Utilization of high-content imaging for the study of lung fibrosis

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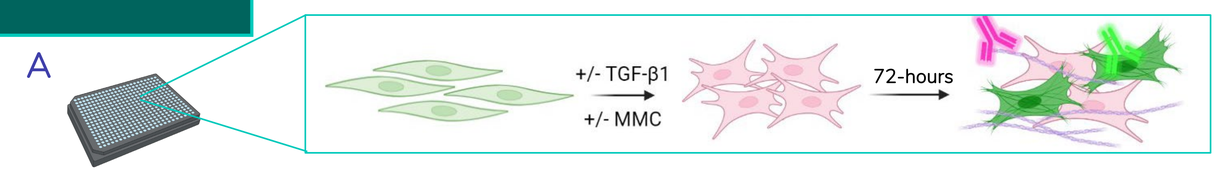
FMT is a pathologic mechanism of fibrosis

- Inhalation of toxic microparticles can damage lower airway epithelial cells. Sustained microinjury of the epithelium results in aberrant epithelial-fibroblast communication via the release of fibrotic stimuli, including TGF-β1.
- TGF-β1 drives the phenotypic transition of fibroblasts to myofibroblasts (FMT). Activated myofibroblasts are responsible for the excessive deposition of extracellular matrix (ECM) proteins, a characteristic feature of fibrosis. This leads to irreversible damage to the lung architecture and function. Therefore, new anti-fibrotic therapies are needed to suppress disease progression.

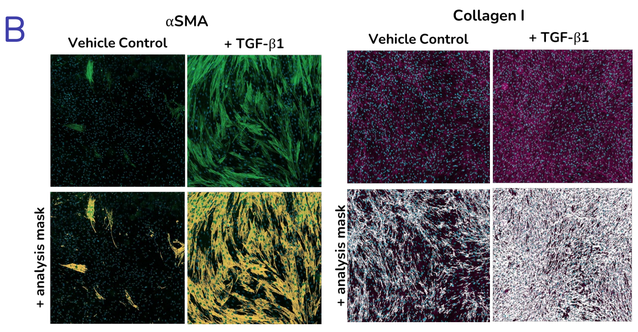
Modelling FMT processes *in-vitro* using high-content imaging enables compound screening to assist anti-fibrotic drug-discovery.

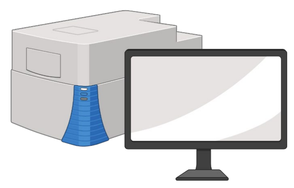


Primary human lung fibroblasts (HLF) are seeded into 384-well plates.

Cells are pre-incubated with anti-fibrotic test articles prior to TGF-β1 stimulation in the presence a of macromolecular crowder (MMC).

Immunocytochemistry and high content imaging are used to quantify the expression of fibrotic proteins.





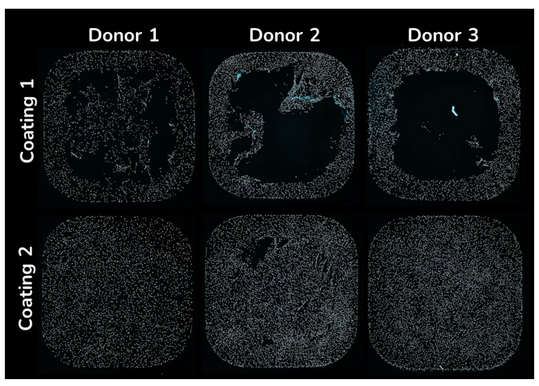
Segmental analysis masks allow quantification of the expression of phenotypic markers and ECM proteins .

Figure 1: High throughput assay setup.
A) Schematic outlining the process of the FMT assays.
B) Representative images showing the segmental analysis masks used for quantification of fibrotic proteins. Examples shown: α-smooth muscle actin (α-SMA) and collagen I.

