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

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:**

<table>
<thead>
<tr>
<th>day</th>
<th>0</th>
<th>2</th>
<th>9</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>initiation medium</td>
<td>olate – palmitate – fructose</td>
<td>olate – palmitate – inducers</td>
<td>spheroid formation</td>
<td>steatosis</td>
</tr>
</tbody>
</table>

- 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

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.