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BioIVT PHASEZERO® Research Services
Development of a human 3D in vitro model of liver fibrosis

Caroline Freathy, ACTC May 2018, Cardiff

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Why liver fibrosis ?

- Liver disease 5th biggest cause of death in UK
- Many liver diseases result in development of fibrosis
- No effective treatments = unmet clinical need
- Liver disease market $8-13bn, growing at ~4-8% CAGR[1,2]
- Pharma focus on anti-fibrotics
- Animal models mixed reviews – importance of translational R&D
- What is the right approach ?

1 BCC Research, 2012
2 Transparency Market Research, 2014

Current models

- Animal models remain widely used
  - Efforts to standardize the range of different *in vivo* fibrosis models

- Various groups trying to develop *in vitro* models
  - Animal or human ?
  - Primary cells or iPSC-derived ?
  - 2D or 3D ?
  - Matrix/scaffold or matrix-free ?

3Rs Impact

- Many different animal models to mimic various liver insults resulting in fibrosis
  - Typically mice, genetically modified
- Remain gold standard for modelling the complex interactions between key cell types
- One key scientific concern – species differences in inflammatory response
- Key welfare concern – led to focus on optimized approaches and SOPs for animal experimentation supporting liver fibrosis [3]
- Plethora of different animal models + increased research focus = opportunity to develop suitable *in vitro* alternatives

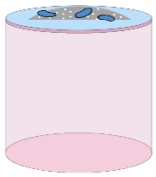
3 Liedtke *et al*., Fibrosis & Tissue Repair. 2013, 6(19)

Challenges

- Complex pathogenesis
  - multiple cell types, matrix re-modelling
- Multiple triggers / causes
  - cholestasis, steatosis, hepatitis, alcohol, cancer, inherited disorders
- Apparent *in vitro* "inertia" in deposition of ECM[4]
- Trying to model something *in vitro* that can take many months to manifest *in vivo*

4 Chen & Raghunath, Fibrogenesis & Tissue Repair, 2009

3D co-culture model – our approach



ORGANDOT™

- Primary cells
- Transfer to a semi-permeable membrane and culture at air liquid interface
- Re-aggregation of the cells over time in culture
- 3D microtissues recapitulate original tissue architecture and functionality

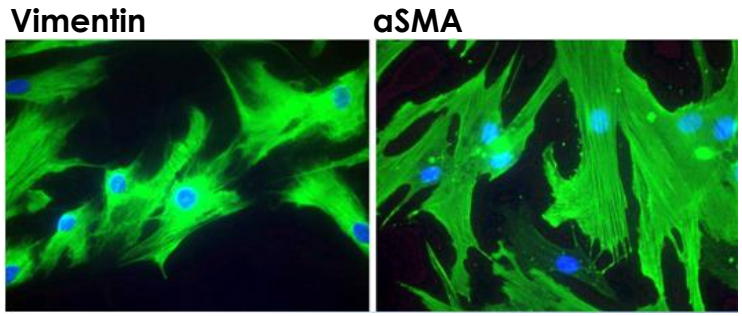
- Multicellular, organotypic model (hepatocytes, Kupffers, stellates)
- Establish stimulus / insult that is common to many causes
- Clinically relevant markers / endpoints
- Fit-for-purpose for specific applications
- Ultimate goal – expedite identification and development of new anti-fibrotic therapies
- Secured funding from Innovate UK for a 12 month feasibility study with Prof Neil Hanley at the University of Manchester

