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VALIDATION OF A 3D BIOPRINTED MODEL OF AIRWAY SMOOTH MUSCLE – A NOVEL TOOL TO STUDY ASTHMA PATHOGENESIS

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Background:
Asthma is one of the most common chronic childhood diseases and is the leading cause of youth hospitalisation in Canada. Exaggerated airway narrowing caused by abnormal contraction of airway smooth muscle (ASM) represents a hallmark of the disease. However, ASM function is routinely studied in 2D models that are inadequately replicate the complex *in-vivo* 3D micro-environment. Utilizing a technique called 3D-bioprinting, we have produced an experimental model of ASM that more accurately reproduces real tissue.

Objective:
To evaluate the functionality of cells in 3D and establish the biologic relevance of 3D over 2D models.

Methods:
Human ASM cells ($2.5*10^7$cells/mL) were mixed with bio-ink consisting of 0.25-1%w/v alginate, 5mg/ml fibrinogen and 0.5mg/ml collagen-1, and printed following a computer-aided-3D-design with the RX-1 bioprinter. After printing, constructs were incubated with thrombin (1.25U, 30minutes). We assessed cell health, viability and morphology with LDH assays, Hoechst/propidium-iodide and filamentous-actin staining respectively. Lumen compaction was tracked by live cell microscopy and expression of ASM relevant genes measured by qPCR.

Results:
Bioprinted constructs maintained structural integrity and exhibited high cell viability (>80%) for up to 14 days in culture. Histology revealed development of filamentous actin fibres (>90% cell spreading, although actin filaments were heterogeneously organized). Lumen compaction plateaued at 30% after approximately 7 days. Compared with 2D cultures, cells in 3D had a higher mRNA abundance of myosin, but lower vimentin and matrix metalloproteinase-3 (all $p<0.05$).

Conclusion:
We have developed a flagship tool suited for investigating ASM dysfunction in asthma. Our 3D model is mechanically robust, supports cell viability and spreading. However, heterogenous alignment of actin fibers could indicate a breakdown of bio-ink components. Moving forward, inhibitors of such breakdown (e.g. aprotinin) will be included in constructs.