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Precision-cut liver slices as an ex-vivo model for human NAFLD show hepatic progenitor cell activation and fibrogenesis

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On behalf of the EPoS consortium

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disease occurring in the absence of excessive alcohol consumption that ranges from isolated hepatic triglyceride accumulation (steatosis); through hepatic triglyceride accumulation plus inflammation and hepatocyte injury (non-alcoholic steatohepatitis, NASH); and ultimately progresses to fibrosis/ cirrhosis and potentially hepatocellular carcinoma. Although rodent models are widely used to investigate chronic liver disease, there are still questions about how representative such models are of human disease.

AIM

The current study aimed to establish an ex-vivo human model to understand interactions between different cell types during liver damage.

METHOD

250 µm thick precision-cut liver slices were cultured in a novel bioreactor and culture plate system that rocks the slices to mimic hepatic blood flow (Figure 1). Healthy rat liver tissue (n=4) was used to optimise and test the novel system. Medium was collected daily to analyse protein secretion using ELISA and tissue was harvested on days 2, 4 and 6 for qPCR and histopathological analysis. Normal human liver tissue samples (n=5), collected following surgical resection of colorectal cancer metastasis, were obtained after informed consent from patients at the Freeman Hospital, Newcastle-upon-Tyne, UK. After 24h in culture human liver slides were loaded with a mixture of oleic, palmitic and linoleic acid for 48h to induce lipid loading and processed for RNA sequencing and histochemistry. Differentially expressed genes were analysed using Qiagen's Ingenuity Pathway Analysis software. Medium was collected daily and analysed using high-performance electrochemiluminescence multiplex immunoassays.

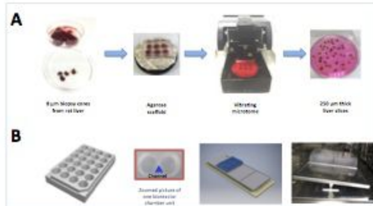

Figure 1. Method Overview. (A) 8 µm wide biopsy cores were processed into 250 µm thick liver slices. (B) The slides were grown in custom-made 12-well plates using a novel bioreactor system.

RESULTS

Compared to a static system, the flow generated by the rocking bioreactor significantly increased cell viability and albumin production in the rat precision-cut liver slices and preserved tissue morphology as the bile ducts, the central venules and the sinusoids remained intact for up to 7 days in culture (Figure 2). After 72h the human liver samples showed a loss of hepatocyte-related genes (e.g. *GSTA1, ALDH2, GCDH, IGFBP3, IDO2*), and a strong increase in extracellular matrix (e.g. *COL1A1, MMP12, LOX, ITGB5, PDGFRB*) and hepatic progenitor cell (e.g. *EPCAM, CD24, KRT19, HEYL, LAMC2*)-related markers. Ingenuity pathway analysis stratified 'Granulocyte Adhesion and Diapedesis, and 'Hepatic Fibrosis' among the top canonical pathways, while TGFB1 is a potential upstream regulator (Figure 3). Network analysis linked progenitor cell features with immune signalling (e.g. *CCL2, CXCL5*) (Figure 3). Histopathological assessment confirmed early fibrosis and collagen deposition in the human samples after 72h *in vitro* while none was detected at time zero. In addition an expansion of the hepatic progenitor cell compartment and parenchymal congestion was observed (Figure 4). Fat loading aggravated the process of bridging fibrosis, and focally induced steatosis and ballooning of the remaining hepatocytes. RNA sequencing data suggested involvment of mTOR signalling. Soluble CCL2 was increased over time, while IL6 was reduced at 72h.

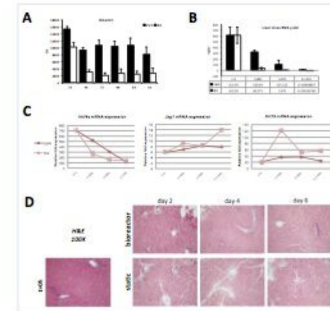

Figure 2. Comparison of the bioreactor with the static system using healthy rat liver tissue. (A) The bioreactor resulted in higher soluble albumin levels in the cell culture medium (B) as well as a higher RNA yield after extraction at different time points. (C) qPCR analysis showed a gradual decline in Hnf4a expression and a stable expression of Krt19 and Jag1 in samples grown with the bioreactor compared to the static system. (D) Tissue morphology, as the bile ducts, the central venules and the sinusoids, remained intact in the bioreactor system compared to the static system.

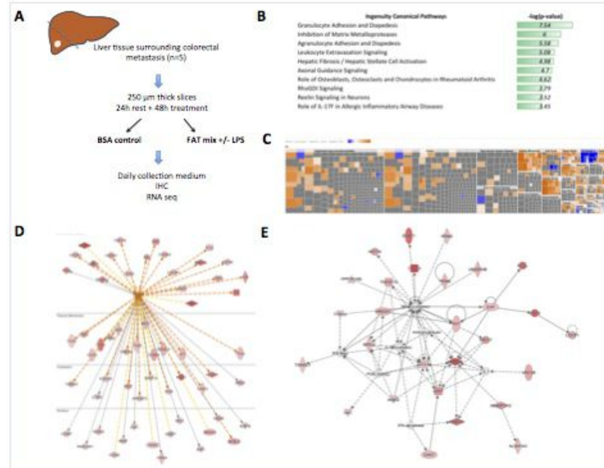

Figure 3. RNA sequencing of human liver slice samples (n=5) grown for 72h in the bioreactor. (A) Experimental design. (B) Top canonical pathways when analysing the 300 up-regulated genes (72h compared to t=0h). (C) Top diseases and Biofunctions. (D) TGFB1 as upstream regulator. (E) Top network 'Cell Death and Survival, Cancer, Organismal Injury and Abnormalities'.

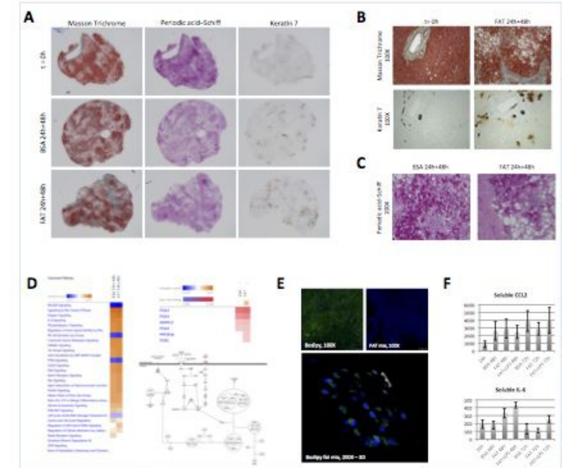

Figure 4. Fat loading of human liver slices. (A) Histopathological analysis of human liver slices fat loaded for 48h (24h rest + 48h loading) compared to the control, bovine serum albumin (BSA). (B) Fat loading aggravated the process of bridging fibrosis and progenitor cell activation, and (C) focally induced steatosis and ballooning of the remaining hepatocytes. (D) Comparison of the 300 up-regulated genes of the fat loaded samples and controls suggested involvment of mTOR signalling. (E) Confocal imaging of a human liver slice loaded with fluoresecent fat Bodipy.
(F) Medium was collected daily and measured for protein expression. Soluble CCL2 was increased over time compared to untreated at 24h, while IL6 was reduced at 72h.

CONCLUSIONS

Human precision-cut liver slices can be used to model features of non-alcoholic fatty liver disease *in vitro*.

CONTACT INFORMATION

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