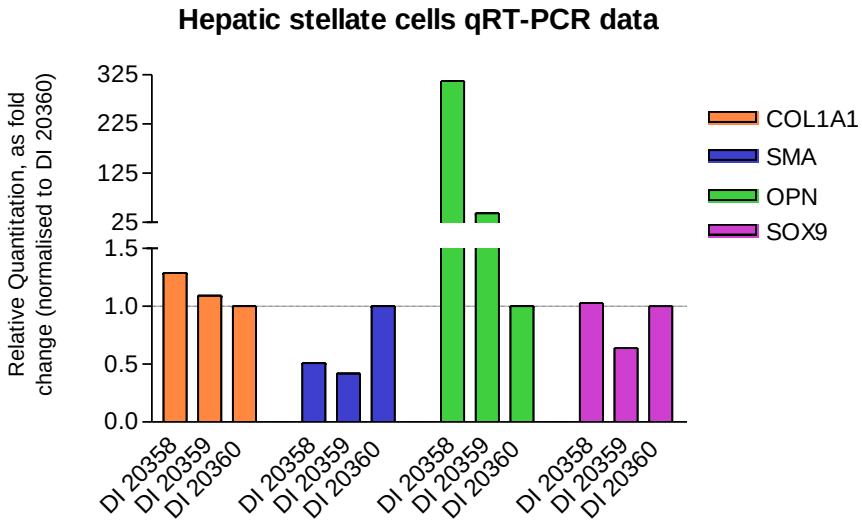
Study design

- Establish and demonstrate longevity of liver co-cultures (>21 days)
  - Cell viability and function: ATP, P450 activity, albumin secretion, LPS stimulation
  - Optimization of co-culture conditions: optimal cell number, co-culture medium
- Isolation and characterization of human hepatic stellate cells
- Select and optimize fibrogenesis endpoints
  - IHC: collagen I, collagen III, and markers of activated stellate cells (e.g. αSMA)
  - Secreted markers: pro-collagen, hyaluronic acid, osteopontin
  - Fibrogenic gene expression: COL1A1, COL3A1, CTGF, OPN, SOX9, SMA, FAP, MMP2, MMP9, TIMP1 and TIMP2.
- Establish an inflammatory / fibrotic insult
- Test compounds / drugs for potential inhibition of fibrogenesis

Hepatic stellate cells

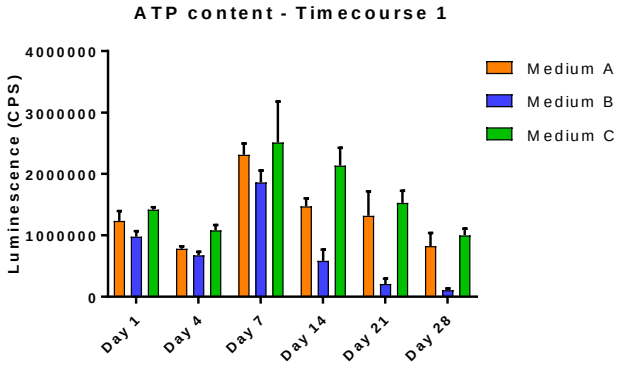
- Activate under standard 2D culture conditions to a myofibroblast-like phenotype
- Characterization (IHC and qRT-PCR)

