Development of an iPSC-derived hepatocyte and hepatic stellate cell co-culture model for the study of Metabolic-dysfunction Associated Steatohepatitis (MASH) *in vitro*

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INTRODUCTION

Metabolic dysfunction-associated steatohepatitis (MASH), the advanced stage of metabolic dysfunction-associated steatotic liver disease (MASLD), is characterised by lipid accumulation, oxidative stress, and excessive accumulation of extracellular matrix proteins (fibrosis). Despite the severity, there is currently only one licenced treatment against MASH, mainly due to disease complexity and lack of predictive pre-clinical models. We hypothesised that the development of iPSC-derived hepatocyte (Ulti-HEP) and hepatic stellate cell (Ulti-HSC) co-cultures can offer a suitable and predictive platform for MASH modelling.

MATERIALS AND METHODS

Wild-type iPSCs were differentiated to Ulti-HEP1 and Ulti-HSC, and cell functionality was determined by qPCR, immunocytochemistry, western blotting, colorimetric assays, and ELISA. Ulti-HEP and Ulti-HSC were co-cultured and differences in lipid accumulation and collagen secretion between monocultures and co-cultures were measured by BODIPY staining and ELISA. Ulti-HEP/Ulti-HSC co-cultures were treated with a mixture of free fatty acids (FFA), prior to treatment with 10 μM Firsocostat and 5 μM Alk5i to investigate the effect of pharmacological intervention in reversing steatosis and collagen secretion, respectively.

