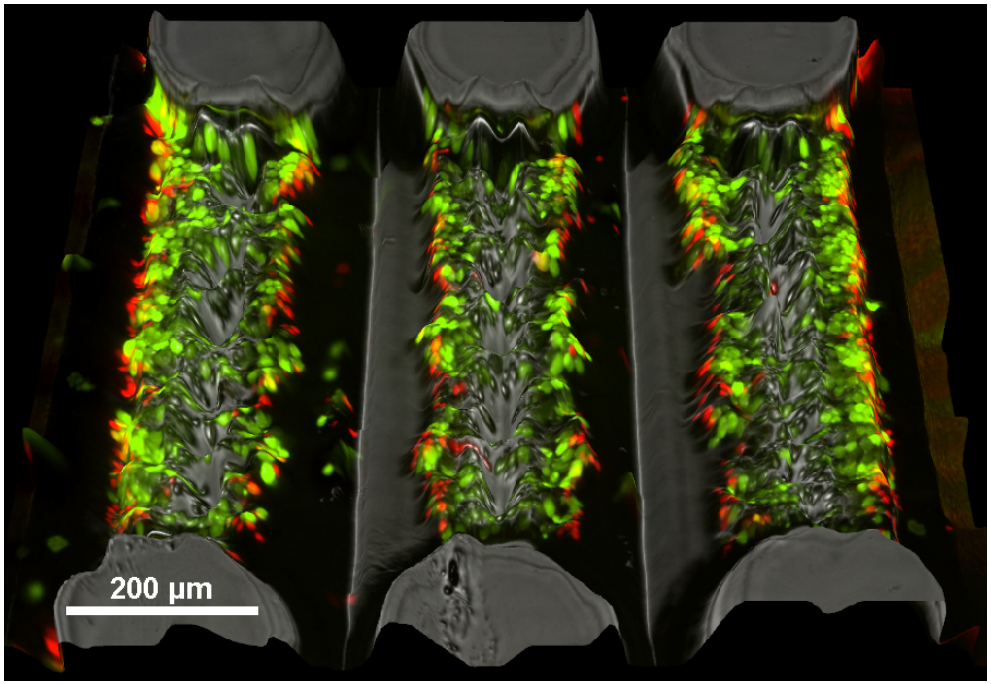


Dr. Martin Stelzle

martin.stelzle@nmi.de

Artificial micro organs“- neue zelluläre Testsysteme in der Medikamentenentwicklung

Natural and Medical
Sciences Institute
at the University of Tübingen

Drug safety and liver toxicity

Los Angeles Times

Diabetes Drug Rezulin Pulled Off the Market

By:
David Willman
LA Times Staff Writer
March 22, 2000

*"…The FDA has concluded that Rezulin use has "possibly or probably" resulted in **90 liver failures, including 63 deaths** and seven nonfatal organ transplants. …"*

Pfizer's Thelin Withdrawn Due to Fatalities

Dec 16, 2010
By: Stephanie Sutton
ePT--the Electronic Newsletter of Pharmaceutical Technology

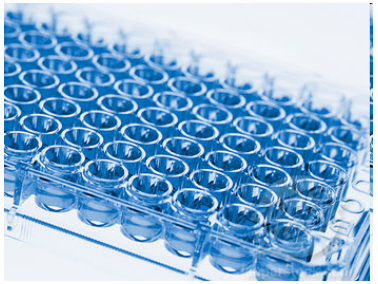
*"Following **two cases of fatal liver injury**, Pfizer has voluntarily withdrawn Thelin (sitaxentan) from the worldwide market and discontinued all ongoing trials. The drug had been approved in the European Union (EU) , Canada, and Australia for the treatment of pulmonary arterial hypertension (PAH). …."*

Why use artificial micro organs for safety assessment?

in vivo

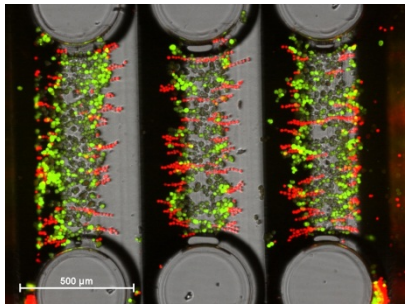
+ *in vivo* environment
- relevance to human
- imaging/read-out
- handling

2D/3D cell culture



+ human cells
+ handling
+ imaging/read-out
- *in vivo* relevance

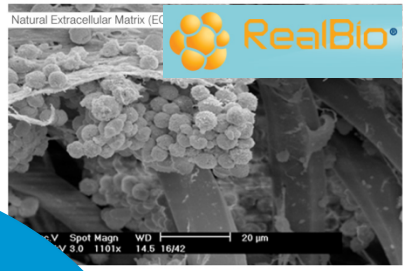
artificial micro organs

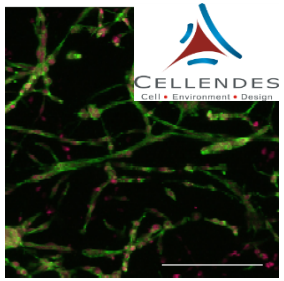


+ human cells
+ handling
+ imaging
+ *in vivo* relevance

Towards *in vivo*: commercial 3D cell culture approaches

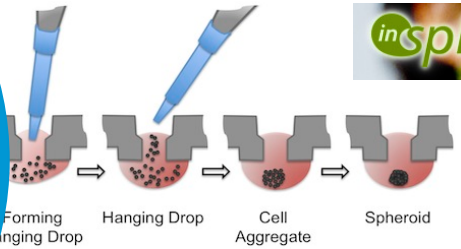
- **3D-matrices / hydrogels**
  (BD Bioscience, Cellendes, RealBio, …)



