Development of a diet-induced disease-mimicking *in vitro* model of non-alcoholic steatohepatitis / fibrosis

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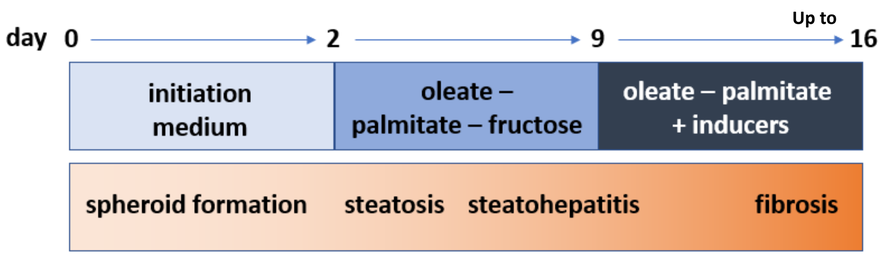
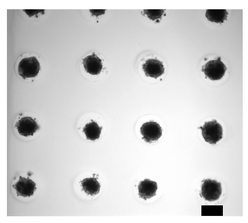
Background

Non-alcoholic fatty liver disease (NAFLD), characterized by hepatocyte steatosis, is the most common form of chronic liver disease and may progress towards development of non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma. Currently no effective therapeutic treatment is available to halt or reverse progression of NAFLD, partly due to the absence of translational cell models.
We present data on induction of steatosis, modulation of steatosis by prototype compounds and profibrotic cell activation in a 3D liver spheroid model using primary human cells.

Methods

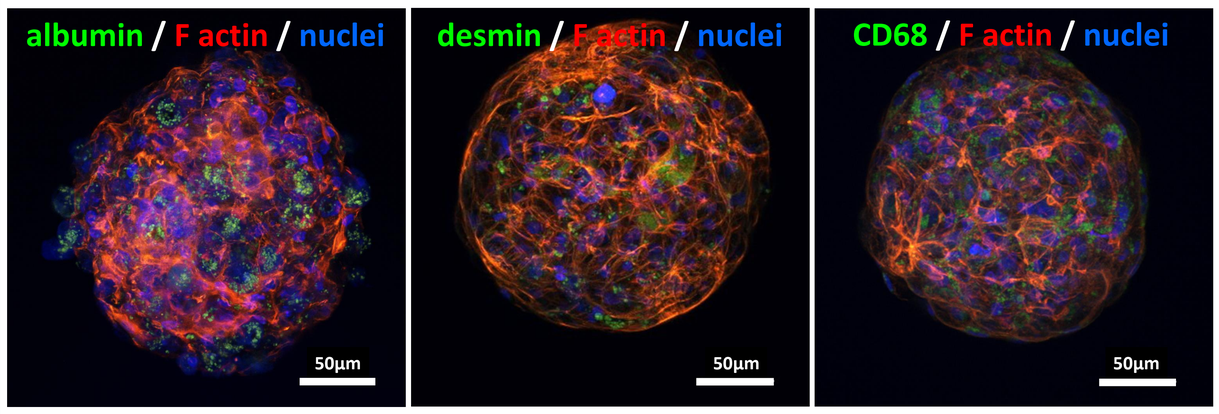
General culture and treatment scheme:





