Fibrosis Technology Overview

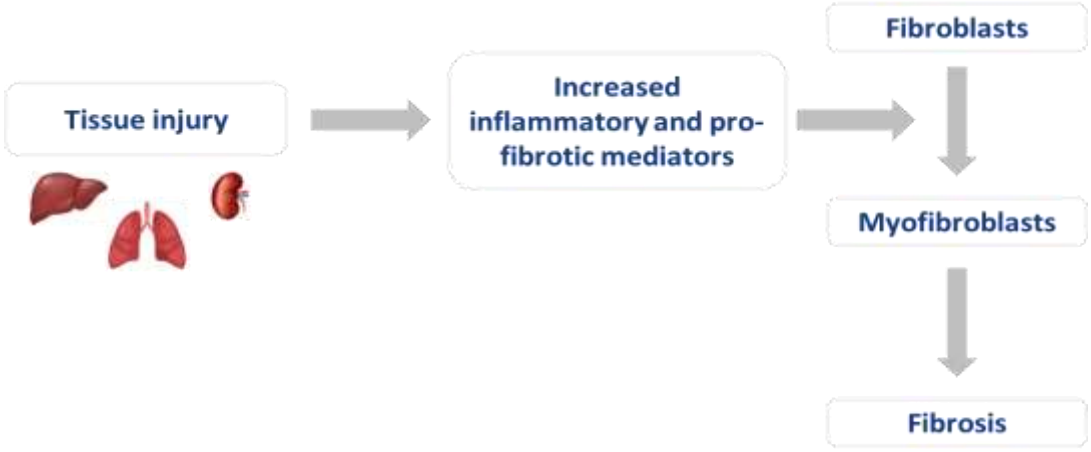
The evaluation of test materials for their potential to cause tissue damage by induction of fibrosis or for their potential anti-fibrotic activity

Service overview

Fibrosis is a complex, multi-faceted disease process that is associated with the de-regulation of key enzymes that maintain the local cellular environment. This dysregulated fibrotic cascade results in the excessive production and deposition of extracellular proteins that impair normal tissue and can lead to tissue dysfunction, damage and disease. AvantiCell Science (ACS) isolates and cultures primary fibroblasts from tissues obtained with full ethical permission and has developed a primary cell-based model measuring fibrotic-activity, that offers a flexible and scalable analysis of fibrotic and anti-fibrotic activity, high reproducibility and low limits of detection.

Markers to be analysed are entirely customisable. ACS analysis measurements include;

- Cell fibrotic status by immunofluorescent (IF) staining
- Intracellular and secretory protein analysis using enzyme-linked immunosorbent assay (ELISA)
- Key fibrotic gene expression analysis via real-time PCR (qPCR)



Summary

ACS fibrosis models include those for the study of renal fibrosis and idiopathic pulmonary fibrosis (IPF), using interstitial fibroblasts isolated from human kidney and human primary IPF lung. Current isolates were prepared in-house by ACS and tissue was dissected and dissociated by proteolytic digestion.

