ASCCT-ESTIV and JSAAE Joint Webinar
12/11/2023

Accurate Evaluation of Hepatocyte Metabolisms on a Novel Oxygen-Permeable Material with Low Sorption Characteristics

Chemical System Engineering
Sakai-Nishikawa Lab
masaki@chemsys.t.u-tokyo.ac.jp

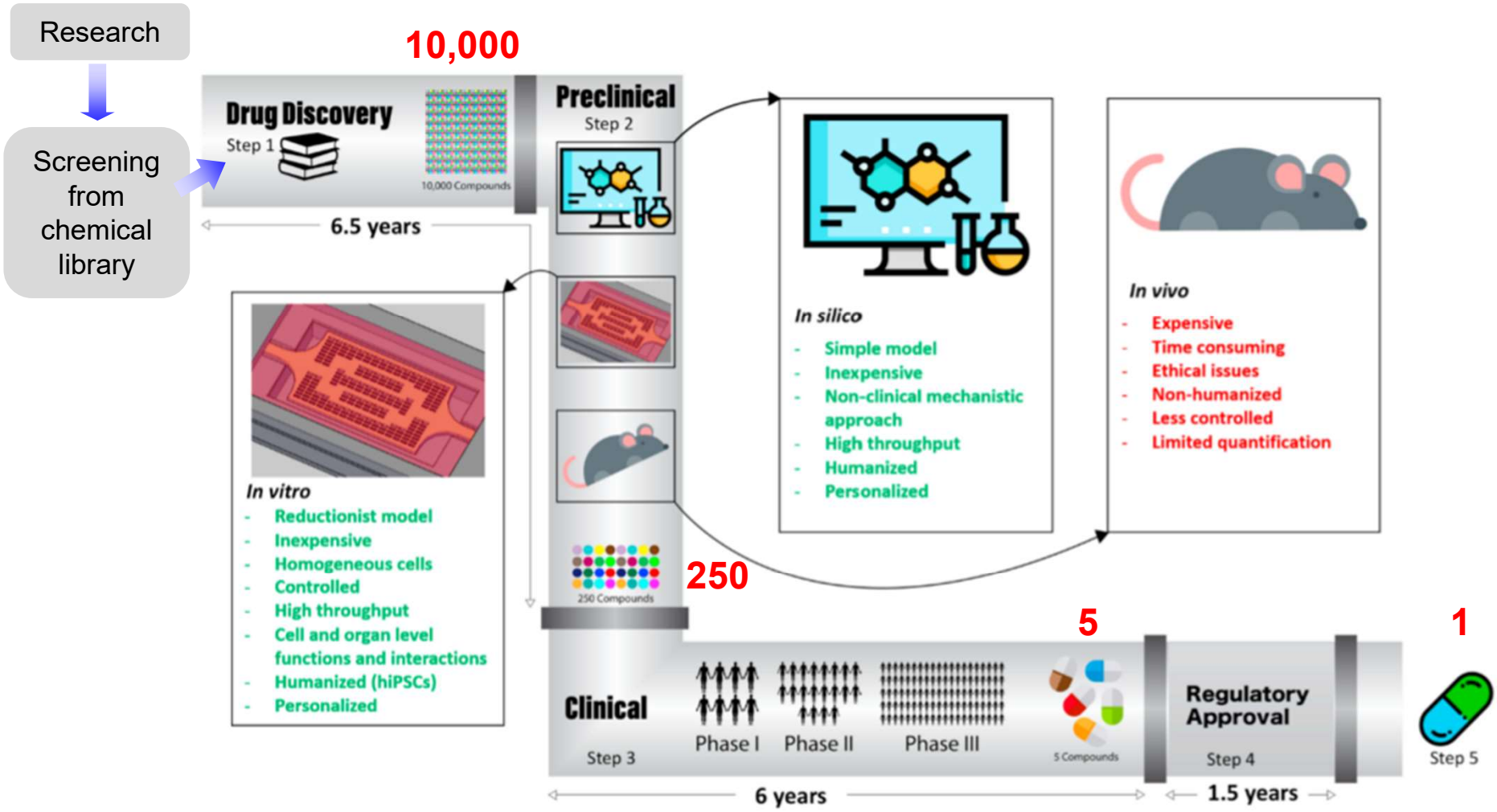
Masaki Nishikawa

東京大学
THE UNIVERSITY OF TOKYO

Organs and Biosystems Engineering
Sakai-Nishikawa Lab.
Institute of Industrial Science, The University of Tokyo

2

Background: Drug development & Preclinical research



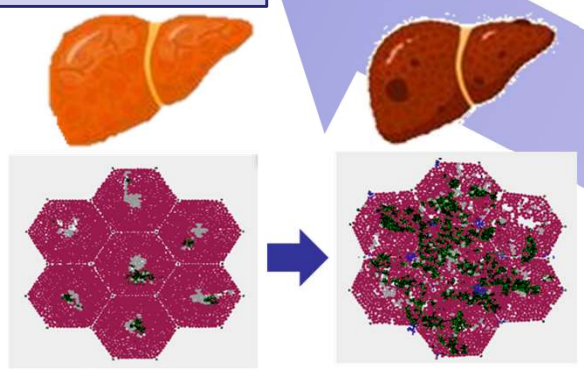
~ 15 years & nearly $1 billion

Accurate prediction of effects and toxicities in humans (*Animal Free*)

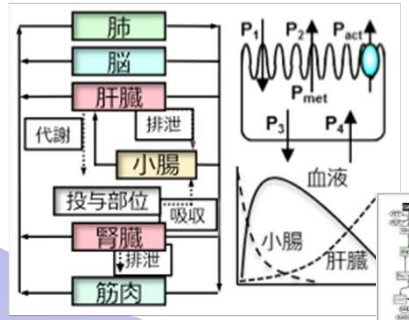
Savoji, *et al.*, *Biomaterials*, 2019

in silico Numerical Models

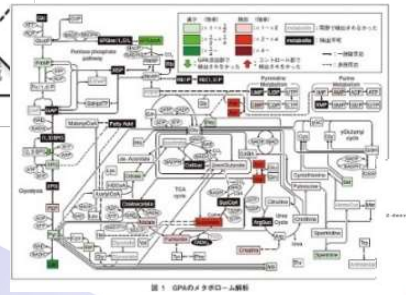
Liver diseases



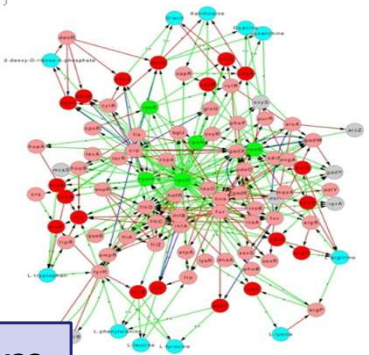
Organs



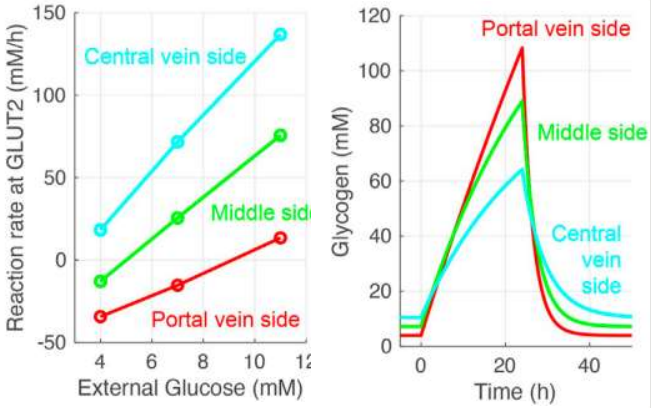
Cells

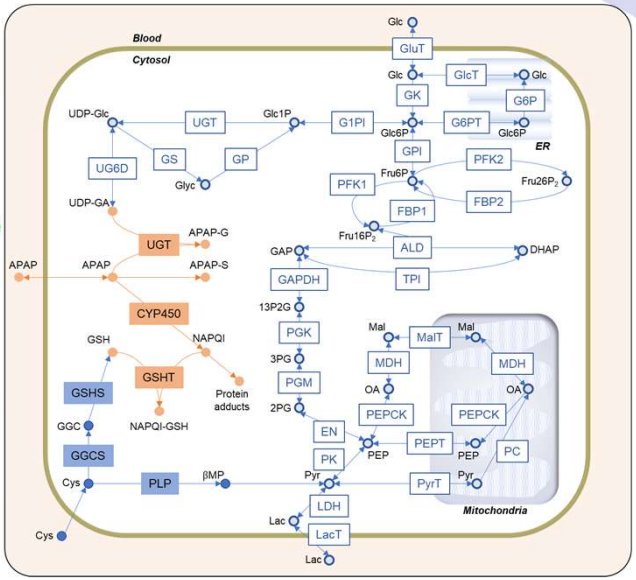


Molecules

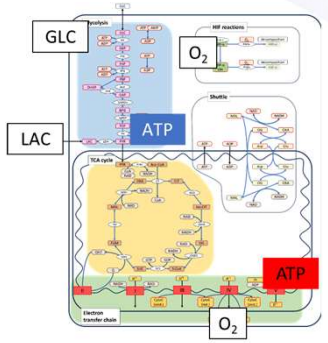


Hepatic metabolisms（Glucose & APAP）





Energy Metabolism



Yoshizawa *et al.*, *Sci Rep.*. 2022.
Maeda, *et al.*, *Front Pharmacol.*,. 2022.

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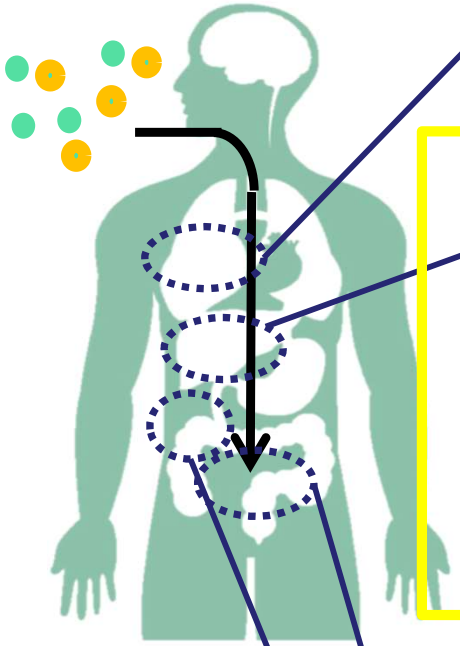
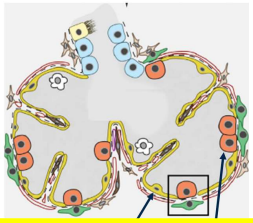
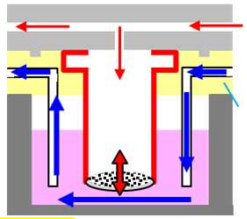
in vitro
MPS / cell culture models

