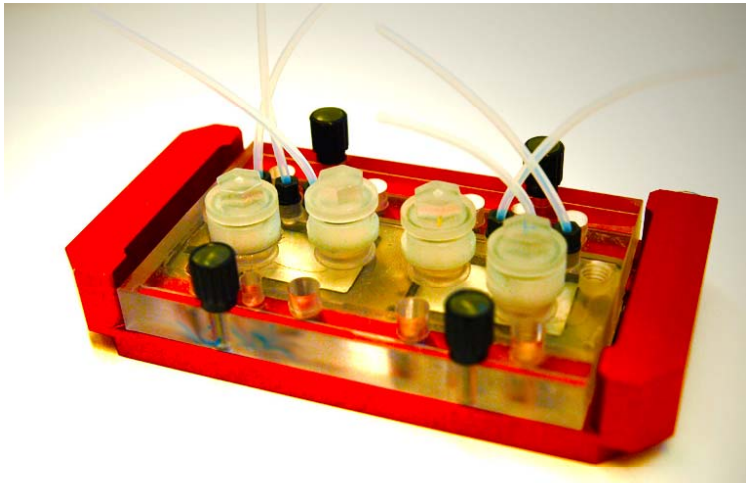
“Human-on–a-chip” concepts to implement in vitro repeated dose substance toxicity testing predictive to human exposure

EUSAAT Congress
LINZ 2012



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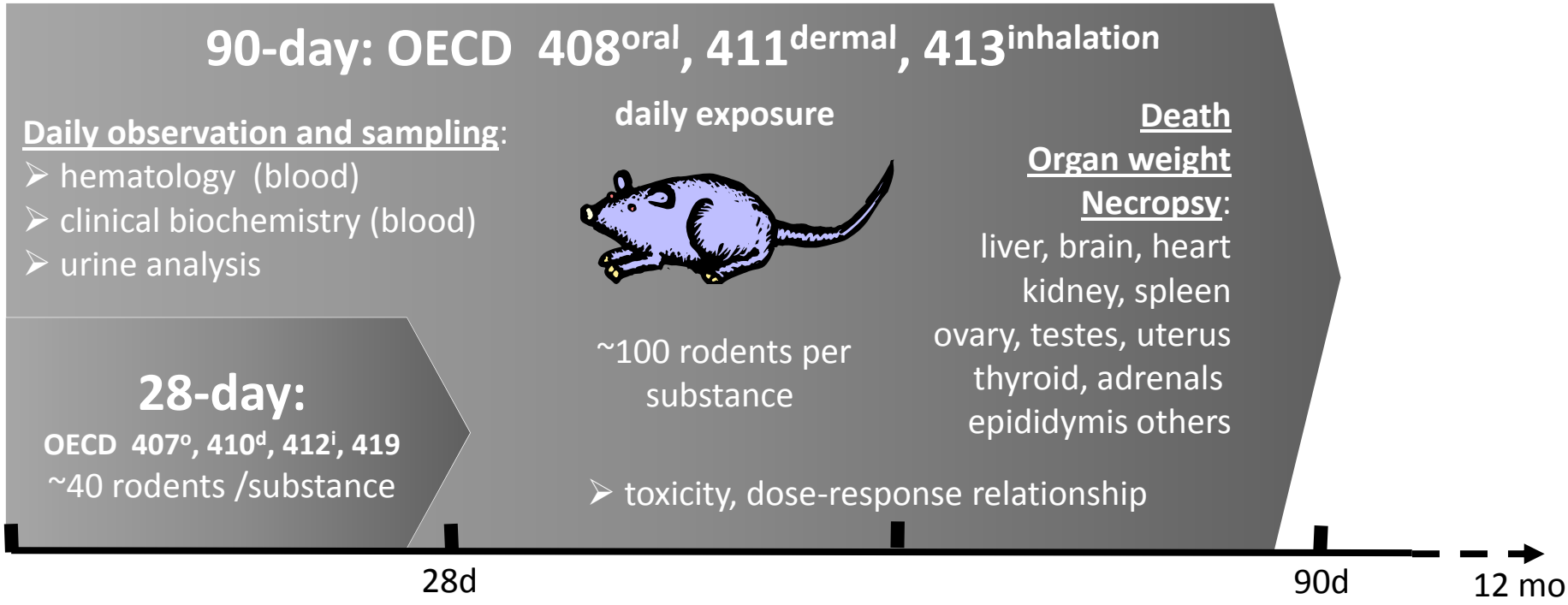
Technische Universität Berlin

