Modeling NAFLD Using 3D Bioprinted Human Liver Tissue

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Background

Nonalcoholic fatty liver disease (NAFLD) is a chronic condition that originates as lipid accumulation within hepatocytes (steatosis) and progresses into nonalcoholic steatohepatitis (NASH), characterized by lipid accumulation, inflammation, oxidative stress, and fibrosis. NAFLD is now recognized as the most common cause of chronic liver disease, with a prevalence of 25% worldwide, and is projected to become the leading indication for liver transplant by 2025. Despite decades of research, the mechanisms of NAFLD progression, therapeutic approaches and non-invasive diagnostics are still resoundingly absent. The study of steatosis and NASH has traditionally utilized rodent models, which are time consuming to generate and do not fully recapitulate the complex phenotypes associated with the human disease. Furthermore, current 2D cell culture models lack relevant liver cell types and have limited utility due to rapid loss of cell viability and function. Thus, there is a significant need for a more predictive human multicellular 3D *in vitro* model to study the progression of steatosis into NASH.

Methods

ExVive™ Human Liver Tissue, a human *in vitro* 3D bioprinted liver model comprising primary human hepatocytes, hepatic stellate cells, and endothelial cells, exhibits a complex multicellular architecture similar to that of native liver and retains metabolic competence and liver-specific functions for at least 4 weeks in culture. To mimic the proposed pathogenesis of NASH via a "Two-Hit Hypothesis", base ExVive Human Liver Tissue was supplemented with Kupffer cells to achieve immune competency then exposed to steatogenic cues via a nutrient overload approach of simple sugars and fatty acids, followed by inflammatory stimulation using prototypical inducers.

Technology Overview

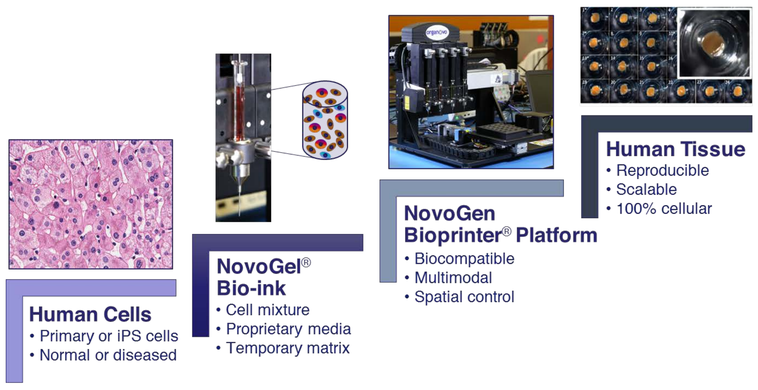
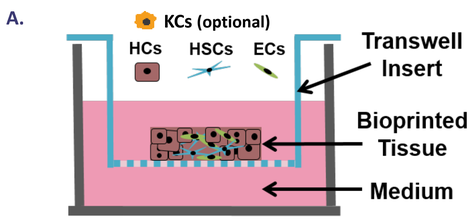
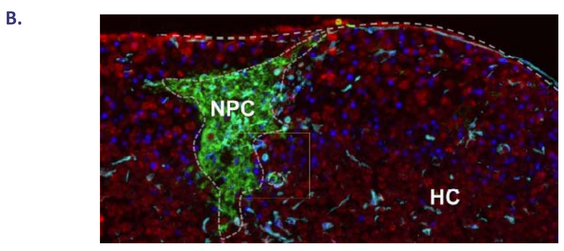


Figure 1: 3D human tissue development using the NovoGen Bioprinter® Platform**.** Cells reside in heterogeneous and architecturally structured 3D environments *in vivo*. Using the proprietary NovoGen Bioprinter Platform, Organovo builds 3D tissues through automated, spatially-controlled cellular deposition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





