Utilization of a 3D Bioprinted Liver Tissue Model to Evaluate the Antifibrotic Effects of an ALK5 Inhibitor in a TGFβ-induced Model of Hepatic Fibrosis

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Abstract

Compound induced chronic liver injury can lead to initiation of profibrotic processes resulting in sustained production of growth factors and profibrotic cytokines where inflammation, tissue remodeling and repair pathways are activated simultaneously to counteract the injury. Evaluation of potential antifibrotic therapies are limited using conventional non-human animal models, due to their inability to accurately reflect complex *in vivo* human biology, while 2D models lack the multicellular complexity and life span required to study fibrosis progression and regression. Utilization of a human 3D-bioprinted liver tissue model (ExVive™ Human Liver Tissue) comprised of primary hepatocytes, hepatic stellate cells (HSCs), and endothelial cells, which can model TGFβ induced fibrosis [Norona, *et al*. (2016) Tox Sci. 154(2):354-367], enables a mechanistic interrogation with an anti-fibrotic compound. In this study, galunisertib, a small molecule ALK5 (TGFβR1 kinase) inhibitor, was used to evaluate pathway-specific blockage of TGFβ-induced fibrogenesis. The co-administration of galunisertib with TGFβ prevented the characteristic features of TGFβ-induced fibrosis, including upregulation of collagen deposition, phosphorylated SMAD2/3, and TIMP-1. Increased HSC activation was observed only in the TGFβ-induced fibrosis model, demonstrated by α-smooth muscle actin (αSMA) labeling and upregulation of ACTA2 transcript. Tissue and hepatocellular health remained stable following treatment with galunisertib, as shown by LDH release, viability, and albumin production which remained similar to vehicle levels, suggesting prevention of TGFβ induced tissue damage. These results demonstrate that a progressive *in vitro* model of liver fibrosis can be utilized to interrogate disease-associated pathways, and establish proof of concept for application of the model for preclinical evaluation of certain classes of anti-fibrotic drugs.

Technology Overview

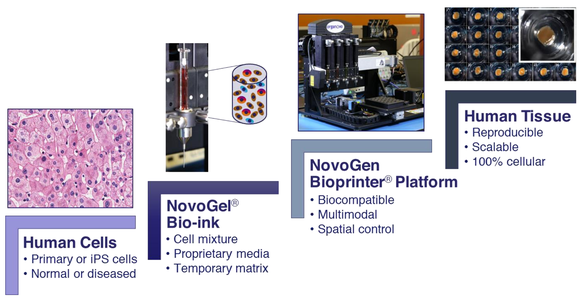


Figure 1: 3D human tissue development using the NovoGen Bioprinter® Platform. Cells reside in heterogeneous and architecturally structured 3D environments *in vivo*. Using the proprietary NovoGen Bioprinter Platform, Organovo builds 3D tissues through automated, spatially-controlled cellular deposition to better recapitulate native tissue structure and function.

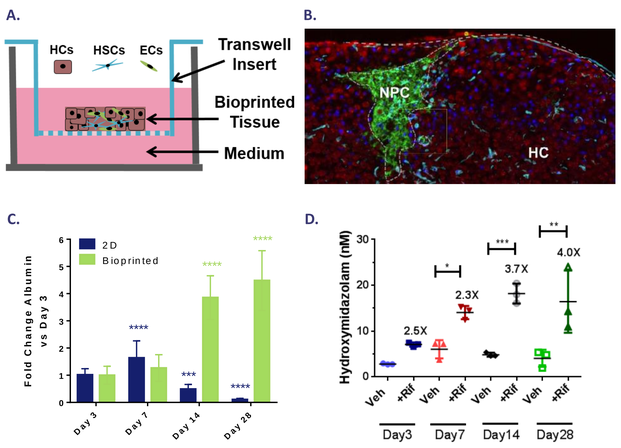


Figure 2: (A) Schematic of ExVive Human Liver Tissue, comprised of primary human hepatocytes (HCs), hepatic stellate cells (HSCs), and endothelial cells (ECs) bioprinted in a compartmentalized geometry onto the membranes of 24-well transwell culture inserts. **(B)** Representative immunofluorescence image of 3D human liver tissue showing distinct zones of non-parenchymal cells (NPC) in green and parenchymal (HC) cells in red. [Norona, *et al*. (2016) Tox Sci. 154(2):354-367]. **(C)** ExVive Liver tissue exhibits sustained hepatocyte function as indicated by albumin levels versus standard 2D hepatocyte culture, as well as sustained CYP3A4 activity **(D)** (2D = matched hepatocytes, grown on collagen 1 coated plates). [Nguyen, *et al.* (2016) PLoS One. 11(7):e0158674].