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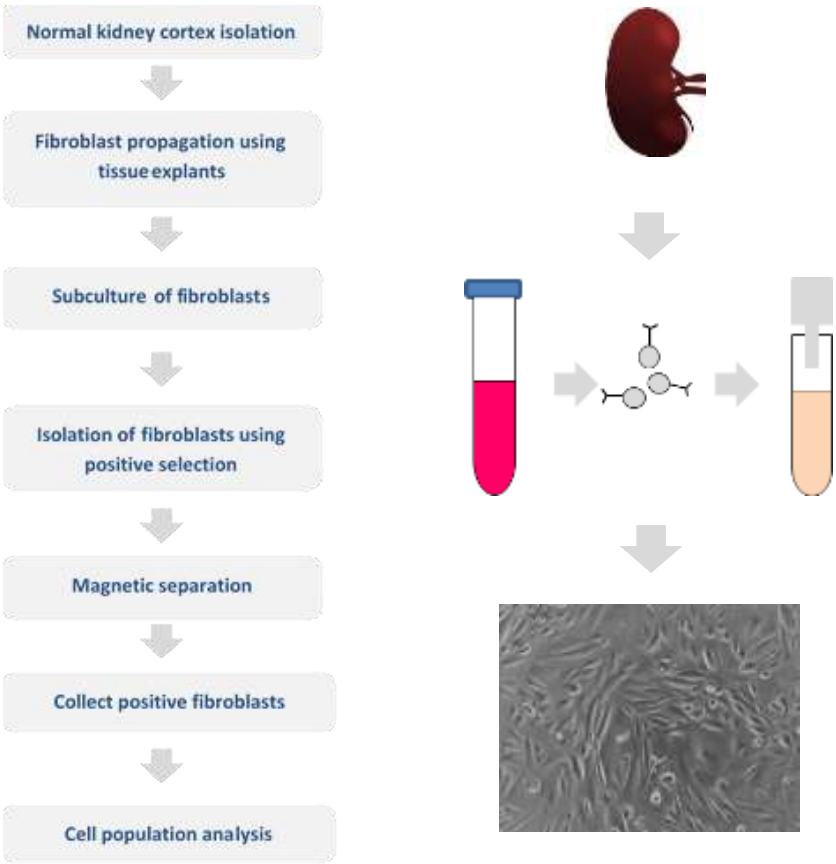
Application: Renal Fibrosis

Renal fibrosis is characterized by excessive production and deposition of extracellular matrix (ECM) proteins, such as alpha smooth muscle actin (αSMA) and collagen, resulting in structural damage, functional impairment and, finally, end-stage renal disease.

TGFβ is as a key mediator of protein production and structure of the renal ECM. It has been identified as pivotal to the progression of renal fibrosis.

Isolated human primary renal fibroblasts were challenged with pro-fibrotic transforming growth factor beta (TGFβ) and anti-fibrotic, selective TGFβ-inhibitor IN1130. Visible morphological changes were observed following treatment with TGFβ. IF staining further highlighted cell populations positive for αSMA and human collagen 1 alpha 1 (Collα1). ELISA was used to measure secreted matrix metalloproteinase-2 (MMP-2) and Pro-CollagenIα1 (ProCollα1).

Fibroblast Isolation: Human Primary Renal Fibroblasts (ACS catalogue code KF-HN-051) were isolated from healthy donor kidney tissue, obtained with full ethical permission. Tissue was mechanically dissociated into small explants which were cultured under conditions optimised for primary kidney fibroblasts. Fibroblasts were recovered in cells out-grown from the explants. A fibroblast population was isolated using fibroblast specific protein 1 (FSP-1) immuno-magnetic selection. Following limited population expansion, cells were cryopreserved at -150°C. The cell population was analysed by flow cytometry to confirm fibroblast phenotype and activation status.



Renal fibroblasts are selected from tissue explant out-growth via immunomagnetic separation. Fibroblast cell surface marker expression was confirmed via flow cytometry.

