Rapid *in vitro* modelling of cardiac fibrosis and anti-fibrotic drug screening using macromolecular crowding

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BACKGROUND

- Cardiac fibrosis is driven by the activation of fibroblasts to myofibroblasts and excessive Type I collagen deposition, stiffening the heart.
- Conventional *in vitro* models of fibrosis often take weeks to deposit a measurable collagen matrix.
- 'Scar-in-a-jar' (SIJ) models of fibrosis integrating macromolecular crowding (MMC) and TGF-β are established for pulmonary fibroblasts [1] but remain unexplored for human cardiac fibroblasts (CFs).
- Traditional microscopy lacks the ability to measure cell volume. Using quantitative phase imaging (HoloMonitor® M4), we generated label-free, 3D volumetric data over time to ask: Is cell volume a biomarker of fibrosis?

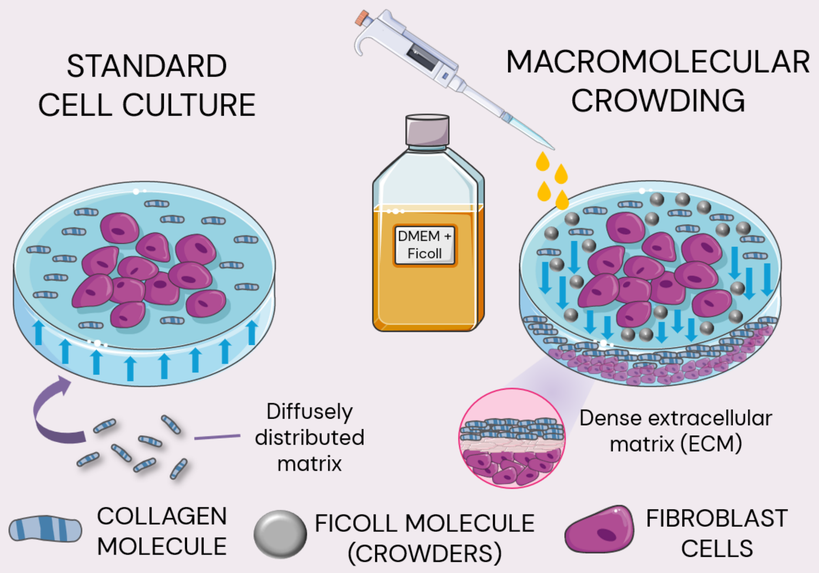
METHODS

Scar-in-a-jar experimental timeline:

Day 1: Seed CFs* in growth medium (DMEM + 10% FBS)

Day 2: Switch to assay medium (containing MMC, TGFβ, drug)

Days 2-7: Incubate for ECM deposition & Holomonitor recording



TGFβ drives **excessive collagen** production.

Ficoll creates a crowded **macromolecular environment.**

Result: Accelerated formation of a dense, organised **fibrotic scar**.

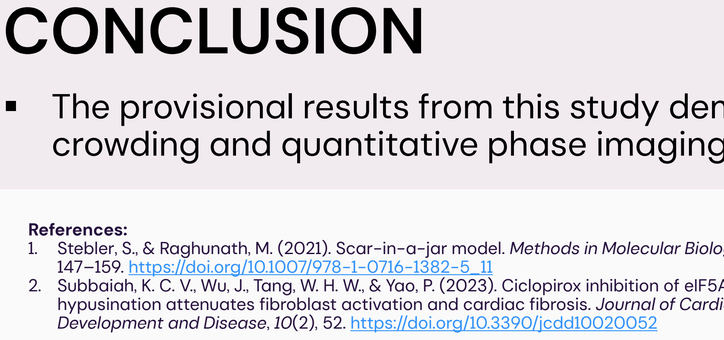
* Foetal CFs purchased from ScienCell Laboratories (United States), donor information is 21 weeks gestation, sex unknown.

Pharmacological screening (to validate the assay):

- Ciclopiroxolamine (CPX): A synthetic antifungal and anti-cancer candidate drug that is shown to inhibit TGFβ CF proliferation, activation and collagen expression [2].
- CZ415: A selective mTOR inhibitor (effective in pulmonary fibrosis SIJ but untested on CFs).