Modeling Induction and Prevention of Fibrosis

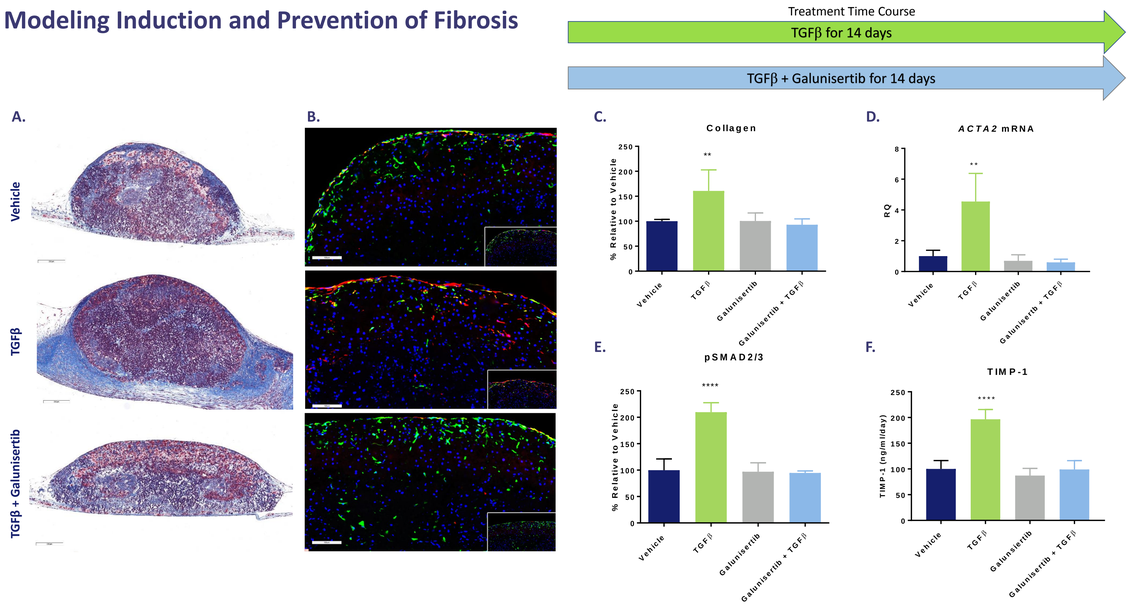


Figure 3: Galunisertib treatment blocks TGFβ-induced collagen deposition, stellate activation, and TGFβ-pathway specific biomarkers in ExVive Human Liver Tissue. Tissue was treated for 14 days with and without galunisertib co-treatment. **(A)** Trichrome staining for collagen deposition. **(B)** Alpha-smooth muscle actin (red) and desmin (green) immunofluorescence following 14 days of treatment. **(C)** Collagen quantification via Sirius Red-Fast Green assays on 15 µM tissue sections taken from tissue adjacent to the histological image. Dye was extracted and quantification was performed on a spectrophotometer. **(D)** ACTA2 transcript quantified by qRT-PCR. **(E)** SMAD2/3 phosphorylation quantified by ELISA. **(F)** TIMP-1 secretion quantified by ELISA. Data plotted as mean values ±1 SD, **p<0.01, ****P<0.0001 by ANOVA with Dunnett's multiple comparisons.

Modeling Induction and Regression of Fibrosis

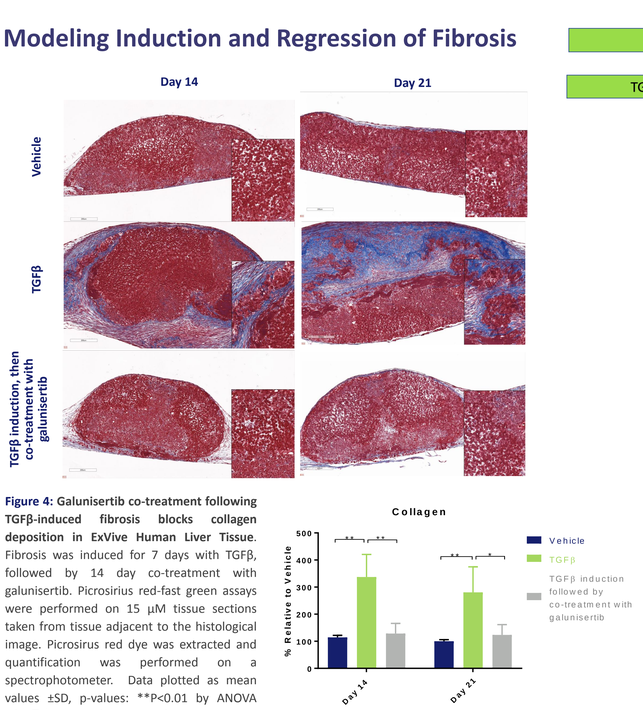


Figure 4: Galunisertib co-treatment following TGFβ-induced fibrosis blocks collagen deposition in ExVive Human Liver Tissue. Fibrosis was induced for 7 days with TGFβ, followed by 14 day co-treatment with galunisertib. Picrosirius red-fast green assays were performed on 15 µM tissue sections taken from tissue adjacent to the histological image. Picrosirus red dye was extracted and quantification was performed on a spectrophotometer. Data plotted as mean values ±SD, p-values: **P<0.01 by ANOVA with Tukey's multiple comparisons.