Optimization of culture conditions for assay robustness

A

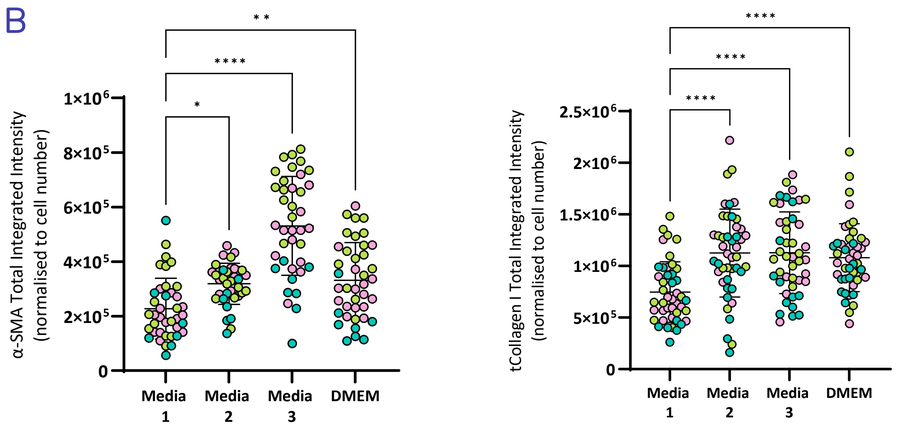
Culture plate coatings were compared to assess fibroblast adherence and reduce assay variation



- Significant cell detachment following mechanical plate washing was observed for coating 1 whilst coating 2 improved assay robustness.

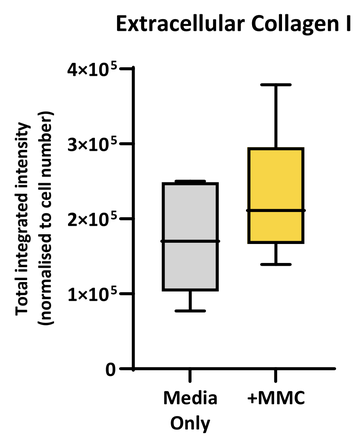
B

Different media conditions were compared to achieve a substantial and consistent assay window



- Media 3 promotes FMT, as indicated by the increased expression and quantification of α-SMA and collagen I following stimulation with TGF-β1.

C



- The inclusion of a macromolecular crowder (MMC) in the assay media promotes the secretion and deposition of extracellular matrix proteins, such as collagen I, a key protein shown to be upregulated in lung fibrosis.

Figure 2: FMT assay conditions have been optimized to ensure reproducibility and achieve the greatest assay window to allow the study of potential anti-fibrotic therapeutics.
A) 384-well plates were coated with two different coatings and HLF were seeded into wells at an equal density. Cells were fixed and stained using Hoechst 33342 to detect cell nuclei representative images shown of whole well x4 magnification. B) Four assay medias were compared to determine which assay media promotes FMT to provide a robust and significant assay window. HLF were stimulated with TGF-β1 in four different assay medias. Shown is total integrated intensity normalized to cell number for a-SMA (left) and collagen I (right) of HLF (N=3 donors, each colour point represents a donor) stimulated with 1ng/mL TGF-β1. Statistical analysis performed; one-way ANOVA with Dunnett's multiple comparisons test compared to Media 1. C) Inclusion of a macromolecular crowding agent (MMC) in the culture media promotes deposition of extracellular matrix and shows an increase in the baseline expression of extracellular collagen I.