TISSUSE
Emulating Human Biology

Repeated dose toxicity testing
- status quo-



2012

OECD 453
combined chronic toxicity/carcinogenecity

A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing

D. Basketter et al, ALTEX 29, 1/12, pp 1-91

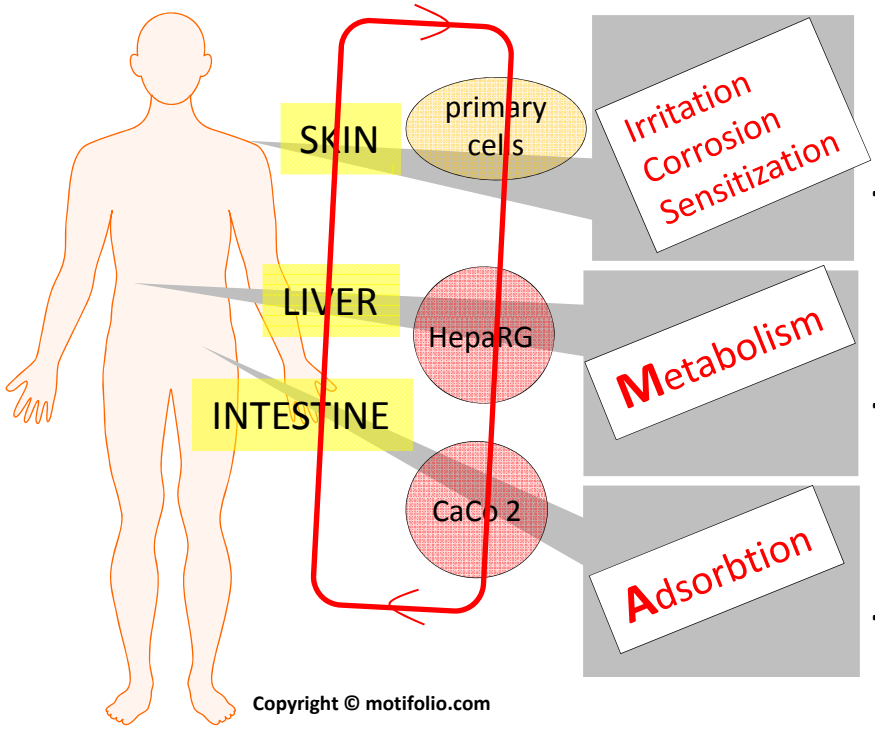
→ integrated testing strategy (ITS)

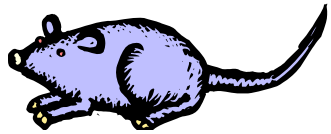
Integrated testing strategies

human *in silico* + human *in vitro* + animal *in vivo*

Roadmap questions:
„ .. what constitutes an appropriate model for each organ system, i.e., what makes a heart a heart, a liver a liver etc.“

Roadmap request:
„more physiological culture conditions such as homeostasis, oxygen supply, cell density…“





Adsorbtion
Distribution
Metabolism
Excretion
Toxicity

Molecular initiation → subcellular → cellular → organ → organism

Pathway of Toxicity (PoT)

Mode of Action (MoA)

Adverse Outcome Pathway (AOP)

Our development objectives

- **dynamic 3D multi-tissue culture - MOC**

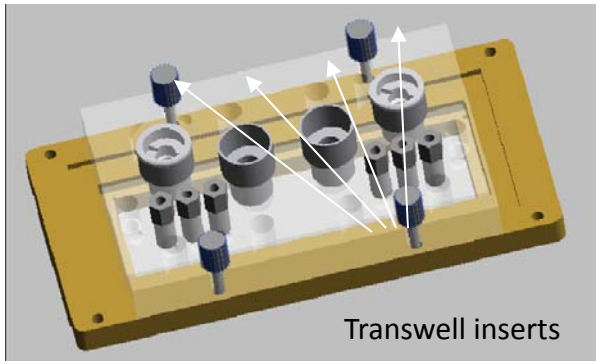
  (optimized oxygen supply, mechanical stress, dynamic protein gradients, “shared” medium ..)