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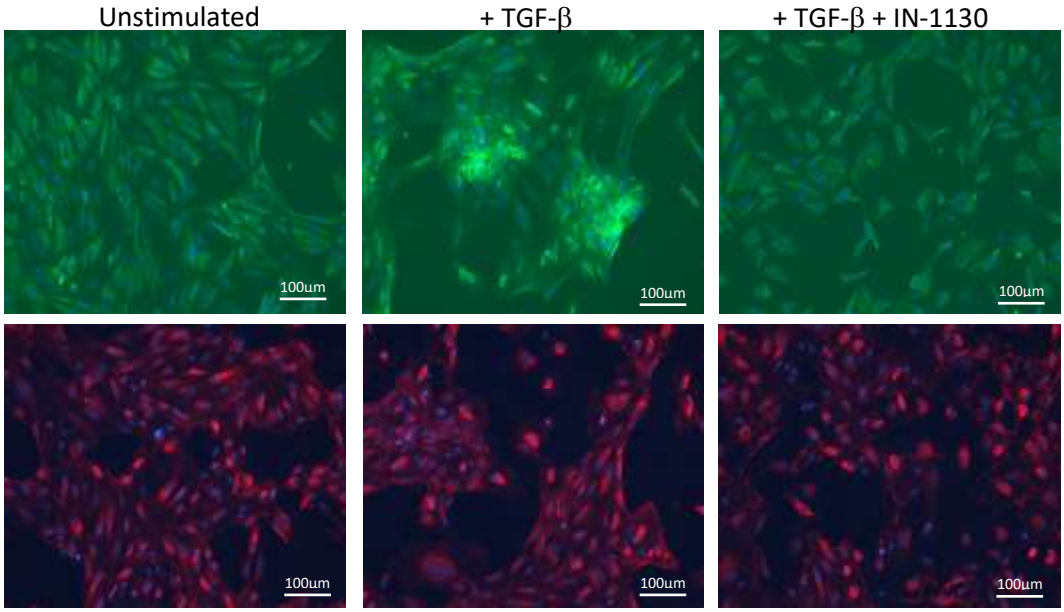
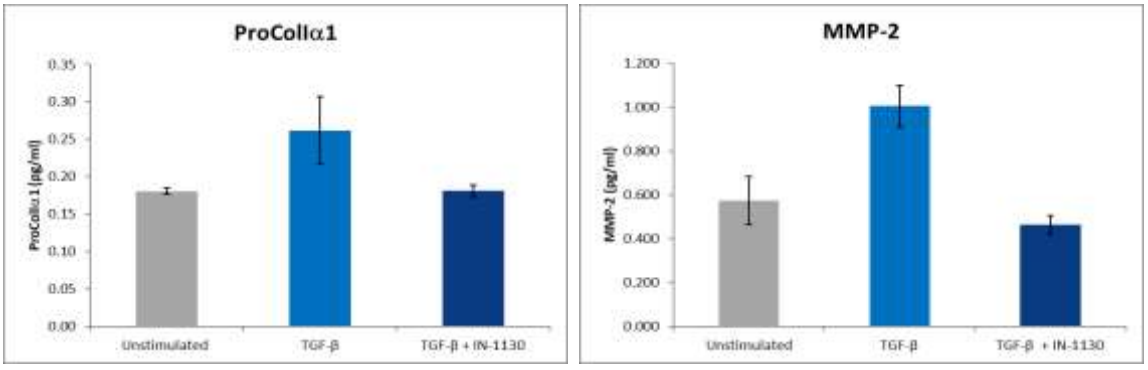
Fibrosis Analysis: Fibrosis analysis was performed using renal fibroblasts cultured in 96-well culture plates for 24 hours prior to treatment with pro-fibrotic TGFβ and/or anti-fibrotic, selective TGFβ-inhibitor IN1130 for 72 hours. Cells were stained using anti-αSMA and anti-Collα1 antibodies, which detect myofibroblasts and fibrogenesis, respectively. The presence of activated myofibroblasts, and subsequent production of collagen, is a recognised predictor of fibrosis. TGFβ is known to play a major role in the production of myofibroblasts, consequently producing increased levels of collagen and fibrosis.



Green: αSMA Blue: DAPI Red: Collα1

Immunofluorescence staining of renal fibroblasts measured after 72 hours stimulation with TGF-β or inhibition with IN-1130.

Cell challenge with pro-fibrotic TGFβ increased the proportion of cell population positive for αSMA and Collα1. The increase in cells expressing these markers was reduced in the presence of the selective TGFβ-inhibitor, IN1130. Collα1 was detected in increased levels in TGFβ stimulated cells compared to unstimulated and IN1130 treated cells. ELISAs were utilised to measure the levels of MMP-2 and procollα1 protein secretion into the extracellular environment. TGFβ-treatment induced the secretion of MMP-2, while IN1130 inhibited this response.



ELISA analysis of renal fibroblasts supernatants measured after 72 hours stimulation with TGFβ or inhibition with IN1130.