TGF-β1 drives FMT in normal and IPF donors

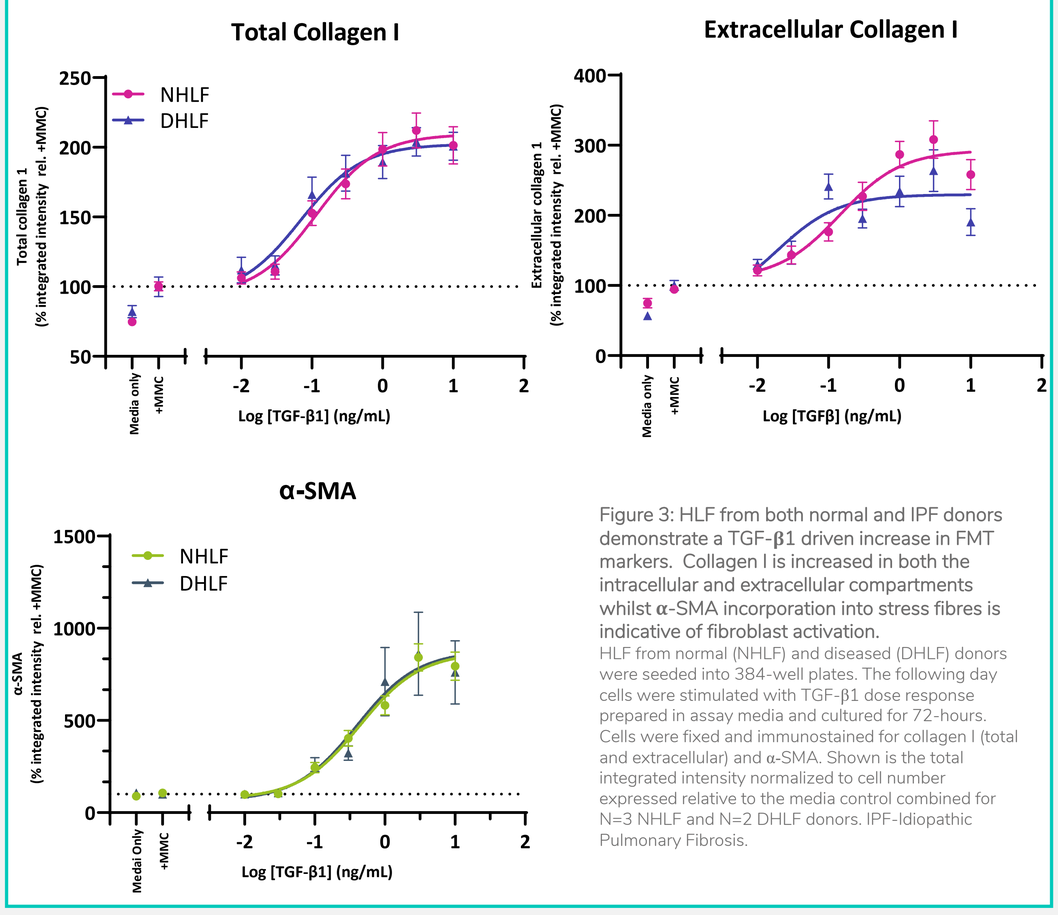
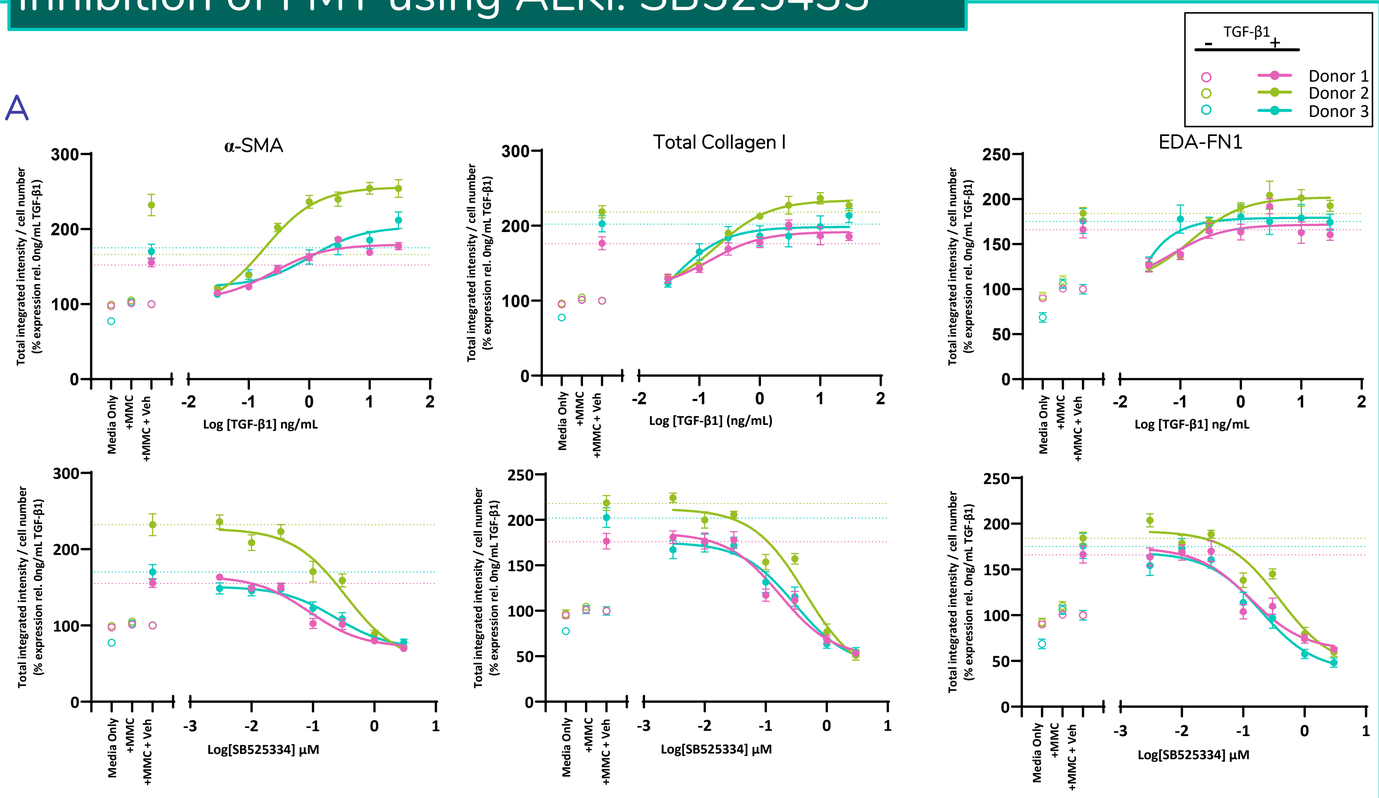


