High-content imaging assays enable the study of the fibrotic processes: fibroblast-to-myofibroblast transition (FMT) and epithelial-to-mesenchymal transition (EMT).

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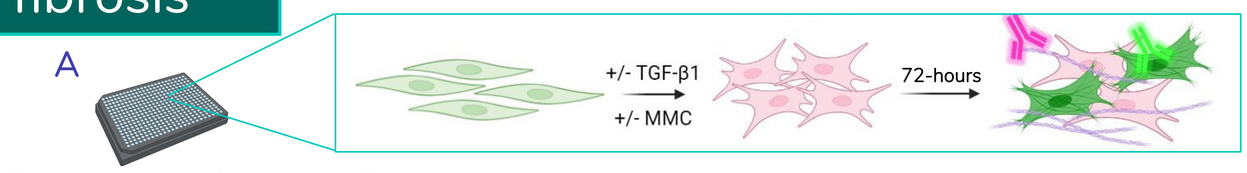
FMT and EMT are pathologic mechanisms of fibrosis

- Excessive and abnormal deposition of extracellular matrix (ECM) is a characteristic feature of fibrotic lung disease, such as idiopathic pulmonary fibrosis (IPF).
- Repetitive injury to lower airway epithelial cells drives epithelial-to-mesenchymal transition (EMT). Release of fibrotic stimuli, including TGF-β1, results in aberrant epithelial-fibroblast communication. And drives the phenotypic transition of fibroblasts to myofibroblasts (FMT). Both processes potentiate ECM deposition causing irreversible damage to the lung architecture and function.

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



Primary human lung fibroblasts (HLF) or primary human small airway basal cells (SAEC) seeded into 384 well plates.

Pre-incubated with test articles prior to TGF-β1 stimulation in the presence a of macromolecular crowder (MMC).

Immunocytochemistry and high content imaging were used to quantify expression of FMT and EMT markers.

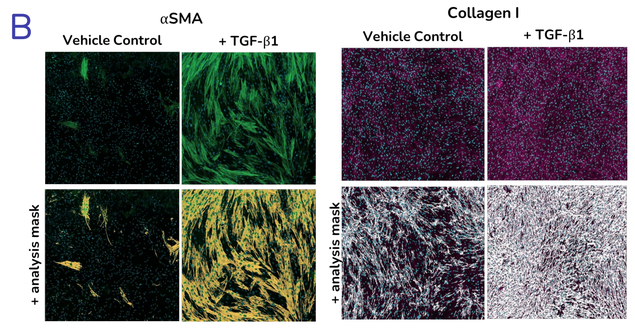
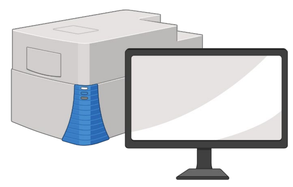


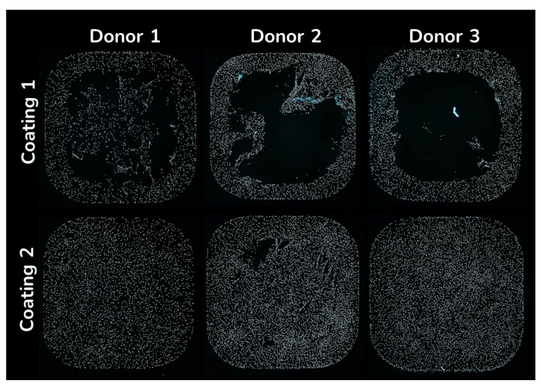
Figure 1: High throughput assay setup.
A) Schematic outlining the process of FMT and EMT assays. B) Representative images showing the segmental analysis masks used for quantification of markers. Examples shown: α-smooth muscle actin (α-SMA) and collagen I.



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

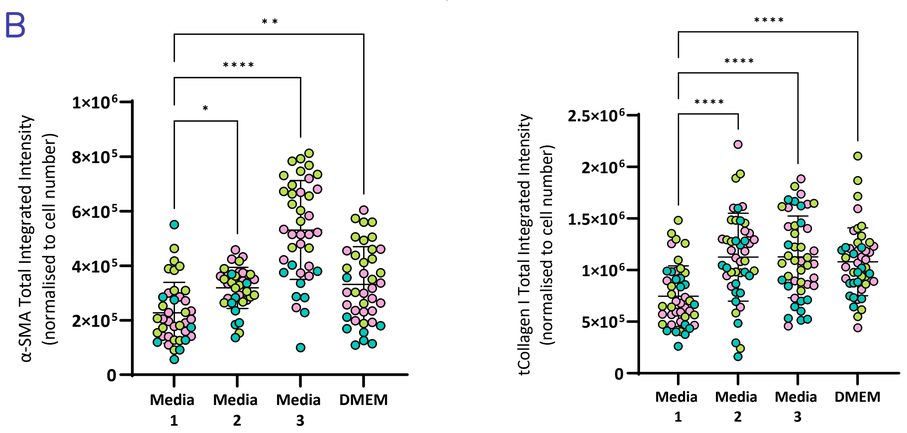
Optimization of culture conditions for assay robustness

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.

