FULLY HUMAN IN VITRO GUT-LIVER MODEL OF NON-ALCOHOLIC STEATOHEPATITIS

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MATERIAL & METHODS

Cryopreserved human hepatocytes and Kupffer cells (Life Technologies) and hepatic stellate cells (isolated by Krista Rombouts - UCL) were seeded together into LiverChip® wells. Cells were cultured in chemically defined lean or fat media containing standard supplements, including physiological concentrations of glucose, insulin, ± 600 µM free fatty acids. Media was changed on wells every 2-3 days.

Production of IL-6, TNF-α, pro-collagen (R&D systems) and albumin (Assay Pro) were all measured by ELISA. Kupffer and stellate cells were transduced with Adenoviruses expressing eGFP and mCherry (Vector Labs) (MOI: 25) prior to seeding into LiverChip®. Microtissues were imaged on a Nikon Eclipse Ti-E fluorescence microscope to demonstrate localization of HK and HSC, representative images are shown at 40x magnification.

RESULTS

Figure 1 – LiverChip® Hardware

A) LiverChip® is a 12 well perfused cell culture system. B) Media flows through the engirdled scolloid diffusion barriers to each microtissue. The speed and direction of flow can be adjusted using an electronic controller.

Figure 2 – Triple-culture of Hepatocytes, Kupffer and Hepatic Stellate cells

PHH, HK and HSC were cultured in the LiverChip® platform and cultured together for 4 days. Prior to seeding HK and HSC were transduced with adenoviruses expressing eGFP and mCherry respectively. Scaffolds containing microtissues were imaged using fluorescence microscopy to demonstrate localisation of HK and HSC, representative images are shown at 40x magnification.

Figure 3 – Triple-culture loaded with fat induces inflammatory and fibrotic NASH characteristics

PHH, HK and HSC were cultured in LiverChip® platform for fourteen days and in either lean or fat conditions. A) 4q production and B) pro-collagen-1 were measured in the culture medium. C) Expression of Sclerostin marker genes was analysed, normalised to GAPDH housekeeping gene and compared to monolayers of PHH cultured in the same media conditions. Data are mean ± SD, n = 3.

Figure 4 – Stimulation with Lipopolysaccharide further enhances NASH phenotype of triple-culture

PHH, HK and HSC were cultured for 4 days under fat and lean conditions in the LiverChip® platform. After 8 days some of the cultures were stimulated with 0.5 ng/ml LPS for 2 – 3 days. A) IL-6 production, B) TNF-α production, C) Albumin production and D) pro-collagen-1, were all measured in the cell culture medium. Data are mean ± SD, n = 3.

Figure 5 – T12 multi-organ MPS hardware.

The T12 plate is a 12 well perfused cell culture system, that allows that culture of three-dimensional PHH cultures alongside a second organ. The system has a reduced working volume ~400 µl to allow for scaled interactions between organs. A) Media is recirculated in each well by two micropumps which move the media through the T12 containing scaffold. B) Transwells in the top of each well can be dosed with cytokines or inflammatory stimuli. C) LiverChip® scaffold was used to culture 3D liver in the T12 plate, each scaffold cultures 600k PHH.

Figure 6 – Functional Caco-2-gut model can be damaged by inflammatory stimulus

Caco-2 cells were dosed on transwells for 30 days. A) Expression of key genes was analysed during maturation. Data were normalised to GAPDH/housekeeping gene and compared to freshly dosed controls. B) TEER measurement for epithelial integrity. C) Matured Caco-2 cells were combined with PHH in an MPS platform and dosed with LPS 7-HC. 7-HC absorption and metabolism was determined by fluorescence. D) Matured Caco-2 cells were dosed with recombinant inflammatory cytokines and TEER measured after dosing. Data are mean ± SD, n = 3.