- **miniaturization to smallest possible scale**
- **optimized fluid to tissue ratio**
- **standardized easy to handle bioreactor and chip format**
- **equally supporting cell line and primary tissue culture**

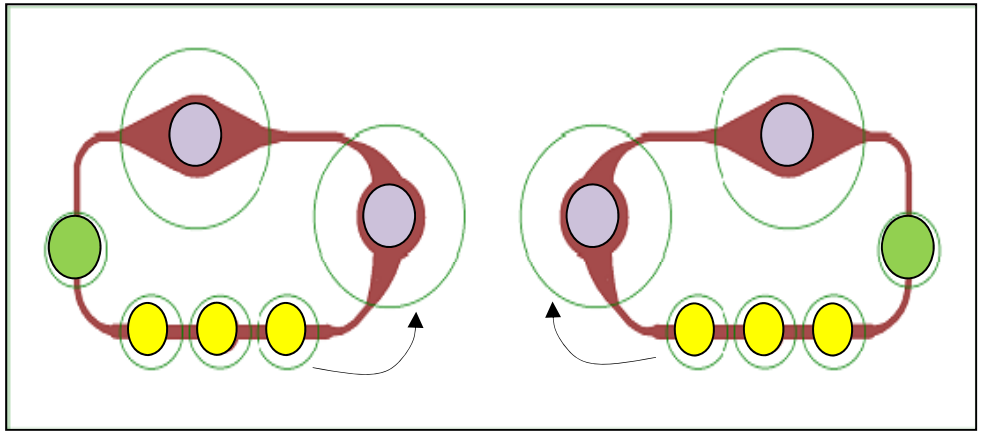
The Multi-Organ-Chip (MOC) technology

Laboratory bioreactor

- **two peristaltic micro-pumps per chip**
- **two media perfusion circuits per chip**
- **adopted to use of Transwell-inserts**
- **two MOC´s (4 circiuts) per bioreactor**



MOC´s of microscopic slide-format



peristaltic micro-pump

Transwell inserts

injection port

28-day MOC culture experimental setup

3D tissue preparation and chip loading

HepaRG cells + stellate cells (12 : 1)

