ORGAN FUNCTION ON-A-CHIP: TOWARDS NOVEL TRANSLATIONAL IN VITRO MODELS FOR HEALTHY AND DISEASED LIVER AND GUT

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IMAGINE.....A WORLD OF PERSONALIZED THERAPY

Molecular Profiling

Prognostic Markers
- Markers predictive of drug sensitivity/resistance
- Markers predictive of adverse events
Drug development process: ineffective, very costly (many drugs fail in clinical stage)

- Marketed drugs – general for large population
- Need for pre-clinical models that mimic populational differences

CURRENT DRUG DEVELOPMENT PIPELINE

- Drug candidates
- Preclinical models
- Clinical – patients in trial
OUR SOLUTION

DRUG CANDIDATES → STEM CELL BASED MODELS → CLINICAL – PATIENTS IN TRIAL

- Selection of drug candidates effective for small group of patients
- Improved design of clinical trial, e.g. larger or selected target group

Population on-a-chip

Precision medicine!
POPULATION ON-A-CHIP

- mimic human populational differences in preclinical in vitro models
- disentangle complex interactions (between different cell types, or between environmental pathogens and body cells)
- add complexity where needed
- test effects of interventions (foods, chemicals, drugs, biologicals and assess their toxicology, kinetics and efficacy).
- reduce number of test animals (societal demand)

ORGAN ON A CHIP: INCREASING COMPLEXITY
POPULATION ON-A-CHIP: 2 USE CASES

Personalized translational *in vitro* model of the GI tract

Personalized translational *in vitro* model of Non-Alcoholic SteatoHepatitis (NASH)
NON-ALCOHOLIC STEATOHEPATITIS (NASH)

Prevalence estimate based on US + 5 big EU countries (Germany, France, UK, Italy, Spain).

HCC: 250,000
NASH related liver transplantation
From 2020 >50% due to NASH

Healthy subject

NASH patient

~147 million
~25 million
~8 million
PRE-CLINICAL ANIMAL NASH MODELS REVEALED
KEY PROCESSES

**RNAseq-Transcriptome**

- Cholesterol biosynthesis
- LXR/RXR pathway
- FXR/RXR pathway
- DC-maturation
- Leukocyte extravasation signalling
- T-cell signalling
- NF-kB signalling
- NRF2-mediated oxidative stress
- Hepatic Fibrosis
- Stellate cell activation
- TNO fibrosis gene signature
A translational 3D in vitro NASH/fibrosis model representing the populational differences and having its application in pre-clinical research of pharmaceutical interventions (cure, prevention)

Criteria:

- 3D co-culturing of hepatocytes and stellate cells
- “chronic” induction by fatty acids (steatosis in hepatocytes, fibrosis by stellate cells)
- pathways representing the patient situation
- validation by testing compounds
- human iPSC’s are used for stratification and personalization
A translational *in vitro* model of liver disease (i.e. NASH) representing the populational differences and having its application in pre-clinical research of pharmaceutical interventions (cure, prevention).
INDUCTION OF STEATOSIS & FIBROSIS

Steatosis and fibrosis induction in HU-H7 cells and stellate cells
HEPATOCYTE AND STELLATE CELL CULTURE

- Set-up 3D co-culture of primary hepatocytes and primary stellate cells in hanging drop
- 3D spheroid culture in cellulosic sponges

Huh7 cells in cellusponge

Primary hepatocytes in cellusponge

Bright field  DAPI

Ananthanarayanan et al. 2014, Molecular Pharmaceutics
# LIVER ON-A-CHIP

## Co-cultures of HuH-7 and hepatic stellate cells

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<thead>
<tr>
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<th>Unstimulated control</th>
<th>TGF-β stimulated</th>
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<tbody>
<tr>
<td><strong>Vimentin</strong></td>
<td><img src="image1" alt="Unstimulated" /></td>
<td><img src="image2" alt="TGF-β stimulated" /></td>
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<tr>
<td><strong>αSMA</strong></td>
<td><img src="image3" alt="Unstimulated" /></td>
<td><img src="image4" alt="TGF-β stimulated" /></td>
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## Stem cell derived hepatocytes

![Images of stem cell derived hepatocytes](image5)
TGFβ induced collagen production in 3D co-cultures of hepatocytes and stellate cells in scaffolds
FROM SCREENING TO POPULATION ON A CHIP

Predictive NASH/fibrosis 3D in vitro model

Precision NASH/fibrosis 3D in vitro model
POPULATION ON-A-CHIP: 2 USE CASES

Personalized translational *in vitro* model of the GI tract

Personalized translational *in vitro* model of Non-Alcoholic SteatoHepatitis (NASH)
GUT FUNCTION ON-A-CHIP

WHAT ARE WE AIMING FOR?