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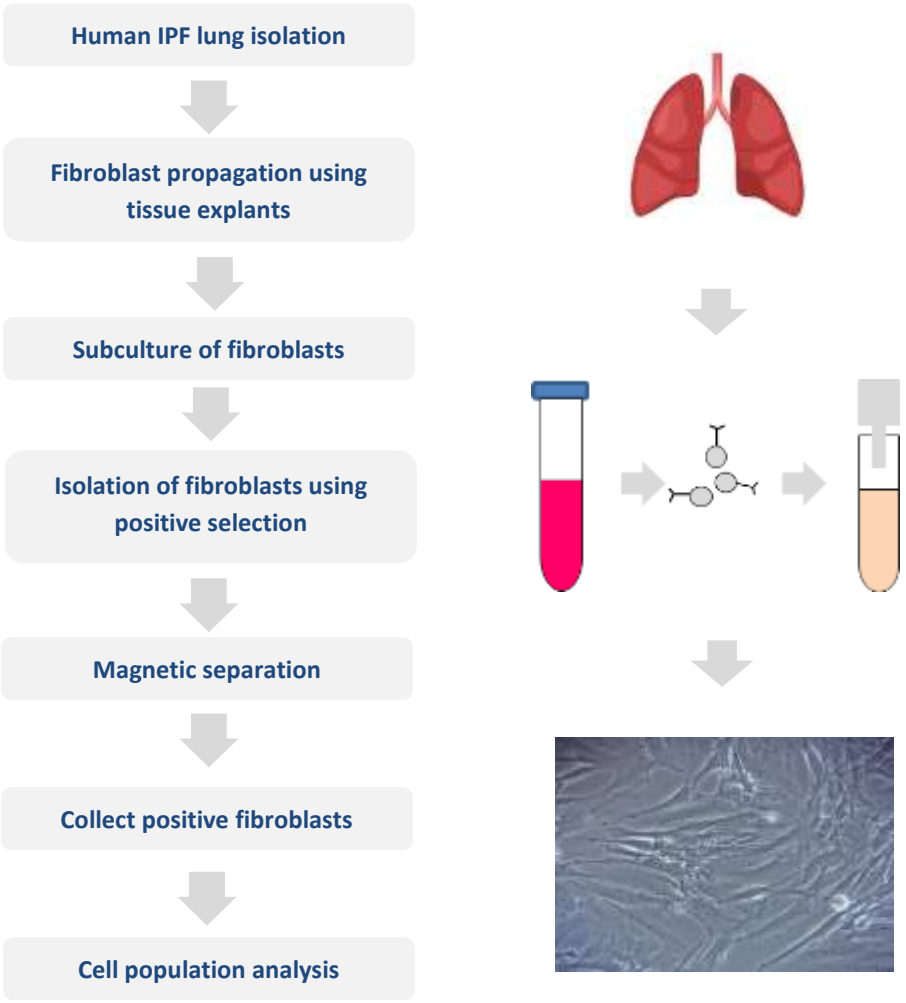
Application: Idiopathic Pulmonary Fibrosis

Idiopathic Pulmonary Fibrosis (IPF) is a progressive lung disease of unknown etiology and poor prognosis. Disease markers include epithelial and immune cell activation, subsequent myofibroblast accumulation and disproportionate deposition of ECM.

Isolated human primary IPF fibroblasts were challenged with pro-fibrotic TGFβ and anti-fibrotic, selective TGFβ-inhibitor IN1130. Visible morphological changes were observed following treatment with TGFβ. IF staining further highlighted cell populations positive for αSMA. qPCR gene expression analysis was used to measure upregulation of genes COL1A1 (collagen I) and MMP-1 (matrix metalloproteinase -1).

MMPS are a family of zinc-dependent enzymes that are known for breaking down the components of the extracellular matrix and are believed to play a role in the pathogenesis of fibrosis.

Fibroblast Isolation: Human Primary Idiopathic Pulmonary Fibrosis Lung Fibroblasts (ACS catalogue code LF-HD-087)



IPF lung fibroblasts are selected from tissue explant out-growth via immunomagnetic separation. Fibroblast cell surface marker expression was confirmed via flow cytometry.