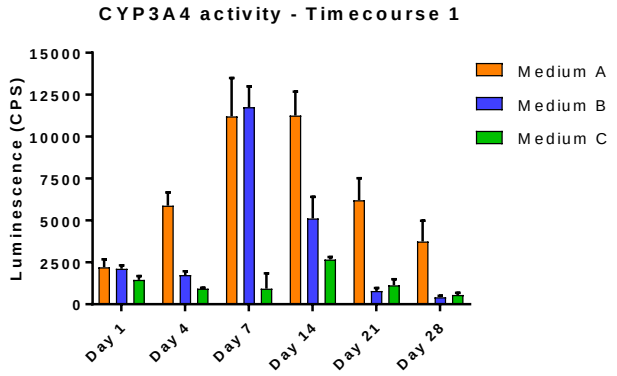


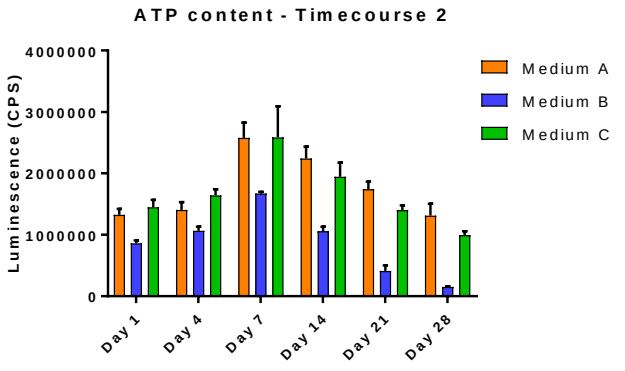


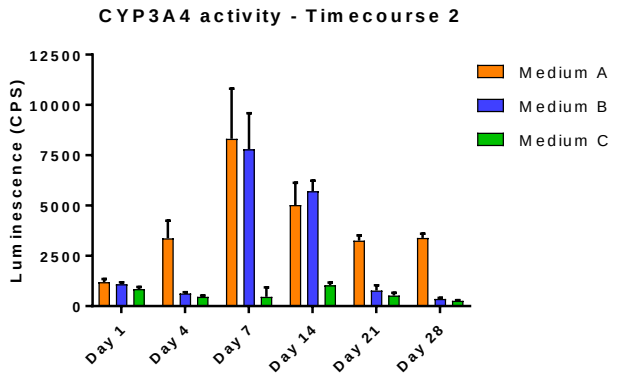
ORGANDOT – establishing liver co-cultures

- Co-cultures (human hepatocytes, Kupffers, stellates) viable for up to 4 weeks in culture
- Importance of co-culture medium selection



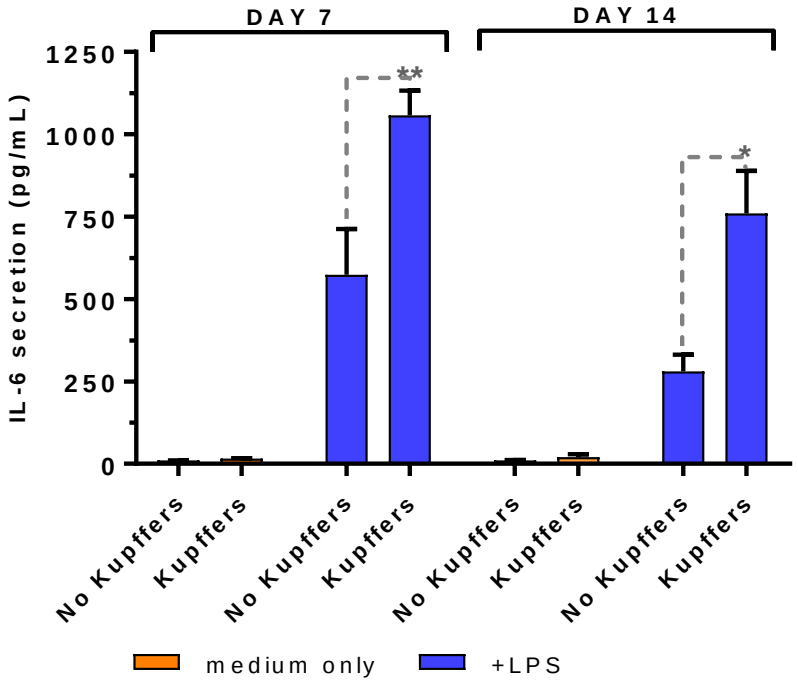






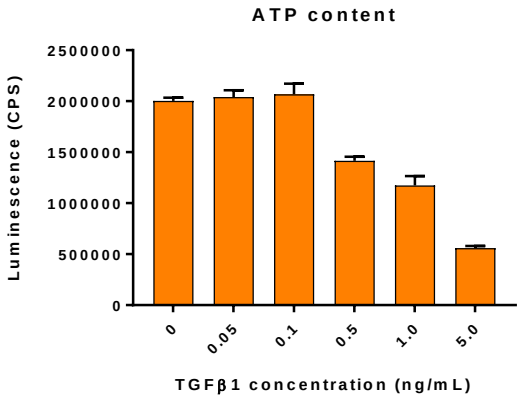
ORGANDOT - functionality of Kupffer cells