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



Media 3 permits the greatest TGF-β1 mediated induction of α-SMA and Collagen 1 expression.

Figure 2: FMT assay conditions have been optimized to ensure reproducibility and achieve the greatest assay window.

A) 384-well plates were coated with two different coatings then Normal 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 test which provides the greatest 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 1 (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 1.

* Work in development

TGF-β1 drives FMT in normal and IPF donors

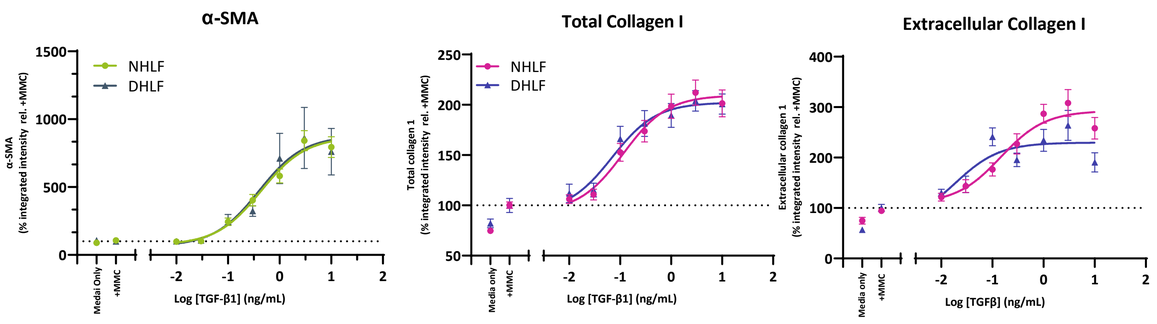


Figure 3: HLF from both normal and IPF donors demonstrate a TGF-β1 driven increase in FMT markers. Collagen 1 is increased in both the intracellular and extracellular compartments whilst α-SMA incorporation into stress fibres is indicative of fibroblast activation.
HLF from normal (NHLF) and diseased (DHLF) donors were seeded into 384 well plates. The following day cells were stimulated with TGF-β1 dose response prepared in assay media then 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.

Inhibition of FMT using ALKi: SB525433*

* Work in development

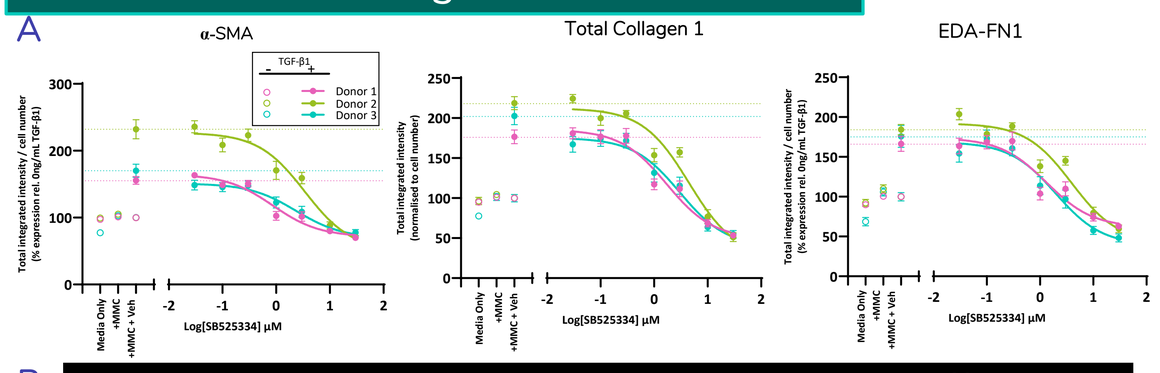


Figure 4. Multiplexed detection and quantification of fibrosis proteins including EDA-variant of Fibronectin (EDA-FN1)*
Normal HLF (N=3 donors) were seeded into 384 well plates. Cells were pre-treated for 1 hour ± SB525334 over 7-point dose-response prepared in assay media, then stimulated with TGF-β1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

