Bioprinted Human Tissues for Toxicology and Disease Modeling

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Abstract

Fully human 3D bioprinted tissues with spatially-controlled architecture enable biochemical, genetic, and histologic interrogation following exposure to modulators of interest, making them valuable *in vitro* tools for toxicology and disease modeling. We have developed ExVive™ Human Liver Tissues, comprised of primary hepatocytes, hepatic stellate cells and endothelial cells, which exhibit histological and functional similarity to native liver, with sustained viability (ATP, albumin) and *CYP3A4* activity over four weeks. The liver tissues have proven useful in preclinical toxicology assessment, especially in situations where chronic exposure, bioactivation, interactions among several cell types or tissue level phenotypes are critical components of the toxic response. Treatment of ExVive tissues with valproic acid resulted in decreases in ATP and GSH and the accumulation of cytoplasmic vacuoles within the hepatocytes reflective of a steatotic phenotype. Fibrosis is particularly difficult to model outside of the tissue context as it involves several processes driving disease progression including inflammation, tissue remodeling, and compensatory repair. Treatment of ExVive tissues with methotrexate or TGF-β1 revealed fibrillary collagen deposits similar to that seen in clinical biopsies and gene expression changes were consistent with stellate cell activation and extracellular matrix deposition. Transient cytokine production reflective of a wound-healing response was observed, with time- and treatment-dependent alterations in immunomodulatory and chemotactic cytokines such as IL-6 and MCP-1. The ability to reproduce the interplay of different functional cell types within the tissue context allows for the detection of toxicity mechanisms involving complex phenotypes which are not easily modeled in conventional cell cultures

Introduction

- **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.**

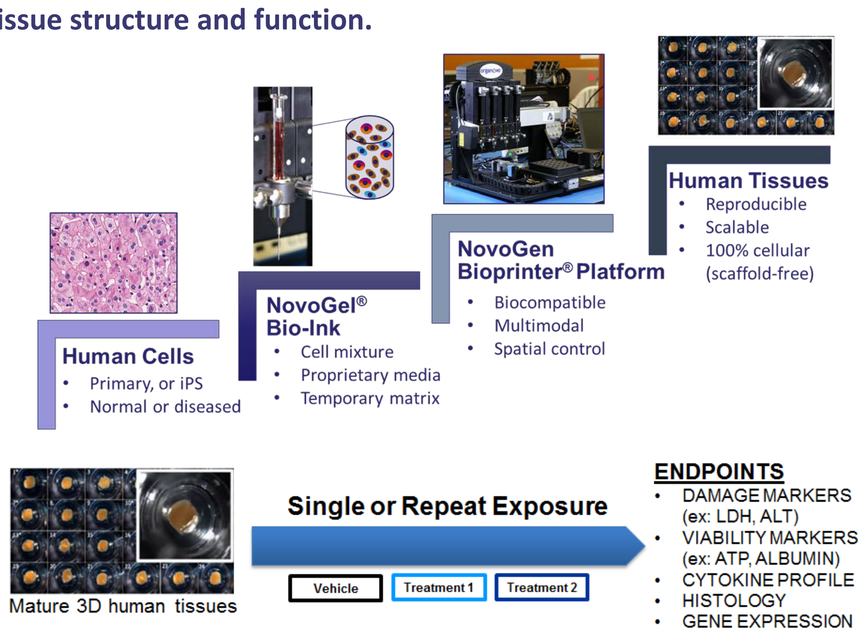


Figure 1: 3D human tissue development using the NovoGen Bioprinter® Platform, and a sample experimental workflow.