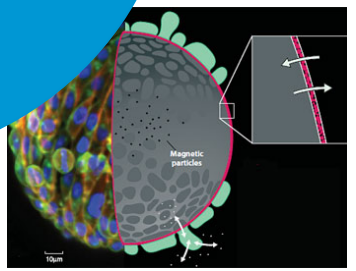


- **spheroids**
  inSphero, n3D Bioscience

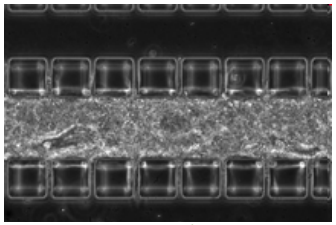
3D ≠ organ like

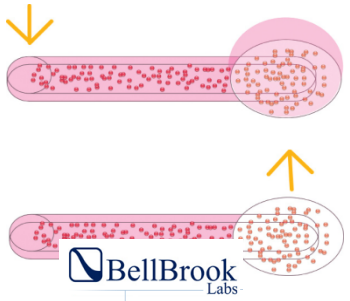


- **3D cell carriers**
  Global Cell Solutions & Hamilton



- **Microfluidic / bioreactors**
  CellAsics, Hµrel, QuasiVivo, BellBrook Labs





Strategy: organ like cell cultures - closer to *in vivo* reality

Mimicking *in vivo* environment:

3D architecture

cell-cell interactions

cell-matrix interactions

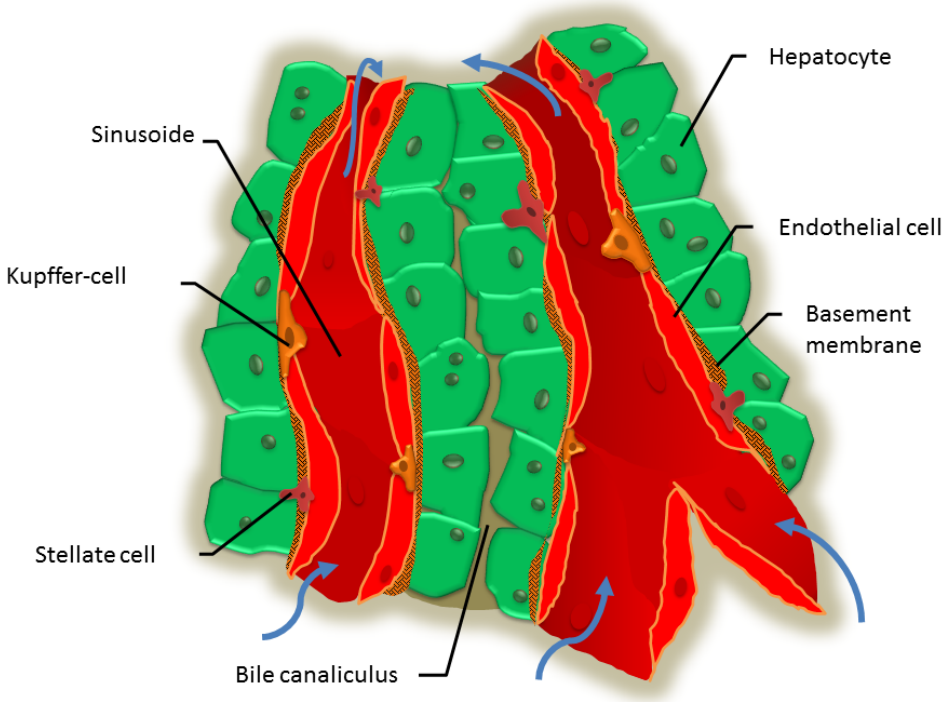
perfusion

concentration gradients

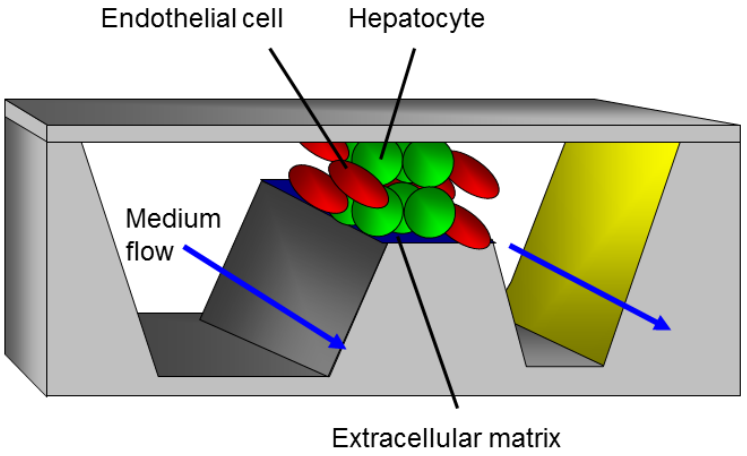
Mimicking the liver sinusoid in HepaChip®

NMI achieving results

human liver:



HepaChip®:

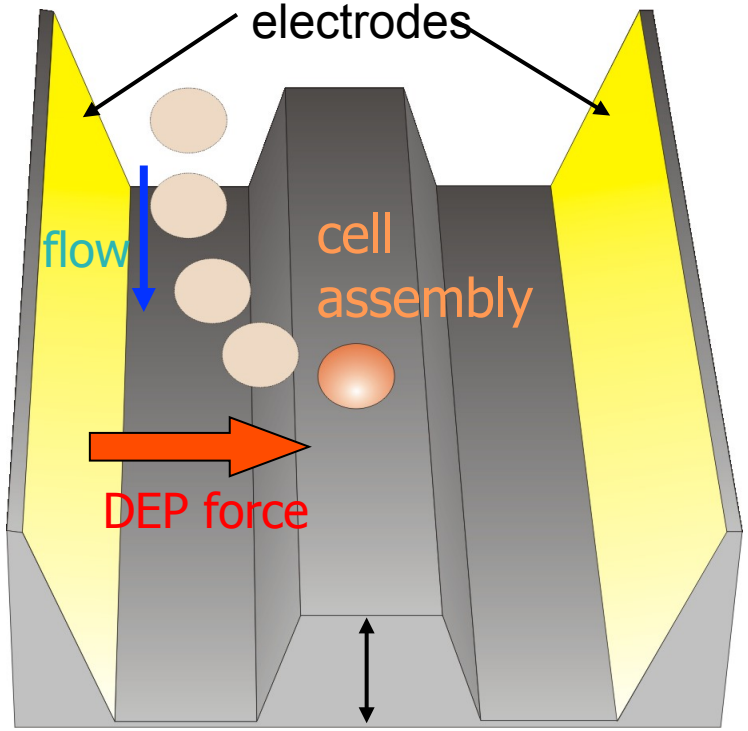


- active assembly of viable cells
- organ like architecture
- perfusion
- shear forces
- extracellular matrix

