Three-dimensional human cell culture model for studying Non-Alcoholic Fatty Liver Disease

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Non-Alcoholic Fatty Liver Disease (NAFLD) is a growing concern worldwide and is set to become the most prevalent cause of chronic liver disease. NAFLD describes a range of disease states from liver steatosis, which is the common and most common though to stage liver disease, cirrhosis and liver cancer. There are currently no FDA approved drugs for the treatment of NAFLD and there is an interest in developing better models for understanding disease progression and the molecular pathways underlying the disease (1).

Currently available pre-clinical models, although useful, suffer from a number of limitations and differ in regard to the degree of hepatic curvature and metabolic alterations associated with disease development (2). Culturing with acellular matrix generally utilise hepaticocyte carcinoma-derived cell lines, which lack many of the biochemical properties of primary hepatocytes and fail to represent the liver microenvironment and disease pathology (3).

Utilise the perfused cell culture system LiverChip®, to develop a fully human in vitro NAFLD model. Culture primary human hepatocytes in three dimensions to mimic the liver microarchitecture and support hepatic function over extended periods of culture, allowing the continual accumulation of fat deposits in the hepatocytes.

OBJECTIVES

• Fat and lean culture conditions were developed for the culture of PHH for up to two weeks.

• Fat-free pre-treatment induced comparable fat and micro-scanning electron microscopy drops were observed in tissues.

• Fat accumulation reduces transcriptional and proteomic changes that correlate with clinical observations from NAFLD patients, including upregulation of CYP2E1 expression and induction of fibrinogen production, with changes in adipokine levels.

• Fat loading reduces metabolic capacity of PHH, effecting numerous P450 enzymes and the continual accumulation of fat deposits in the hepatocytes.

• PHH were cultured in fat or lean conditions for 7 days before total RNA was extracted and gene expression was compared using the Fatty Liver RT2 profiler PCR Array. Adipokine expression changes were validated by a fold change ≥1.8 and P < 0.05. Results are an average of three hepatocyte donors (n=3).

• Fat change in expression in fat vs lean condition was greater for genes (n=3).

• Total RNA was extracted using Triuret (Ambion) and RNA was converted to cDNA using the high capacity cDNA synthesis kit (Life Technologies). Gene expression profiles were quantified in equivalent cDNA samples using Fatty Liver RT2 PCR profiler arrays (Qiagen) and Metformin were dosed for 7 days after 7 days pre-culture in fat/lean conditions.

• Fat accumulation was measured by Oil Red O staining of fixed micro-tissue. Oil Red O stain was removed from tissues and quantified by absorbance at 515 nm. Total protein content of acellular matrix was measured by BCA assay (Thermo). Free fatty acid content in media was measured by BCA assay (Thermo). Adipokines (profilin, angiogenin, TNF, IL-8, IL-6) were used to compare conditioned media samples. Data is a mean ± SD of six donors (n=3), P < 0.05.

• PHH were cultured in fat or lean conditions for 7 days A) CYP3A activity was measured in three donors by CYP3A-Glo assay. B, C) P450 activity of cells was compared using an LC-MS/MS cocktail assay. D) PHH were dosed with 10 µM diclofenac and samples of culture media analysed by LC-MS/MS. Data is a mean (n=3), P < 0.05.

• Cells were treated for 7 days in the continued presence/absence of fat. Fat content was measured by Oil Red O staining and normalised to total protein content. Data is a mean ± SD of six donors (n=3), P < 0.05.

• Accumulation of fat deposits in the hepatocytes.