Figure 3: HLF from both normal and IPF donors demonstrate a TGF-β1 driven increase in FMT markers. Collagen I is increased in both the intracellular and extracellular compartments whilst α-SMA incorporation into stress fibres is indicative of fibroblast activation.
HLF from normal (NHLF) and diseased (DHLF) donors were seeded into 384-well plates. The following day cells were stimulated with TGF-β1 dose response prepared in assay media and cultured for 72-hours. Cells were fixed and immunostained for collagen I (total and extracellular) and α-SMA. Shown is the total integrated intensity normalized to cell number expressed relative to the media control combined for N=3 NHLF and N=2 DHLF donors. IPF-Idiopathic Pulmonary Fibrosis.

Inhibition of FMT using ALKi: SB525433*

* Work in development

A



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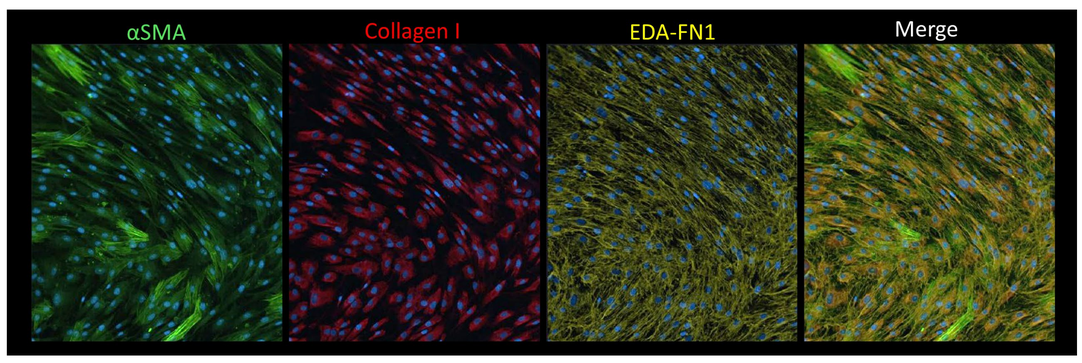

Figure 4. Multiplexed detection and quantification of fibrosis proteins αSMA, collagen I, and the EDA-splice variant of Fibronectin (EDA-FN1)*
Normal HLF (N=3 donors) were seeded into 384-well plates. Cells were pre-treated for 1 hour ± SB525334 over 7-point dose-response prepared in assay media prior to stimulation with TGF-β1. Cells were cultured for 72-hours, fixed and stained using multiplexed antibodies directed against α-SMA, total collagen I and EDA-fibronectin was performed. Quantification showing % change in total integrated intensity relative to the vehicle control for each marker normalised to cell number (A) and representative images of stained FMT markers in TGF-β1 stimulated cells (B). TGF-β1 dose response (top) and inhibition with ALK5i SB525334 (below). Dashed line represent TGF-β1 spiked vehicle control. Error bars represent ±SEM. n=2 experimental repeats, n=12 technical replicates

Newcells Biotech's FMT-assay allows the evaluation of anti-fibrotic therapeutics

Newcells Biotech's FMT assay utilises high-content imaging to determine the effect of therapeutics on disease relevant markers of fibroblast activation (α-SMA) and ECM protein expression (collagen I and EDA-FN1).

Further details available online or contact us at:
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