iPSC-DERIVED Ulti-HEP AND Ulti-HSC CAN BE SUCCESSFULLY CO-CULTURED IN 2D FORMAT

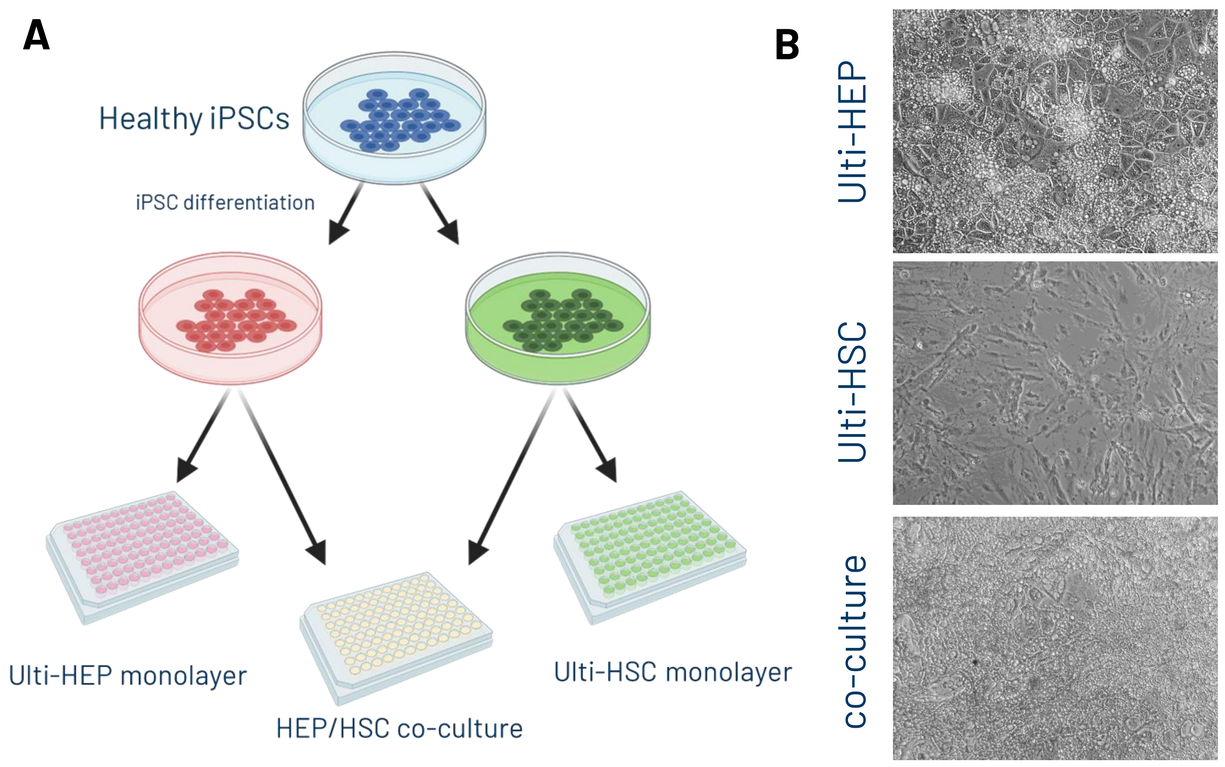

Figure 1. A) Simplified schematic of the experimental design towards the development of DefiniGEN Ulti-HEP/Ulti-HSC co-culture cell systems. **B)** Representative brightfield pictures showing the morphology of DefiniGEN Ulti-HEP, Ulti-HSC, and HEP/HSC co-cultures.