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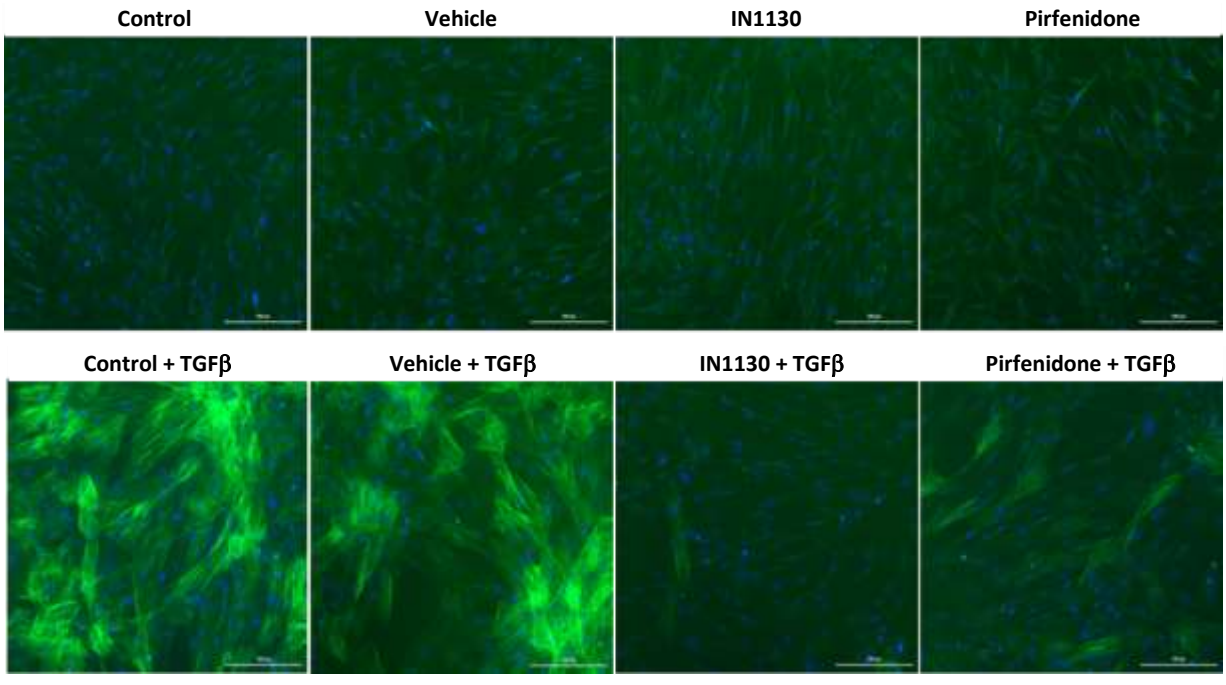
were prepared and isolated in-house from tissue obtained with full ethical permission. Tissue was mechanically dissociated into small explants which were cultured under conditions optimised for primary kidney fibroblasts. Fibroblasts were recovered in cells out-grown from the explants. Fibroblasts were recovered in cells out-grown from the explants Cells expressing fibroblast specific protein 1 (FSP-1) were isolated using immuno-magnetic selection, after which fibroblast phenotype and activation status was confirmed via flow cytometry. Following limited population expansion, cells were cryopreserved at -150°C.

Fibrosis Analysis: IPF lung fibroblasts were cultured in 96-well or 24-well plates and model utility was evaluated via cell challenge with test materials known for their fibrotic or anti-fibrotic potential. Analysis was based upon fibroblast to myofibroblast conversion by cell population analysis since the presence of activated myofibroblasts, and subsequent production of collagen, is a recognised predictor of fibrosis.

Cells were serum-starved overnight prior to compound challenge, and were challenged for 72hr in the presence or absence of TGFβ. Challenge compounds were IN1130, a highly selective small molecule kinase inhibitor of TGFβ signaling and suppressor of fibrosis, and Pirfenidone.

Pirfenidone is a synthetic anti-fibrotic and anti-inflammatory agent that downregulates the production of growth factors and procollagens I and II. It is used to treat idiopathic pulmonary fibrosis.

Immunolabelling and fluorescence imaging revealed that IPF fibroblasts showed a clear up-regulation of αSMA following treatment with TGFβ. Distinctive morphological changes are also observed, with cells displaying classic myofibroblast characteristics. Treatment with the TGFβ inhibitor, IN1130, blocks this effect and treatment with Pirfenidone also significantly reduced the fibrotic response.