ExVive™ Liver tissues model clinically relevant toxic phenotypes

Tissue Characterization

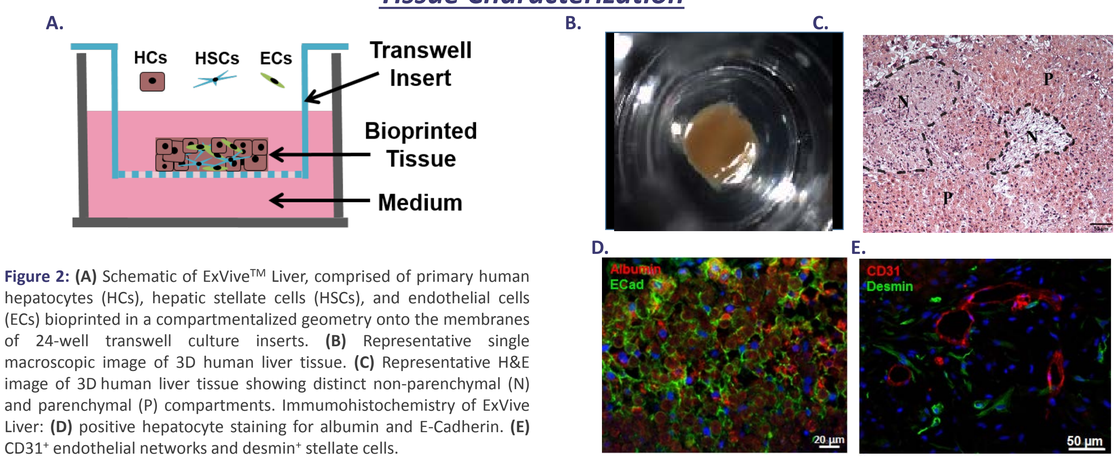


Figure 2: (A) Schematic of ExVive™ Liver, comprised of primary human hepatocytes (HCs), hepatic stellate cells (HSCs), and endothelial cells (ECs) bioprinted in a compartmentalized geometry onto the membranes of 24-well transwell culture inserts. **(B)** Representative single macroscopic image of 3D human liver tissue. **(C)** Representative H&E image of 3D human liver tissue showing distinct non-parenchymal (N) and parenchymal (P) compartments. Immumohistochemistry of ExVive Liver: **(D)** positive hepatocyte staining for albumin and E-Cadherin. **(E)** $CD31^+$ endothelial networks and $desmin^+$ stellate cells.

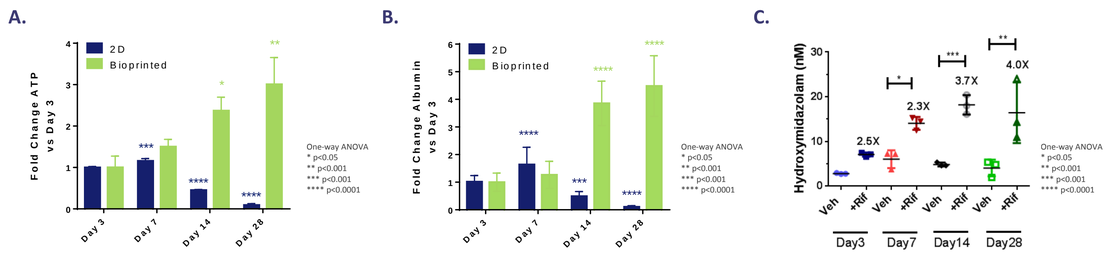
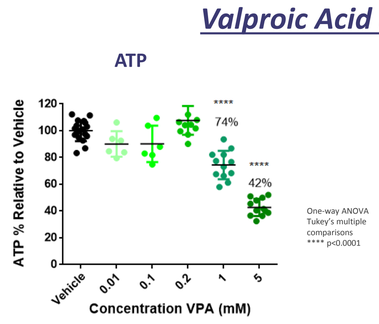
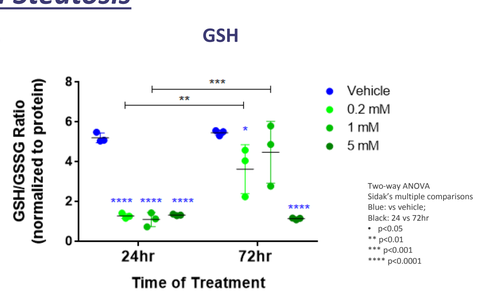


Figure 3: ExVive Liver tissues exhibit sustained viability as indicated by ATP levels **(A)** and sustained hepatocyte function as indicated by albumin levels **(B)** versus standard 2D hepatocyte culture, as well as sustained *CYP3A4* activity **(C)**. 2D = matched hepatocytes, grown on collagen 1 coated plates).