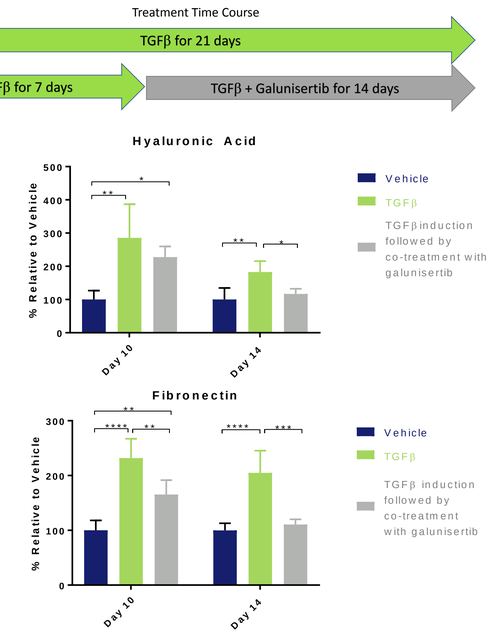


Figure 5: Galunisertib treatment inhibits TGFβ-induced hyaluronic acid and fibronectin secretion in ExVive Human Liver Tissue. Fibrosis was induced for 7 days, followed by 7 days of co-treatment with galunisertib. Data plotted as mean values ±SD, p values: *P<0.05, ** < 0.01, ***<0.001, **** < 0.0001 by ANOVA with Tukey's multiple comparisons.

Hepatocellular and Tissue Health Maintained Throughout Treatment with ALK5 Inhibitor

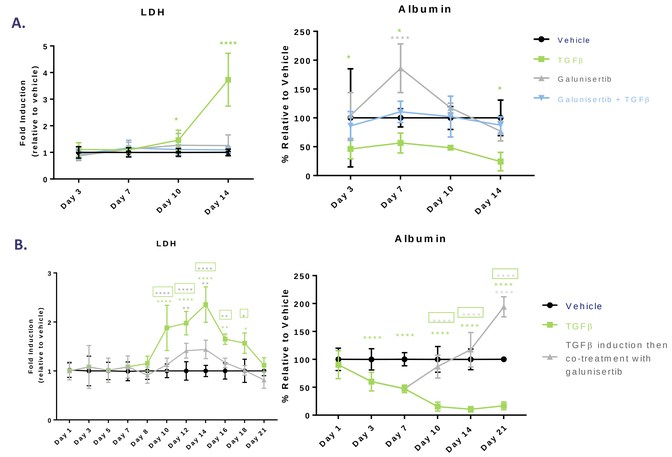


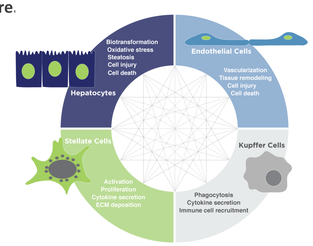
Figure 6: Galunisertib treatment of ExVive Human Liver Tissue blocked TGFβ-induced increases in LDH and decreases in albumin secretion. (A) Tissues were co-treated with TGFβ and galunisertib for 14 days. **(B)** Tissues were co-treated with galunistertib following 7 day induction with TGFβ. Galunisertib helps to rescue hepatocyte health and cell damage, assessed by quantifying albumin and LDH in supernatants. Data plotted as mean ±SD, p-values : *<0.05, ** < 0.01, ***P<0.001, **** < 0.0001 by ANOVA with Dunnett's multiple comparisons.

Summary

ExVive Human Liver Tissue is able to model liver fibrosis, evidenced by increased collagen deposition and activation of pro-fibrotic pathways with increased phosphorylation of SMAD2,3, TIMP-1 secretion, and fibronectin secretion. Co-administration of an ALK5 inhibitor with TGFβ prevented the histopathological characteristic features of TGFβ-induced fibrosis, including collagen deposition and stellate activation. Hepatocellular and tissue health biomarkers such as albumin and LDH demonstrated inhibition of TGFβ-induced changes following co-treatment with galunisertib. Co-administration of an ALK5 inhibitor reduced pro-fibrotic biomarkers after 7 days of TGFβ-induced fibrosis and prevented ECM deposition, assessed histologically.

These results demonstrate the ability of ExVive Human Liver Tissue to:

- **Evaluate potential mechanisms of injury and interrogate disease-associated pathways.**
- **Model complex, chronic injury, enabling the discovery of novel therapeutics, biomarkers, and safety assessment of drugs in a disease-relevant background.**
- **Model induction and progression of fibrosis, through fibrogenesis, tissue remodeling, and compensatory hepatocellular mechanisms which require interactions of multiple cell types in an appropriate tissue architecture.**
- **Provide assessment of morphological changes in tissues following treatment via histology to aid in elucidating mechanisms of action.**
- **Recapitulate native human liver biology *in vitro*, with sustained function and viability, enabling mechanistic insights into the phenotypes that progress over time.**



References

- Norona, *et al.* (2016) Toxicological Sciences. 2016, Vol.154, No. 2.
- Nguyen, *et al.* (2016) PLoS One. 11(7):e0158674.

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