Ulti-HEP/HSC CO-CULTURES EXPRESS HEPATOCYTE AND STELLATE CELL MARKERS

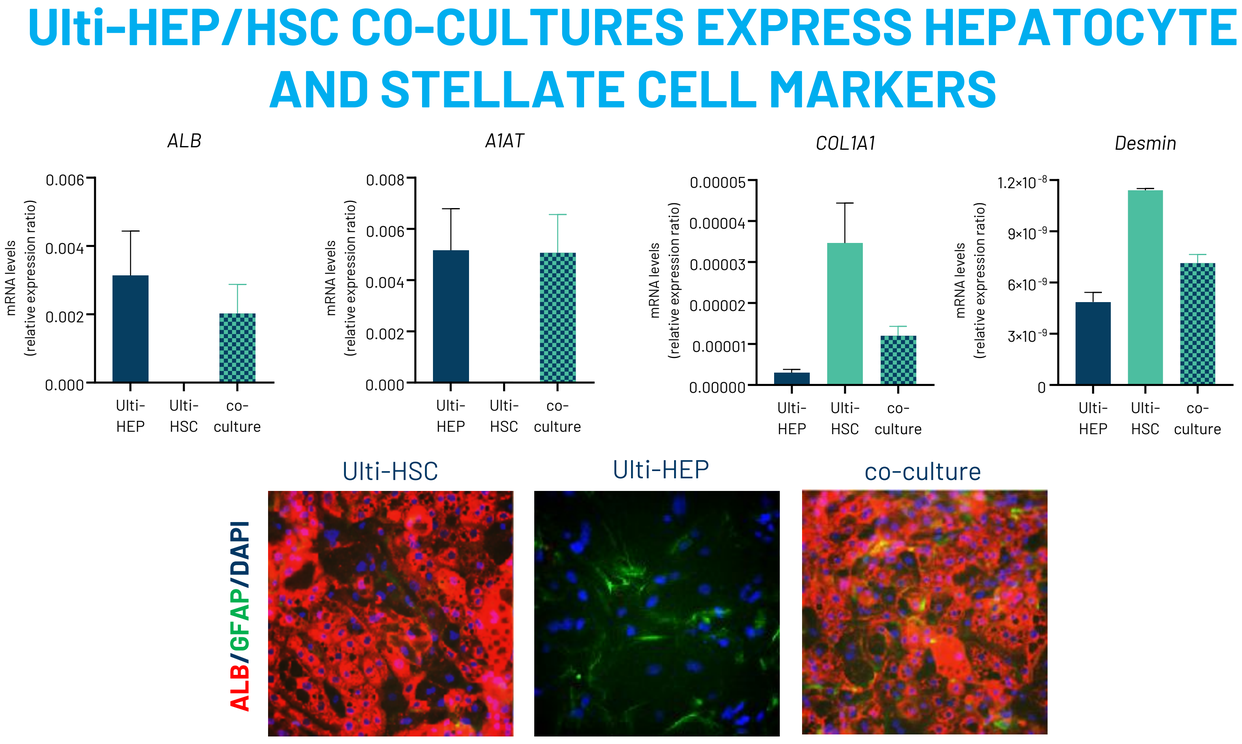

Figure 2. A) mRNA levels of the hepatocyte markers albumin (ALB) and alpha-1-antitrypsin (A1AT) and hepatic stellate cell markers collagen (COL1A1) and Desmin in DefiniGEN Ulti-HEP, Ulti-HSC, and Ulti-HEP/HSC co-cultures. B) Representative immunocytochemistry pictures showing expression of the hepatic stellate cell marker GFAP and hepatocyte marker albumin (ALB) in in DefiniGEN Ulti-HEP, Ulti-HSC, and Ulti-HEP/HSC co-cultures. Data are presented as mean±SEM of n=2 independent experiments. mRNA expression data were normalized to 18S rRNA.

ACTIVATED Ulti-HSC DRIVE HEPATIC STEATOSIS AND FIBROSIS IN Ulti-HEP/HSC CO-CULTURES AND THIS CAN BE PHARMACOLOGICALLY REVERSED

