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Designing a 3D-bioprinted Experimental Model to Study the Effects of Structural Defects in the Pathophysiology of Persistent Pulmonary Hypertension of the Newborn

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Background:

One in every 500 live births results in persistent pulmonary hypertension of the newborn (PPHN). Structural defects, including increased vascular stiffness, are known contributors to PPHN pathogenesis, however studying these structural defects using traditional experimental methods presents challenges.

Objective:

We have designed functional 3D-bioprinted tissues with modifiable stiffness using cells encapsulated within an alginate-based bioink. We hypothesize that our model replicates structural defects observed in PPHN, enabling us to demonstrate that increased tissue stiffness leads to irregular pulmonary arterial smooth muscle (PASM) contraction and vascular remodelling.

Methods:

The stiffness of structures was controlled by adjusting the bioink alginate (0.25-1.0% w/v) content. PASM ($2.5x10^{7}$-$4.0x10^{7}$ cells/mL) were mixed in bioinks and 3D tissue structures were printed from predetermined designs (8 or 15 mm rings with or without structural framework). Brightfield imaging assessed structural integrity and tissue compaction while viability and cellular organization were determined using Hoechst/Propidium iodide and filamentous actin (f-actin) staining respectively. All the structures were compared to each other.

Results:

All printed structures were physically robust. F-actin staining on 8mm rings showed greater cell elongation and cell-cell contact within ~36 hours. These structures compacted and shrank over 12 days, further validating cell-cell and development of tension within the structures. Conversely, in 15mm rings f-actin staining showed rounded cells with minimal cell contact and compaction. Cell viability typically fell 48 hours after printing, and then plateaued for both ring sizes. Rings with structural framework exhibited intense f-actin staining with little compaction. Similar observations were made across the stiffness range.

Conclusion:

Cell elongation and cell-cell contact were observed in our structures but excessive compaction may cause the rings to collapse. Including a structural framework eliminates this issue while maximizing cell elongation. Ongoing optimization will allow us to test the effects of vascular stiffness on PPHN pathophysiology, and may present new therapeutic targets to tackle PPHN.