

3D Printing of Bioengineered Human Cardiac Stem Cells

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Introduction: MYH6 gene variants are associated with Hypoplastic Left Heart Syndrome (HLHS), a complex form of Congenital Heart Disease (CHD). CRISPR/Cas9 is a new gene-editing technology that our lab has successfully utilized to genetically modify HLHS patient-derived induced pluripotent stem cells (iPSCs). Three-dimensional bioprinting of human cardiac cells is an even newer developing technology with the potential to more closely mimic human cardiac tissue than cells cultured in a standard monolayer dish. **Hypothesis/Goal:** The goal for this project is to develop a new protocol for 3D-bioprinting of HLHS-patient-derived iPSCs that have been differentiated into beating cardiomyocytes. **Methods:** An alginate/gelatin-based bioink was developed with which to 3D-print the cells. The alginate (3%) and gelatin (7%) were sterilized as powder under UV radiation and mixed in cell culture media at 37°C. The cells were harvested with accutase (for iPSCs) or trypsin (for cardiomyocytes) and recovered in media with ROCK inhibitor (which prevents cell death). Collagen (an extracellular matrix protein) was added into the cell suspension. Cells and ink were placed in two separate syringes, and by using a sterile connector, the two solutions were mixed gently and homogeneously. The cell/bioink mixture was placed in the bioprinter dispenser and printed at 30°C. The printed discs were crosslinked with 100mM CaCl₂, which was quickly replaced with culture media. **Results:** HLHS patient-derived iPSCs were successfully printed and survived. The discs were checked daily for 11 days, and the cells continued to survive and grow as a cluster. iPSC-derived differentiated cardiomyocytes were also successfully printed and maintained.

Awards Won:

NASA: First Award of \$2500