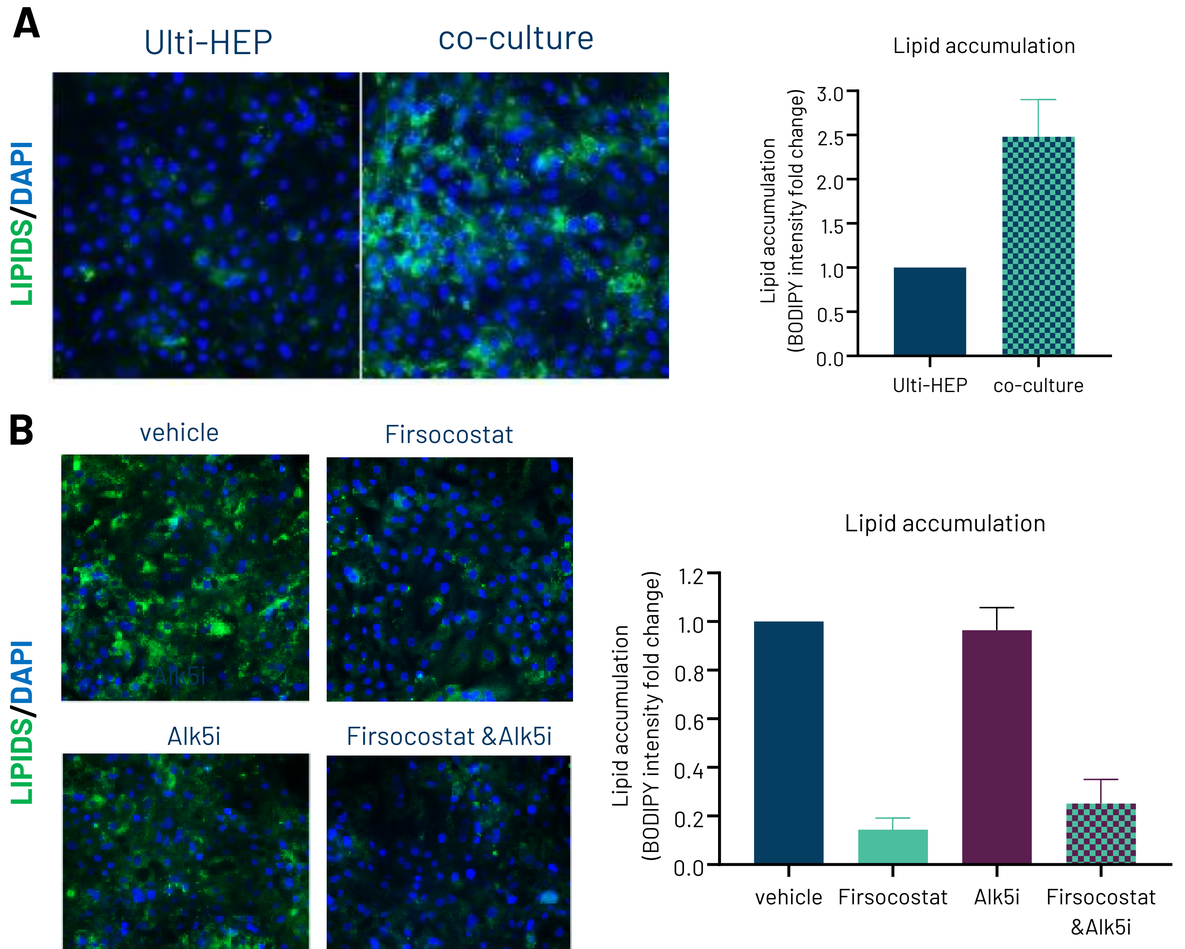

Figure 3: A) Representative immunocytochemistry pictures and quantification of lipid accumulation in Ulti-HEP and Ulti-HEP/HSC co-cultures. **B)** Representative immunocytochemistry pictures and quantification of lipid accumulation in Ulti-HEP/HSC co-cultures following treatment with either vehicle, Firsocostat (10 μM), Alk5i (5 μM), or combination of Firsocostat and Alk5i for 5 days. **C)** Collagen secretion in DefiniGEN Ulti-HEP and Ulti-HEP/HSC co-cultures. **D)** Collagen secretion in Ulti-HEP/HSC co-cultures following treatment with either vehicle, Firsocostat, Alk5i, or combination of Firsocostat and Alk5i for 5 days. Data are presented as mean±SEM of n=2-3 experiments.

FATTY ACID TREATMENT DRIVES STEATOSIS IN Ulti-HEP/HSC CO-CULTURES, AND THIS CAN BE PHARMACOLOGICALLY REVERSED

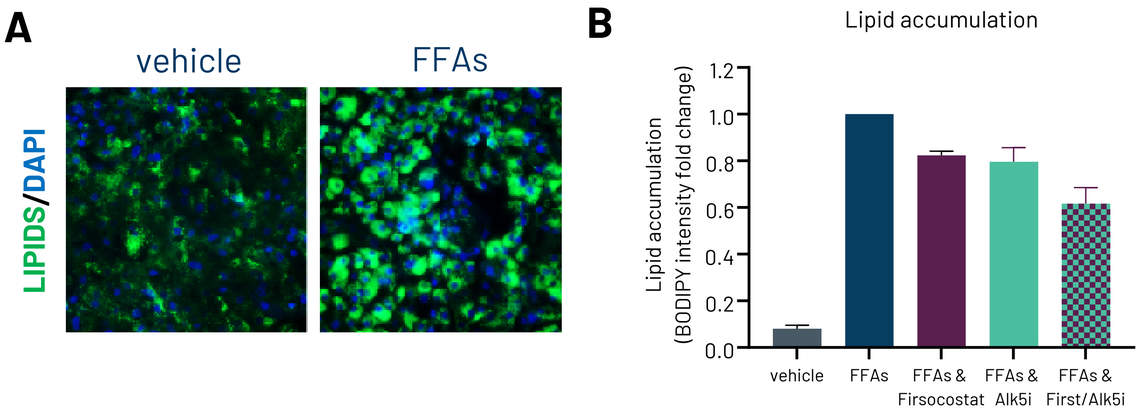

Figure 4: A) Representative immunocytochemistry pictures of lipid accumulation in Ulti-HEP and Ulti-HEP/HSC co-cultures pre- and post- free fatty acid (FFA) treatment. **B)** Quantification of lipid accumulation in FFA-treated Ulti-HEP/HSC co-cultures following treatment with either vehicle, Firsocostat (10 μM), Alk5i (5 μM), or combination of Firsocostat and Alk5i for 5 days. Data are presented as mean±SEM of n=2 experiments.

CONCLUSION

We have successfully produced iPSC-derived hepatocytes and hepatic stellate cells with comparable functionality to that observed in primary human cells. By co-culturing the two cell types, we have generated a novel model that recapitulates the MASH phenotype *in vitro*, offering an efficient pre-clinical platform for the large-scale efficacy screening of novel therapeutics against the disease.