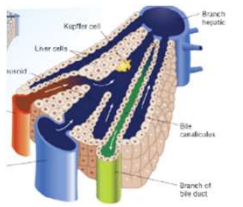


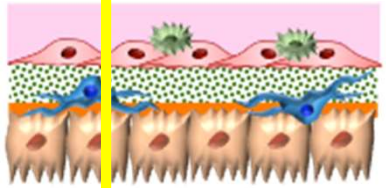
Lung

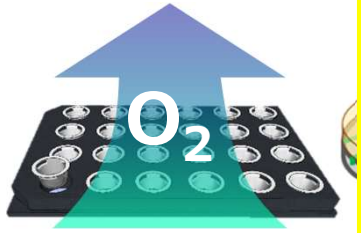


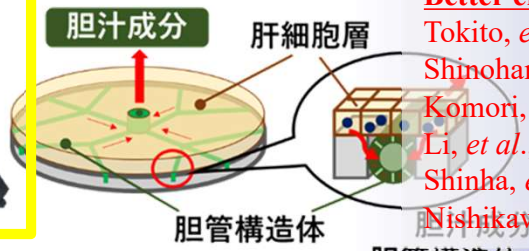


Liver

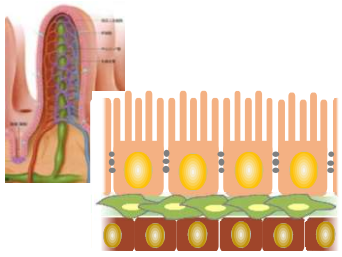


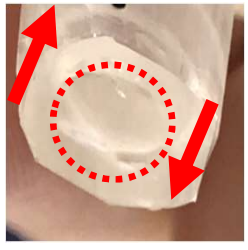


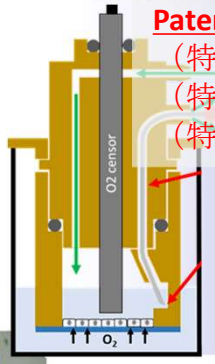




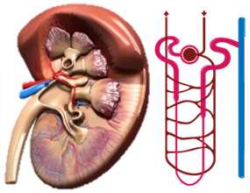
Intestine

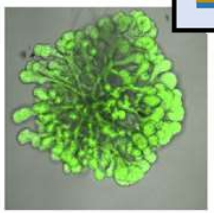






Kidney





Better cells

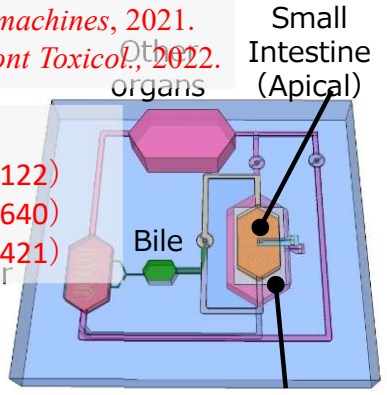
Mihara, *et al.*, *Cells*, 2019.
Scheidecker, *et al.*, *Front Bioeng Biotechnol.*, 2020.
Vadivelu, *et al.*, *Adv Biol.*, 2021.
Choi, *et al.*, *Cells*, 2021.
Torizal, *et al.*, *Commun Biol.*, 2021.
Khadim, *et al.*, *Int J Mol Sci.*, 2022.
Torizal, *et al.*, *J Tissue Eng Regen Med.*, 2022.
Danoy, M *et al.*, *Fundam Toxicol Sci.*, 2022.
Torizal, *et al.*, *Sci Rep.*, 2022.
Gong, *et al.*, *Biotechnol Bioeng.*, in press.

Better environments

Tokito, *et al.*, *J Biosci Bioeng.*, 2021.
Shinohara, *et al.*, *Sci Rep.*, 2021.
Komori, *et al.*, *Bioelectrochem*, 2021
Li, *et al.*, *Appl Sci.*, 2021.
Shinha, *et al.*, *Micromachines*, 2021.
Nishikawa *et al.*, *Front Toxicol.*, 2022.

Patents

（特願：2021-046122）
（特願：2022-116640）
（特願：2022-164421）



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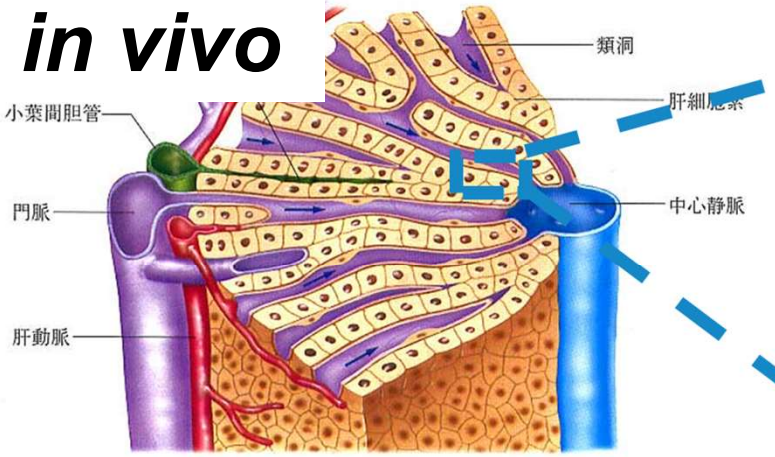
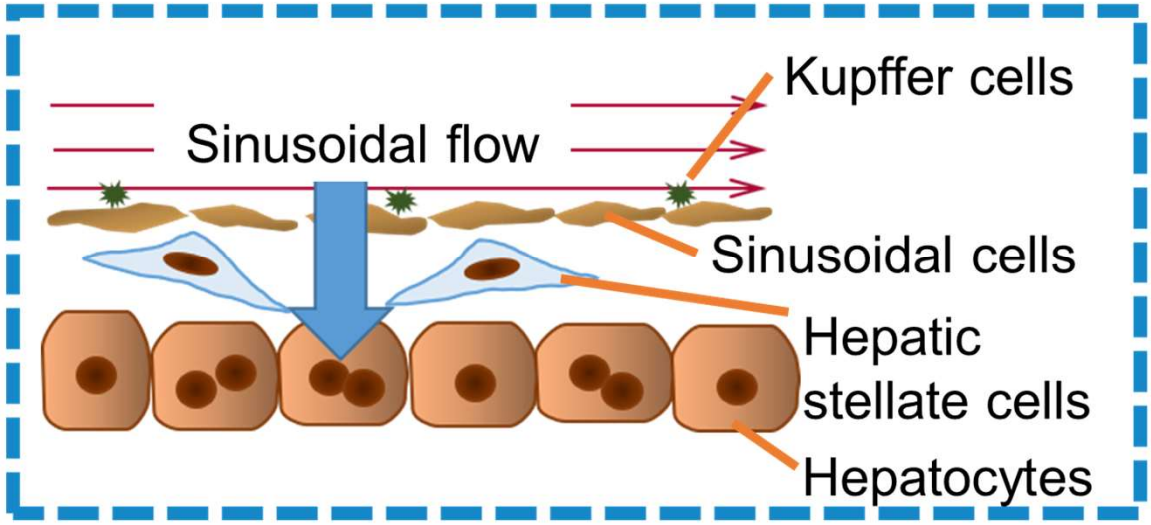
in vitro liver models

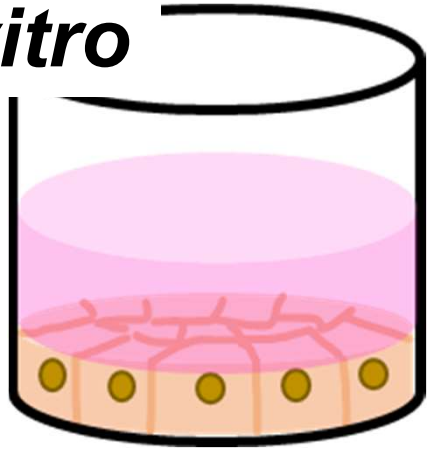
One of the most important organs for drug development

in vivo





in vitro

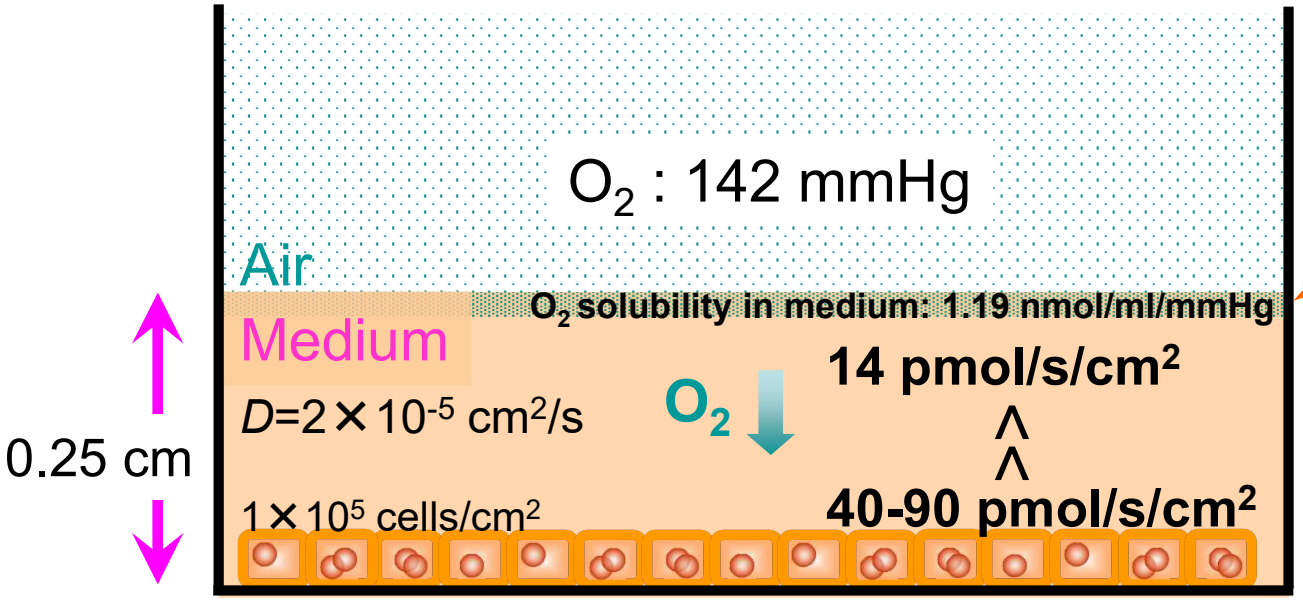


Conventional Tissue culture polystyrene (TCPS)

Insufficient factors:

- ✓ **Material exchanges (O_2, nutrients, growth factors, metabolites, etc.)**
- ✓ **Cell-cell interactions (Hepatocytes and non-parenchymal cells)**

Limitation of Oxygen Supply in Conventional Culture



TCPS plate

From medium surface

Gas-liquid interface:

170 nmol/ml-medium

Cell layer:

0 nmol/ml

Fick's low of diffusion

$$F[\text{mol cm}^2 s^{-1}] = -D\frac{(pO_{2\text{air}} - pO_{2\text{cell}})}{z}$$