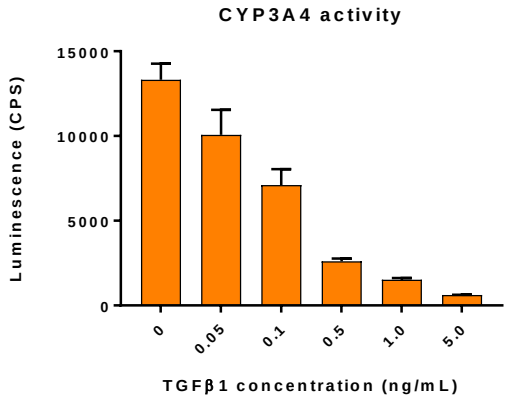
- Maintenance of Kupffer cell functionality in liver co-cultures (LPS-stimulated IL-6 secretion)

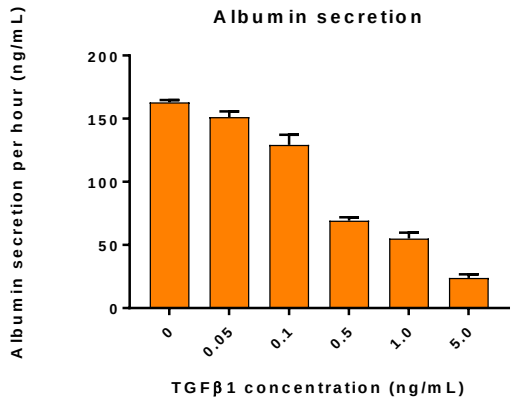


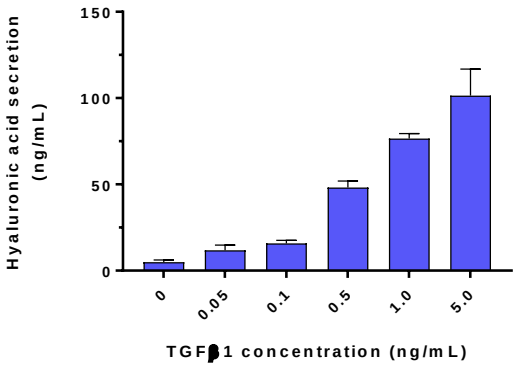
ORGANDOT – triggering a fibrotic phenotype

- Significant effect on viability and functionality of co-cultures following exposure to TGFβ1
- Secretion of hyaluronic acid (HA) – clinically relevant serum marker of advanced liver fibrosis