Stelzle et al. DE102008018170B4

Schuette et al. Biomed. Microdev. 2011

High reproducibility, automated assembly



restriction of channel
cross section

advantages of <u>dielectrophoresis</u>:

selection of viable cells

→ use of cryopreseved cells

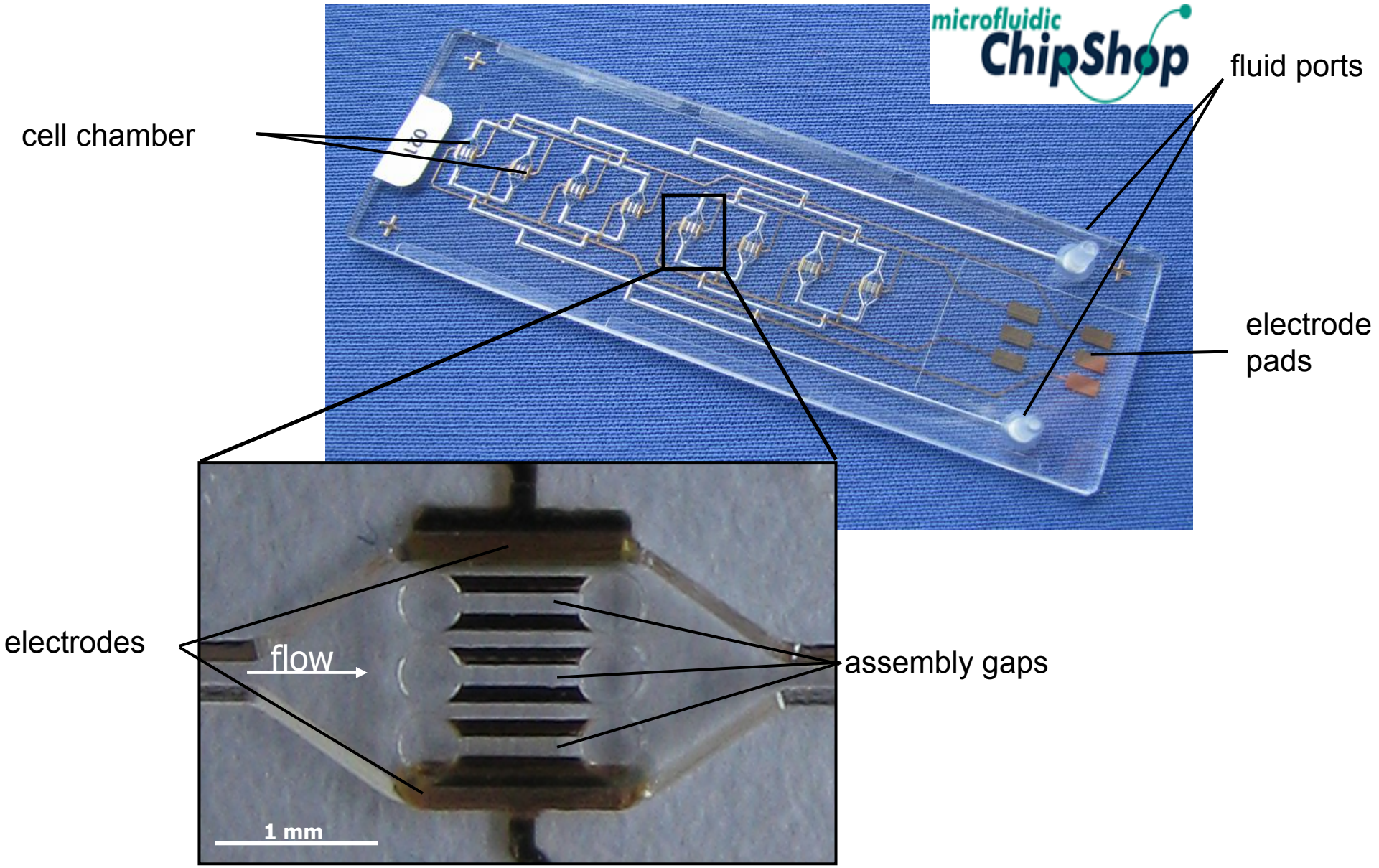
positioning of cells

→ organ like structure

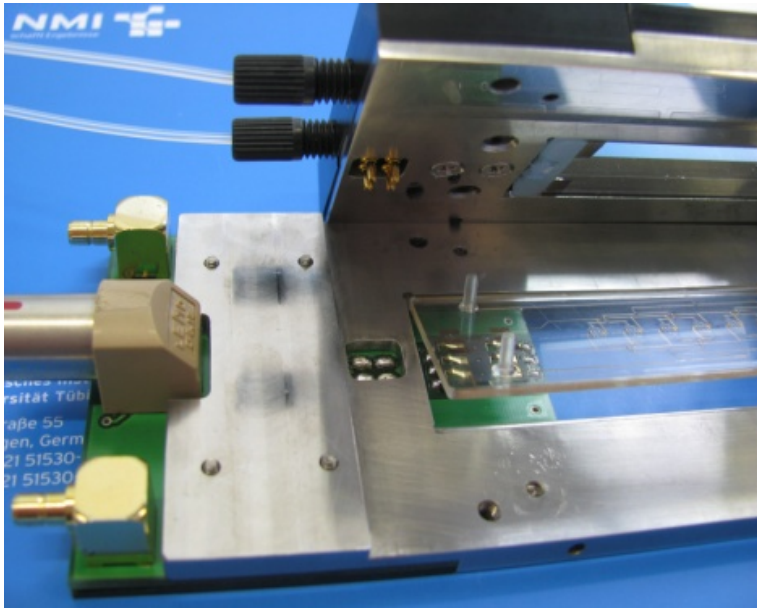
Stelzle et al. WO2009121555(A3)

