Ex-vivo human 3D NASH model as a screening-based discovery approach for selecting and prioritizing drug candidates

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Background:
Non-alcoholic fatty liver disease (NAFLD) is an emerging chronic liver disease characterized by hepatic steatosis that often progresses into steatohepatitis (NASH). Poor translation of animal studies to humans have resulted in a lack of approved NAFLD/NASH-specific drug therapies. We have modeled NAFLD/NASH *ex vivo* using human microtissues technology as a high-throughput tool for drug discovery.
We present here a novel human 3D *in vitro* NASH model, which incorporates primary hepatocytes, Kupffer cells, liver endothelial cells, and hepatic stellate cells, for high-throughput-compatible drug efficacy testing.

Methods:
We generated liver microtissues by culturing human primary hepatocytes, Kupffer cells, liver endothelial cells, and hepatic stellate cells in InSphero plates. Upon exposure to defined lipotoxic and inflammatory stimuli, including free fatty acids and LPS in media containing high levels of sugar and insulin, this 3D NASH model displayed pathophysiologically relevant features within 10 days of treatment. The methods for assessing characteristic markers for NASH included accumulation of intracellular triglycerides (bioluminescent assay), secretion of pro-inflammatory cytokines/chemokines (Luminex), and secretion of pro-collagens type I and III (HTRF/ELISA). Quantification of fibrosis based on Sirius Red-stained tissue slices was performed using the PharmaNest imaging platform.

Results: We observed increases in intracellular triglyceride content and the secretion of proinflammatory (e. g. IL-6, IL-1b, TNF-a) and profibrotic (e.g. IL-10, GRO-a, IP-10, MCP-1) cytokines/chemokines in the NASH-treated tissues as compared to the untreated controls. Further, we detected increased fibril collagen deposition, and increased secretion of procollagen type I/III peptides under NASH conditions. Whole transcriptome analysis of NASH-treated tissues versus control revealed activation of pathways and differential regulation of genes associated with lipid metabolism, inflammation, and fibrosis induction. Treatment with the anti-TGF-β antibody and ALK5i (TGF-βRI inhibitor) concentration dependently decreased secretion of pro-collagen type I/III. Decreased deposition of fibril collagens based on quantification of fibrosis of Sirius Red-stained tissues was observed in the presence of anti-TGF-β antibody and ALK5i. The results from the biochemical readouts of the NASH-treated tissues with drug clinical candidates (Selonsertib and Firsocostat) were in line with clinical observations.

Conclusions: In summary, this high-throughput and compatible 3D human NASH model represents a promising approach for NASH drug candidate efficacy selection early within the drug discovery process.

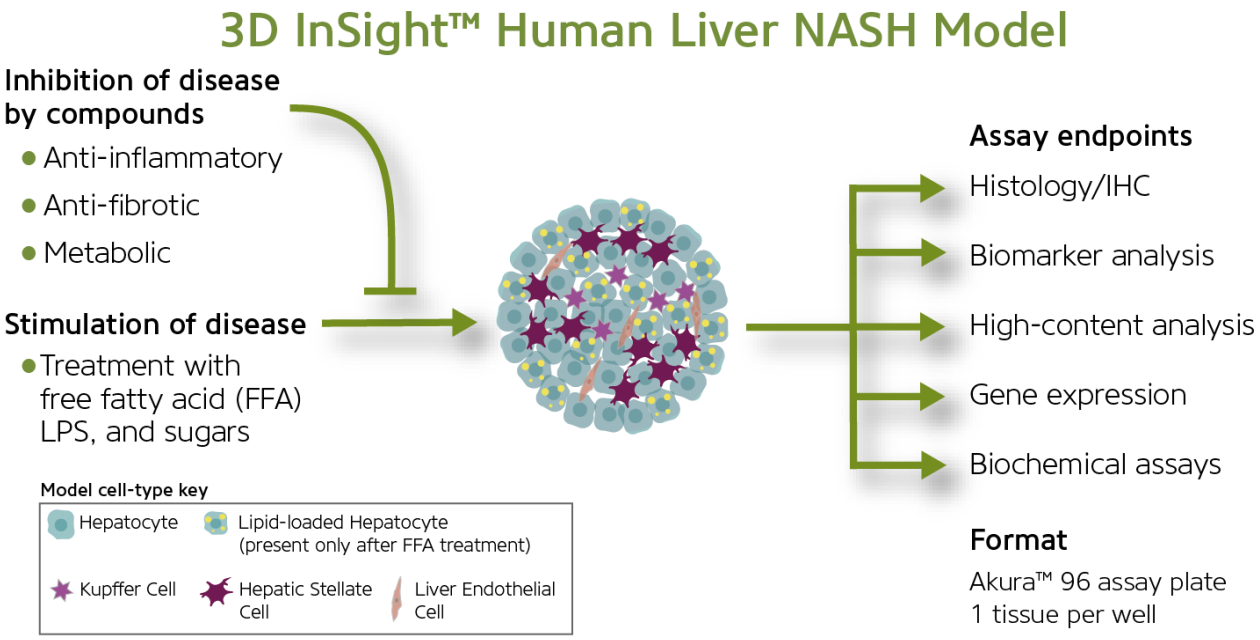

Figure 1. Human primary hepatocytes, Kupffer cells, stellate cells and liver endothelial cells were seeded and aggregated to form liver microtissues in Insphero plates. A specific metabolic and inflammatory stimulation led to the recapitulation of human NASH hallmarks by measuring specific endpoints.