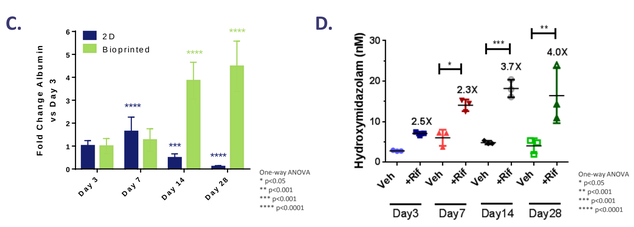


Figure 2: **[A]** Schematic of ExVive Human Liver Tissue, comprised of primary human hepatocytes (HCs), hepatic stellate cells (HSCs), and endothelial cells (ECs) bioprinted in a compartmentalized geometry onto the membranes of 24-well transwell culture inserts. **[B]** Representative immunofluorescence 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 **[D]** sustained CYP3A4 activity. 2D = matched hepatocytes, grown on collagen 1 coated plates.

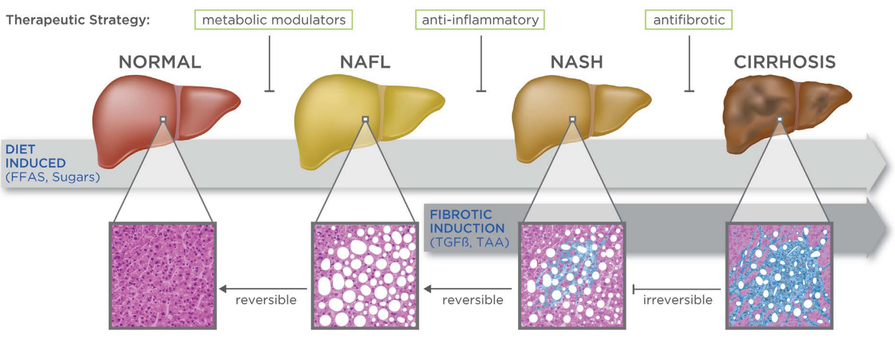


Figure 3: NAFLD is a complex, progressive disease that is driven by multiple "hits" which induce altered metabolic pathways and a pro-inflammatory environment within the liver. With its multiple cell types and complex 3D architecture, ExVive Liver Tissue allows for histological characterization in parallel with biochemical analysis, enabling the visualization of patterns of tissue damage analogous to those seen in clinical samples. Shown in the figure (grey arrows) are strategies employed to induce relevant disease phenotypes in the model.

Steatosis Induction in ExVive Human Liver Tissue

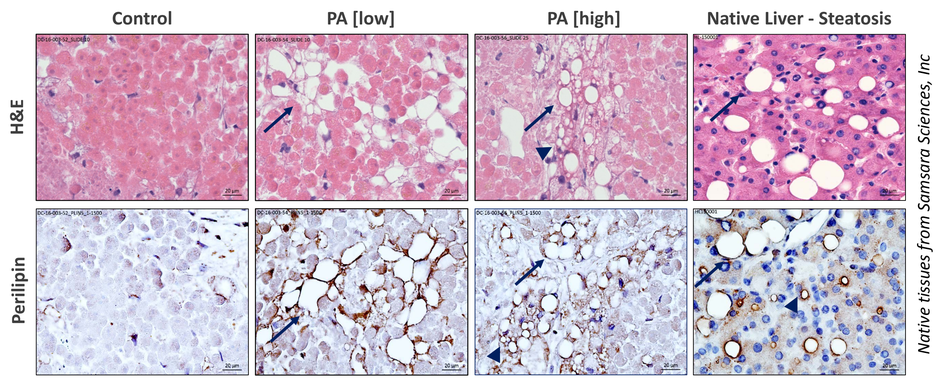


Figure 4: Palmitic acid (PA)-treated tissues exhibit increased incidence of putative lipid vesicles. H&E staining of PA treated tissues shows both macro- (arrows) and micro- (arrowheads) vesicular phenotypes. Perilipin staining of lipid vesicles further confirms the steatotic phenotype.

