A Novel 3D Liver Microtissue Model for Studying Steatosis In Vitro

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Introduction

Fatty liver disease (steatosis) is the most common chronic liver disease in the world, affecting all racial, ethnic, and age groups without sex predilection. Steatosis is characterized by an excessive accumulation of lipids in hepatocytes. Progressive forms of steatosis lead to end-stage liver disease such as fibrosis and cirrhosis, making it a major clinical concern.

Herein, we describe an in vitro model for studying steatosis based on 3D InSphero™ Human Liver Microtissues (hLiMT). The model represents an adaptation of an established spheroid-based human liver co-culture test system, which was shown to maintain prolonged viability and liver-specific functionality in comparison to 2D cultures.

This hLiMT-based steatosis model is based on the incubation of human liver microtissues with oleic and/or palmitic acid and displays a distinct and quantifiable macro- and/or microvesicular accumulation of lipid droplets within the hepatocytes. It has the potential for future applications in drug efficacy and toxicity testing.

Quantification algorithm and characterization of steatotic phenotype

Figure 1. Workflow of lipid quantification. Micro-, macrovesicular and mixed phenotypes after incubation with oleic acid, palmitic acid or oleic palmitic acid.

- OA: Oleic acid
- PA: Palmitic acid
- OA/PA: Mixed oleic/palmitic acid

Figure 2. Time dependent lipid accumulation in liver microtissues treated with oleic acid, palmitic acid and oleic acid/palmitic acid (2:1 ratio).

Time dependent lipid accumulation

A Control B OA C OA/PA D PA

Day 3
- Control
- OA
- OA/PA
- PA

Day 7
- Control
- OA
- OA/PA
- PA

Day 14
- Control
- OA
- OA/PA
- PA

Figure 3. Viability of treated microtissues and quantification of lipid accumulation. ATP content values of microtissues after 3, 7 and 14 days incubation with oleic acid (A), palmitic acid (B) and oleic palmitic acid (C). (D) Quantification of lipid accumulation after 5 days incubation with oleic acid and oleic palmitic acid. Quantification of lipid accumulation after 7 days incubation with oleic acid (D) and oleic palmitic acid (F). Data is represented as mean ± S.D. Statistical significance is always referred to the BSA control (**p<0.01, *p<0.05, student’s t-test, n=3 microtissues per condition). BSA: Bovine serum albumin, OA: oleic acid, PA: palmitic acid, OA/PA: oleic acid palmitic acid

Lipidomics

Figure 4. Lipidomics analysis of tissues treated with oleic acid, palmitic acid and oleic acid/palmitic acid (2:1 ratio).

- OA: Oleic acid
- PA: Palmitic acid
- OA/PA: Mixed oleic/palmitic acid

Dynamic of steatosis

Figure 5. After loading with oleic acid, the steatotic phenotype persists for 1, 7 and 14 days when culturing microtissues in regular maintenance medium without lipid supplementation. (A) Confocal images of microtissues stained for nuclei (blue) and lipid droplets (green). (B) ATP content values of microtissues (C) Quantification of lipid accumulation after 1, 7 and 14 days post induction. Data is represented as mean ± S.D. (N=3 per condition).

Summary and Conclusions

- A steatotic phenotype in 3D human liver microtissues is induced upon exposure to physiological relevant dietary free fatty acids.
- A custom-fit algorithm is able to quantify hepatic lipid droplet accumulation.
- A microvesicular and/or macrovesicular lipid droplet phenotype is observed upon incubation with oleic acid, palmitic acid or a combination of both.
- Lipidomics analysis confirmed increased concentrations of di- (18:1/18:1) and triglycerides (18:1/18:1/18:1) in microtissues upon treatment with oleic acid or oleic acid/palmitic acid compared to medium and BSA control.
- In untreated, BSA only treated and palmitic acid treated microtissues, comparable concentrations of triglycerides (14:0/16:0/16:0) are observed.
- This model is suited to study the formation as well as the prevention of steatosis.
- Given the persistence of steatosis after removing lipid supplementation, efficacy testing of anti-steatotic drugs is possible in a serum-free environment.

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