Diffusion Coefficient **Concentration Gradient**

Oxygen supply

∧

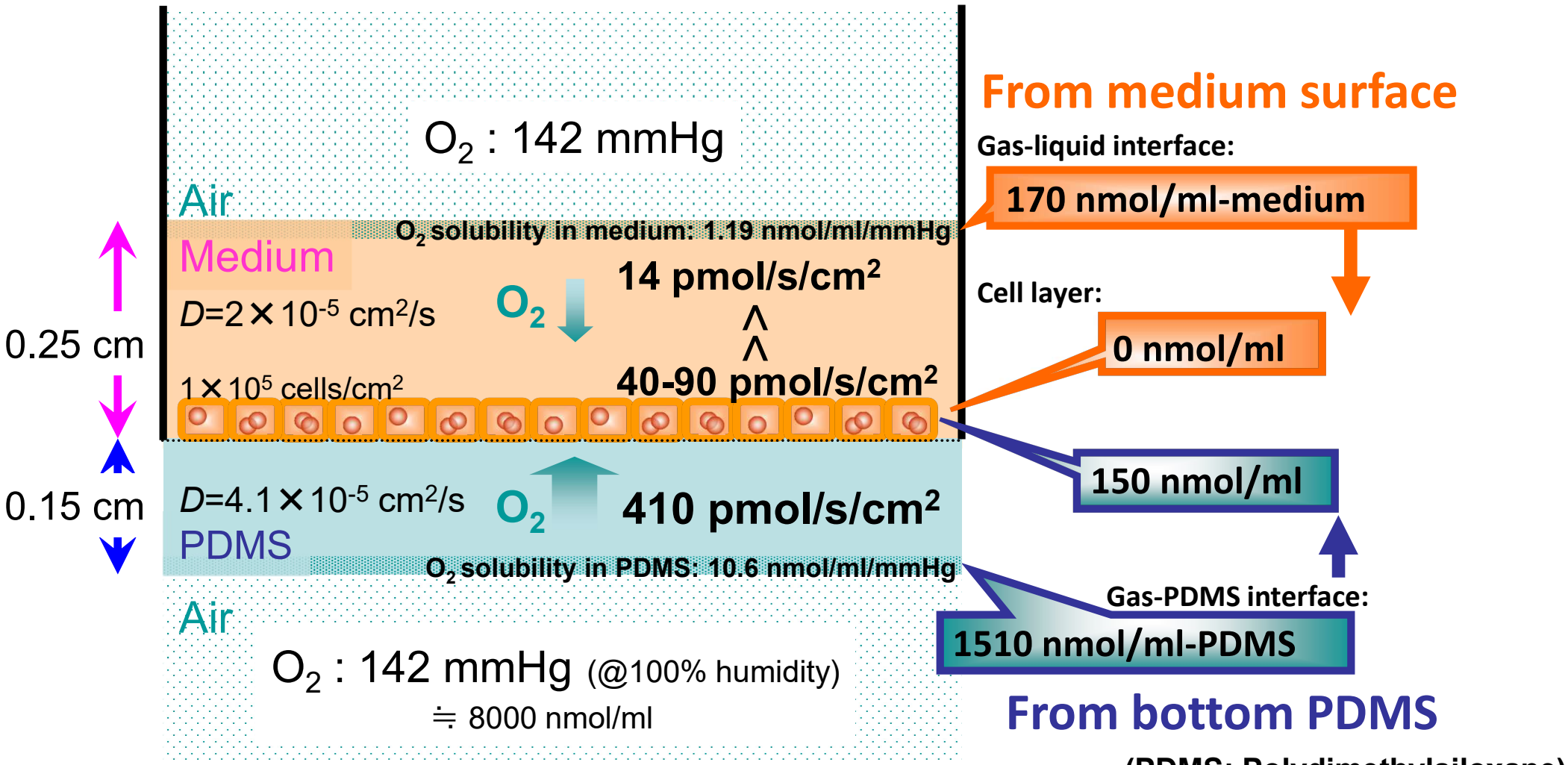
∧

Oxygen demand

Oxygen Shortage

Limited Function

Direct oxygenation through O_2 permeable materials



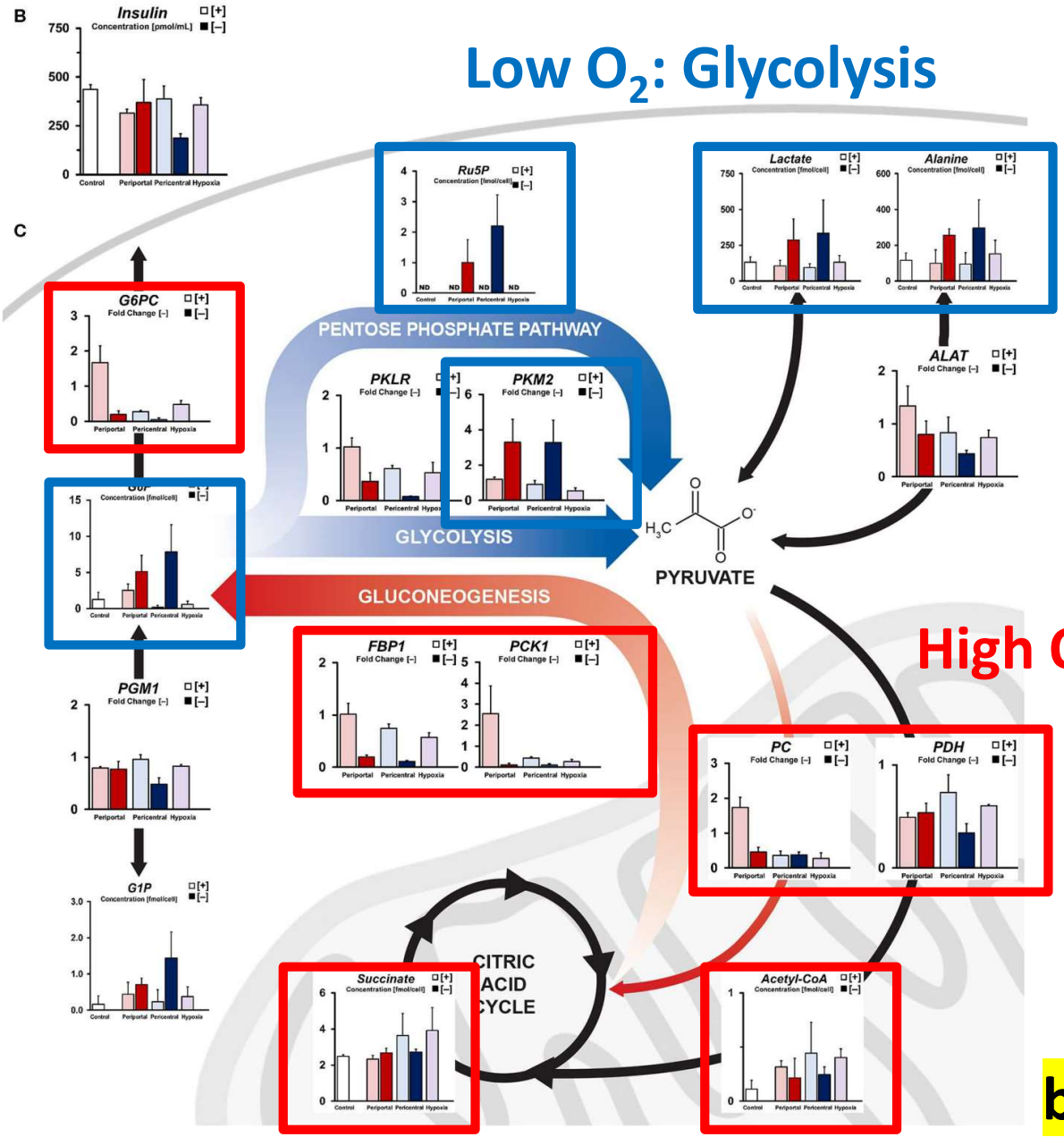
A solution to the limitation of oxygen supply

- **simple**
- **effective during cell attachment ➡ High cell density**

Nishikawa, *et al.*, ***Biotechnol Bioeng.,*** 2008; Nishikawa, *et al.*, ***J Biotechnol.,*** 2008

Metabolome Analysis of Oxygen Effect

Scheidecker, *et al*., *Front bioeng biotechnol*., 2020

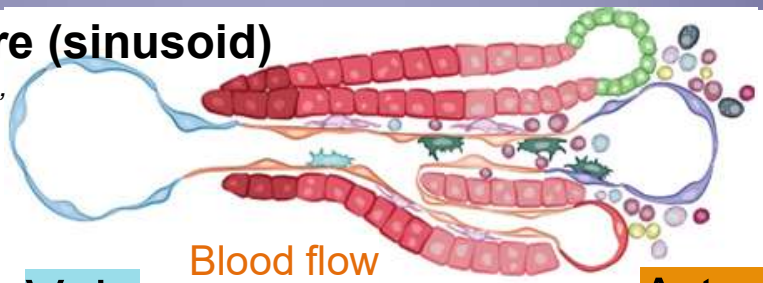


Energy production by aerobic respirations

Transcriptome Analysis of Oxygen Effect

Liver microstructure (sinusoid)

Adopted from MacParland, *et al.*, *Nat. Commun.*, (2018)

