Bioprinting Methods 101

There are three common bioprinting methods: inkjet, stereolithography (SLA), and extrusion. Extrusion bioprinting is the most widely used method of the three types due to its lower cost, versatility, and ability to handle a larger range of biomaterials. Extrusion bioprinting most closely resembles fused deposition modelling (FDM) in traditional 3D printing, with a bioink being dispensed through a syringe to build a 3D structure layer-by-layer (Zhang et al., 2021). Resolution for this method is lower than inkjet and SLA printing, extrusion-based bioprinting allows for the creation of larger constructs with structural integrity, which is the best method to create scaffolds to support cell growth (*Extrusion vs. DLP 3D Bioprinting - Explanatory Comparison*, 2023). As such, this project utilizes extrusion based bioprinting in the form of a modified Prusa MK4S 3D printer.

FRESH Bioprinting

Freeform Reversible Embedding of Suspended Hydrogels Bioprinting (FRESH) bioprinting is a subset of extrusion bioprinting which specializes in bioprinting soft gelling biomaterials (*FRESH Bioprinting Enables More Complex Geometries*, n.d.). FRESH supports these softer biomaterials by printing in a support bath as a compatible bath supports the extruded bioink on all sides, allowing the ink to rapidly go through a gelation process. This method enhances cell viability by providing a supportive, customizable aqueous environment that can include cell culture media and growth factors to promote cell vitality.

Hydrogels Used as a Support Bath in Bioprinting

The support bath used in bioprinting is most commonly a hydrogel, as it provides an environment that closely mimics the extracellular matrix (ECM) found in biological tissues. The extracellular matrix is a complex network that surrounds cells and supports cells within a tissue, providing a physical scaffold for cells within tissues. Thus, changes in the ECM's mechanical properties directly affect cells. One example is a stiffer ECM promoting excessive proliferation, while a softer ECM inhibits cell division (Wu et al., 2023). Hydrogels mimic the ECM through primarily being composed of water, supporting cell viability, and mimicking conditions found in human tissues. Hydrogels are also easily modifiable by adjusting a chemical property, crosslinking, or encapsulating another cell within its matrix, as well as structurally sound enough to provide a support scaffold for larger 3D tissue constructs. Due to these properties, they act as an ideal

support for softer bioinks when extruded from the syringe, allowing them to stay in place until successfully cured.

Sodium Alginate Used as a Bioink in Bioprinting

Within bioprinting, the bioink acts as the "filament" in standard 3D printers of which the construct is printed out of. Bioink selection is crucial as it dictates the rheological and biocompatible properties of the final construct. In this case, sodium alginate was chosen as the bioink of choice in bioprinting. Previous studies have shown the success of sodium alginate for direct bioprinting as it supports cell growth and exhibits high biocompatibility, thereby presenting it as a viable option for printing tissues (Bociaga et al., 2019).

Section II: Specific Aims

This proposal's objective is to develop a 3D bioprinted microenvironment to facilitate planaria stem cell proliferation in a controlled and observable environment. As planaria stem cell division and differentiation during regeneration share similarities with cancer cells exhibiting rapid division, neoblast proliferation within a wounded planarian serves as a model for cancer cell metastasis. The long-term goal is to develop a microenvironment for cell growth that is easily replicable, cost-effective, and provides a platform to study anticancer therapies through planaria biology. The central hypothesis of this proposal is that sodium alginate bioink printed in a calcium support bath will be able to foster cell division and proliferation of neoblasts within a cut planarian. The work we propose here will help create a more effective model compared to those currently used.

Specific Aim 1: Create a bioprinted model that supports planarian life.

Specific Aim 2: Use the model to study planarian neoblast proliferation.

Specific Aim 3: Determine most effective manufacturing parameters for the model (Tian et al., 2021). The expected outcome of this work is to create a bioprinted structure that supports planarian neoblasts and promotes cell growth in a way consistent with previously published research.