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Development of a disease-mimicking model for NASH and fibrosis in a triple cell-type, spheroid-based liver-on-chip platform with microfluidics

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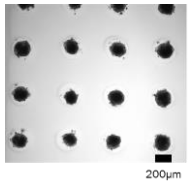
Background

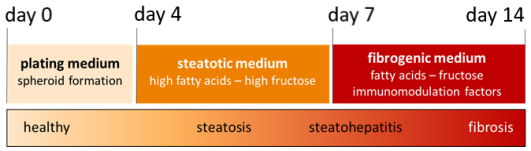
Despite ongoing efforts there is currently no effective therapeutic treatment available for non-alcoholic steatohepatitis (NASH). Several drug candidates have failed clinical trials due to lack of efficacy, underlining the need for predictive preclinical models.

To this end we developed a disease-mimicking in vitro model which closely resembles the pathophysiology of liver fibrosis induced by lifestyle factors.

Methods

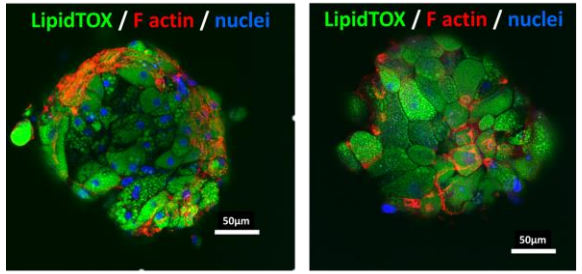
- Primary human hepatocytes, Kupffer cells, and stellate cells were cultured in a matrix-free environment resulting in formation of multiple uniformly-sized spheroids.





- Fatty acids, carbohydrates, inflammatory and immuno-modulatory factors were used at physiological concentrations to recapitulate disease development and progression of NASH.
- A novel, customized liver-on-chip was developed in-house. Spheroids were cultured under static conditions or subjected to continuous pump-driven flow for 2 weeks.
- The effect of different experimental drugs on disease development was examined.
- Steatosis was measured by LipidTox accumulation. Expression of secreted protein markers was determined by specific ELISA's or multiplex assays. Collagen deposition was examined using a quantitative protein assay.

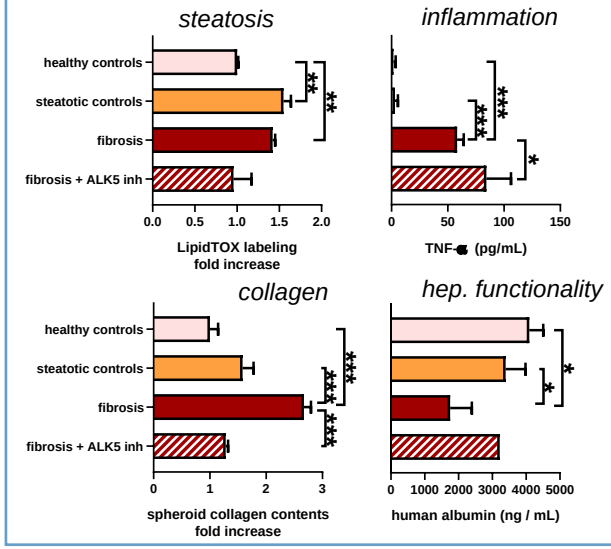
Effect of microfluidics on steatosis



Static conditions | Flow conditions

Microfluidic flow conditions resulted in a more homogenous distribution and size of lipid droplets as compared to static culture conditions where droplet size was more variable.

In vitro NASH-fibrosis disease induction



Spheroids (n=3 pools of 96 spheroids each) were maintained for 14 days in base (healthy) or steatotic medium, or after induction of fibrosis with or without an ALK5 inhibitor (Ly-364947, from day 7 - 14).

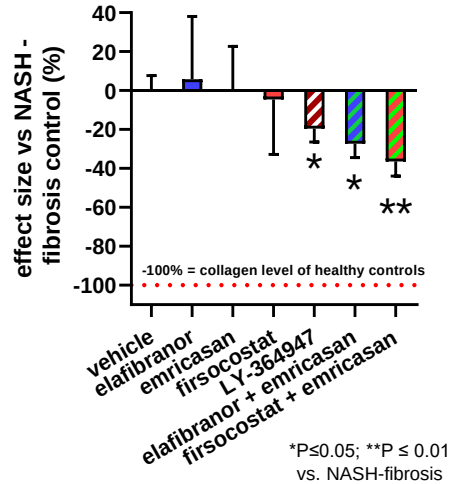
*P≤0.05; **P ≤ 0.01; ***P ≤ 0.001

Effect of experimental NASH drugs on collagen deposition – single and combination treatment

Spheroids were treated with the indicated drugs from day 8 to 14 of disease induction.

Collagen deposition was measured in pooled samples.

Collagen levels were plotted as effect size relative to NASH-fibrosis (0%) and healthy controls (-100%).



*P≤0.05; **P ≤ 0.01 vs. NASH-fibrosis vehicle control

Conclusions

- We present a disease-mimicking cell model for NASH and fibrosis that results in collagen production under static and flow conditions.
- The model is responsive to pharmacological interventions. We will further investigate the effect of microfluidic flow and experimental drugs currently in clinical trials as single or combination treatment.

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