Domainex
Enrich your Medicines Pipeline

# "Scar-in-a-jar": *in vitro* assay for the quantification of key markers of fibrosis

White Paper

domainex.co.uk

## Introduction

Fibrosis is a pathological wound repair response caused by the irreversible formation of excessive scar tissue. Fibrosis is characteristic of many disease phenotypes and occurs through the accumulation of myofibroblasts in extracellular matrix (ECM) rich lesions at the site of the wound. High throughput and quantitative *in vitro* assays for the development of anti-fibrotic agents is therefore of high value to drug discovery.

A recent publication[1] has demonstrated that a pseudo-3D cell culture of fibroblasts can provide a model for fibrosis using the macromolecular crowding effect of the highly branched polysaccharide Ficoll. This promotes the formation of ECM structures and fibroblast activation upon exposure to pro-fibrotic factors such as transforming growth factor β (TGF-β).

Domainex has confirmed that stimulation of human fibroblasts with TGF-β increases these hallmarks of fibrosis in this "scar-in-a-jar" system in a 96 well format. We have also validated pharmacological modulation of these responses with a TGF-β receptor 1 inhibitor.

## Methodology

Methods are based on Good *et al.* (2019)[1]. Human dermal fibroblasts (HDFs) were grown in 96 well plates and stimulated with TGF-β1 in the presence of Ficoll and L-ascorbic acid for 72 hours. Test inhibitors were incubated for 3 hours prior to TGF-β1 stimulation. Cells were fixed with formaldehyde, and then blocked (with BSA) and permeabilised (with Tween-20). Cells were then stained with fluorescent antibodies against the fibrotic markers; collagen I or α-smooth muscle actin (α-SMA) (red fluorescence). Cells were nuclear stained with SYTOX™ Green (green fluorescence). Image acquisition and fluorescence quantification was performed using an IncuCyte S3. Quantification used fluorescence area, normalised to nuclear count, and was normalised as % change from control untreated wells.

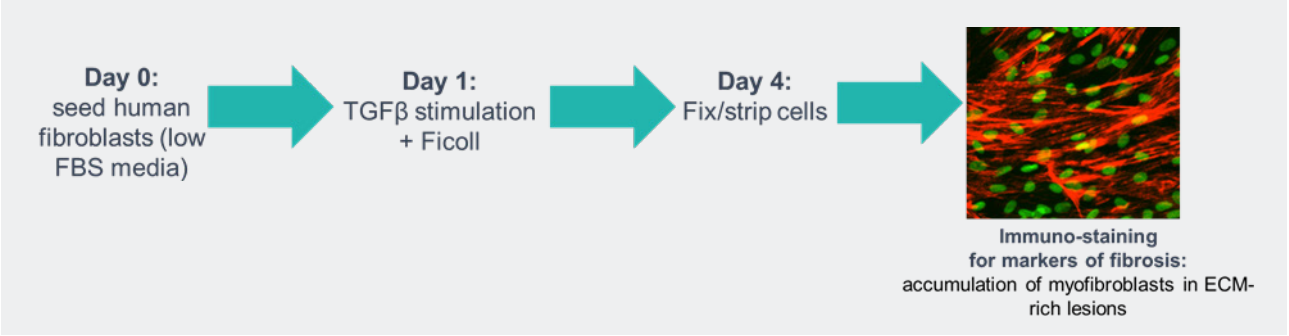
## Example Data

ECM deposition after TGF-β1 stimulation can be quantified using collagen I staining. As shown in Figure 2, TGF-β1 showed a concentration dependent increase in collagen deposition (as quantified by red fluorescence) with an $EC_{50}$ of 72 pg/ml.

In a parallel experiment, myofibroblast activation was quantified using α-SMA staining (Figure 3), where TGF-β1 also showed a robust stimulation with an $EC_{50}$ of 530 pg/ml.

Pharmacological modulation of the fibrosis readouts was investigated using the TGF-β receptor 1 (ALK5) small molecule inhibitor SB 525334. This compound caused complete inhibition of TGF-β1-mediated stimulation of both collagen I (Figure 4) and α-SMA (Figure 5) staining with respective $IC_{50}$ values of 310 and 250 nM.