Analysis methods:

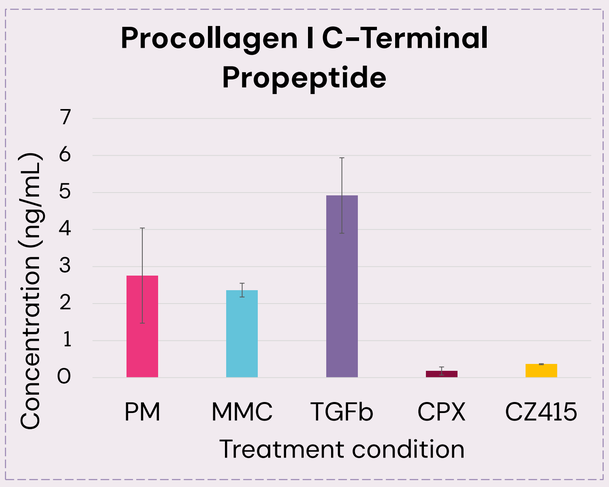
- **Morphological features via HoloMonitor® M4** – run for 6 days, image every 30 mins.
- **Immunocytochemistry** (stain for nucleus and **αSMA**) + fluorescence imaging.
- **ELISA** for type 1 **collagen** deposition.



Holomonitor:

- Provides time-lapse sequences of cells inside the incubator.
- Records over **30 morphological features** for every cell, including optical thickness for 3D volumetric profiling that standard microscopy cannot achieve.

RESULTS



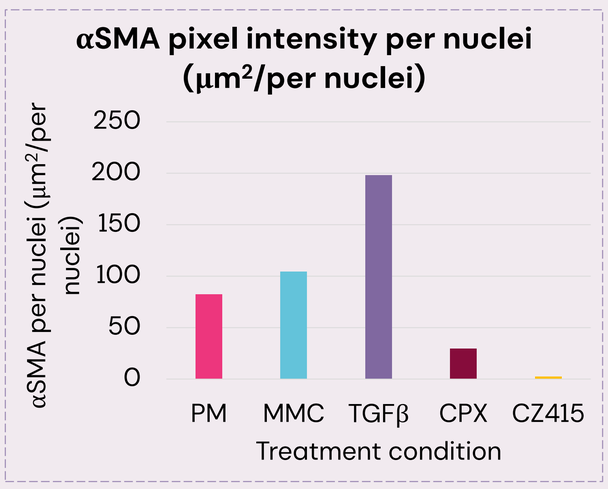


Figure 1: TGFβ drives rapid myofibroblast activation and collagen deposition under MMC. TGFβ halts proliferation while upregulating secreted Procollagen I C-Terminal Propeptide (*left*, ELISA) and αSMA expression (*right*). αSMA staining was quantified via a custom Python pipeline utilising Otsu binarization and morphological filtering to extract total positive area normalised to nuclear count.

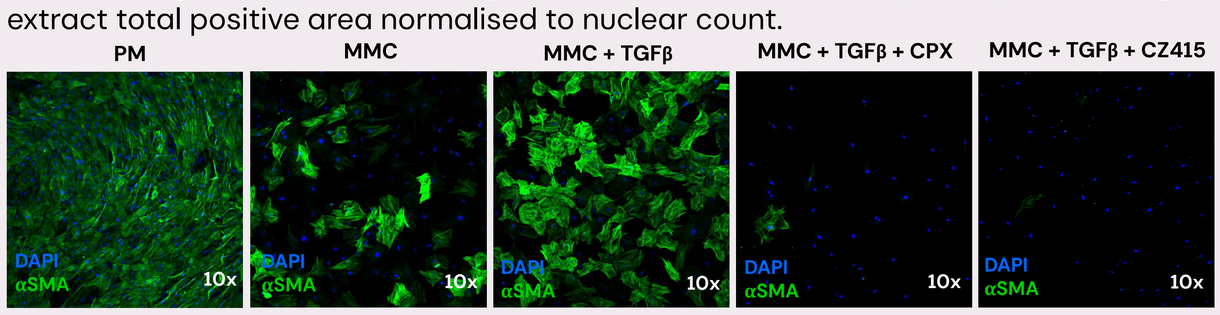
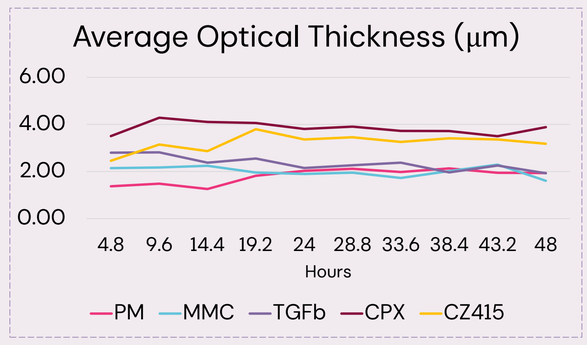


