“Scar-in-a-jar”: in vitro assay for the quantification of key markers of fibrosis

Alison Holiday, Sarah Whipple, Daniel Hothersall and Trevor Askwith

Domainex Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden, CB10 1XL

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 (Figure 1). 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 have 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.

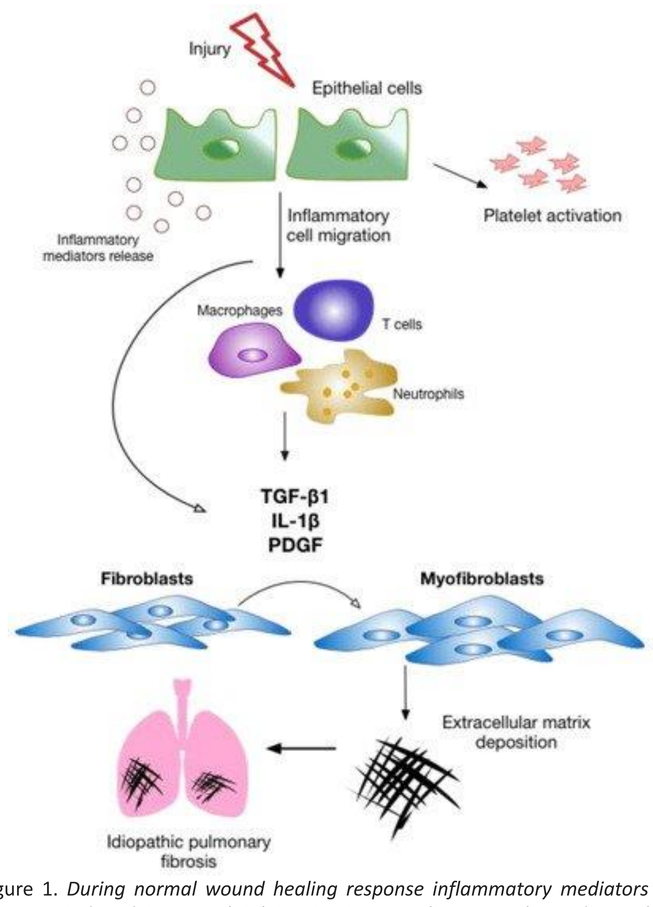


Figure 1. *During normal wound healing response inflammatory mediators are secreted and trigger platelet activation resulting in enhanced vessel permeability for the recruitment of leukocytes. The release of pro-fibrotic cytokines e.g. TGF-β1 mediates the activation and recruitment of fibroblasts and their differentiation into myofibroblasts and the release of ECM components to promote wound healing. In Fibrosis, aberrant wound repair leads to irreversible formation of excessive scar tissue.*[2]

Method: 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 (BSA) and permeabilised (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.

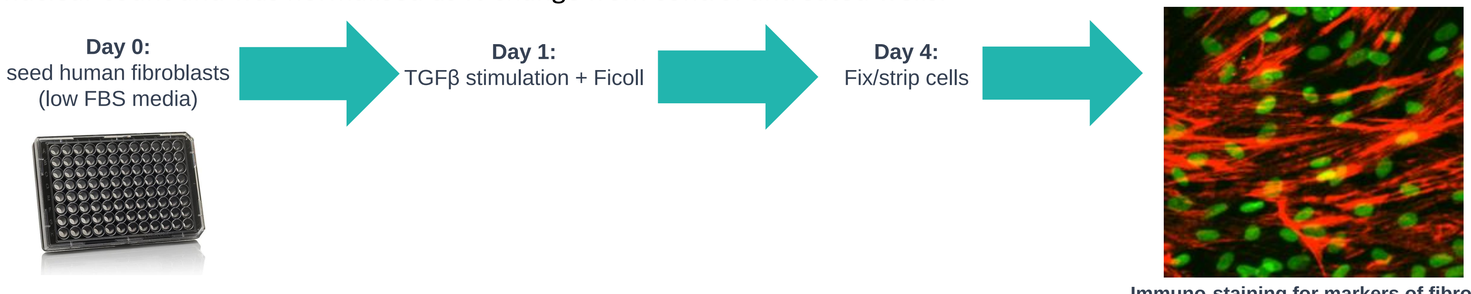
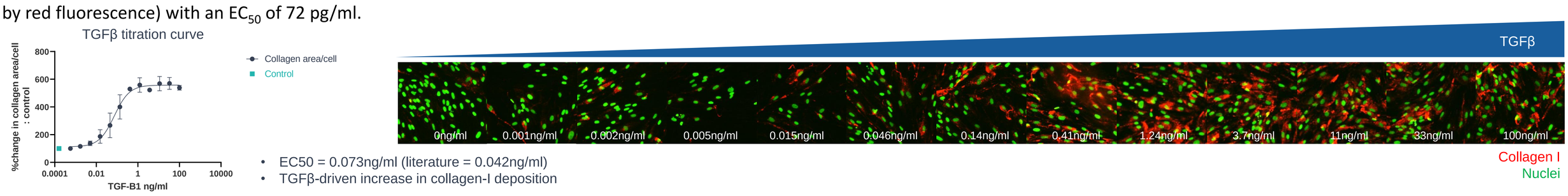