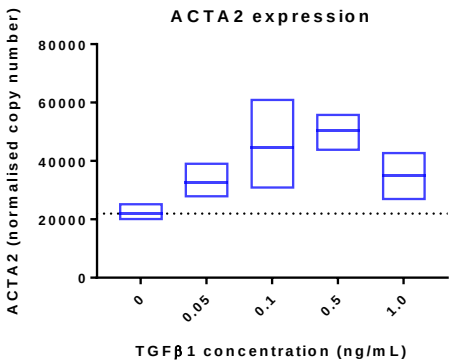


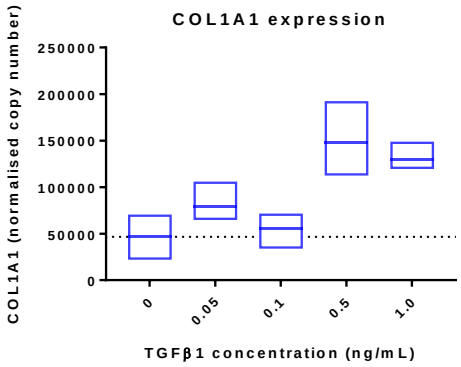


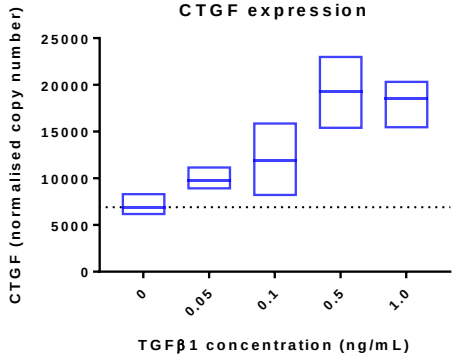


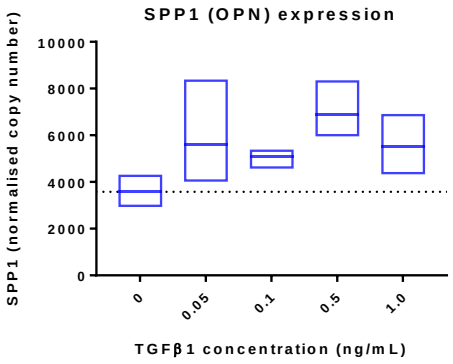
ORGANDOT – fibrogenic gene changes

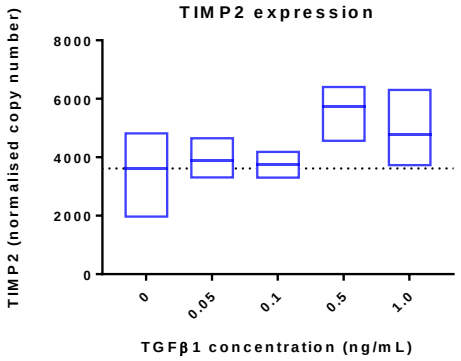
- Increase in ACTA2 (SMA), COL1A1, CTGF, SPP1 (OPN), and TIMP2 gene expression
- Negligible effect on MMP2 gene expression