Schuette et al. Biomed. Microdev. 2011

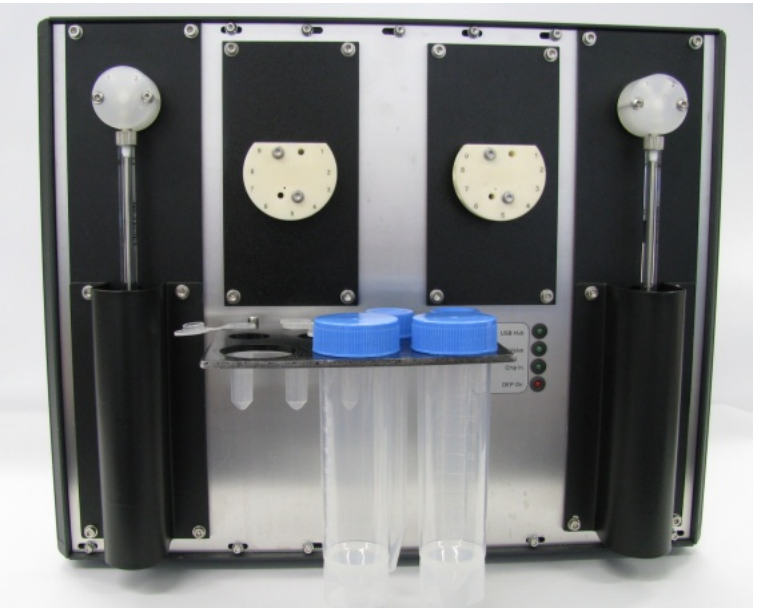
Injection moulded micro chips



Microfluidic controller

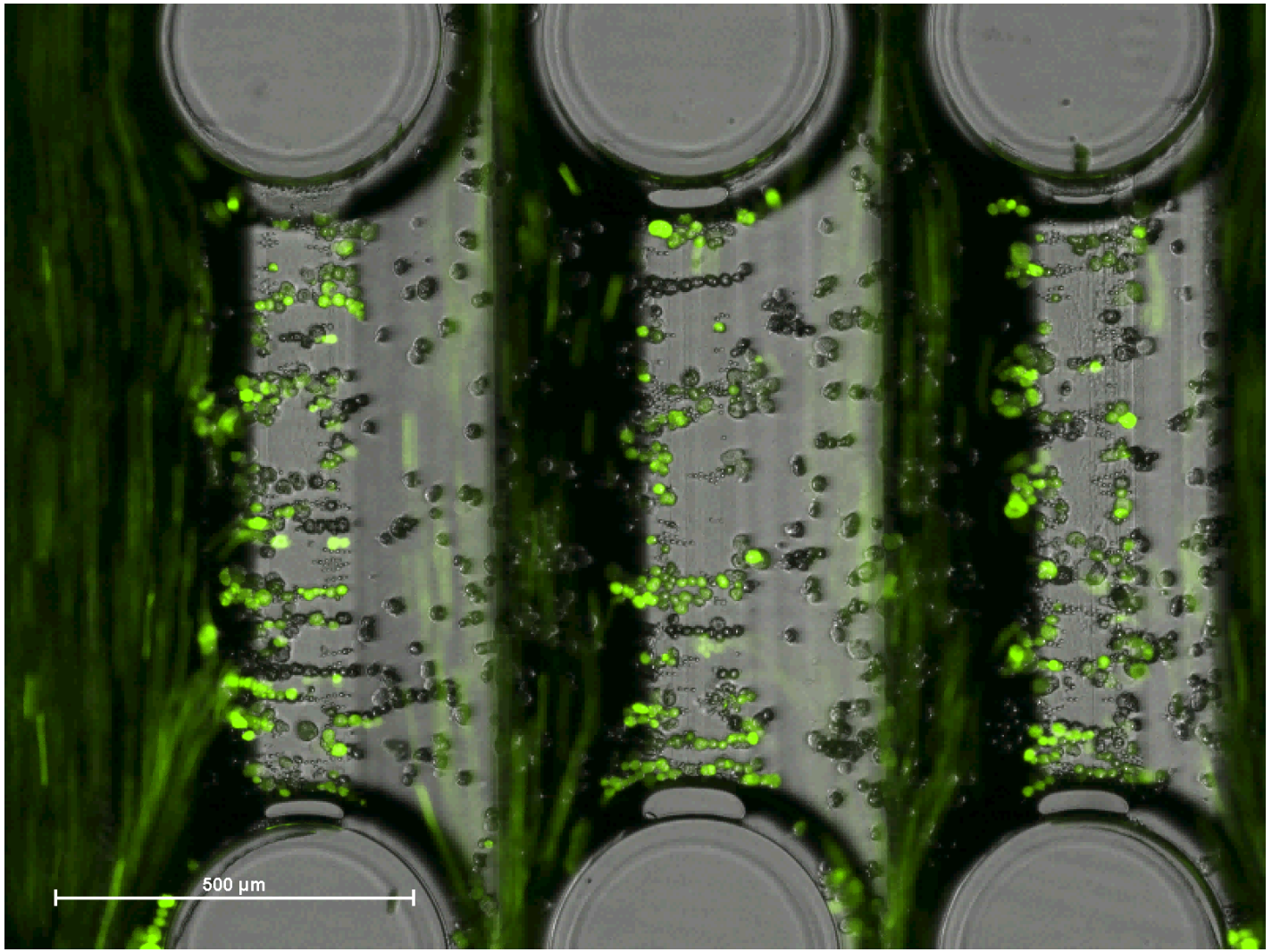


Chip carrier



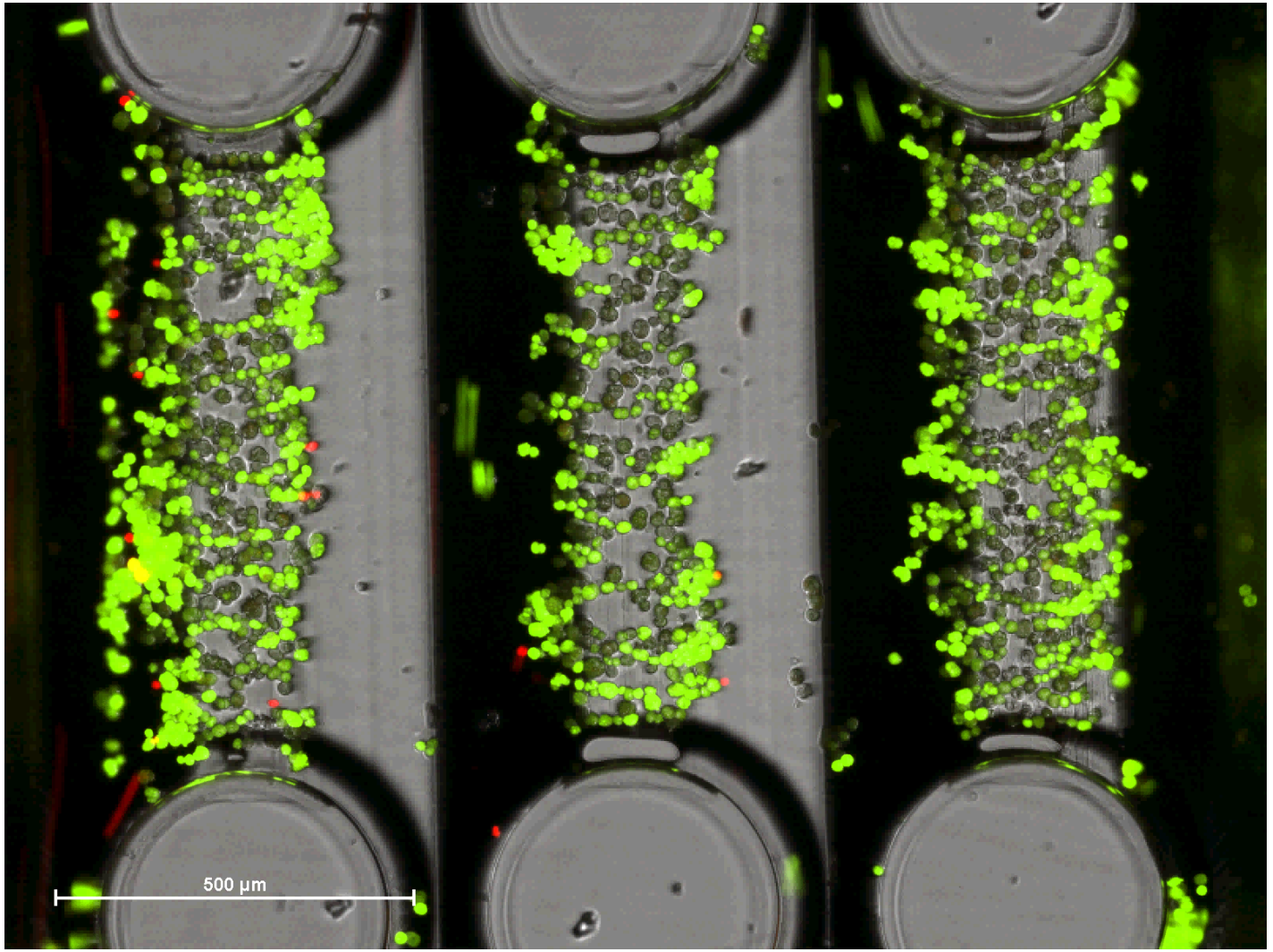
Control unit

Assembly of viable hepatocytes



staining: Calcein AM

Assembly of *in vivo* like sinuosid