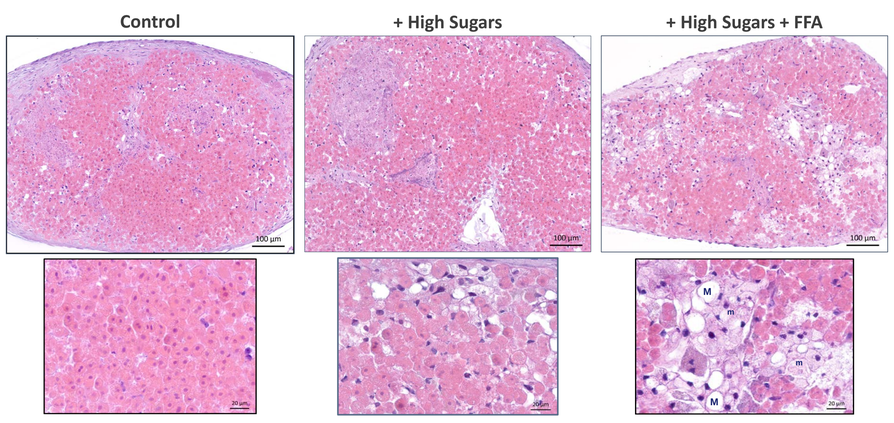


Figure 5: Tissues treated with high sugars resulted in increased presence of steatosis which was further enhanced with the addition of free fatty acid (FFA). M = macrovesicular steatosis, m = microvesicular steatosis

NASH Induction in ExVive Human Liver Tissue

Figure 6: Progression of steatosis to inflammation with fibrosis can be observed following incorporation of Kupffer cells (KC) and treatment with palmitic acid (PA) in conjunction with lipoplysaccharide (LPS). **[A]** Increased activation of hepatic stellate cells (by α-SMA staining) is apparent, similar to native tissue samples. **[B]** Trichrome staining reveals increased fibrosis (F = regions of increased collage staining in blue) and steatosis (arrows), similar to native tissue samples.

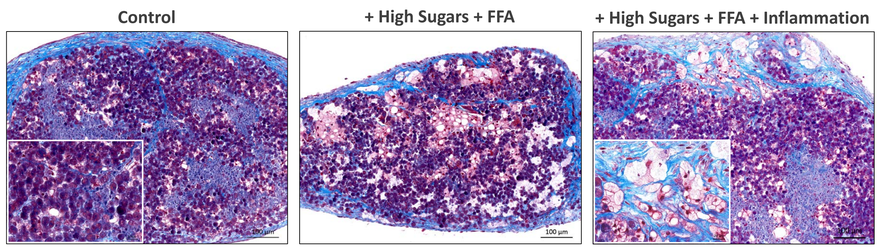


Figure 7: Trichrome staining reveals increased fibrosis and hepatocellular degeneration in tissues treated with both high sugars + FFA and inflammation.

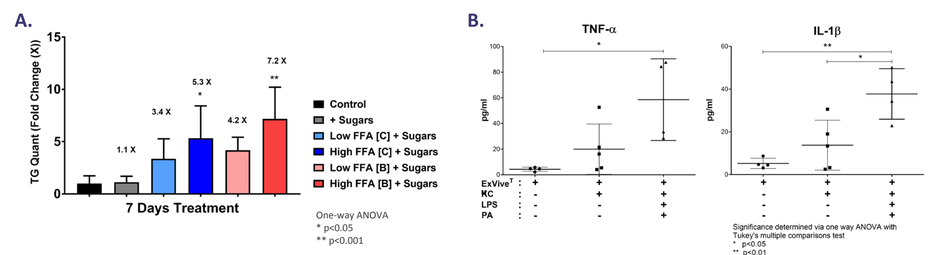


Figure 8: **[A]** Immune competent (+ Kupffer cells) tissues treated with high dose FFA regimens displayed significantly increased triglyceride (TG) accumulation versus control. **[B]** Chronic dosing of immune-competent tissues with inflammatory inducer (LPS) and palmitic acid (PA) on a high sugar background led to significantly increased inflammatory cytokine levels.