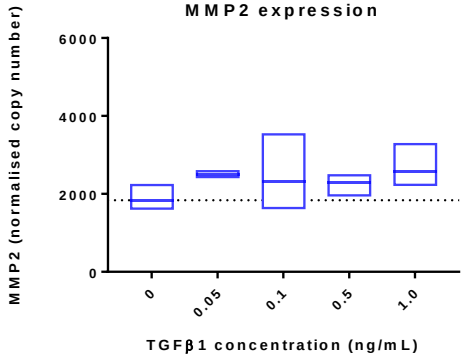






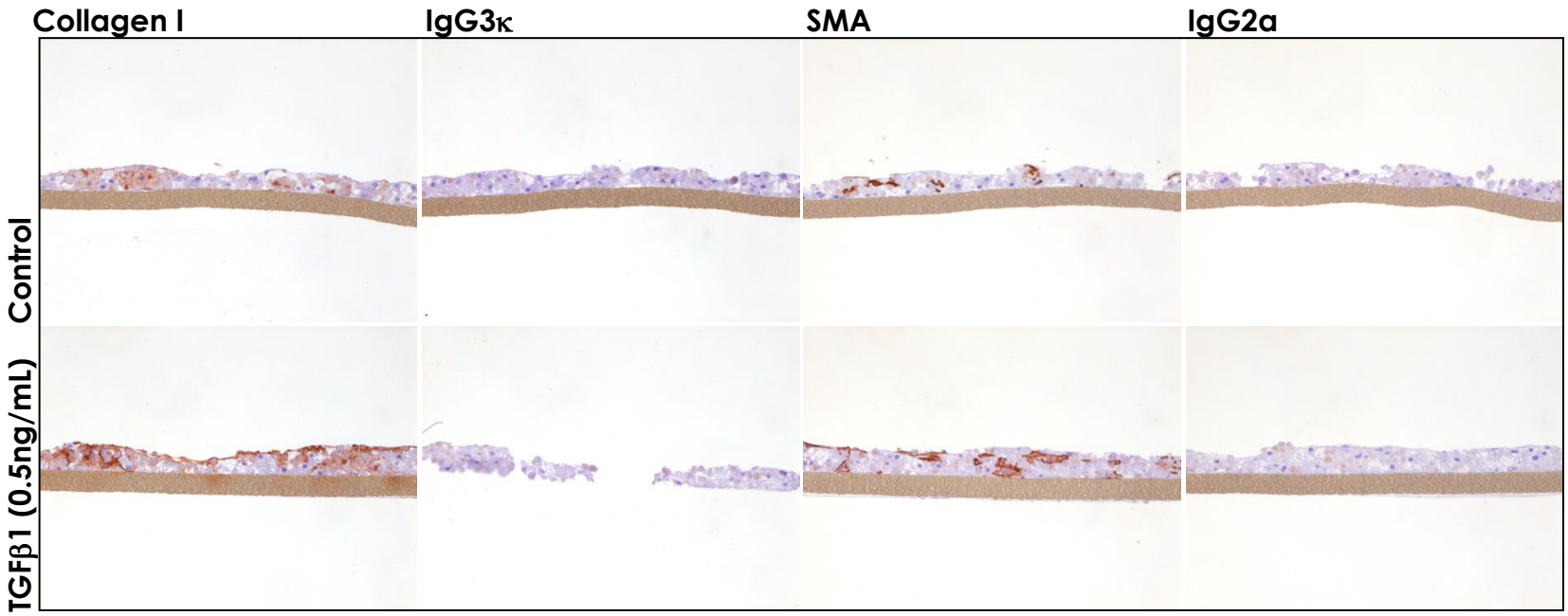






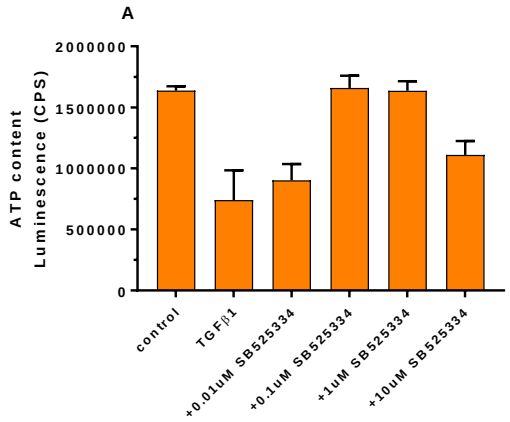
ORGANDOT – collagen I deposition

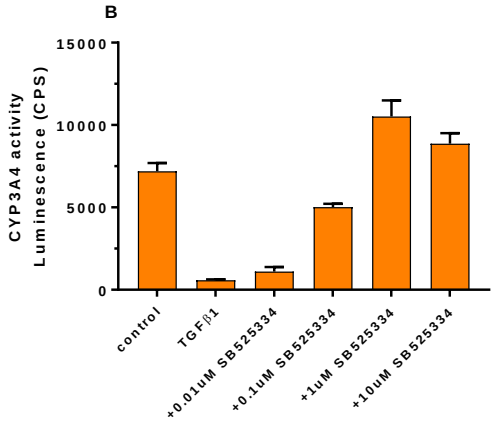
- Increase in collagen I and SMA in TGFβ1-treated liver co-cultures (FFPE sections)

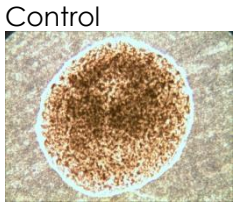


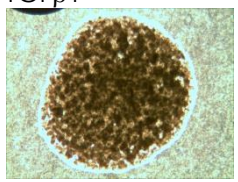
Inhibition of TGFβ1-induced fibrosis

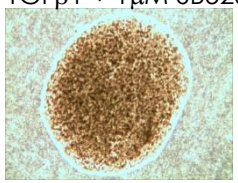
- Detrimental effect of TGFβ1 (0.5ng/mL) on the functionality of the co-cultures and increase in HA secretion prevented by ALK5 inhibitor