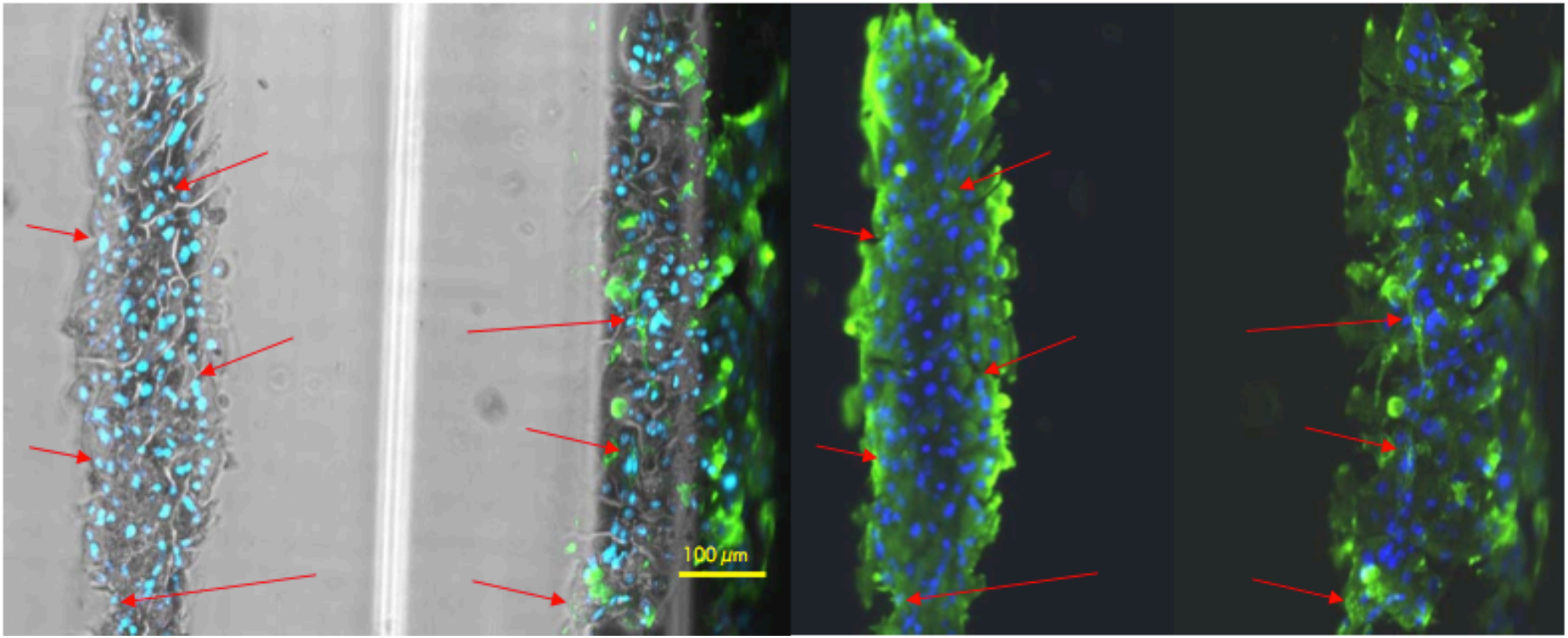


staining: Calcein AM
green: hepatocytes
red: endothelial cells

Schütte et al., Biomed Microdevices, 2011

Preservation of organ like cell arrangement during culture

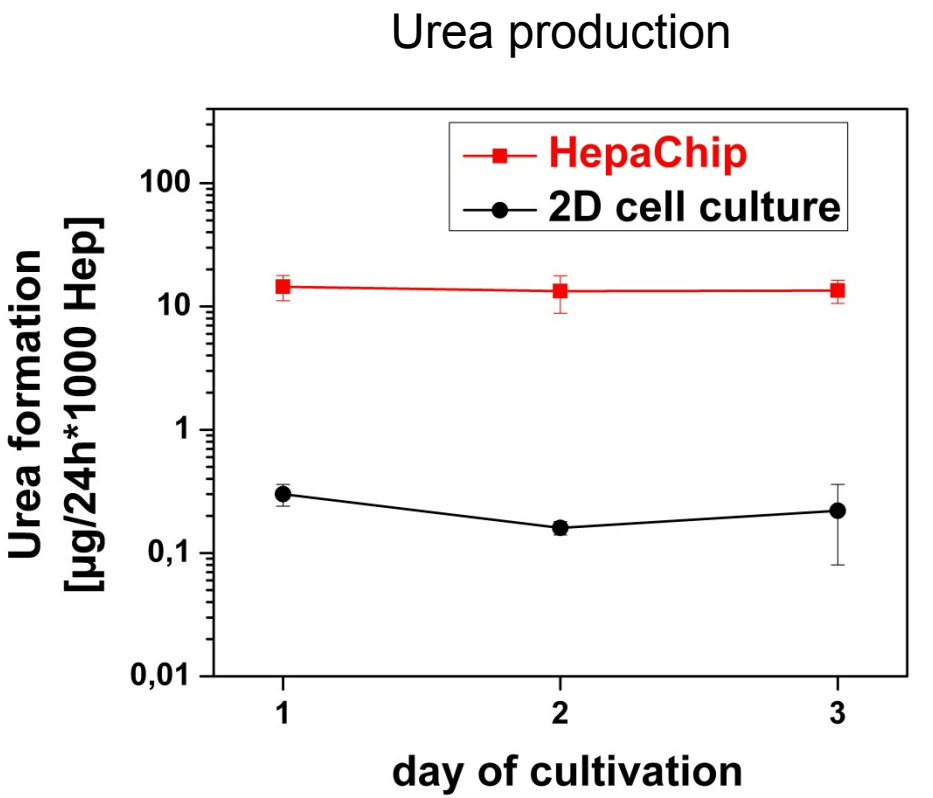
4 days culture, mouse hepatocytes, human endothelial cells