↓

24h — **aggregate formation in AggreWell plates**

↓

4 days — **low-attachment-plate culture**

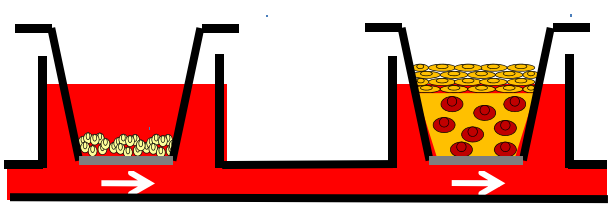
Foreskin donor recruitment

↓

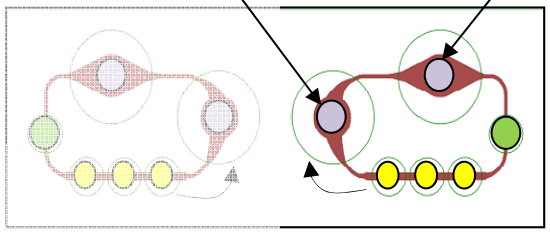
tissue excision and transport to lab

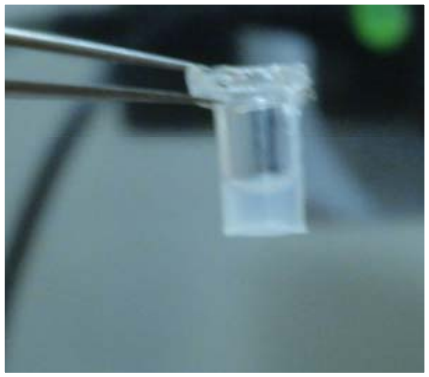
↓

Preparation of sterile punch biopsies



“liver” model “skin” model





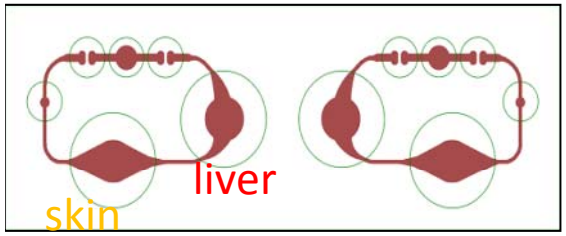
tissue volume: ~30µl
channel volume: ~10µl
total volume: 300µl

28-day MOC culture – time course

Multi-Organ-Chip culture regimen

n = 8 liver-skin circuits
daily feeding (50% medium exchange)

Tissue culture medium:
William's E Medium (Basalmedium)
+ 10% FCS, + 5µg/ml insulin, + 2mM glutamine
+ 5 * 10^{-5} mol/L hydrocortisone hemisuccinate
+ 1% Pen/ Strep



Sample 1:
14 days
1chip (2 circuits)
2 static controls

Sample 2:
21 days
1chip (2 circuits)
2 static controls

Sample 3:
28 days
2chips
4-6 static controls

Daily media exchange (150µl - out of ~300µl)
During the first week media was changed every 12 h

Total of 616 media samples to be anaylzed

28-day MOC culture – ongoing analysis

genes expression (end point determination)

- *TBP, SDHA (references)*
- *Albumin, CPS-1 (secreted proteins)*
- *CYP1A2, CYP2B6, CYP3A4 (Phase I metabolism)*
- *UGT1A1, GSTA2 (phase II metabolism)*
- *MDR1, MRP2, BSEP (transporter)*
- *PP4, DKK3, ZO-1 (others)*