Valproic Acid Induced Steatosis





- **Valproic acid is prescribed for epilepsy, bipolar disorder and migraine**
- **5% - 10% of patients develop ALT elevations**
- **Usually asymptomatic or resolving**
- **Rarer, severe injury occurs with mitochondrial toxicity and steatosis**

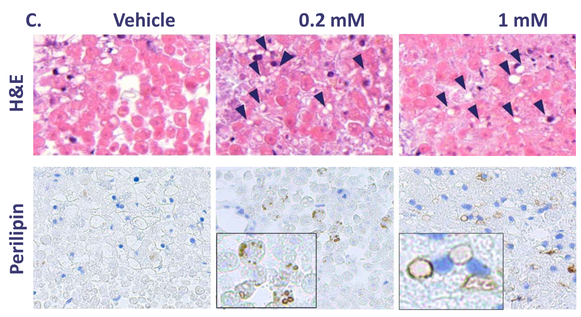
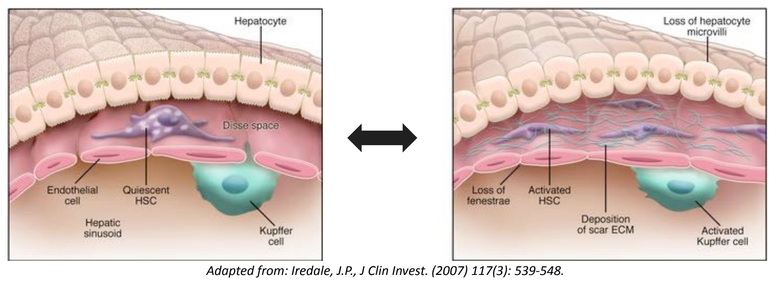


Figure 4: (A) Viability of ExVive liver tissues declines with increasing dose of valproic acid (VPA). **(B)** All doses of VPA induce oxidative stress, evidenced by GSH decline, following 24hrs of exposure, with partial recovery at lower doses by 72hrs. **(C)** H&E staining of VPA treated tissues shows a vesicular steatotic phenotype (arrowheads). Perilipin staining of lipid vesicles further confirms the steatotic phenotype.

ExVive™ Liver tissues model clinically relevant disease phenotypes

Progressive Liver Fibrosis



Adapted from: Iredale, J.P, J Clin Invest. (2007) 117(3): 539-548.

Figure 5: Progressive fibrotic liver injury is orchestrated by complex intercellular interactions among hepatocytes (HCs), endothelial cells (ECs), hepatic stellate cells (HSCs), Kupffer cells (KCs) and recruited bone-marrow derived cells. This interplay between resident and recruited cell types results in the appearance and progression of disease features that are best detected and interpreted in the context of a three-dimensional (3D) tissue environment, including inflammation, fibrogenesis, tissue remodeling, and compensatory hepatocellular regeneration. ExVive Liver tissues contain the key cell types (HCs, ECs and quiescent HSCs), appropriate tissue architecture, and long term maintenance of key signaling pathways required to model induction and progression of fibrosis.

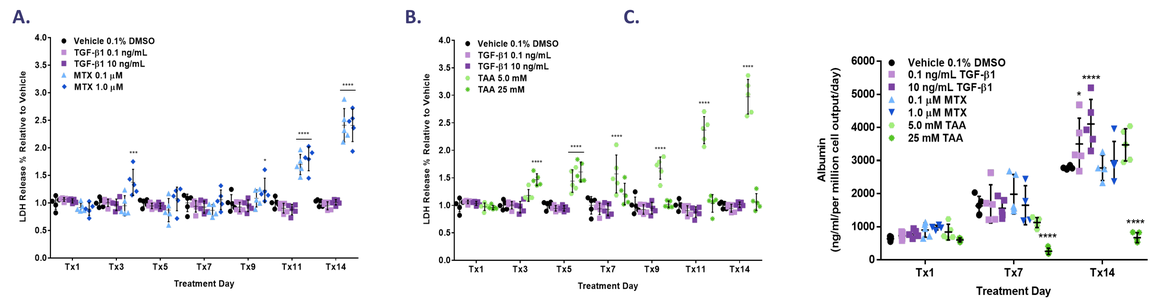


Figure 6: Impact of fibrotic agents on bioprinted liver tissue viability and functionality. 14-day treatment with MTX **(A)** and TAA **(B)** resulted in tissue damage, as evidenced by significant increases in LDH release (n = 9 for Tx1-Tx7, n = 5 for Tx9-Tx14) . Conversely, no overt damage is observed with TGF treatment. The degree of hepatocellular damage as measured by albumin output (**C**; n = 5) at key time points during the treatment period was most pronounced with 25 mM TAA, as demonstrated by a statistically significant decrease in albumin output relative to vehicle treated control. Significance was determined using a one-way ANOVA with post hoc Dunnett's multiple comparisons test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

