Zebrafish as a Model System for Cutaneous Wound-Healing Research. Journal of Investigative Dermatology, 133(6), 1655-1665. doi:10.1038/jid.2013.16

Novoa, B., & Figueras, A. (2011). Zebrafish: Model for the Study of Inflammation and the Innate Immune Response to Infectious Diseases. *Advances in Experimental Medicine and Biology Current Topics in Innate Immunity II,* 253-275. doi:10.1007/978-1-4614-0106-3_15

Poss, K. D., Keating, M. T., & Nechiporuk, A. (2003). Tales of regeneration in zebrafish. Developmental Dynamics, 226(2), 202-210. doi:10.1002/dvdy.10220

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