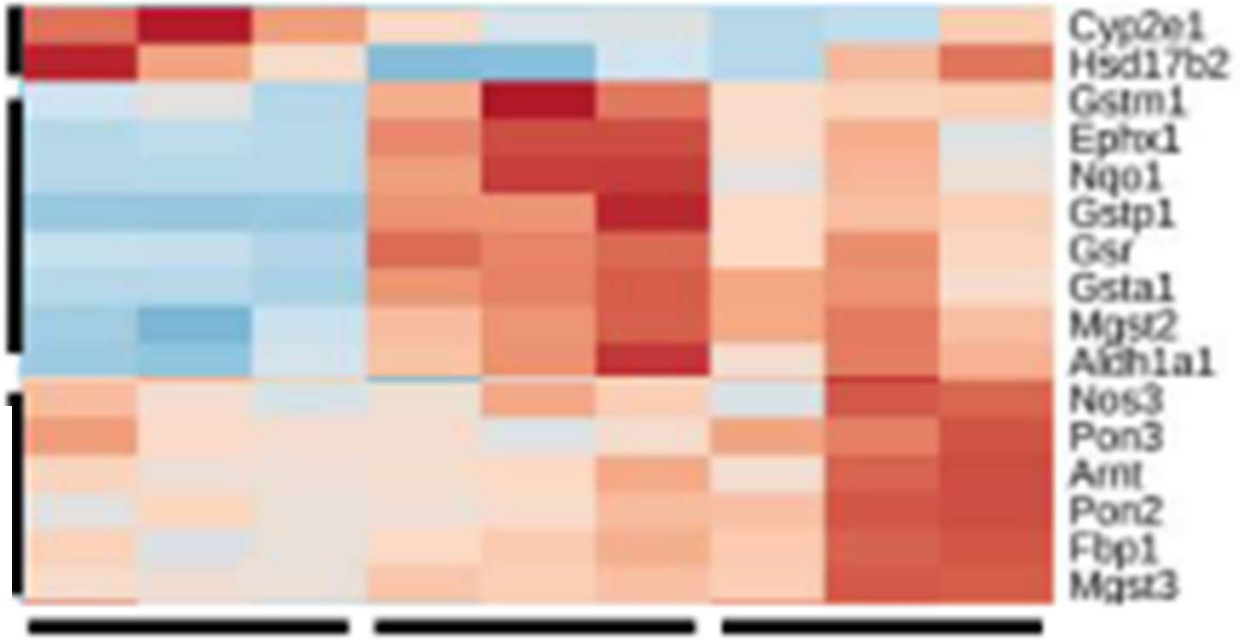


Scheidecker, *et al.*, *Front bioeng biotechnol.*, 2020

Drug metabolism Phase I

Drug metabolism Phase II

Oxygen-gradient dependent genes



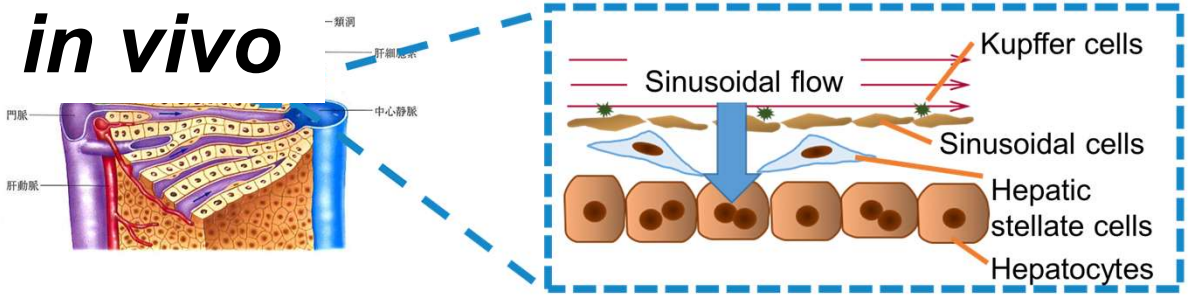
2.5% **5%** **10%**

Vein — **Oxygen tension** — Artery

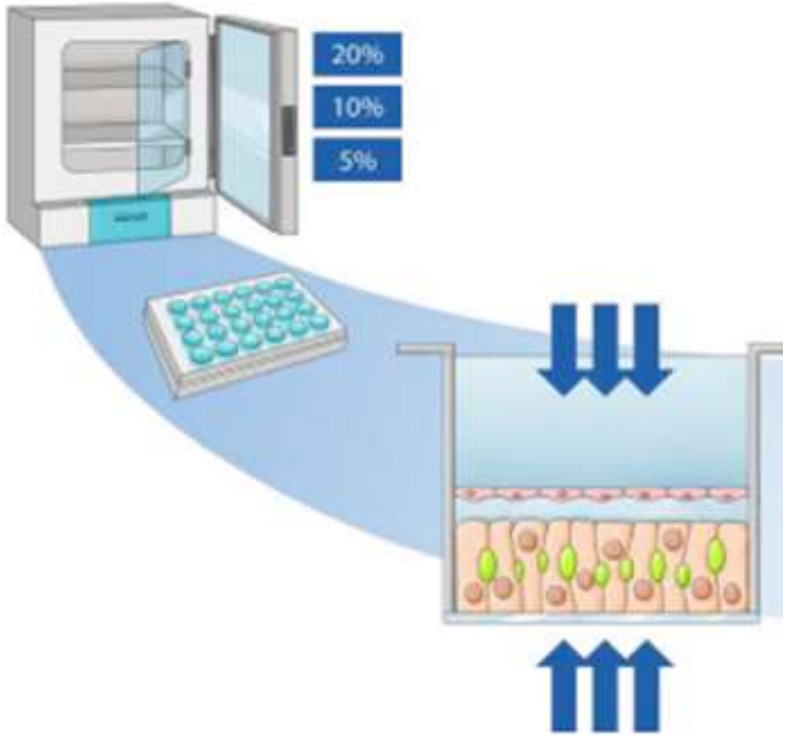
Physiological liver cell phenotypes depend on oxygen flux

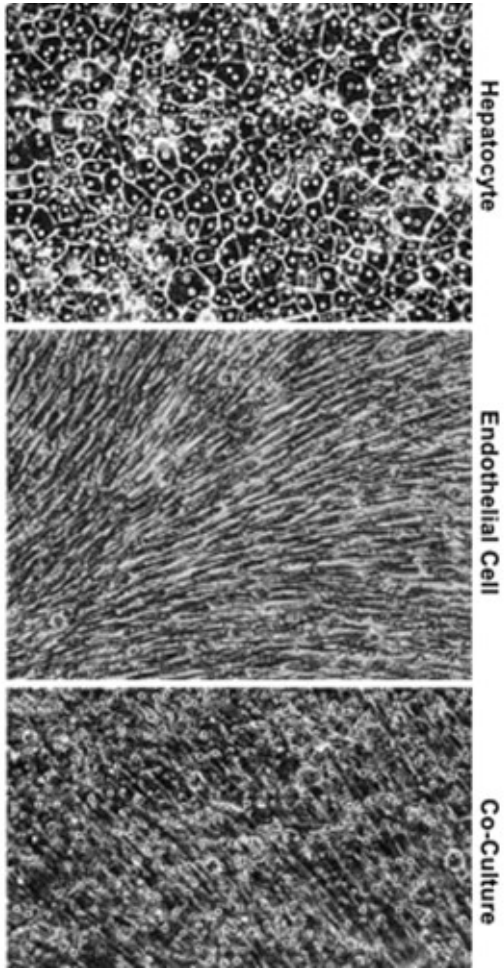
Cell-cell interactions (Hepatocytes and non-parenchymal cells)

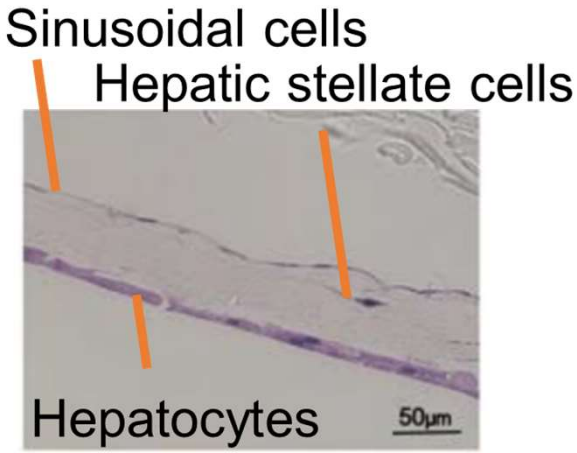
in vivo

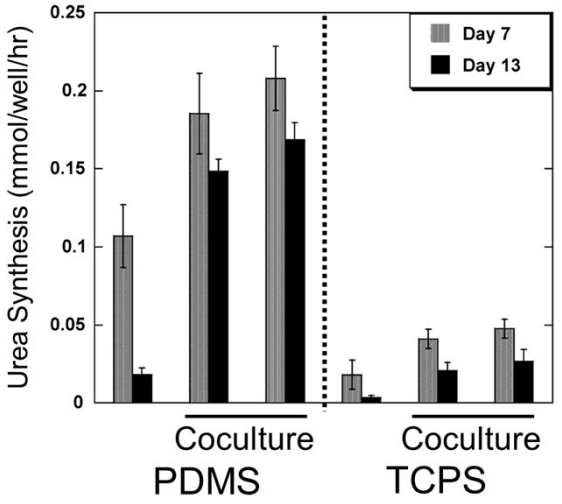


in vitro









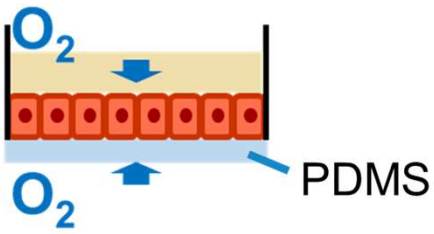
Nishikawa *et al*., *J Biotechnol*., 2008
Danoy *et al*., *Integr. Biol.,* 2018
Scheidecker, *et al*., *Front bioeng biotechnol*., 2020

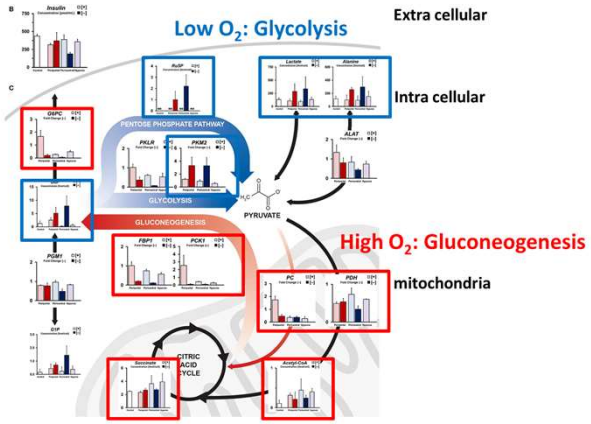
PDMS-based Microphysiolocial systems (MPS)

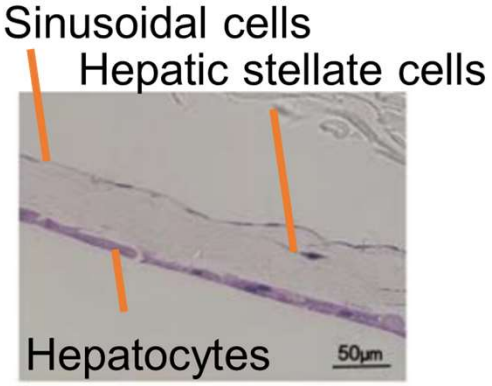
Pros

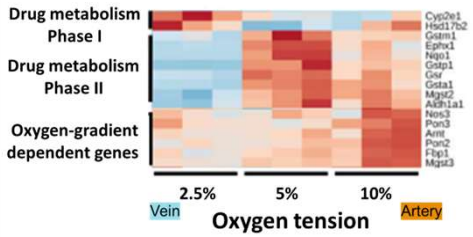
High function

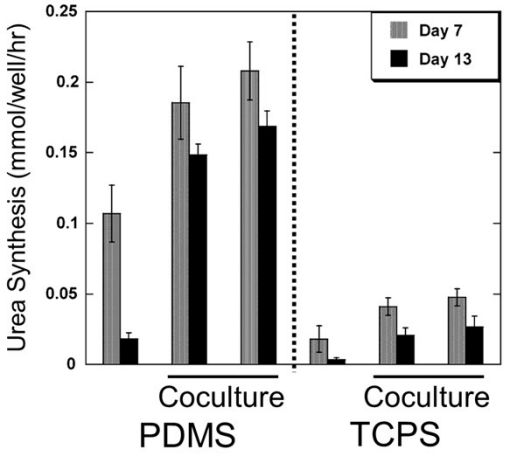
- ✓ O_2 supply
- ✓ cell-cell interactions