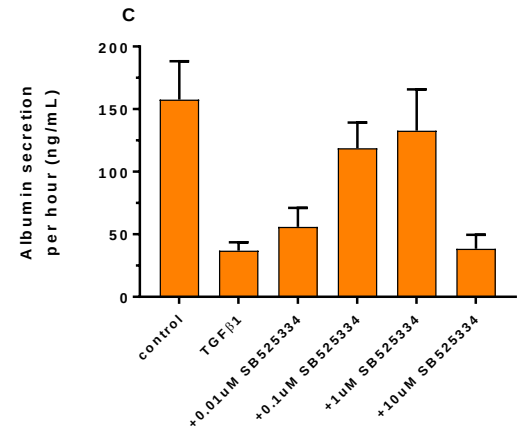


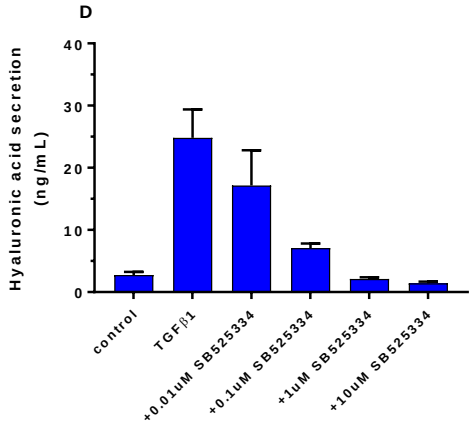






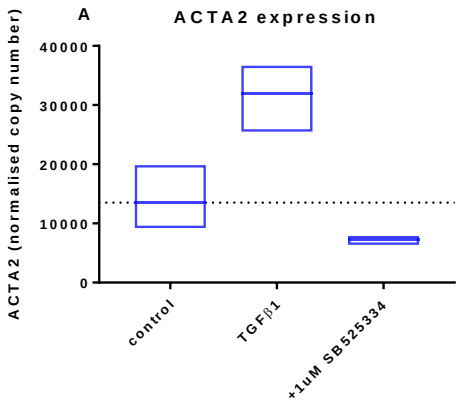


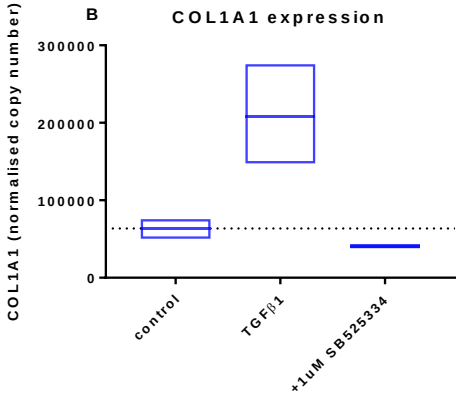


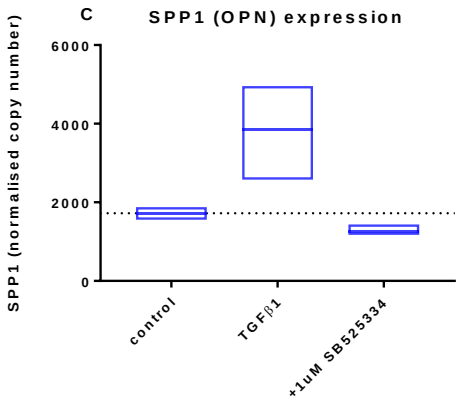


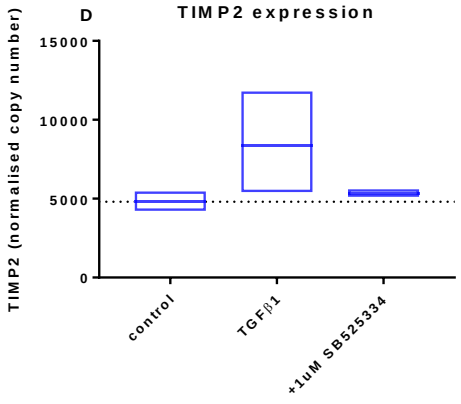
ALK5 inhibitor - fibrogenic genes

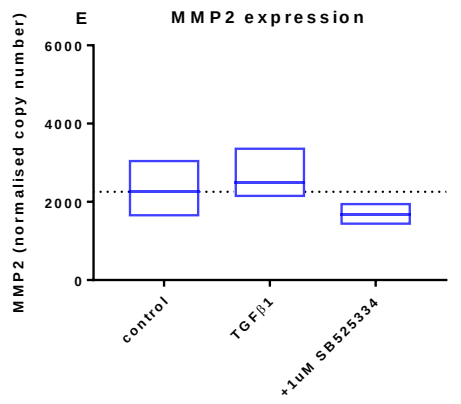
- Increase in ACTA2 (SMA), COL1A1, SPP1 (OPN) and TIMP2 gene expression prevented by ALK5 inhibitor
- Again - TGFβ1 had a negligible effect on MMP2 gene expression