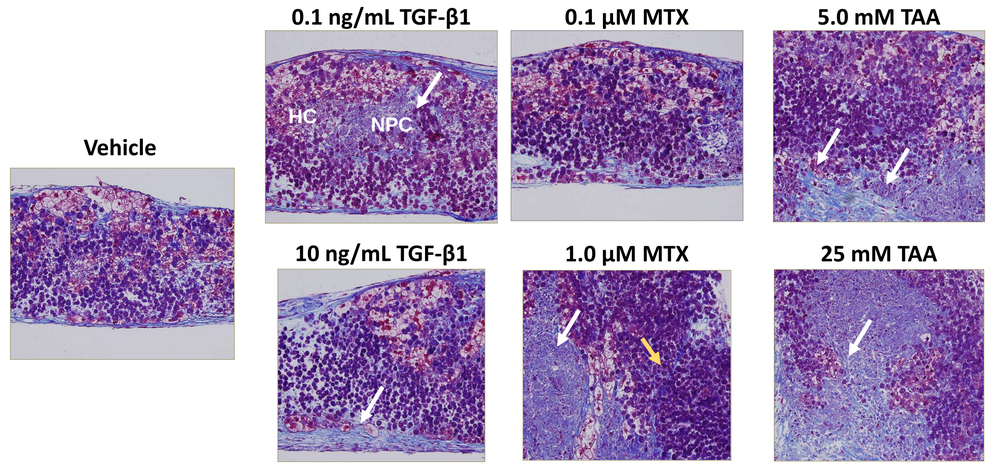


Figure 7: Histological assessment of bioprinted liver. Fixed tissues sections were stained with Gomori's One-Step trichrome to visualize collagen (blue), cytoplasm (pink/purple), and nuclei (dark purple). TGF-β1 treatment caused focal nodular fibrosis (white arrows) in the nonparenchymal compartment (NPC) but generally preserved hepatocellular (HC) mass. MTX caused mild hepatocellular damage and nodular and pericellular fibrosis at lower doses, with evidence of bridging fibrosis (yellow arrow) connecting NPC at 1.0 µM. Treatment with TAA nearly eliminated HCs in the tissues by 14 days, with the majority of tissue replaced by fibrotic scar tissue. Scale bar = 100 µm.

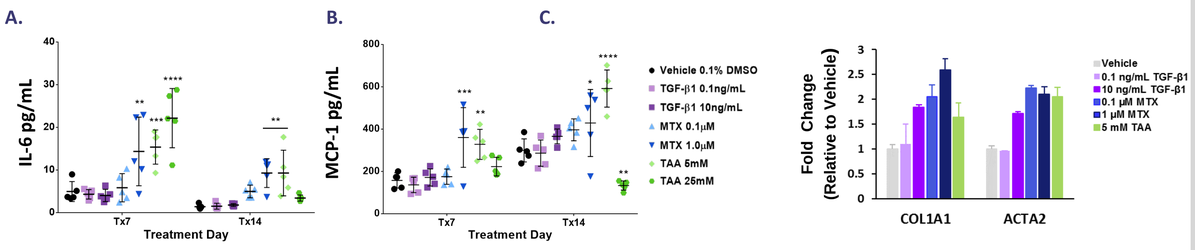


Figure 8: Measurement of cytokine levels at Tx7 and Tx14 (n = 5) showed treatment- and time-dependent differences in immunomodulatory and chemotactic cytokines. **(A)** IL-6 regulates acute phase response proteins in response to injury and was significantly increased at Tx7 for 1.0 µM MTX and both TAA treatment groups. **(B)** Monocyte chemotactic protein-1 (MCP-1), involved in facilitating macrophage/monocyte infiltration to perpetuate an adaptive response to continued insult, increased at Tx14 for MTX and TAA treatment. Significance was determined using a one-way ANOVA with post hoc Dunnett's multiple comparisons test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). **(C)** Time-dependent up-regulation of two fibrosis-associated genes. The levels of fibrogenic markers α-smooth muscle actin (*ACTA2*) and collagen, type 1, α1 (*COL1A1*) were measured using RNA isolated from whole tissue constructs at treatment day 14. Values are represented as fold-change relative to vehicle control. Treatment-induced induction of these genes suggests active fibrogenic processes and provide evidence to support collagen deposition within the tissue constructs.

Summary

- **Organovo's bioprinting platform enables the construction of architecturally correct 3D human tissues through controlled cellular placement without the use of exogenous scaffolding.**
- **ExVive™ 3D Bioprinted Human Liver Tissues recapitulate native liver physiology *in vitro*, with sustained function and viability.**
- **This enables mechanistic insights into phenotypes that progress over time and require multiple cell types in a specific spatial organization, reflecting the true complexity of human biology.**