Modulation of Fibrosis through the TGFβ Pathway in ExVive Liver Tissue

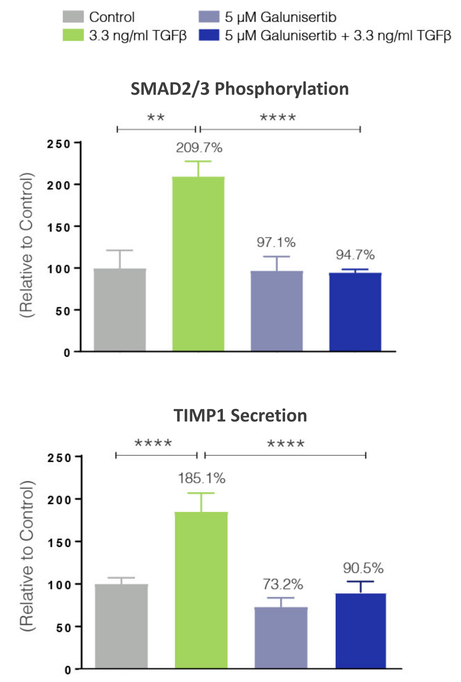


Figure 9: Galunisertib treatment blocks TGFβ-induced SMAD2/3 phosphorylation and TIMP1 secretion. Tissue was treated for 7 days with and without galunisertib co-treatment. Data plotted as mean values ë1 SD, p values: ** < 0.01, **** < 0.0001 by ANOVA.

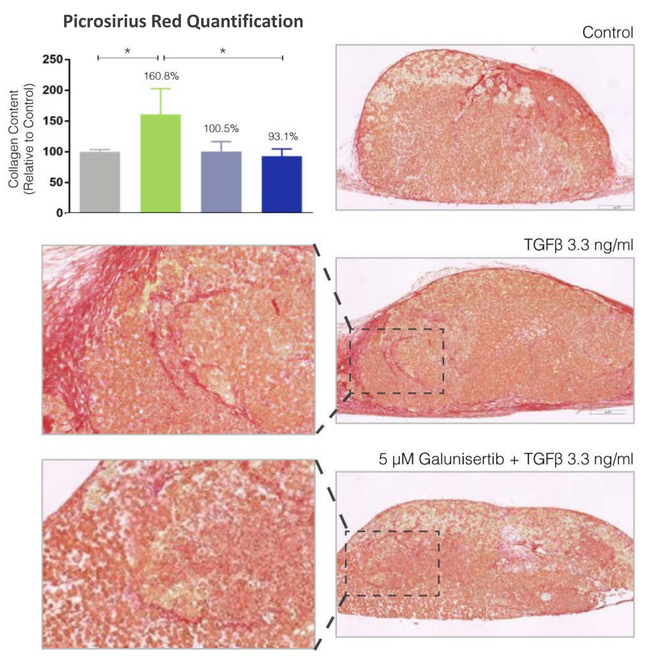


Figure 10: Galunisertib treatment blocks TGFβ-induced collagen deposition. Tissue was treated for 14 days with and without galunisertib co-treatment. Picrosirius red assays were performed on 15 µM tissue sections taken from tissue adjacent to the histological image. Picrosirius red dye was extracted and quantification was performed on a spectrophotometer. Galunisertib treatment blocks TGFβ-induced collagen deposition. Data plotted as mean values ±1 SD, *p <0.05 by ANOVA.

Summary

- Organovo's bioprinting platform enables the construction of 3D human tissue with complex architecture, sustained function and viability.
- ExVive Human Liver Tissue can recapitulate a variety of disease relevant phenotypes including steatosis, inflammation, and fibrosis.
- Inhibition of the TGFβ-induced fibrosis was observed with co-treatment of galunisertib.
- ExVive Liver Tissue holds promise for the study of complex, chronic conditions such as NASH, enabling the discovery of novel therapeutics, biomarkers, and safety assessment of drugs in a disease-relevant background.