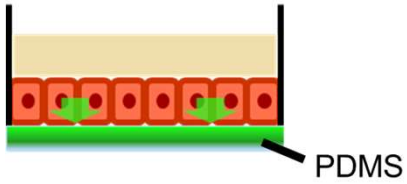


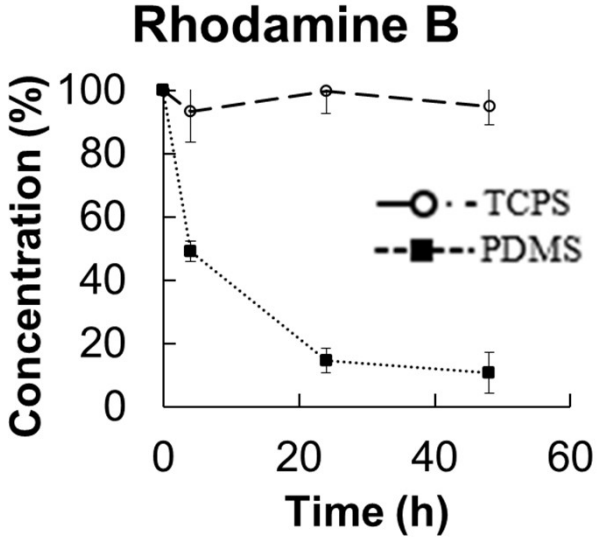




Cons

Sorption





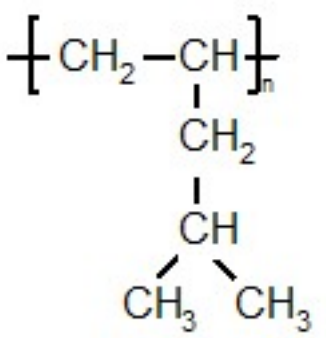
Loss of chemicals compromise the reliability of tests

Nishikawa *et al.*, *J Biotechnol.*, 2008
Danoy *et al.*, *Integr. Biol.*, 2018
Scheidecker, *et al.*, *Front bioeng biotechnol.*, 2020

New Oxygen-Permeable Material with Low Sorption Characteristics

Nishikawa *et al., Front Toxicol.* Jun 6;4:810478. 2022

PMP



poly(**4-methyl-1-pentene**)

- used for gas-permeable packaging
- Low sorption

Mitsui Chemicals

Had not been used for cell culture

➢ O_2 permeability test

O_2 flux was calculated based on the measured O_2 conc. at the cell layer

➢ Sorption tests

Chemical concentrations in the medium were measured.

➢ Hepatocyte culture and functions

Primary rat hepatocytes (1E5 cells/cm^2) were cultured for 7 days.
Albumin secretion, gene expression and CYP activities were measured.

O_2 permeability test

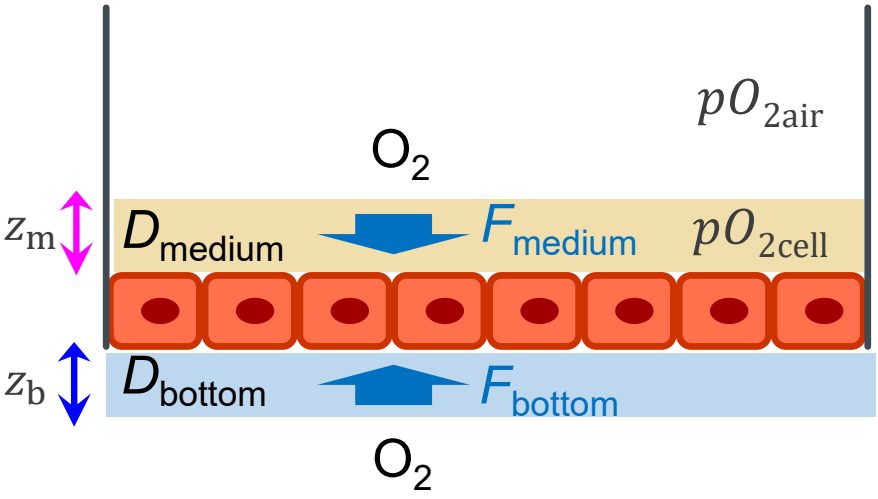
Calculate oxygen consumption rates (= oxygen flux) based on the O_2 concentration at the cell layer (pO_{2cell})

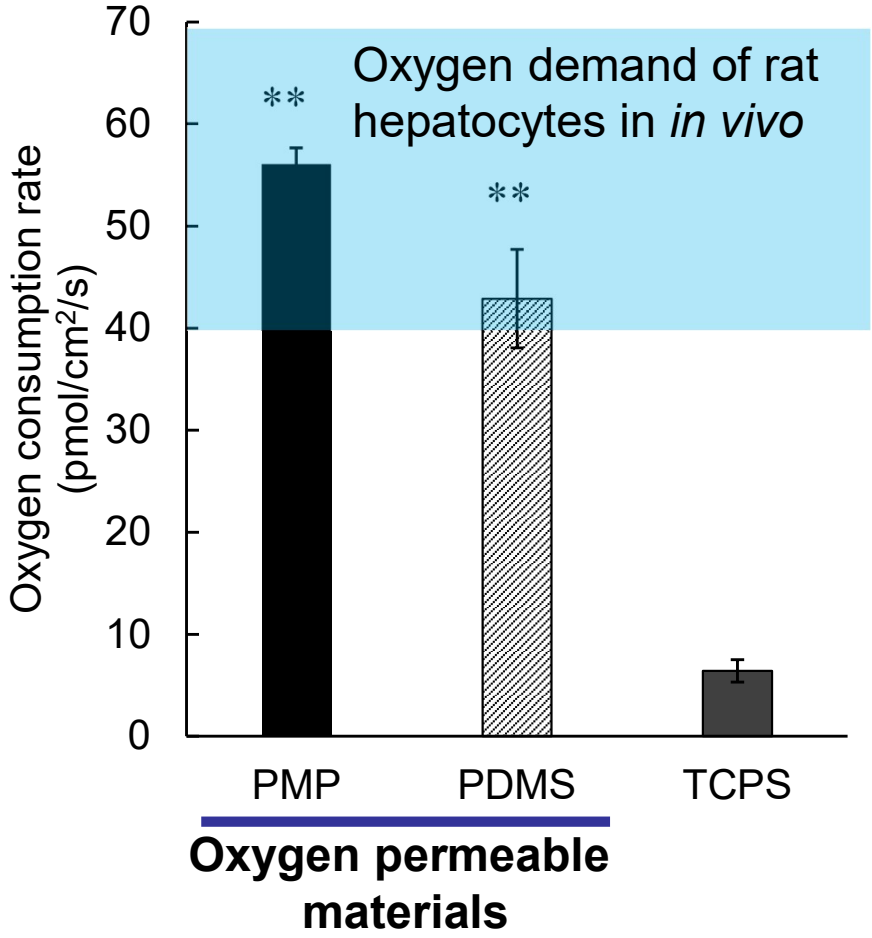
Nishikawa *et al., Front Toxicol.* 2022

Fick's low of diffusion

$$F[\mathrm{mol\ cm^2}s^{-1}] = -D\frac{(pO_{2air} - pO_{2cell})}{z}$$

Diffusion Coefficient **Concentration gradient**

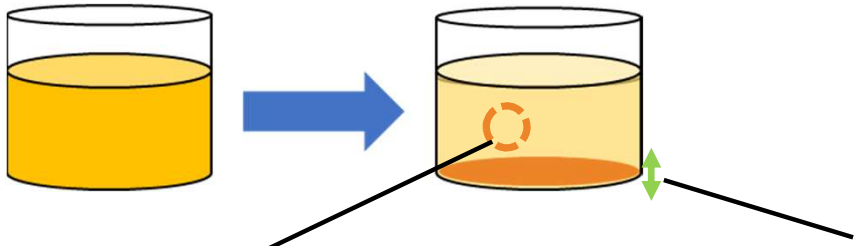




Cell culture on oxygen permeable materials can meet oxygen demand of primary hepatocytes

