Methodology

Role of Student v Mentor

The development of expertise in planaria care was made possible through the support and guidance of Robert Schnittker from the Sánchez Alvarado Lab at the Stowers Institute for Medical Research. Through multiple correspondences, valuable feedback and advice were obtained regarding cell culturing and planarian biology. All aspects of the project—including conceptualization, literature review, experimental execution, and data analysis—were conducted by the student researcher. Since August of 2024, this project has been conducted at the Massachusetts Academy of Mathematics and Science at Worcester Polytechnic Institute and is planned for presentation on February 20th, 2025.

Equipment and Materials

Schmidtea mediterranea were obtained from the Sánchez Alvarado Lab at the Stowers Institute for Medical Research. Planaria were maintained in clear plastic storage containers filled with an Instant Ocean Salt (IOS) distilled water solution, following standard protocols. To facilitate routine water changes, a separate plastic container was designated for waste collection. Planaria were fed hard-boiled egg yolks, and all surfaces were sanitized with a 70% bleach or ethanol solution before and after use.

The sodium alginate and calcium chloride used for bioink preparation, along with the Prusa MK4 i3 3D printer, were pre-existing resources within the Mass Academy lab. The Celestron Digital Imager HD, ImageJ, and a micrometer calibration slide were also used to acquire results.

Bioprinter

By modifying a Prusa MK4 i3 printer, a cost-effective bioprinter can be created. Modifications to the Prusa were 3D printed using ABS filament in accordance with Bessler et. Al in the 2019 ScienceDirect publication *Nydus One Syringe Extruder (NOSE): A Prusa i3 3D printer conversion for bioprinting applications utilizing the FRESH-method*.

Bioink

The sodium alginate bioink and calcium chloride support bath were made in accordance with Bessler et. Al in the 2019 ScienceDirect publication *Nydus One Syringe Extruder (NOSE): A Prusa i3 3D printer conversion for bioprinting applications utilizing the FRESH-method*. However, planaria neoblasts are seeded within the ink. Provided adequate materials, three trials of varying bioink viscosity will be created.

Planaria Care

Upon arrival, *schmidtea mediterranea* were transferred from their shipment bag into a clear plastic rectangular container. Following feeding, they were monitored daily for any signs of lysis.

At the onset of lysis, around 30 healthy planaria were relocated to separate petri dishes to prevent potential contamination from diseased individuals. Water changes were then conducted daily for the main container, involving a complete exchange of the IOS distilled water solution and a manual wipe-down of residual mucus using a paper towel.

Planaria Testing & Fragmentation

A preliminary test of planaria vitality was conducted prior to embedment into the bioprinted construct. Here, three planarians were fragmented into two distinct parts (head and tail) and studied over a course of five days to ensure regeneration consistency like that from pre-existing research (Bartscherer, 2014). After confirming planaria vitality, individuals from the same batch were selected, fragmented, and placed into the bioprinted construct. Prior to fragmentation, planaria were starved for 10 days to minimize gut bacteria, reducing the risk of water contamination and infection post-incision. Planaria were placed on dampened paper towels infused with IOS water atop an ice pack to minimize movement. Fragmentation was performed using sterile razor blades, which were disinfected with 70% ethanol solution before and after each use.

ImageJ

Images of planaria captured using the Celestron Digital Imager HD must include a fully visible calibration tool with a scale of 1 DIV = 0.1 mm. To establish the pixel-to-mm conversion, five independent measurements of 0.01 mm are obtained using the 'Analyze → Measure' function and averaged. The new scale is then set via 'Analyze → Set Scale,' with the measured pixel distance as the average from the previous step and the known distance set to 0.01 mm. This calibration process must be repeated for each image, as potential discrepancies may arise due to slight shifts in planaria positioning upon introduction to the microscope.

Once the scale is calibrated, five length measurements spanning the full body of each planarian are taken, recorded, and averaged as data. Any images in which the planaria are unsuitable for measurement—such as those that are scrunched, curved, partially out of frame, or have an unclear calibration tool—are excluded from analysis as extraneous data.

Waste Disposal

Bleach was added to liquid waste at a final concentration of 10% bleach and left to sit for at least 15 minutes before being disposed of in the lab sink. Bleach was added to biowaste and left until visible cell death occurred before being disposed of in a lab biowaste bin. Solid waste (petri dishes, pipettes, gloves) was disinfected before being disposed of in the lab.

Statistics

After data collection, results were graphed using Excel. Significance for each testing group was determined using a one-way ANOVA test. Comparisons of means for cell regeneration was done in groups (heads and tails of planaria by day) using a two-tailed t-test. A p-value of < 0.05 was considered statistically significant.