extensive tissue histology

- *marker expression*
- *organoid structure*

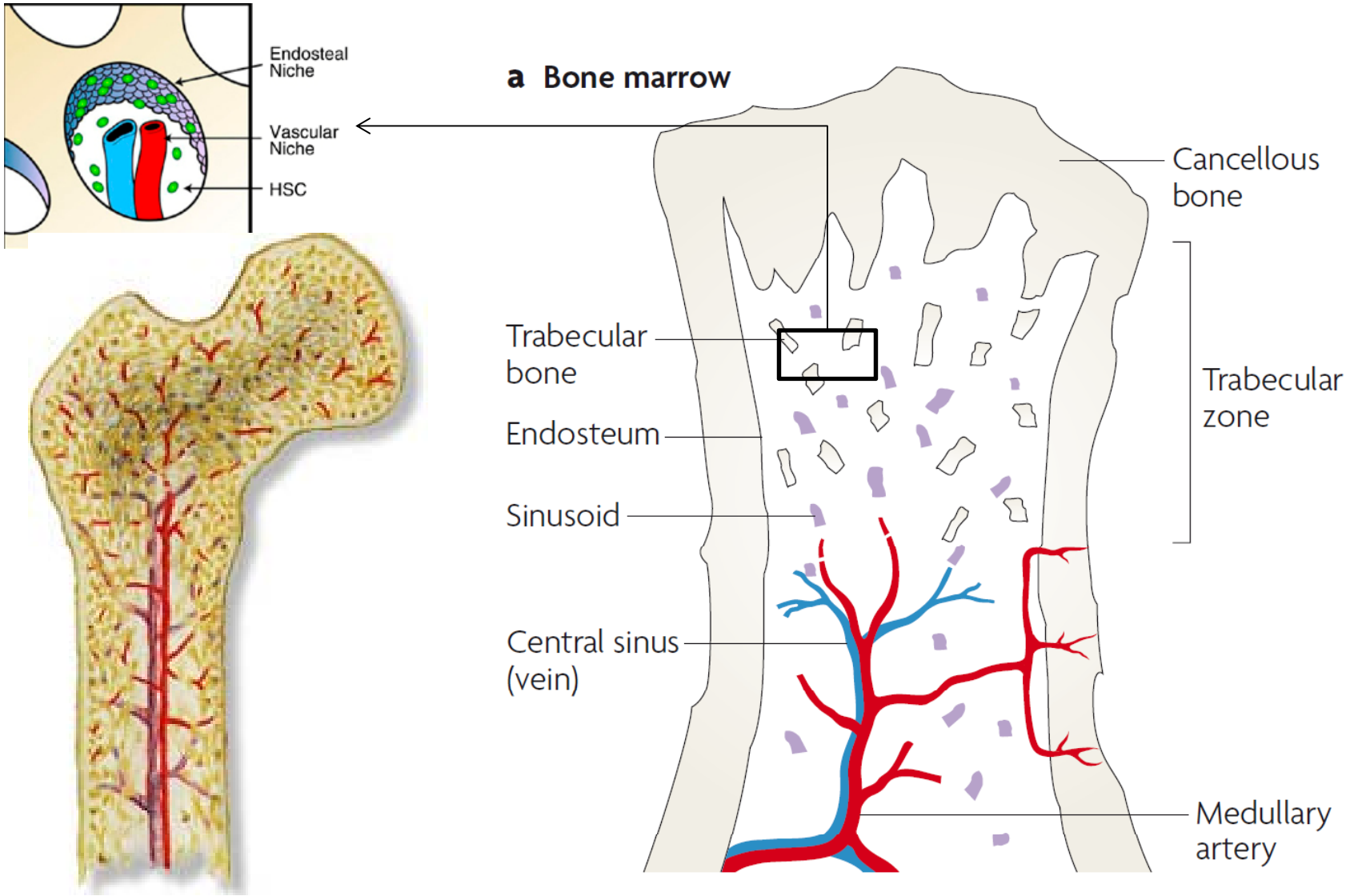
daily medium samples (metabolics)