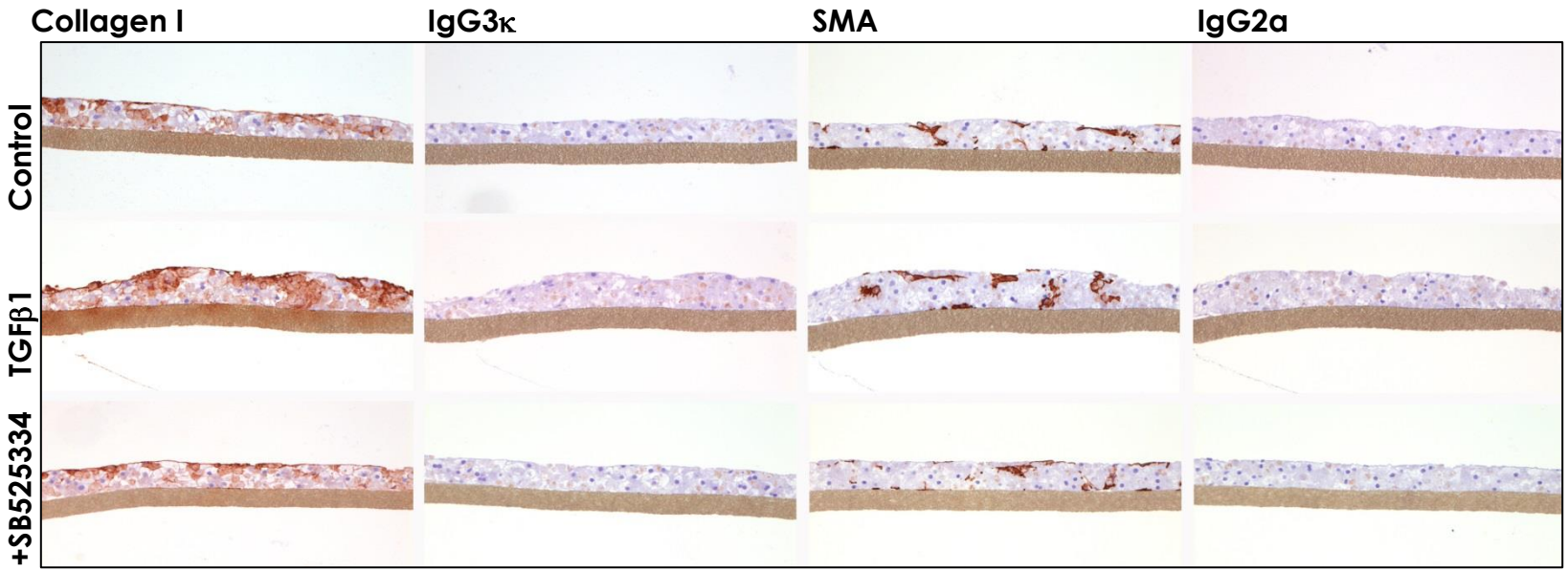






ALK5 inhibitor – collagen I deposition

- Increase in collagen I and SMA expression prevented by ALK5 inhibitor (1μM SB525334)



Summary

- Stable 3D co-cultures of human hepatocytes, Kupffers and stellates established using the ORGANDOT culture platform

- Induction of a fibrotic phenotype with TGFβ1
  - Increase in fibrogenic gene expression, HA secretion, and collagen I deposition

- Preliminary experiments with an ALK5 inhibitor complete
  - Co-administration of an ALK5 inhibitor prevents TGFβ1-induced fibrogenesis

- Next steps :
  - Focus on further development and characterization of the model
  - Select and test additional drugs / modulators of fibrosis for interference with the established endpoints
  - Work back to develop physiologically relevant stimuli to model disease physiology (e.g. steatosis, drug-induced, infection)