- An *in vitro* human intestinal model that can be used to study *populational variation* in (drug) absorption and impact of drugs, nutrition and environment on *gut health*
- Important aspects include:
  - Gut permeability (drugs, antigens, toxins, bacteria)
  - Effect of *microbiome* on gut health/absorption
  - Detection of early immune response
  - Stratification based on microbiome composition and activity → towards my *microbioome* on-a-chip
GUT FUNCTION ON-A-CHIP
APPROACH

ex vivo (human) intestinal tissue model (inTESTine™)

In vitro (human) stemcell derived intestinal organoids
INTESTINE™ – TRANSLATION IN VITRO INTESTINAL MODEL BASED ON TISSUE

- 2-compartmental intestinal model in standard (disposable) multiwell plate
- Up to 96 incubations/study
- Feasible with bio-relevant matrices
- Tissue from human, pigs, minipigs, rat (colon) can be used
- Pig: whole GI tract available, study regional differences

Integrity markers are used in every study
- \[^{[3]}\text{H}\]-mannitol/atenolol (paracellular transport route)
- \[^{[14]}\text{C}\]-caffeine /antipyrine (transcellular transport route)
- FD4, MW 4000 (tissue integrity marker)

Acceptance criteria:
- \(P_{\text{app}}\) C/M or A/A > 3 (jejunum)
- Leakage of FD4 < 1% / h
INTESTINE™ – SOME APPLICATIONS

- **Intestinal absorption of nutrients and drugs**: Processes that determine intestinal absorption of nutrients and drugs can be studied in porcine intestinal tissue mounted in InTESTine™: active transport, metabolism, food-drug effects, excipient-drug effects.
- **Study regional differences** in intestinal absorption (whole GI tract of pigs available).
- **Effect of compounds on excretion of gut hormones** e.g. satiety hormones, serotonin, VIP.
- **Host-microbe interactions** exposure to pathogens, read-out: immune response, transcriptomics, proteomics; bacterial translocation.
INTESTINE™: APPLICATION OF HUMAN INTESTINAL TISSUE

Human intestinal tissue is made available from local hospital
Patients do sign informed consent

Possible to determine (regional) fraction absorbed in human intestinal tissue.
INTESTINE™: APPLICATION OF HUMAN INTESTINAL TISSUE

Possible to determine effect of compound on satiety hormone release in human intestinal tissue.
INTESTINE™ ON-A-CHIP: EXTENDED VIABILITY OF THE TISSUE

Microfluidics

Ex vivo intestinal segment
INTESTINE™ ON-A-CHIP: EXTENDED VIABILITY OF THE TISSUE

Sustained viability of the intestinal tissue, up to 24 hours after incubation, was demonstrated by:
- maintained high percentage of intracellular LDH (90%)
- remained barrier integrity (<0.5% FD4 leakage/h)
- proper functionality and histology of the tissue after 24h of incubation.
GUT FUNCTION ON-A-CHIP
APPROACH

ex vivo (human) intestinal tissue model (inTESTine™)

In vitro (human) stemcell derived intestinal organoids
CULTURING INTESTINAL ORGANOIDS FROM STEM CELLS

In vitro stem cell derived intestinal organoids

INTESTINAL ORGANOID CULTURED AS MONOLAYER

Hoechst
F-actin
lysozyme
Muc2
Villin
Chromogranin A

Permeable membrane coated with collagen

TEER measurement
2D cultured organoids

Transwell insert
Cell monolayer
Permeable membrane
Vectorial transport

0 10 20 30 40
0 200 400 600 800

well 1
well 2

Time (days)

TEER (Ω·cm²)

Parenchymal cells
Mucous-secreting cells
Enterocytes
Enteroendocrine cells
INTESTINAL ORGANOID CULTURED AS MONOLAYER

Microfluidics

Organoids on permeable membrane

“my microbiome on a chip”
In vitro stem cell derived intestinal organoids

Permeable membrane coated with collagen

In collaboration with:
Dr. JP ten Klooster
Dr. S.F.C. Vaessen
PROBLEM

CURRENT DRUG DEVELOPMENT PROCESS NOT DESIGNED FOR DEVELOPING PRECISION MEDICINE

DRUG FAILURE!

SOLUTION

DEVELOPMENT OF TOOLBOX TO ENABLE EARLY SELECTION OF DRUGS THAT WORK FOR PART OF POPULATION

STEM-CELL BASED APPROACH

2 USE CASES

GUT FUNCTION ON-A-CHIP
LIVER FUNCTION ON-A-CHIP

STRATIFICATION BASED ON HIPSC USE AND/OR MICROBIOME COMPOSITION
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  - Karin Toet

2-REAL-GUTS - innovation in intestinal models for food research
THANK YOU FOR YOUR ATTENTION!

Voor meer inspiratie:
TIME.TNO.NL