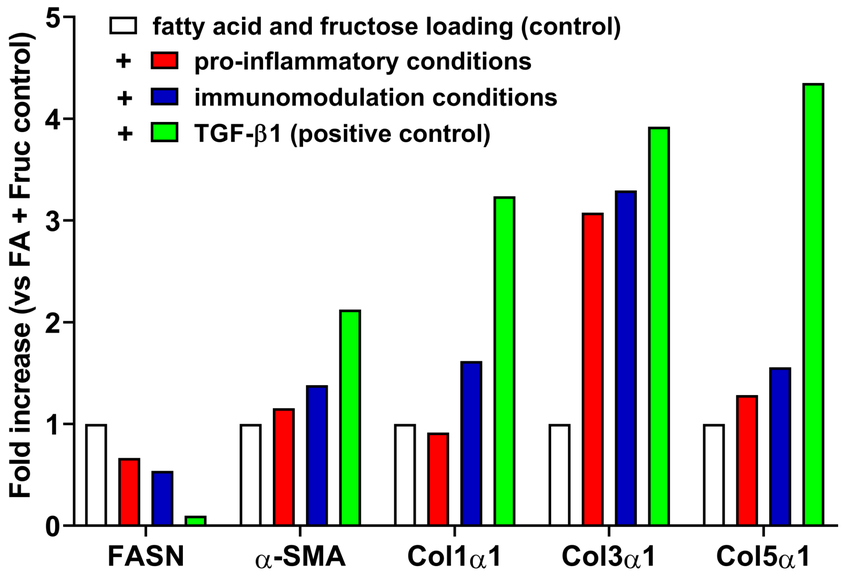
- Primary human hepatocytes, stellate and Kupffer cells were cultured in matrix-free molds (above right, bar = 200µm), resulting in formation of liver spheroids;
- Steatosis was induced by adding BSA-conjugated fatty acids, fibrosis was induced by adding fructose and additional proinflammatory, profibrotic and immunomodulatory triggers;
- Immunostainings desmin for stellate cells, albumin for functional hepatocyte visualisation and LipidTOX for steatosis and F-actin for cytoskeleton protein were imaged by confocal microscopy;
- CYP3A4 activity was measured in medium using a fluorimetric assay; steatotic and fibrotic markers in mRNA isolates with qPCR;
- Cells were treated with pioglitazone (5µM), fenofibrate (30µM) from day 9 of culture and collected on day 12 for further analysis.

Liver spheroid morphology



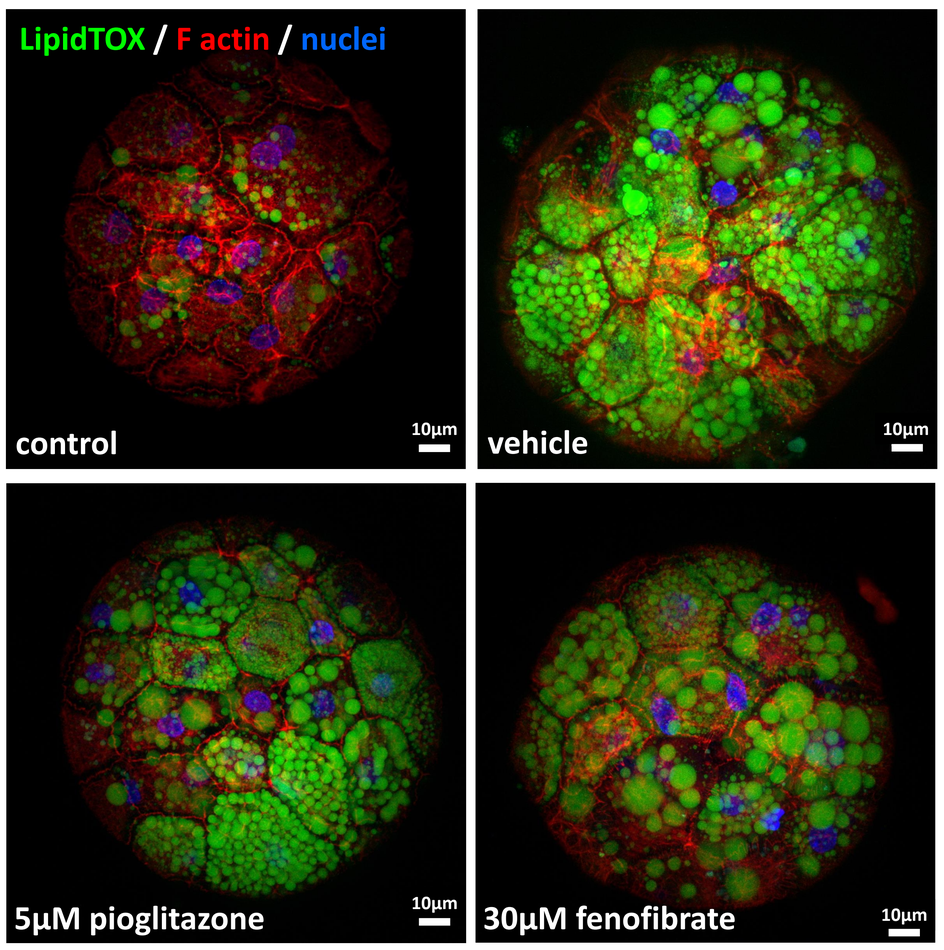
Spheroids cultured for 5 days in culture medium. Immunofluorescent labeling and imaging by confocal microscopy of hepatocytes (left), stellate cells (middle) and Kupffer cells (right) using cell markers as indicated.