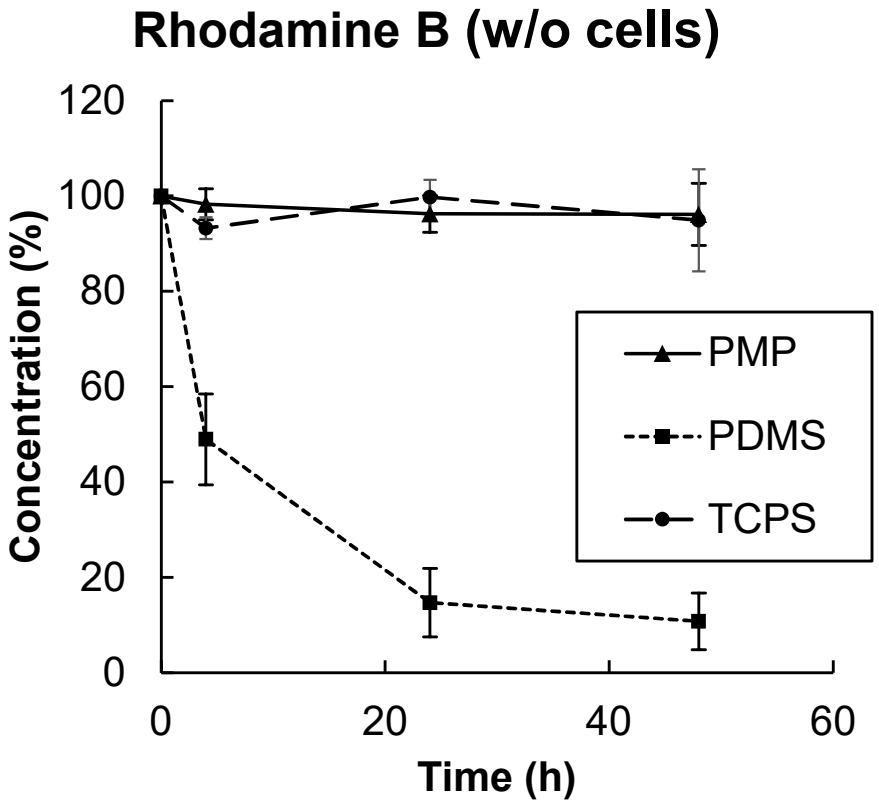
Sorption tests —Rhodamine B —

Nishikawa *et al., Front Toxicol.* 2022

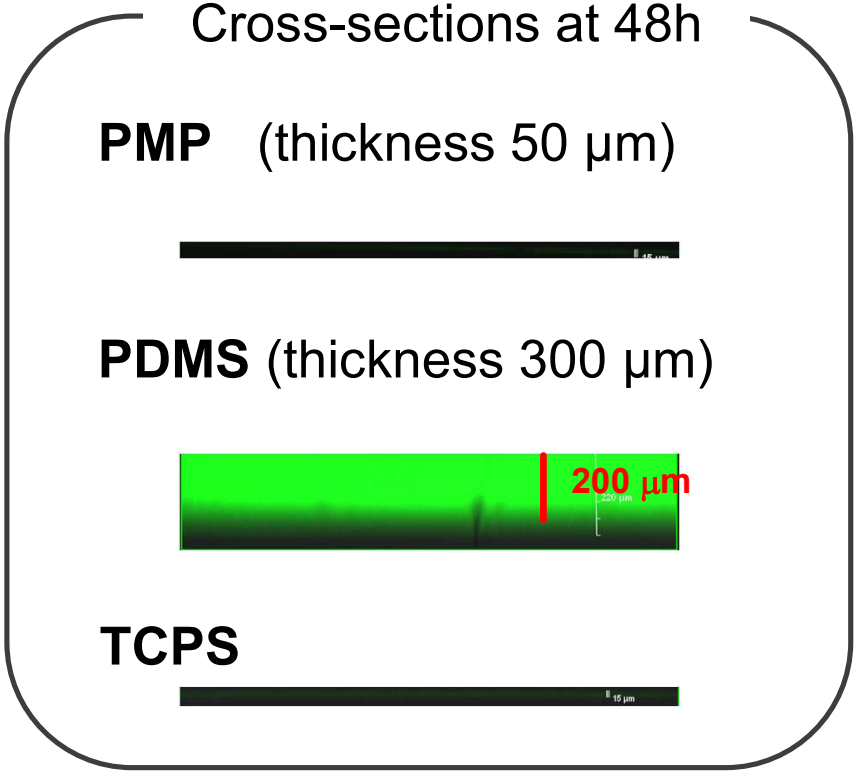


Quantify Rhodamine B conc in supernatants

Observe cross-sections by confocal microscopy



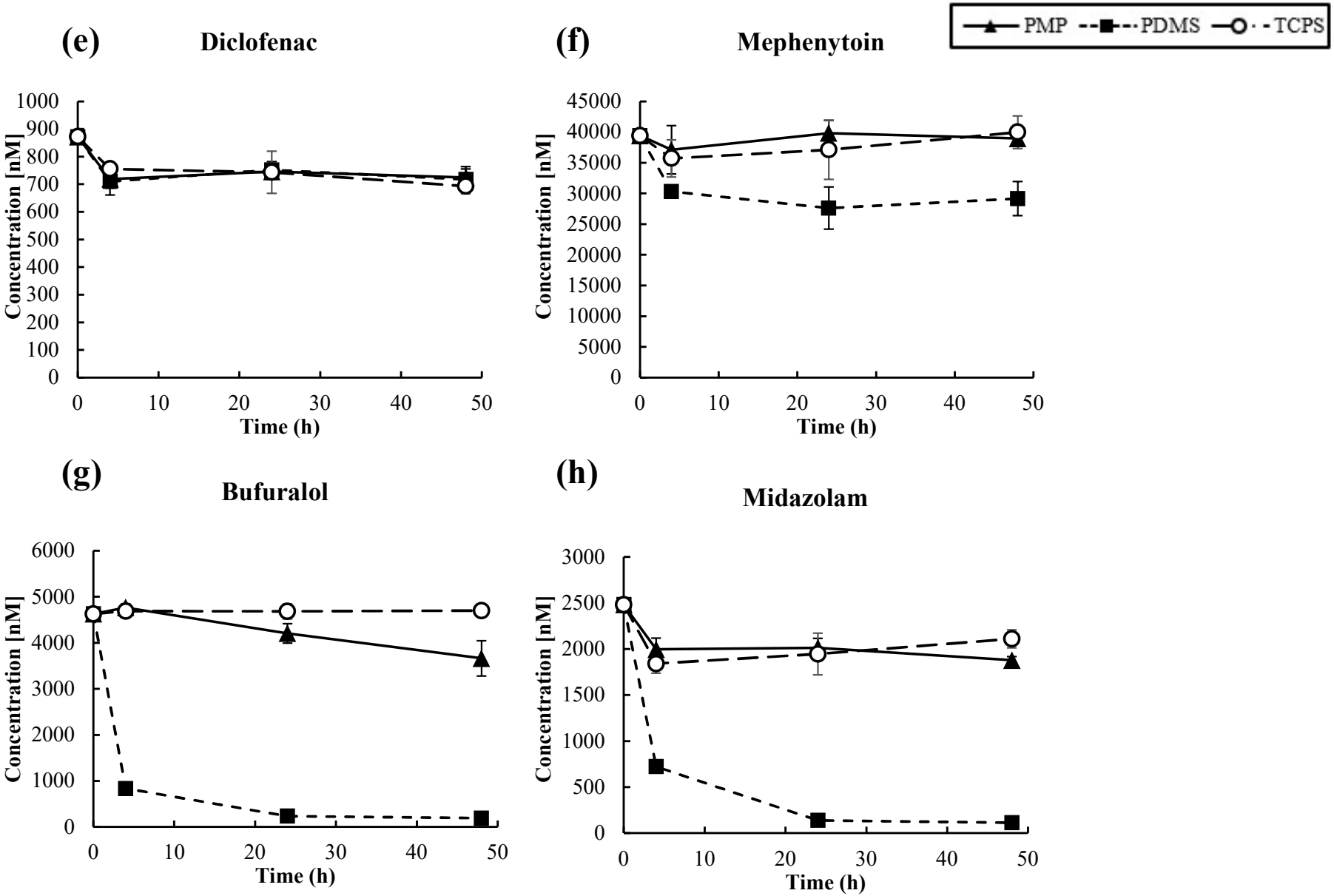
48 h: 90% sorption into PDMS



Sorption into PDMS

PMP showed almost no sorption

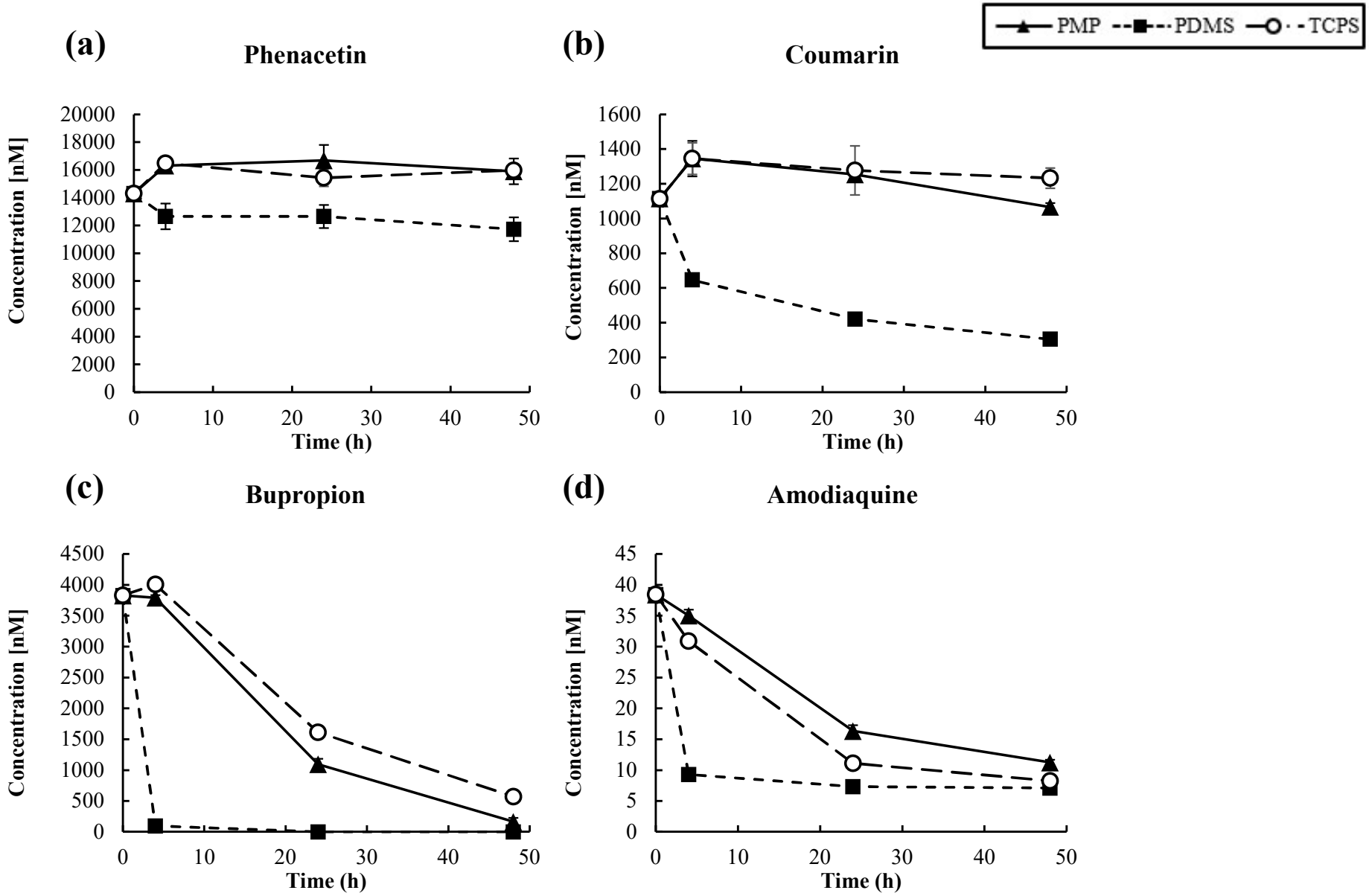
Sorption tests — CYP substrates 1 —



Sorption characteristics of PMP were almost comparable with TCPS

Nishikawa *et al., Front Toxicol.* 2022

Sorption tests — CYP substrates 2 —



Sorption characteristics of PMP were almost comparable with TCPS

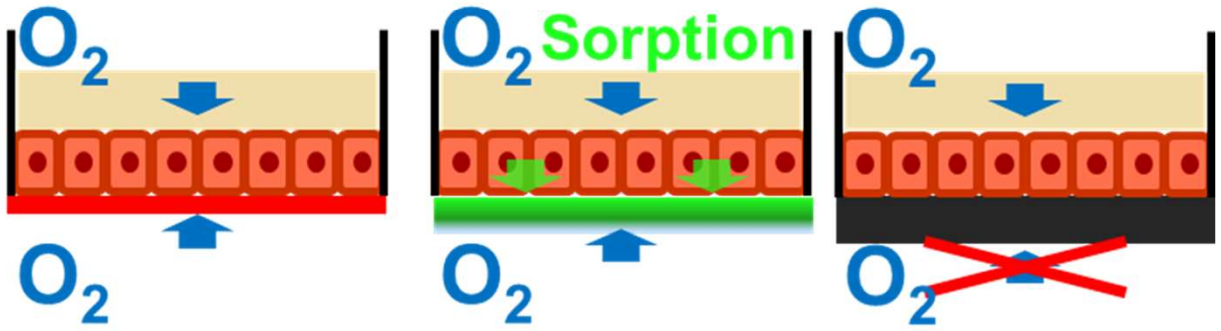
Nishikawa *et al., Front Toxicol.* 2022

Hepatocyte culture and functions

➢ Cell culture conditions

- ✓ Primary rat hepatocytes (1E5 cells/cm^2) were cultured for 7 days
- ✓ PMP and PDMS cultures were in 10% O_2, TCPS culture was in 20% O_2
- ✓ Albumin secretion, gene expression and CYP activities were measured