- *albumin*
- *urea*
- *glucose / lactate*

environmental data

- *oxygen concentration*
- *pH-value*

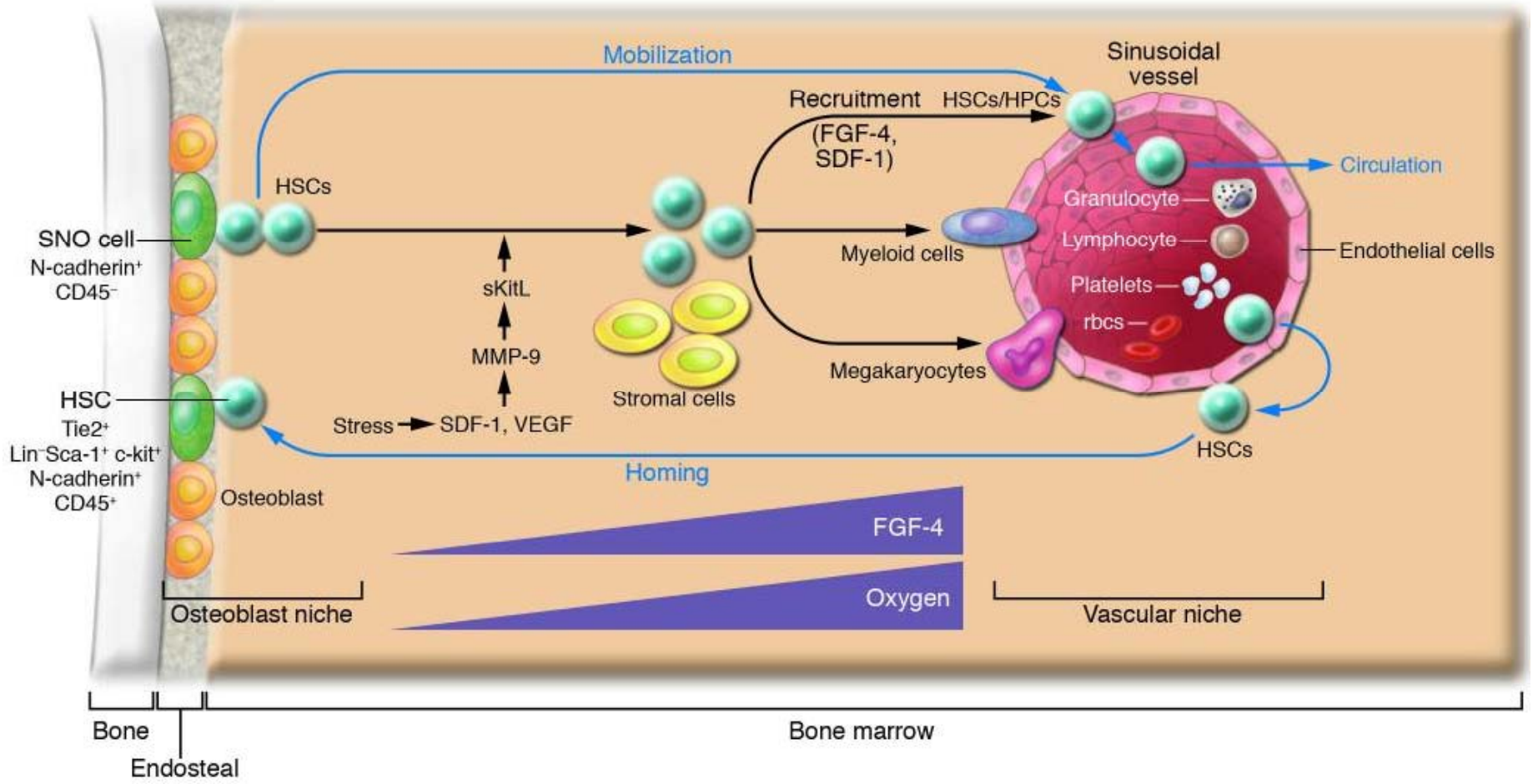
Bone marrow biology



Shiozawa Y, Havens AM, Pienta KJ, Taichman RS.Leukemia. 2008 May;22(5):941-50. Epub 2008 Feb 28
Kiel MJ, Morrison SJ.Nat Rev Immunol. 2008 Apr;8(4):290-301. Epub 2008 Mar 7. Review.

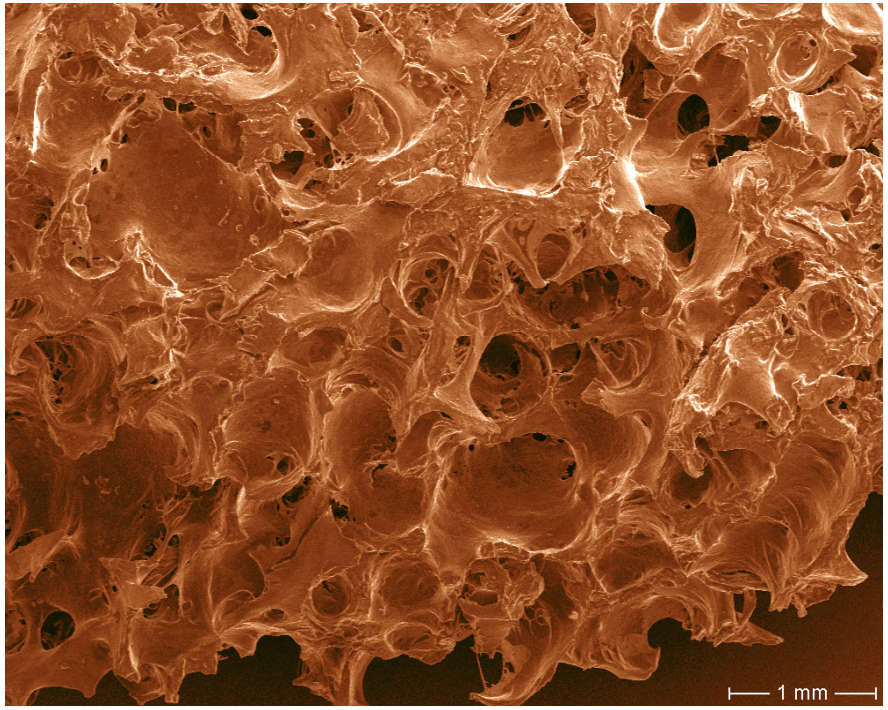
The two types of bone marrow stem cell niches

