Section III: Results

Preliminary Data

In the initial phases of this project, planaria were assessed for viability. Figure 2.1 displays the mean length of the head fragment measured over time, while Figure 2.2 presents the mean length of the tail fragment. Statistically significant differences ($p < 0.01$) are indicated by asterisks. Both graphs illustrate regeneration from Day One to Day Three, demonstrating a correlation between time and growth. This measurement approach was also applied to a second, distinct planarian, which exhibited similar growth patterns, shown in Figures 3.1 and 3.2.

Figures 2.1 and 2.2

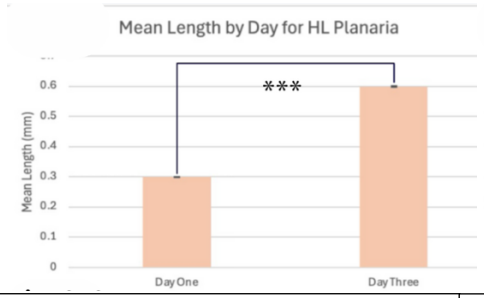

Figure 2.1: Mean length by day for the head of the "lighter" planaria. Asterisks denote statistical significance ($p<0.01$)

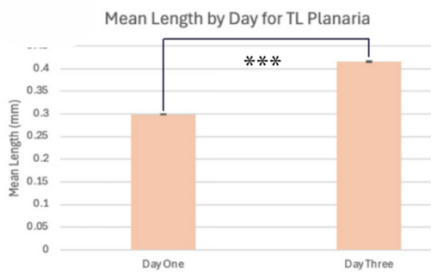

Figure 2.2: Mean length by day for the tail of the "lighter" planaria. Asterisks denote statistical significance ($p<0.01$)

Figures 3.1 and 3.2

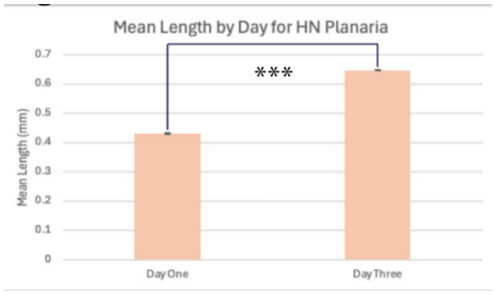

Figure 3.1: Mean length by day for the head of the "newer" planaria. Asterisks denote statistical significance ($p<0.01$)

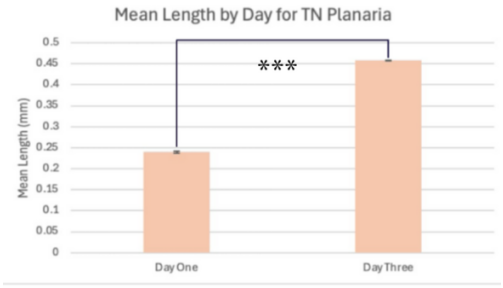

Figure 3.2: Mean length by day for the tail of the "newer" planaria. Asterisks denote statistical significance ($p<0.01$)

Print Stability

Following the initial creation of a print, it is essential to assess its viability for continued use. Due to time and material constraints, this testing process was limited. A visual inspection was conducted to evaluate the print based on three key criteria: (1) visible adhesion of the bioink to confirm structural integrity, (2) tensile strength assessed through stress testing, and (3) a timed trial to measure vitality over a one-hour

period. Out of the total amount of prints made, it is hoped that all of them are viable, however, the researcher found a success rate of 80% after taking factors such as material constraints and human error into consideration.

Bioink, and Cell Dissociation

Test One – Lower Viscosity

Test One examined the regeneration of planaria embedded in a less viscous bioink. After printing, the structure was allowed three minutes to cross-link before being removed from the support bath. Compared to the higher viscosity ink, this formulation resulted in a softer, less stable structure. Following an hour-long viability assessment of the embedded planaria, the print was monitored over five days to evaluate regeneration. As shown by Figure 4.1, the less viscous ink showed a 0.03mm increase with a linear trend. Images and videos of the embedded planaria were captured daily and analyzed using ImageJ, following the procedures outlined in earlier sections of this paper. The measured lengths from each day were averaged and graphed in Excel for further analysis.

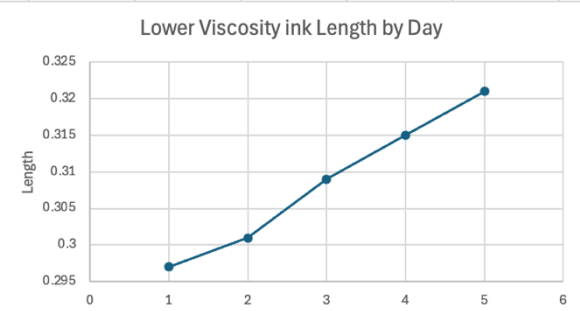

Figure 4.1: Average length of regenerated part (mm) by day

Test Two – Higher Viscosity

Test Two examined the regeneration of planaria embedded in a more viscous bioink. After printing, the structure was allowed five minutes to cross-link before being removed from the support bath. Compared to the higher viscosity ink, this formulation resulted in a harder, more stable structure. Following an hour-long viability assessment of the embedded planaria, the print was monitored over five days to evaluate regeneration. As shown by Figure 4.2, the more viscous ink showed a 0.002mm increase. Images and videos of the embedded planaria were captured daily and analyzed using ImageJ, following the procedures outlined in earlier sections of this paper. The measured lengths from each day were averaged and graphed in Excel for further analysis.