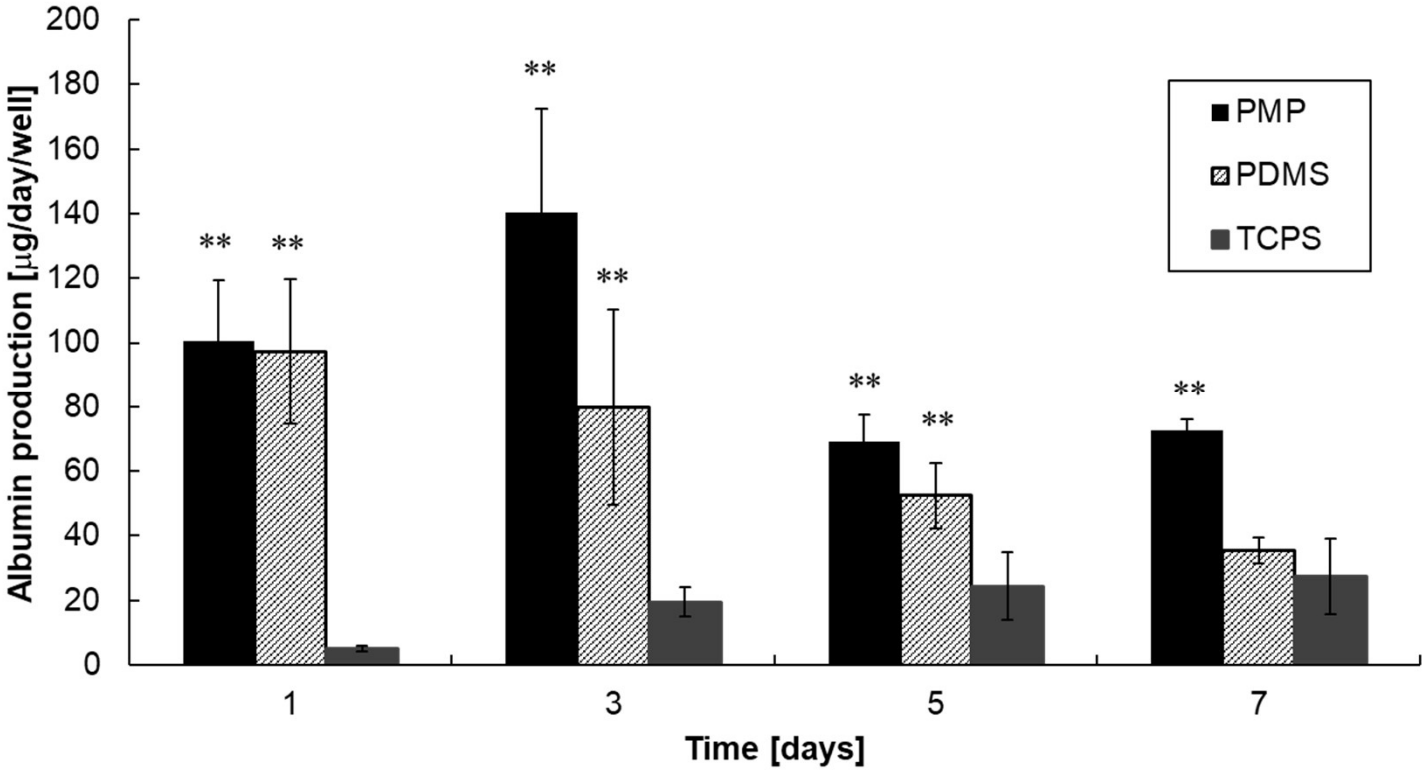
	PMP	PDMS	TCPS
O_2	+	+	—
Sorption	—	+	—

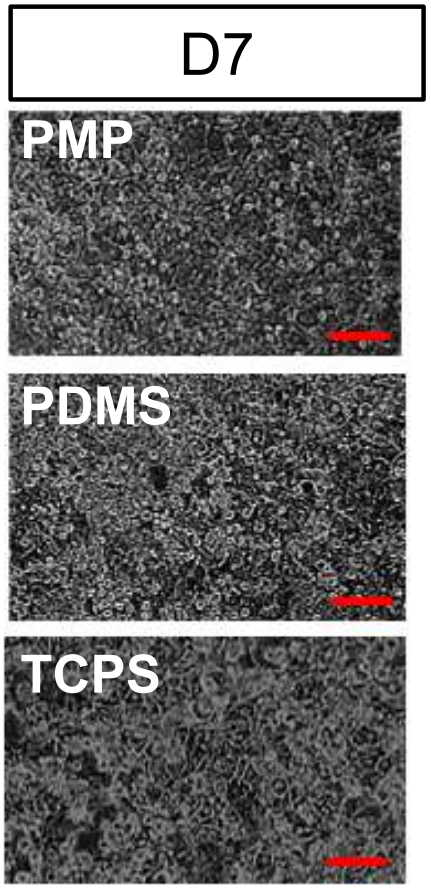


Hepatocyte culture and functions

Nishikawa *et al., Front Toxicol.* 2022

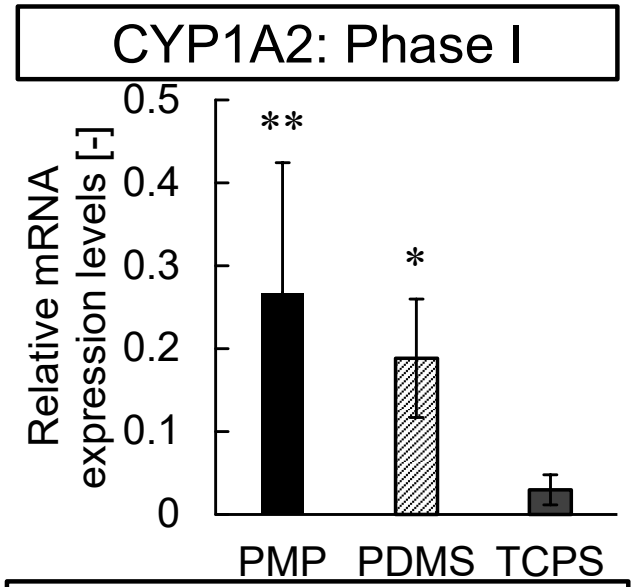
Albumin production rates were measured by ELISA

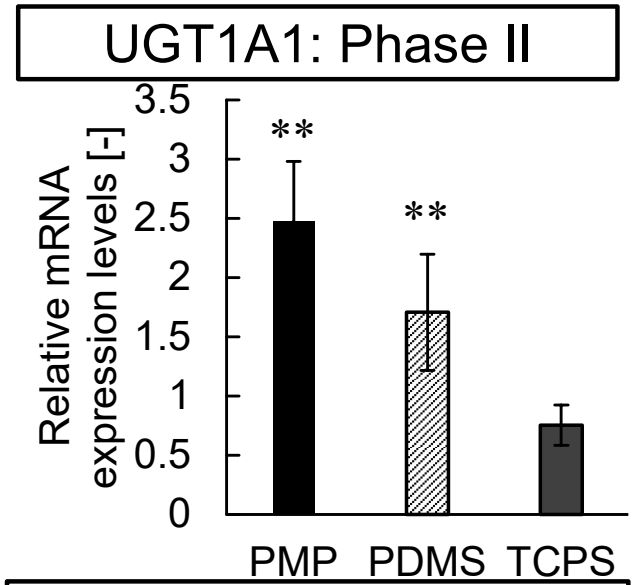


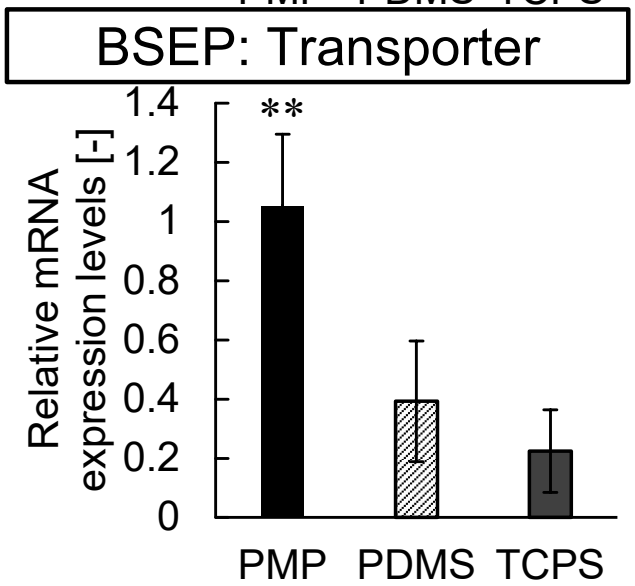


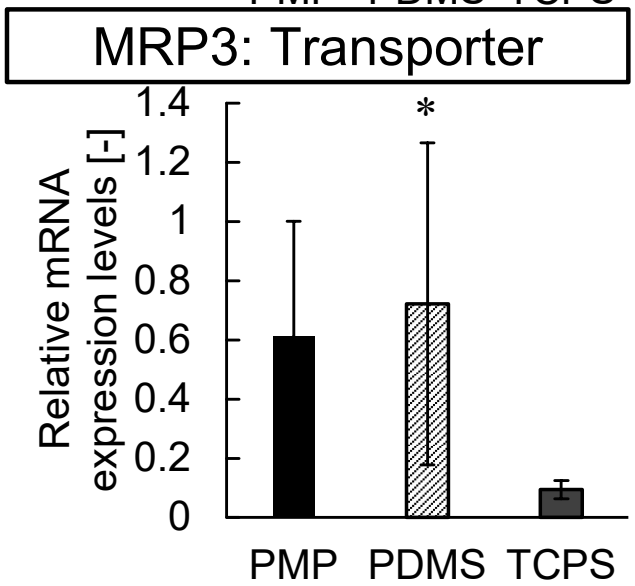
PMP maintained higher albumin production and complete monolayer for 7 days