bone marrow function: production of new blood cells
immunological function (memory B-cells and memory T-cells)

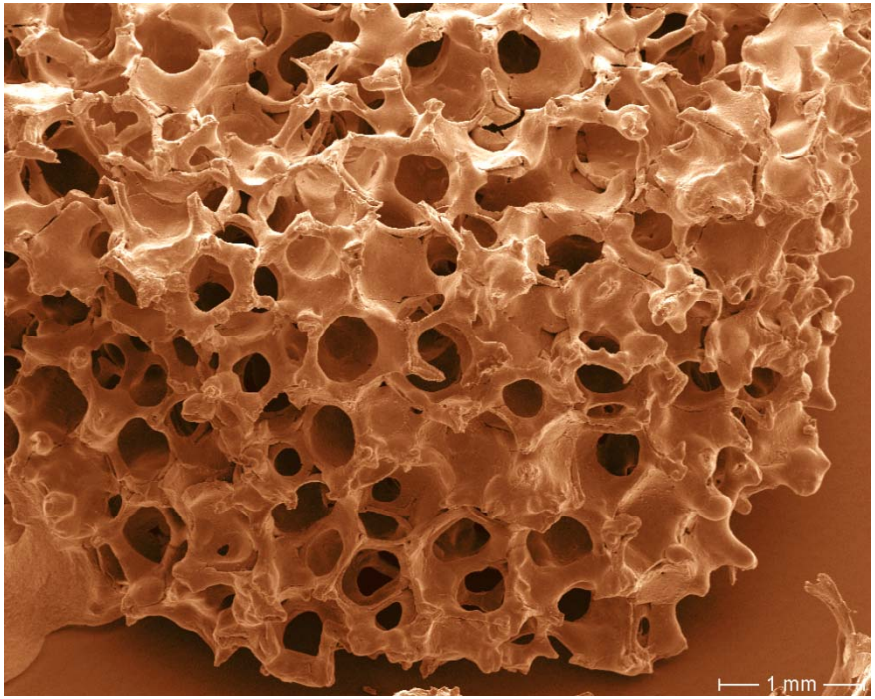


The stem cell niches in bone. Yin T, Li L. J Clin Invest. 2006 May;116(5):1195-201. Review.

Ceramics as 3D scaffold for *in vitro* bone marrow culture



Structure of bone marrow



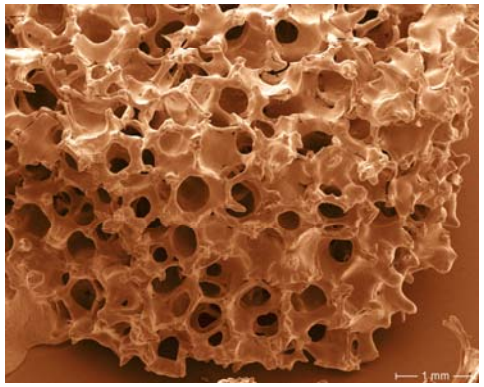
Structure of ceramic carriers

Culture or co-culture of different cell types

Hematopoietic stem cells isolated from bone marrow or cord blood

mesenchymal stem cells

MG 63 osteoblast cell line



→ Static culture

→ Zellwerk ® Bioreactor

→ column based

28-day MOC culture – summary

- **28-day 3D multi-tissue culture is feasible**
- **both cell lines and primary tissues can be used**
- **MOC format supports investigation into different non-standard repeated dose toxicity test designs**