Figure 2. *Schematic of assay methodology*

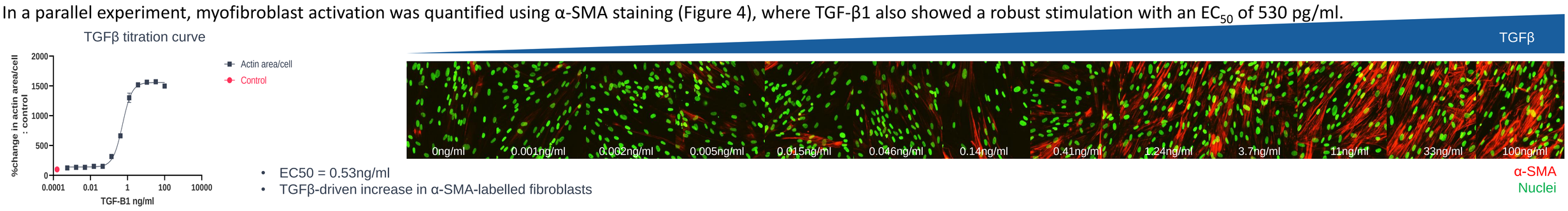
ECM deposition after TGF-β1 stimulation can be quantified using collagen I staining. As shown in Figure 3, TGF-β1 showed a concentration dependent increase in collagen deposition (as quantified by red fluorescence) with an EC_{50} of 72 pg/ml.



- EC50 = 0.073ng/ml (literature = 0.042ng/ml)
- TGFβ-driven increase in collagen-I deposition

Figure 3. *Quantifying ECM deposition using collagen I staining*

In a parallel experiment, myofibroblast activation was quantified using α-SMA staining (Figure 4), where TGF-β1 also showed a robust stimulation with an EC_{50} of 530 pg/ml.



- EC50 = 0.53ng/ml
- TGFβ-driven increase in α-SMA-labelled fibroblasts

Figure 4. *Quantifying myofibroblast activation*

Inhibition of ECM deposition: 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 stimulated of both collagen I (Figure 5) and α-SMA (Figure 6) staining with respective IC_{50} values of 310 and 250 nM.

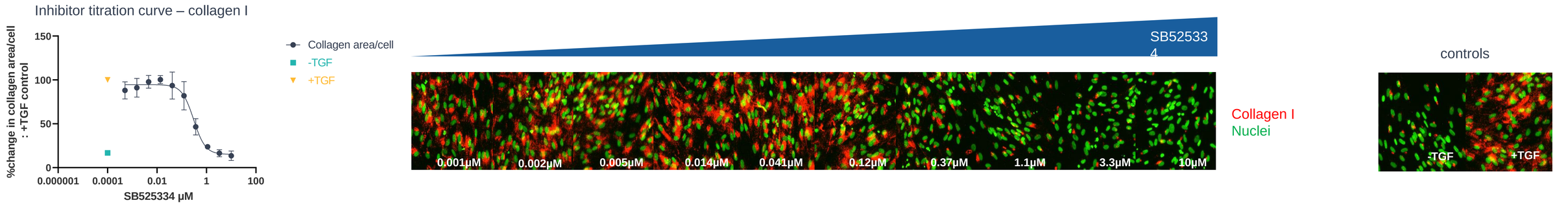


Figure 5. *SB 525334 inhibition of ECM deposition*

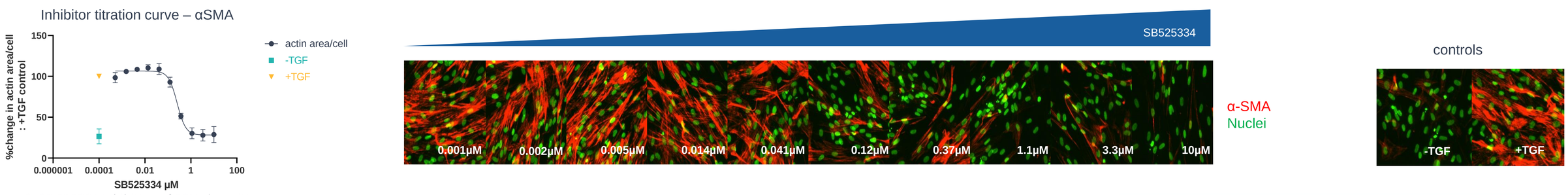


Figure 6. *SB 525334 inhibition of ECM deposition*

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about applying our drug-discovery platform to other targets, please contact:
Catherine.Stace@domainex.co.uk
www.domainex.co.uk

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).
2. Hadjicharalambous, M.R.; Lindsay, M.A. Idiopathic Pulmonary Fibrosis: Pathogenesis and the Emerging Role of Long Non-Coding RNAs. *Int. J. Mol. Sci.* **2020**, *21*, 524

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