Figure 2: ICC images showing αSMA expression (green – αSMA; blue – DAPI for nuclei)



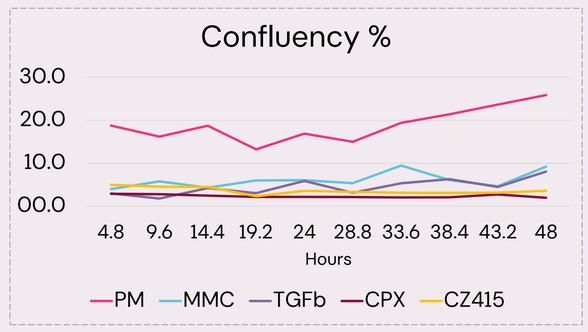


Figure 3: (*Left*) Label-free quantitative phase imaging via the Holomonitor over the first 48 hours. **Top:** Average optical thickness. **Bottom:** Cell confluency.

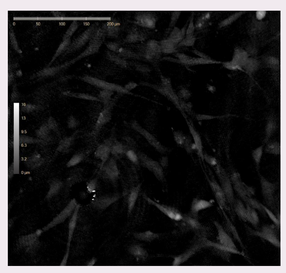


Figure 4: (*Left*) Representative CF phase image. Grayscale gradient indicates optical thickness (0-16µm), enabling 3D volumetric analysis. (*Right*) Scan the QR code for a 6-day timelapse video tracking cell morphology and motility.

DISCUSSION

- **Assay validation:** Combination of MMC and TGFβ successfully induced rapid fibrotic phenotype in CFs, characterised by halted proliferation (verified by HoloMonitor® confluency graph) alongside evidence of myofibroblast activation (αSMA) and ECM deposition (Type I Collagen).
- **Anti-fibrotic efficacy vs. cytotoxicity:** While CPX and CZ415 significantly reduced both αSMA and collagen levels, drug-treated CFs exhibited an unexpected increase in optical thickness. Further assessment of cell viability and dose-response are required to confirm if this reflects true anti-fibrotic structural remodelling or early drug-induced cytotoxicity.
- **Mechanistic validation:** Future experiments will target p-SMAD 2/3 to confirm the direct blockade of the TGFβ signalling cascade to prove these drugs are inhibiting the fibrotic pathway rather than killing the cells.
- **HoloMonitor® optimisation:** High concentrations of Ficoll alter the media's refractive index and degrading holographic resolution. Also, existing segmentation AI struggled to accurately delineate high spread, overlapping myofibroblasts.

CONCLUSION

- The provisional results from this study demonstrate for the first time a 6-day, label-free cardiac fibrosis screening platform utilising macromolecular crowding and quantitative phase imaging.

References:

1. Stebler, S., & Raghunath, M. (2021). Scar-in-a-jar model. *Methods in Molecular Biology*, *2299*, 147–159. https://doi.org/10.1007/978-1-0716-1382-5_11
2. Subbaiah, K. C. V., Wu, J., Tang, W. H. W., & Yao, P. (2023). Ciclopirox inhibition of eIF5A hypusination attenuates fibroblast activation and cardiac fibrosis. *Journal of Cardiovascular Development and Disease*, *10*(2), 52. https://doi.org/10.3390/jcdd10020052