Green: αSMA Blue: DAPI

Immunofluorescence staining of IPF fibroblasts measured after 72 hours treatment with IN-1130 or Pirfenidone in the presence and absence of TGFß.

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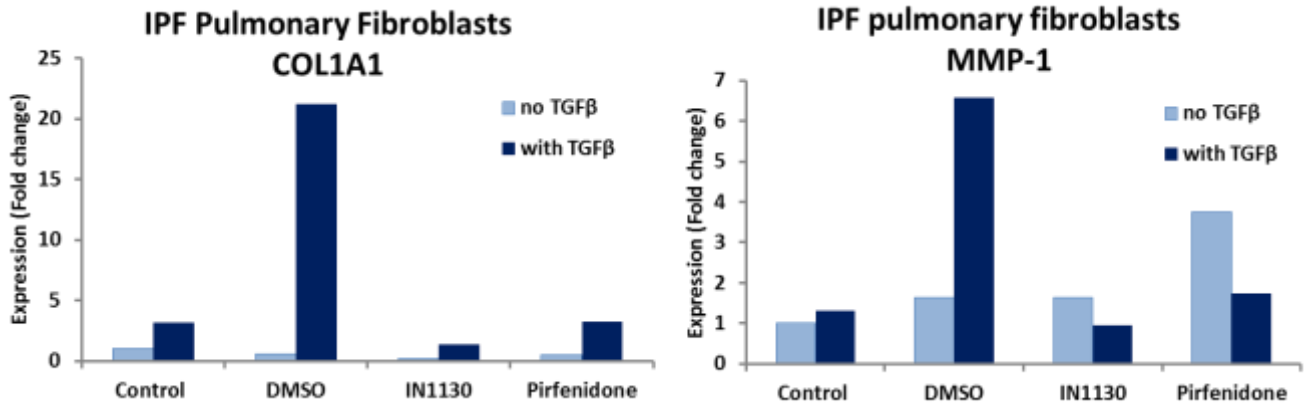
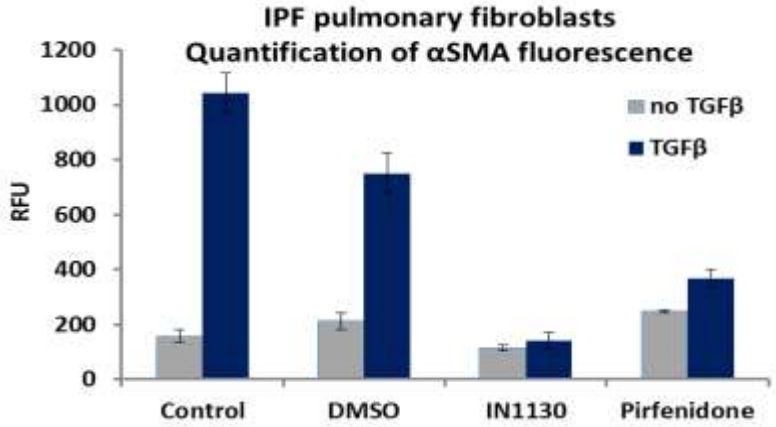


Gene expression analysis of COL1A1 and MMP-1 after treatment with IN1130 and Pirfenidone for 72 hours in IPF fibroblasts.

Following treatment with IN1130 and Pirfenidone, cells were lysed and their RNA was extracted and qPCR analysis measuring the expression of COL1A1 and MMP-1 was performed. Both genes were shown to be induced upon treatment with TGFβ. Co-treatment with IN1130 significantly reduced this response in IPF lung fibroblasts as did Pirfenidone.



Quantification αSMA labelled cells in response to IN1130 and Pirfenidone treatments in IPF fibroblasts. Data expressed as relative fluorescence units (RFU).

This *in vitro* cell-based pulmonary fibrosis assay has been developed using human IPF patient-derived primary fibroblasts in response to TGFβ, IN1130 and Pirfenidone. Models incorporating healthy lung fibroblasts and/or, other pro and anti-fibrotic mediators, are available on request. Additional assay readouts are also available, including immunocytochemistry, secreted protein analysis via ELISA, gene expression analysis and measurement of general cell health indices.

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