Staining: blue: nuclei, DAPI
green: endothelial cells, von Willebrand

in collaboration with University Leipzig

Enzyme function in HepaChip



4000 cells

25 000 cells

10 x

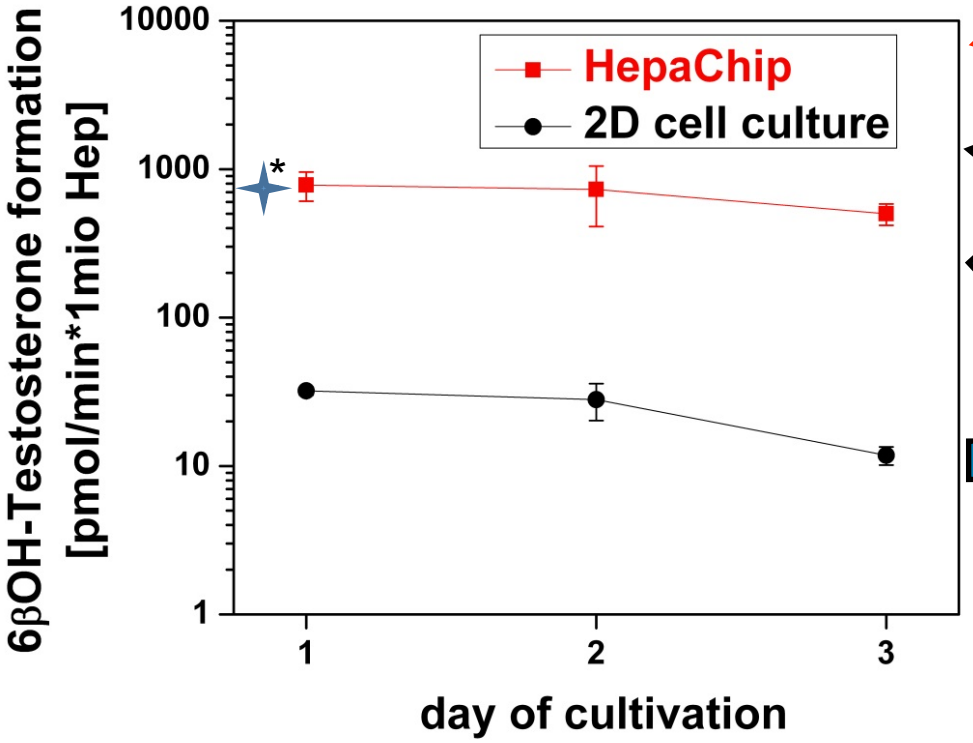
high sensitivity

high functionality

in collaboration with University Leipzig

Organ like enzyme function in HepaChip

CYP 3A4 activity



4000 cells

high sensitivity

25 000 cells