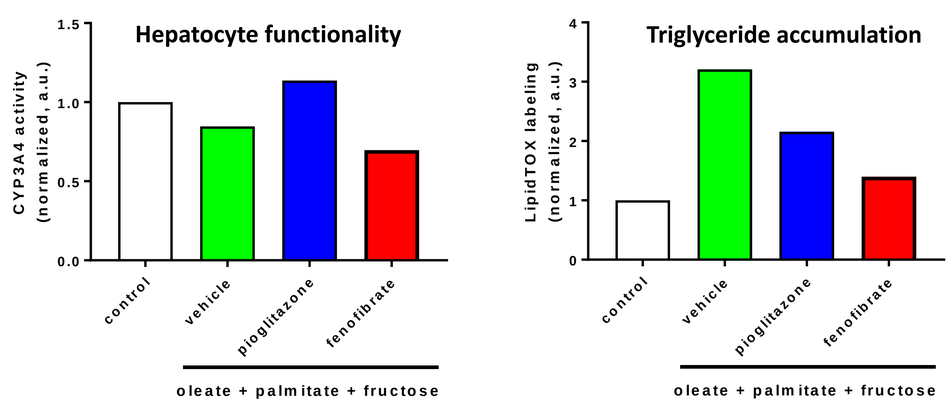
Pro-fibrotic cell activation in steatotic liver spheroids



mRNA analysis focussed on steatotic and fibrotic markers.

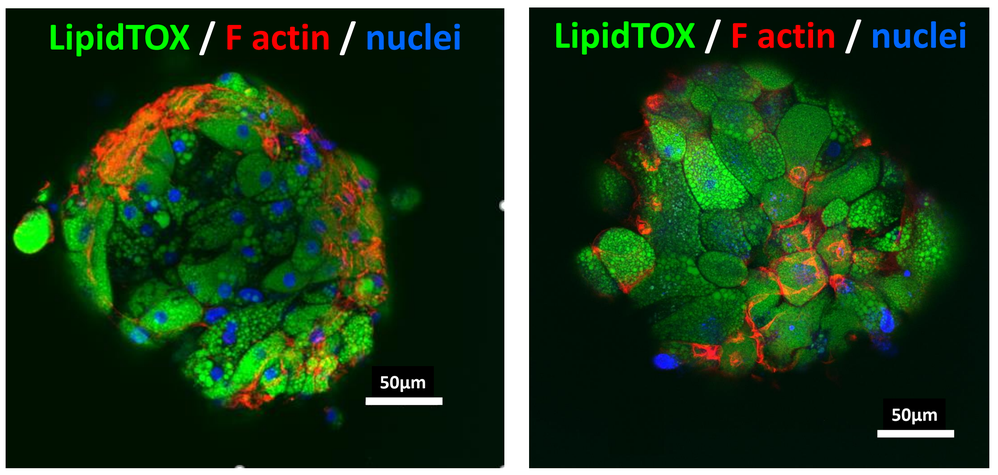
Therapeutic modulation of hepatocyte triglyceride accumulation in spheroids





Spheroids were cultured up to day 9 according to scheme in methods and then treated with pioglitazone or fenofibrate until day 12. LipidTOX labeling of triglycerides was measured by confocal microscopy and fluorometric analysis. CYP3A4 activity was determined to assess hepatocyte functionality.

Static vs. flow conditions on a chip



Static conditions **Flow conditions**

LipidTOX staining at day 16 of steatotic co-culture spheroids under static vs. continuously perfused conditions. Morphology of the hepatocytes under flow conditions is more similar to the in-vivo situation.

Conclusions

- A diet-induced disease-mimicking 3D *in vitro* model, closely resembling the pathophysiology of liver steatosis and early fibrosis was developed;
- The steatosis and steatohepatitis induced by fatty acids and fructose can be modulated by model drugs;
- Proof of principle using a microfluidic chip with flow shows a better morphology (similar to in-vivo) of the hepatocytes

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