* Work in development

SAEC express EMT markers in response to TGF-β1*

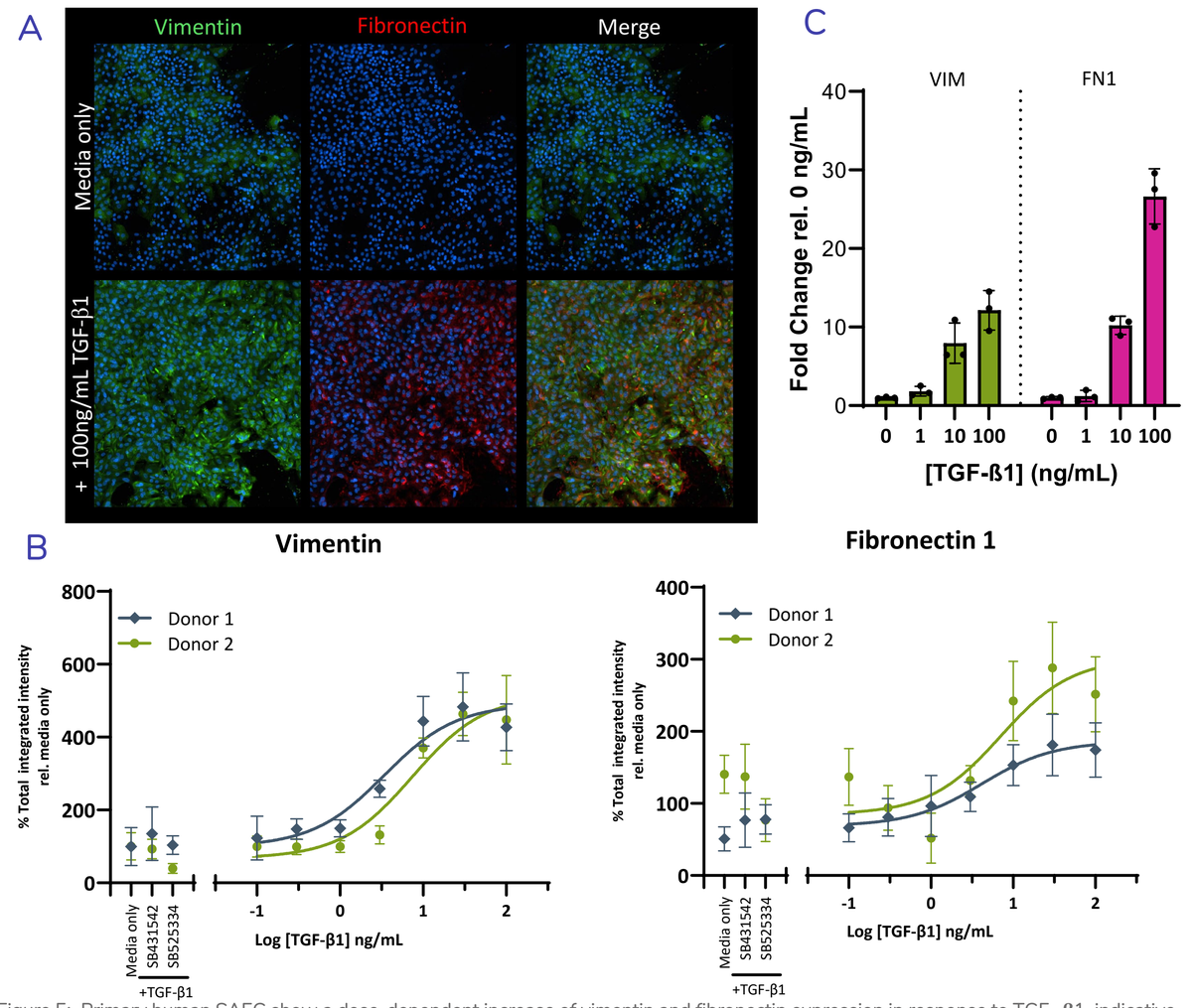


Figure 5: Primary human SAEC show a dose-dependent increase of vimentin and fibronectin expression in response to TGF- β1, indicative of a shift towards a mesenchymal phenotype. SAEC were seeded into plates and cultured until ~80% confluent. Cells were then stimulated with increasing concentrations of TGF-β1 (or co-treated with inhibitory compounds 10µM SB431542 / SB525334) for 72 hours. Cells were either fixed and stained for EMT markers vimentin and fibronectin (A&B) or the RNA collected for RT-qPCR analysis (C). Representative images of vimentin and fibronectin staining and quantification showing % change in total integrated intensity relative to the media only control. Error bars represent ±SD. n=2 experimental repeats, n=6 technical replicates.

High content imaging assays demonstrate key fibrotic processes

Newcells Biotech's FMT assay utilises high-content imaging to determine the effect of therapeutics on fibroblast activation (α-SMA) and ECM protein expression (Collagen 1 and EDA-FN1), and demonstrate the response can be inhibited by ALKi, SB525334. This approach has been applied to demonstrate EMT processes in small airway epithelial cells

Further details available online or contact us at:
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