10 x

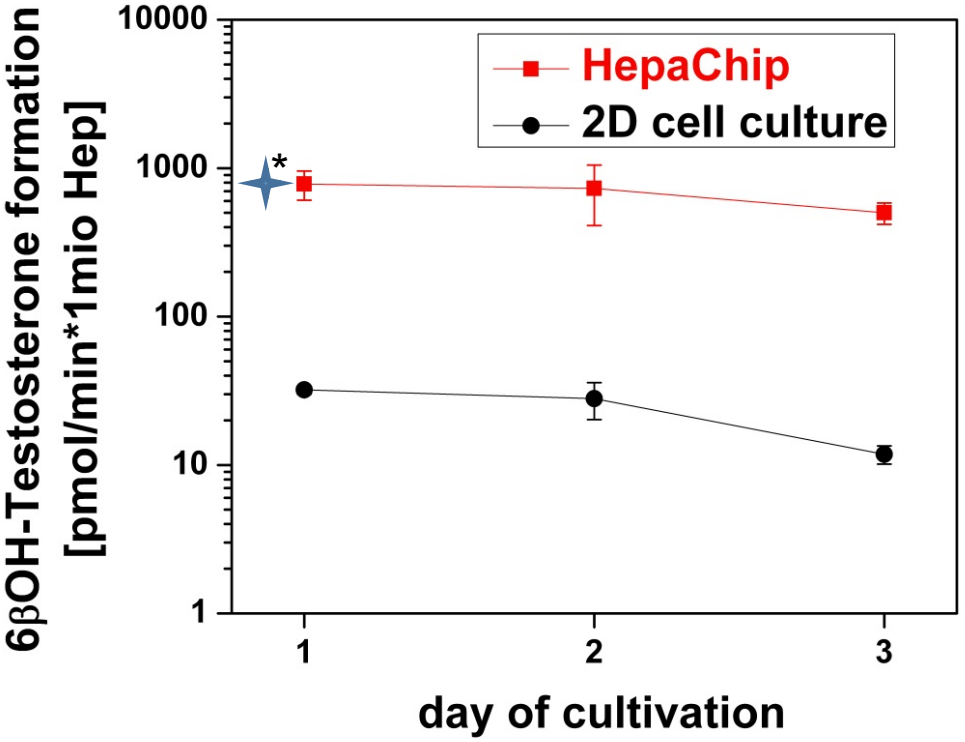
organ like activity

* Freshly isolated mouse hepatocytes (Gebhardt et al. 2003)

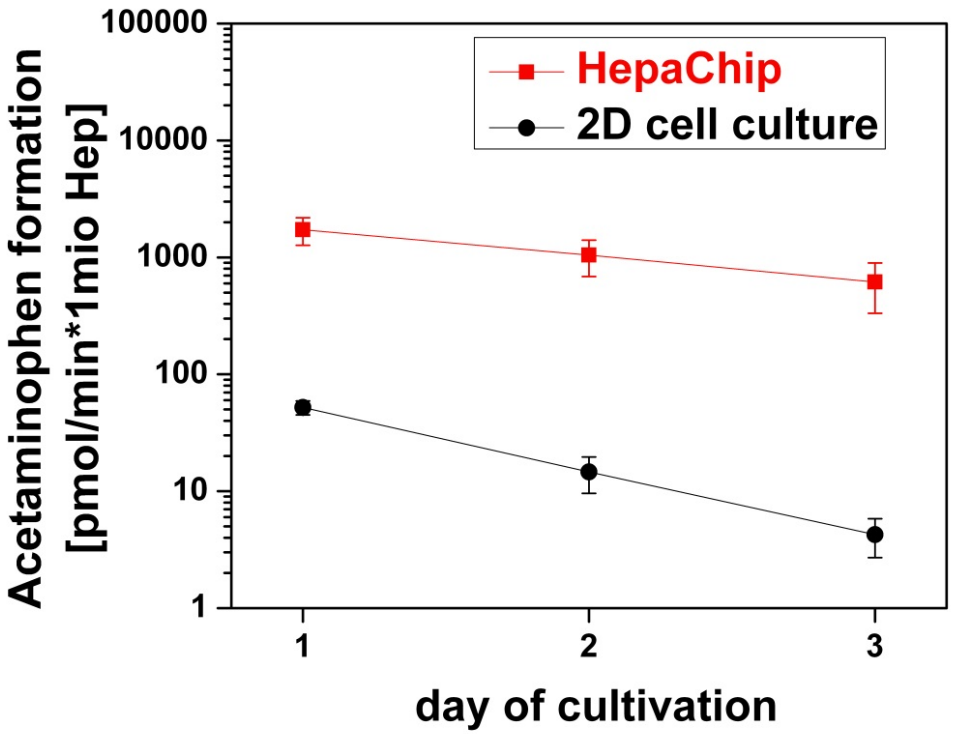
in collaboration with University Leipzig

Enhanced enzyme function in HepaChip

CYP 3A4 activity



CYP 1A2 activity

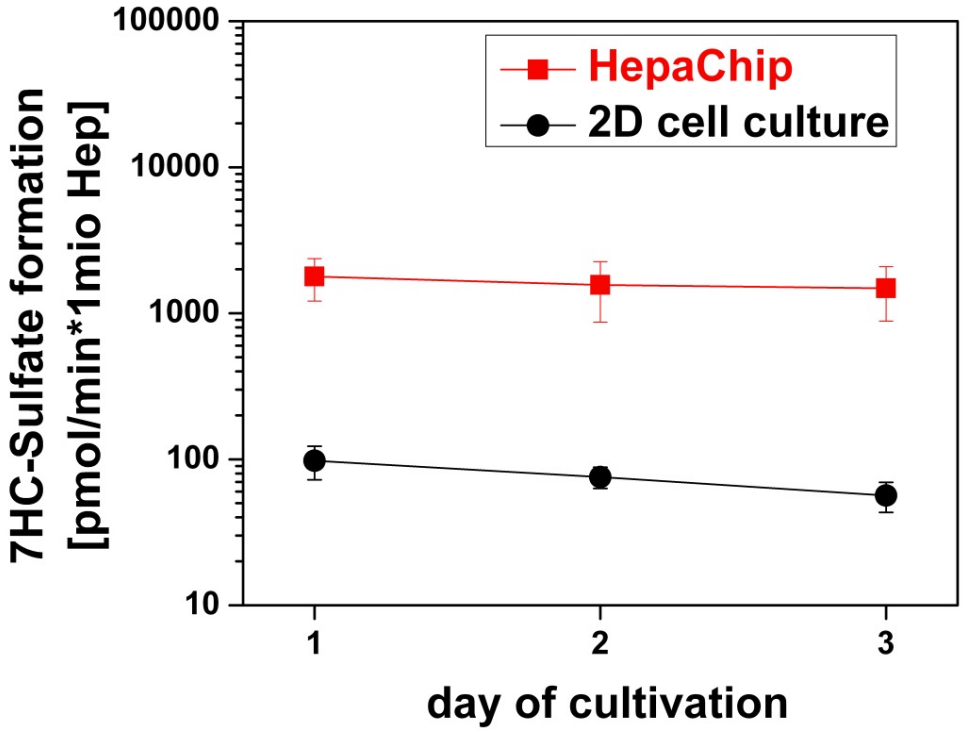


* Freshly isolated mouse hepatocytes (Gebhardt et al. 2003)