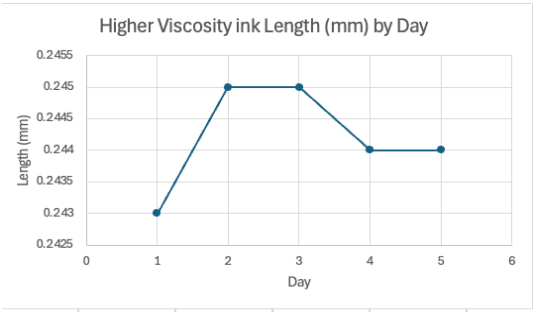

Figure 4.2: Average length of regenerated part (mm) by day

Section IV: Discussion

This study demonstrates that a bioprinted construct can serve as a viable model for cancer metastasis. Although growth within the printed structure was significantly less than that observed outside of it as shown when comparing a 0.3mm/0.2mm increase to that of a 0.03mm increase (Figures 2/Figures 4), this reduced growth can likely be attributed to suboptimal printing parameters. Nevertheless, the fact that growth occurred at all is promising and sufficient for the scope of this experiment, as it indicates that the bioprinted construct can support cellular growth. Much like a study by Huang et al., which explored bioprinted models for tumor cell migration, this work supports the viability of 3D bioprinted environments as physiologically relevant metastatic models. Both studies highlight how mechanical and biochemical factors within a bioprinted construct influence cell behavior. While Huang et al. utilized mammalian cancer cell lines, whereas this study used planaria as a regeneration-based model, demonstrating that even stem cell-driven migration can be influenced by bioink properties. Despite these differences, both approaches reinforce the potential for 3D bioprinting in developing cost-effective, customizable models for cancer research. The following section will address the potential reasons for the observed limitations and explain why these findings remain pertinent.

Control

A comparison of the two planarians within the preliminary data (Figure 2.1 and 2.2) revealed that both exhibited growths as expected; however, the extent of growth was not identical. This finding is significant for future experiments involving planaria embedded in the bioprinted construct, as uniform growth should not be assumed in that context either.

Test One

The results from Test One demonstrate that planaria embedded in the less viscous bioink were able to maintain viability and undergo regeneration over the five-day observation period. The softness of the lower viscosity bioink, due to its reduced cross-linking time, may have influenced both mechanical support and oxygen/nutrient diffusion. While planaria survived the initial hour-long viability trial and the five days that followed, the weaker gel structure likely subjected them to greater mechanical stress when removed from the support bath. This could have affected tissue integrity and movement, potentially impacting long-term

regeneration efficiency. The looser gel structure may have allowed greater diffusion of biochemical factors, potentially altering the microenvironment necessary for optimal neoblast activation.

Test Two

The results from Test Two indicate that planaria embedded in the more viscous bioink were able to maintain viability and undergo regeneration, though with a much slower rate of growth compared to Test One. The 0.002mm increase in length over the five-day period suggests that while regeneration occurred, it was significantly restricted in the higher viscosity environment. This difference from the first test is likely due to the harder and more stable matrix created from the increased cross-linking, which severely limited movement for the embedded planaria. While greater rigidity may have helped preserve the structural integrity of the bioink, it may have also restricted cellular interactions and diffusion of nutrients and oxygen, which are critical for neoblast activation and tissue growth. The vast difference between these two tests also shows the importance of optimizing bioink viscosity for peak regeneration efficiency.

Implications and Applications

This study contributes to the growing field of bioprinting and regenerative medicine by demonstrating how the mechanical properties of bioinks influence cell viability, migration, and tissue regeneration. Although planaria are not a perfect model for cancer, their stem cell-driven regeneration provides valuable insights into cell behavior, proliferation, and directed migration, which are key aspects of metastasis. Beyond offering a potential alternative model for studying cancer cell dynamics, this research also expands our understanding of how bioink viscosity affects cellular processes, particularly stem cell activity and tissue remodeling. By adding to our knowledge of these interactions, this study can contribute to the development of optimized biomaterials for tissue engineering, regenerative therapies, and in vitro disease modeling.

Future Research

Future research should focus on refining and reproducing positive results for this experiment by reprinting constructs and testing again to ensure accuracy, as well as test additional parameters for the model itself to find the ideal framework for cell proliferation. By showing that the success of the printed model is both reproducible and reliable within a larger testing population, bioprinted models of cancer

metastasis can be scaled for high-content, high-throughput screening in the development of anticancer therapies. This reliability also expands the potential applications of bioprinted models beyond metastasis, enabling more accurate representations of the tumor microenvironment. While this study utilized planaria as a biological analogue for metastatic cancer cells due to their rapid cell proliferation and tissue regeneration, it is important to acknowledge the limitations of this model. The extracellular matrix of cancer tumors is composed of numerous factors that this approach was unable to fully replicate using planaria. Therefore, to adapt this technology for cancer cell research, preliminary tests should be conducted using the same procedures established in this study. This technology will be particularly valuable in lowering barriers to cancer research by providing a cost-effective and reliable platform for studying cancer cell behavior and treatment responses. Its accessibility may help expand research opportunities in resource-limited settings, creating innovation in cancer therapeutics and accelerating the development of targeted treatments.