**Figure 1**: Schematic of assay methodology



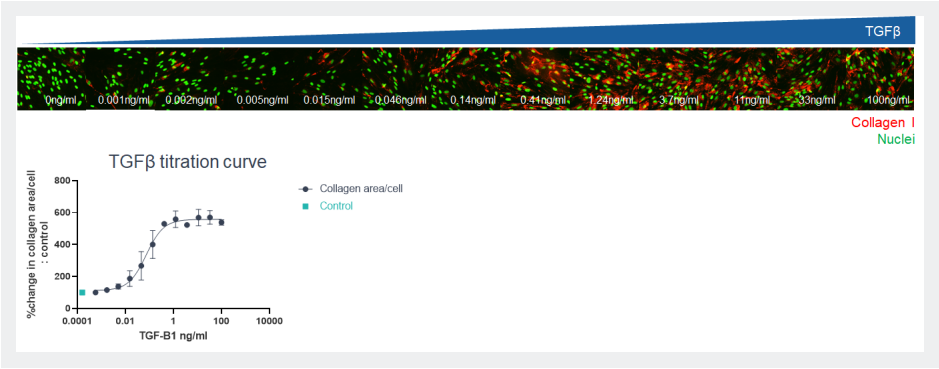



**Figure 2**: Quantifying ECM deposition using collagen I staining

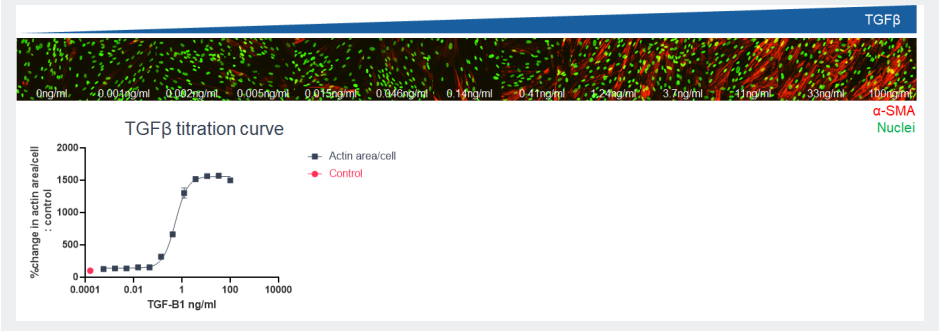



**Figure 3**: Quantifying myofibroblast activation

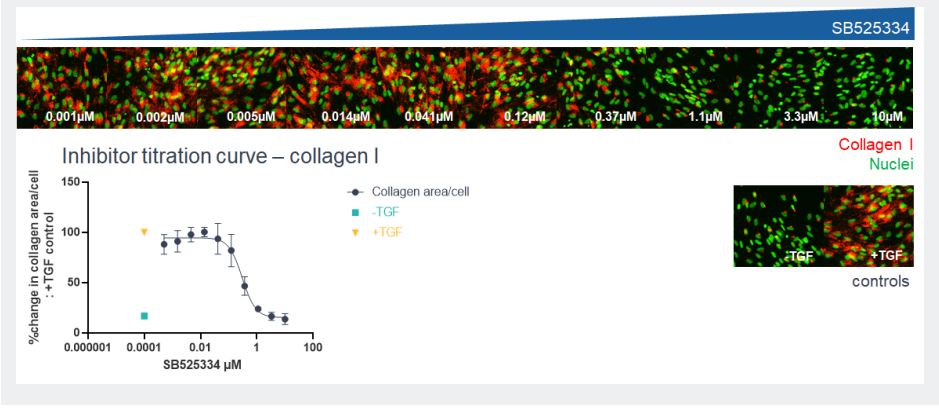



**Figure 4**: SB 525334 inhibition of ECM deposition

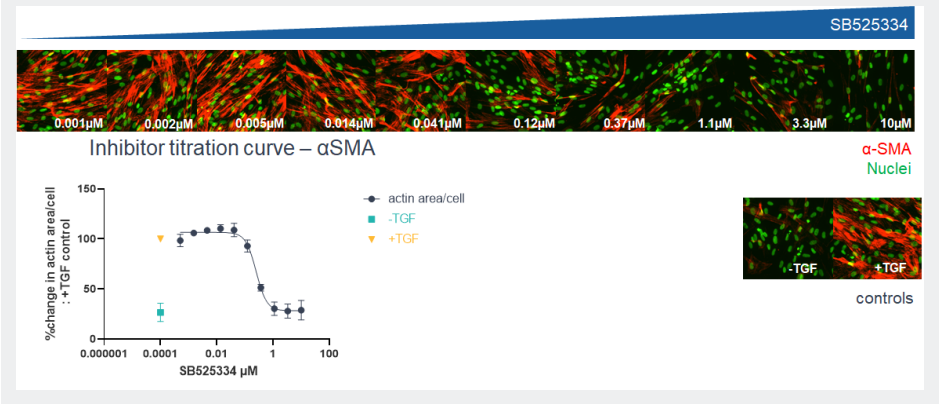



**Figure 5**: SB 525334 inhibition of myofibroblast activation

## Conclusion

The scar-in-the-jar assay is a robust disease relevant readout to measure extracellular matrix deposition. The assay can used for medium throughput compound profiling and can be adapted to model fibrosis in different tissues by altering the source of the cells used.