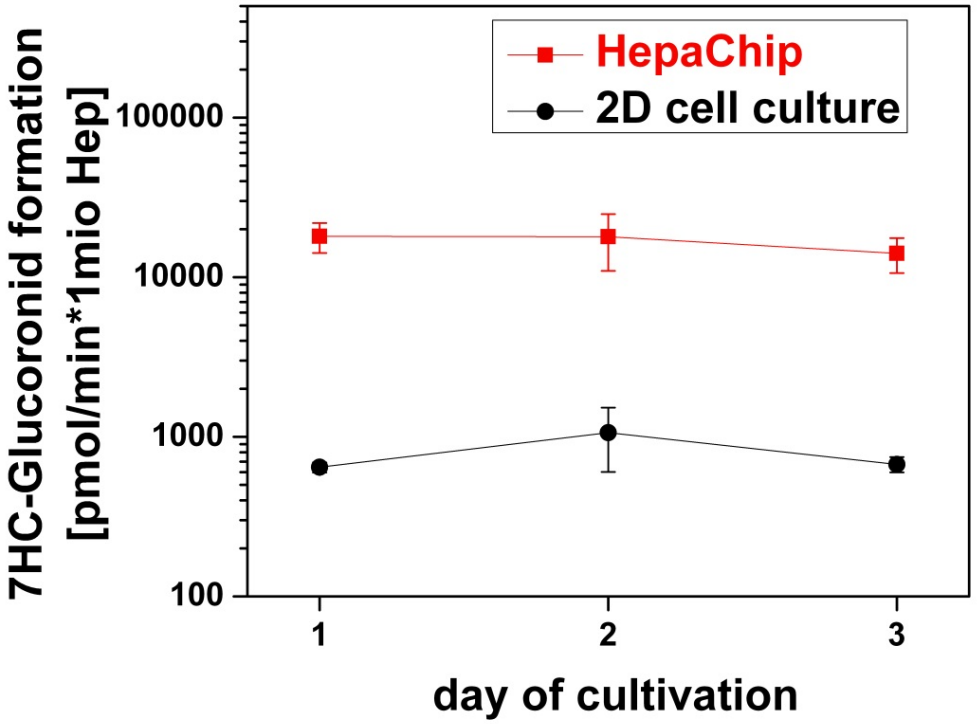
in collaboration with University Leipzig

Enhanced enzyme function in HepaChip

Sulfotransferase



UDP-Glucoronyltransferase



in collaboration with University Leipzig

Status of organ like features in the HepaChip®

- ✓ organ like structure
- ✓ hepatocytes, endothelial cells
  - ➢ Kupffer cells
- ✓ extra cellular matrix
- ✓ organ like perfusion, heating
  - ➢ oxygen/ CO_2
- ✓ organ like metabolism activity
  - ➢ 30 days

Status of HepaChip System

- ✓ bread board systems at collaboration partners: 3
- ✓ multiplexed bread board system at NMI: 1
- ✓ functional cell culture chip
- ✓ method for sinusoid cell architecture assembly
- ✓ protocols to quantify enzyme activity for CYP 3A4, CYP 1A2, SULT, UGT
- ✓ protocols to determine albumin and urea concentrations in effluent

Potential benefits of HepaChip System

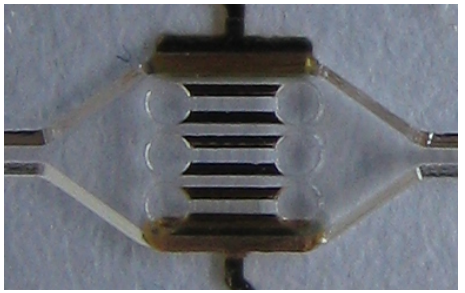
- Primary hepatocytes maintain their full functionality for over thirty days
- Results obtained in toxicity investigations are closer to reality compared to those obtained via 2D cell cultures
- Platform allows continuous measurements of cell vitality and cell activity
- No additional infrastructure is required for cell cultivation
- Strict selection of viable cells only, highly controlled assay conditions, and optimally aligned components and protocols result in consistently trustworthy results

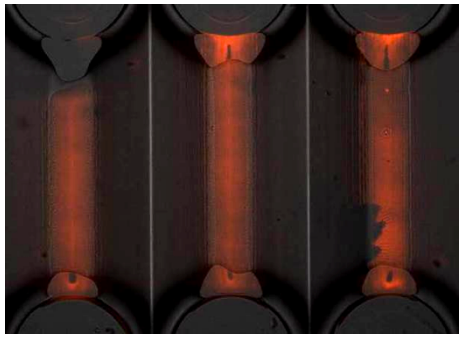
Applications:

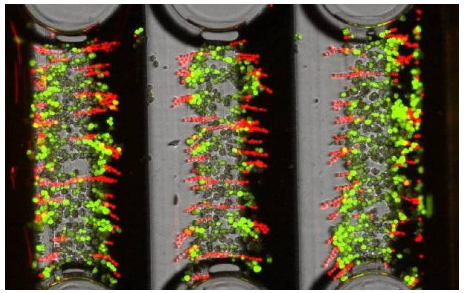
- Toxicity
- ADME
- Drug interaction studies
- Diseased cells
- mechanistic studies

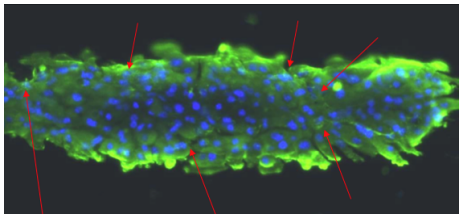
Further artificial organs:

- Blood-brain-barrier
- Kidney
- ….









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