Senior Design / BS Project
May - 2020

3D- Bioprinting of Strategically Engineered Cellular Constructs for Controlled Reproducible Composition

Kate Anderson, Sydney Boyd, Samuel Glover, Spencer Reid
Dr. Dumont, Dr. Agarwal, Andrew Ciciriello
Department of Biomedical Engineering

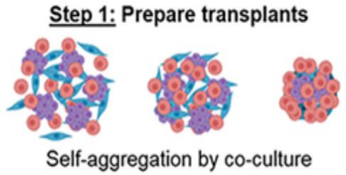
Abstract

3D cell culture systems are able to foster higher levels of tissue organization than traditional 2D systems. Using a 3D bioprinter, we developed a cellular construct composed of mouse embryonic spinal progenitor cells with controlled organization. These constructs will be able to be printed with high reproducibility for high-throughput testing of new cell culture strategies.

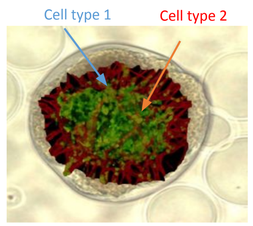
Introduction

In vitro Organ-on-Chip systems have shown promise in simulating the physiology of organs and entire organ systems. Organ-on-Chip systems have been used for investigating cellular interactions and drug discovery. One of the advantages of Organ-on-Chip platforms is the ability to use 3D cell culture which more closely mimics a live cell compared to 2D cell culture systems.

Our team proposed a method for creating 3D multicellular spheroids made up of several different cell types in a controlled manner. These constructs represent a more advanced model of physiology than traditional cell culture, and they have future possibilities as implantable tissues.



Previous experiments using multicellular spheroids allow spheroids to randomly self-assemble with no control over cellular organization.



Our method attempts to combine multiple cell types keeping each cell type in its own area.

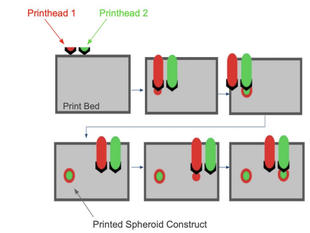
Methods | Design | Analysis

Our developed constructs consist of an alpha and beta prototype. Our materials include:

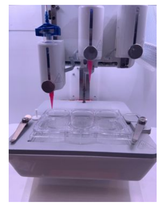
- Cellink BioX 3D bioprinter with pneumatic printheads & 27 G needle
- Prototype G-Code
- 30% Poloxamer - Biocompatible, thermosensitive gel
- 6 µm fluorescently labeled cell-representing beads (alpha prototype) (6mil/mL)
- Mouse embryonic day 14 neural progenitor cells (NPCs) (beta prototype) (2mil/mL)
- Extrusion times of 65,75 ms

In both prototypes, separation of layers, diameter of construct, and viability of beads/cells after printing will be analyzed with fluorescent imaging, Image J, and live/dead assays.

BIoX 3D BioPrinter

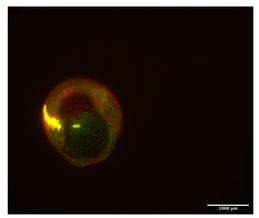


Execution of our BioX GCode. Printer extrudes two separate layers of substrate with beads/cells in a procedural format with heated print bed. Cell/bead composite is extruded and gels upon contact of the printbed.

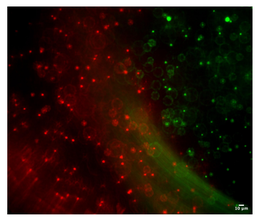


BioX 3D Bioprinter using pneumatic printheads printing onto a 6-well plate

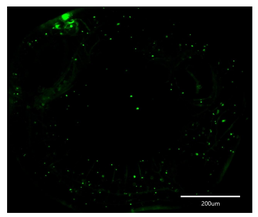
Results



Alpha Prototype using two types of cell-representing beads. Spheroids were printed at 800 X 700 µm.



Alpha Prototype with visible, separate layers between cell-representing beads.



Beta Prototype with viable NPC's after printing, separate layers visible. Courtesy of Dr. Dumont and Andrew Ciciriello.

Conclusion

- This design aimed to fabricate 3D cellular constructs consisting of multiple different cell types in an organized layout in order to advance the field of cellular engineering, with potential applications including, but not limited to biomedical research and islet transplant
- While the cellular printing protocol requires further refinements, our progress represents convincing first steps
- Further refinements include substituting poloxamer for collagen as a thermosensitive print-material, printing with multiple cell types (islets and neural progenitors) in a singular construct, and reducing the size of the constructs

Acknowledgments

We would like to thank our mentors Dr. Dumont, Dr. Agarwal, and PhD candidate Andrew Ciciriello for helping guide and assist us through this endeavor, while giving any needed advice. We would also like to thank Dr. Manns and Dr. Bohorquez for their help in organizing and refining our end goal, along with the assistance they have given us along the way.

References

Anderson, K., Boyd, S., Glover, S., Reid, S. (2020). *3D-Bioprinting of Strategically Engineered Cellular Constructs for Controlled Reproducible Composition.* Unpublished manuscript, University of Miami Department of Biomedical Engineering, Miami, Fl.