### References

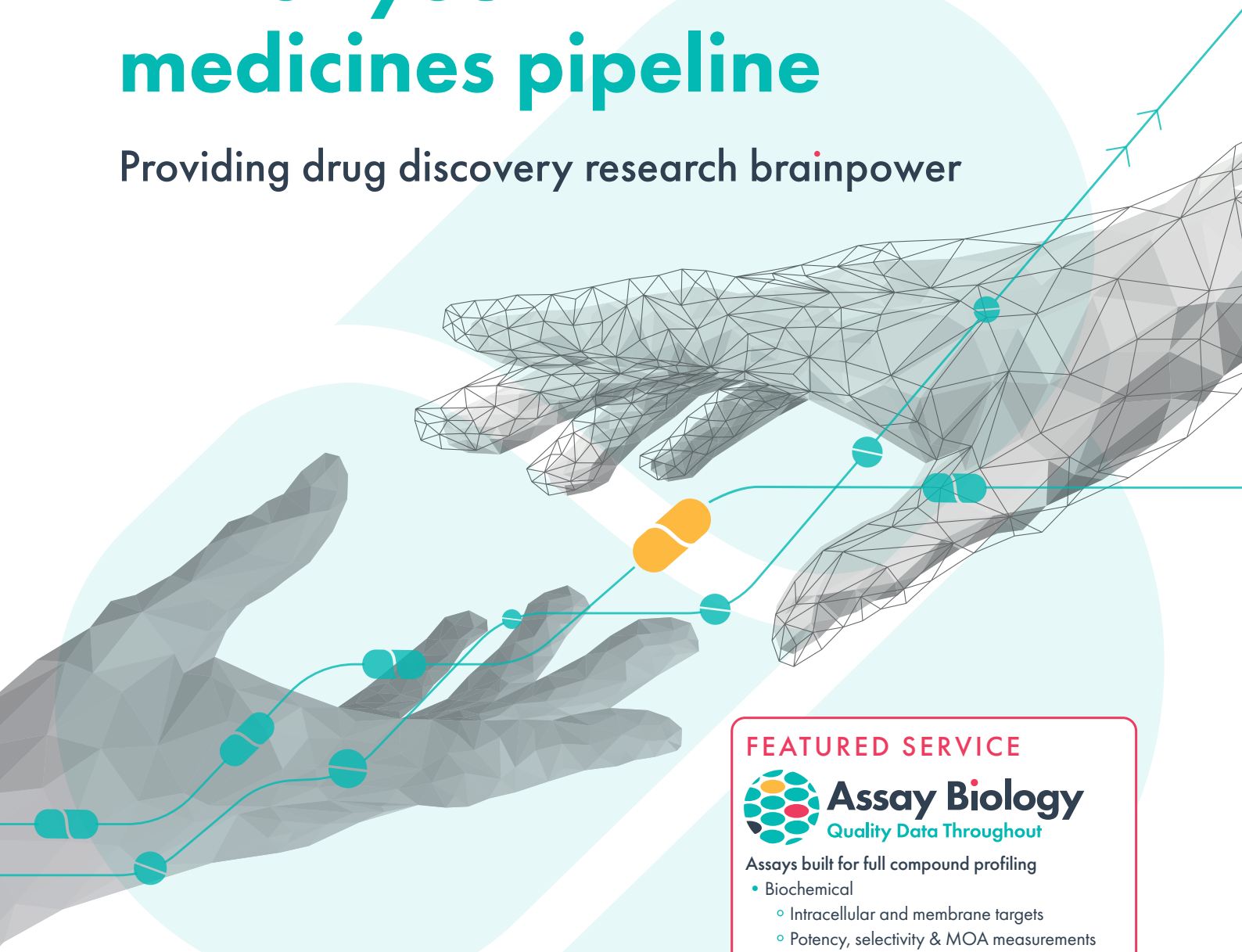
**1**. Good, R.B., Eley, J.D., Gower, E. *et al.* A high content, phenotypic 'scar-in-a-jar' assay for rapid quantification of collagen fibrillogenesis using disease-derived pulmonary fibroblasts. *BMC biomed eng* 1, 14 (2019).

Domainex

domainex.co.uk

# Enrich your medicines pipeline

## Providing drug discovery research brainpower



- Tailored multidisciplinary expertise
- Forming dynamic relationships
- Innovative outcomes for our partners

FEATURED SERVICE

### Assay Biology

Quality Data Throughout

**Assays built for full compound profiling**

- Biochemical
  - Intracellular and membrane targets
  - Potency, selectivity & MOA measurements
- Biophysical:
  - Full suite (MST, GCI/SPR, DSF/nanoDSF, NMR, X-ray crystallography)
  - Affinity, kinetic & structural determinations
- Cellular
  - Cell lines and primary cells
  - Signalling & reporter-based assays
  - On and off target assessments

Based in the Cambridge UK Biocluster

Established in 2001

Contact us today!

enquiries@domainex.co.uk