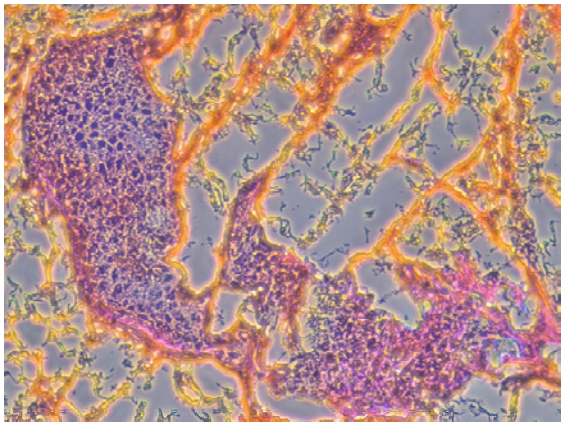
- **complex tissues still suffer from limited nutrition**
- **physiological oxygen supply**
- **unlimited organotypic culture (90-day, 12-months) is impossible at "organ" homeostasis**

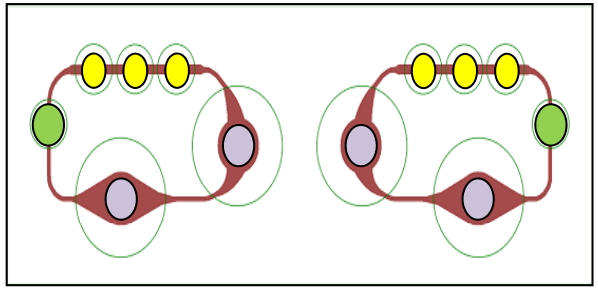
➢ **add human vasculature!**

Next program step

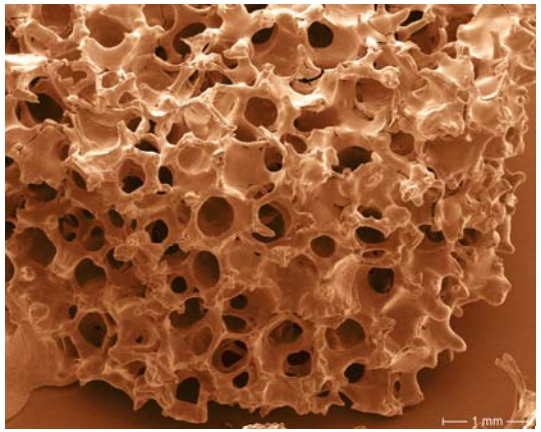
seeding human liver tissues and bone marrow matrices into vascularized dynamic microchips for organoid self-assembly, blood perfusion and long term maintenance .

liver lobuli equivalents

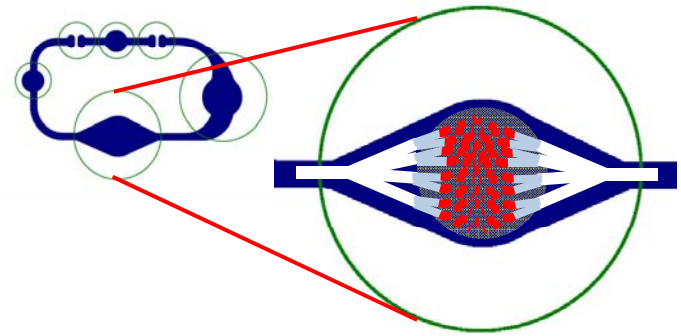




bone marrow unit models



in vascularized chip to induce neo-vascularization



more organoids to create a ADMET – MOC

-gut mucosa
-liver lobili
-skin equivalents
-nephrons tubuli
-alveoli
-bone marrow

combination in perfused culture sustem

Acknowledgements

Institute of Biotechnology

Eva Materne
Ilka Wagner
Reyk Horland
Gerd Lindner
Devasena Kanthi
Katharina Schimek
Roland Lauster






Funding
BMBF: GO-BIO

Fraunhofer IWS, Dresden

Frank Sonntag
Niels Schilling
Udo Klotzbach



Uwe Marx

Thank you for your attention!