Expressions of genes related to drug metabolisms







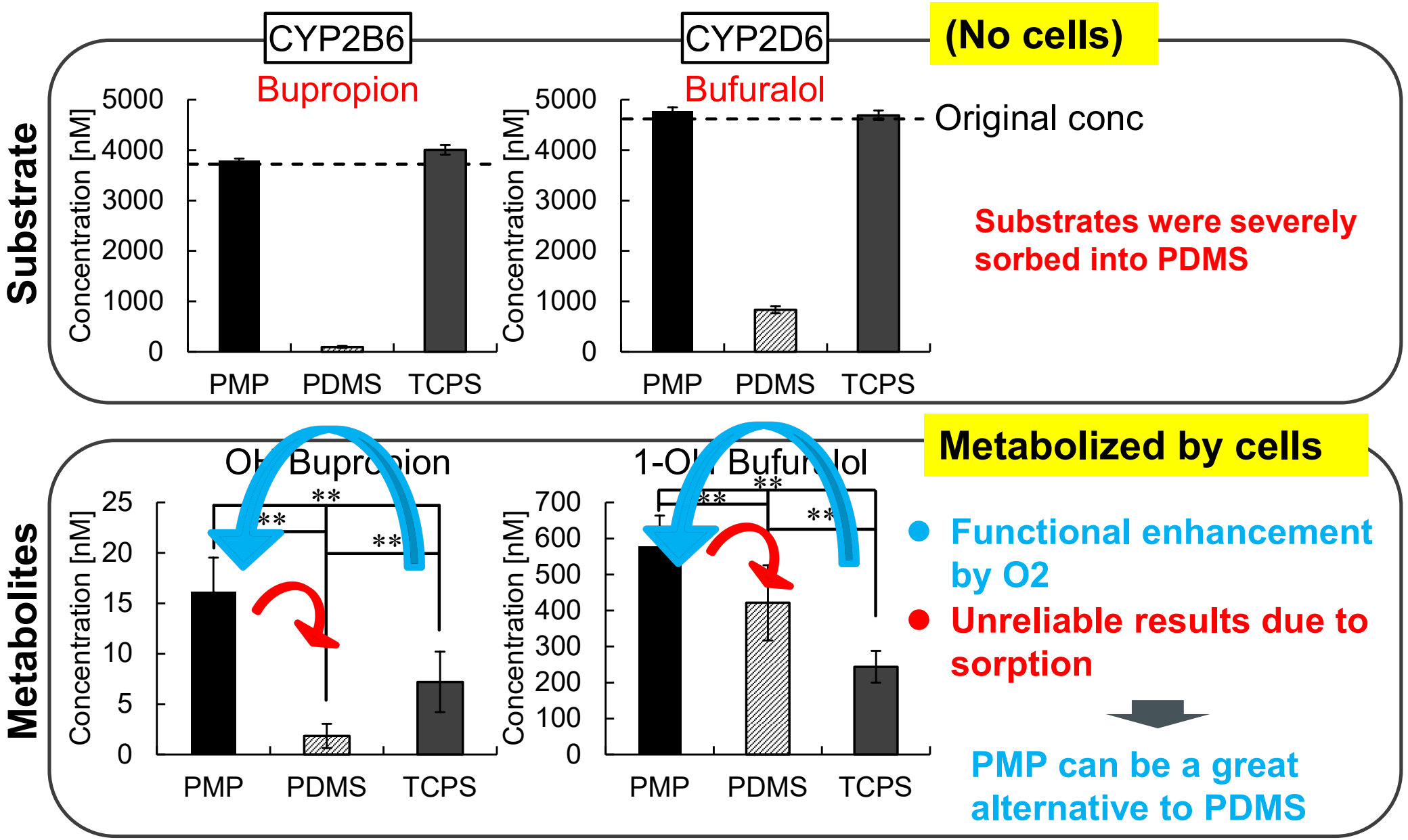


Enhanced by oxygen supply

Nishikawa *et al., Front Toxicol.* 2022

CYP activity tests

Selected results of LC-MS measurements 4 h after substrates exposure



Nishikawa *et al., Front Toxicol.* 2022

Summary of hepatocyte culture on PMP

Sorption to PDMS ➡ PMP

- **High O_2 permeability led to higher cell functions**
- **Low sorption characteristics held test chemicals in medium**
- **The both characteristics made CYP activity tests more reliable**

➡ Accurate evaluation of (rat) hepatocyte metabolism

Nishikawa *et al., Front Toxicol.* Jun 6;4:810478. 2022

Hepatocytes

- **Gold standard** cells available in the market

  Human cryopreserved hepatocytes

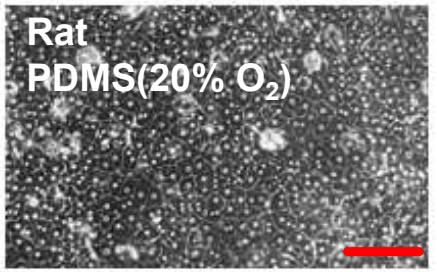
➡ Investigated the effects of direct oxygenation using PDMS

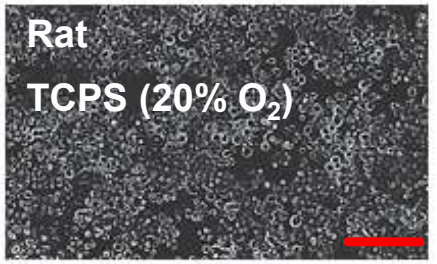
Fresh vs Cryopreserved (Attachment rates)

Fresh rat hepatocytes

More O_2 demands during attachment

➡Direct oxygenation **improved**
the attachment rate (and hepatic function)

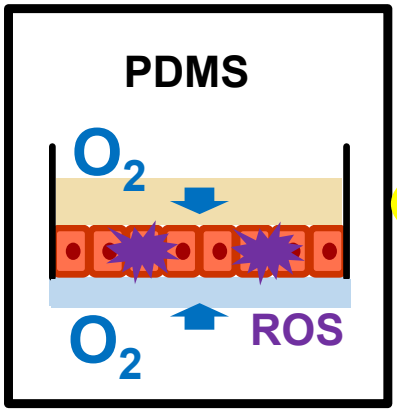


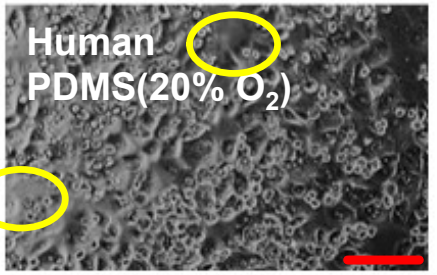


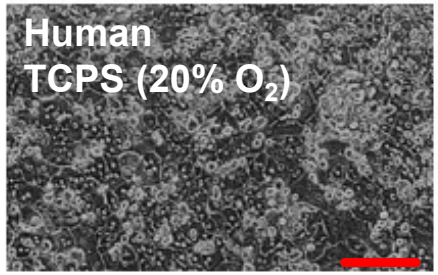
Cryopreserved human hepatocytes

More O_2 demands during attachment

➡Direct oxygenation **exacerbated**
the attachment rate







Hypothesis :

➡Damaged mitochondria during cryopreservation

➡ROS production

O : detached

Scale = 100μm

Modification of inoculation conditions: Reagents and O_2 supply

Inoculation:
Human cryopreserved hepatocytes (2E5 cells/cm²)

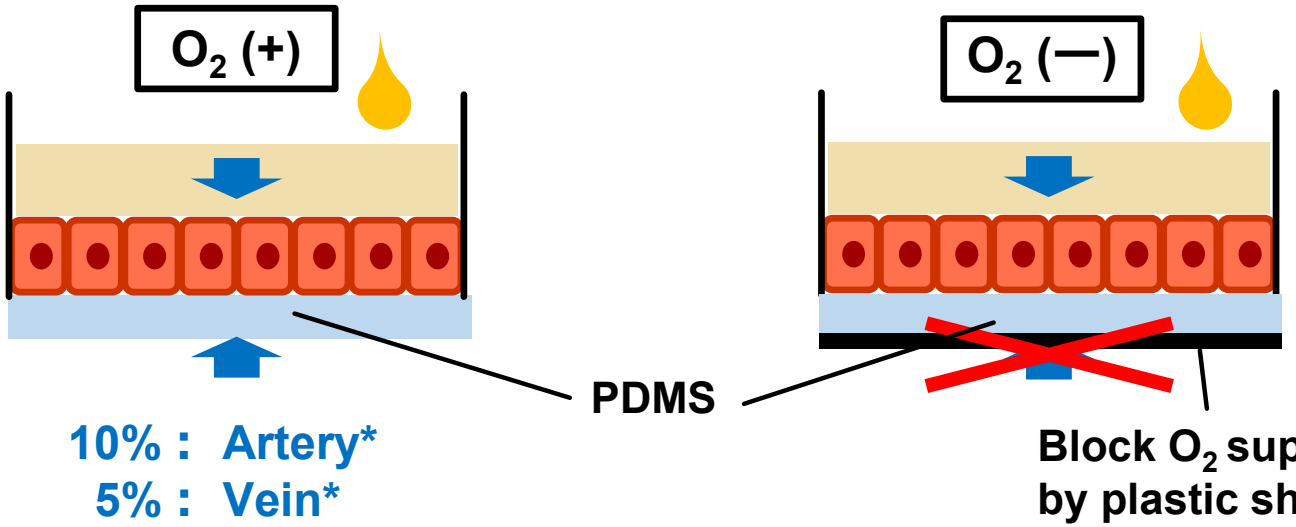
Reagents
Suppress
ROS and Apoptosis

Reagents	Effects
—	—
N-Acetylcysteine	Anti-oxidant
2-Mercaptoethanol	Anti-oxidant
Z-VAD-FMK	Apoptosis inhibitor
Y27632	Apoptosis inhibitor

** added only 12 hrs

O_2 supply
- Meet the demand
- Suppress ROS

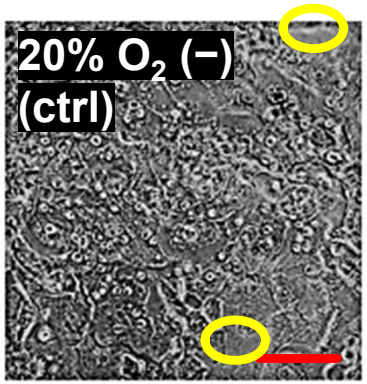
O_2: 20%, 10%, 5%



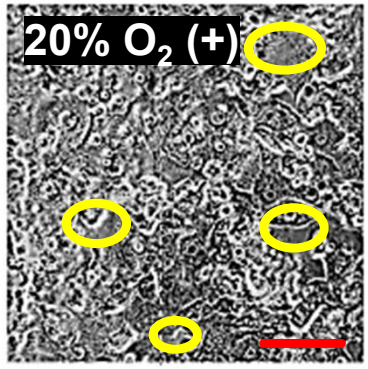
*Scheidecker, *et al.*, *Front Bioeng Biotechnol*. 8:524, 2020

Effects of reagents and O_2 supply on the cell attachment

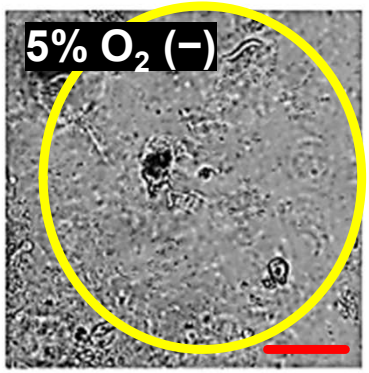
O : detachment Scale: 100μm



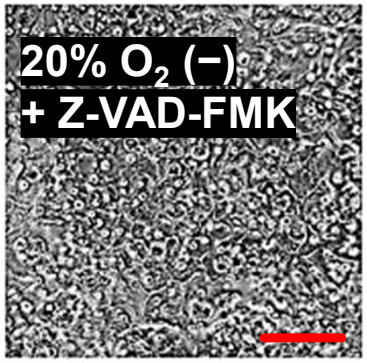
Common condition
➡partly detached

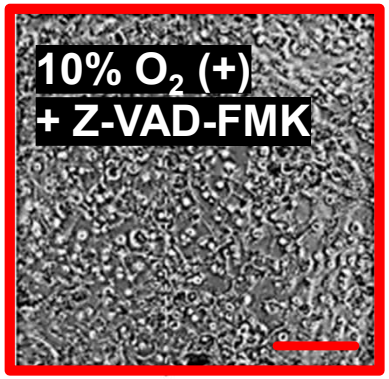


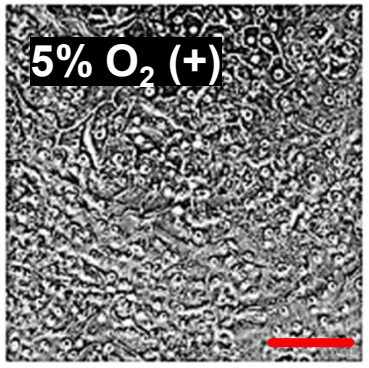
More O_2 supply
➡More detachment



Less O_2 supply
➡No attachment



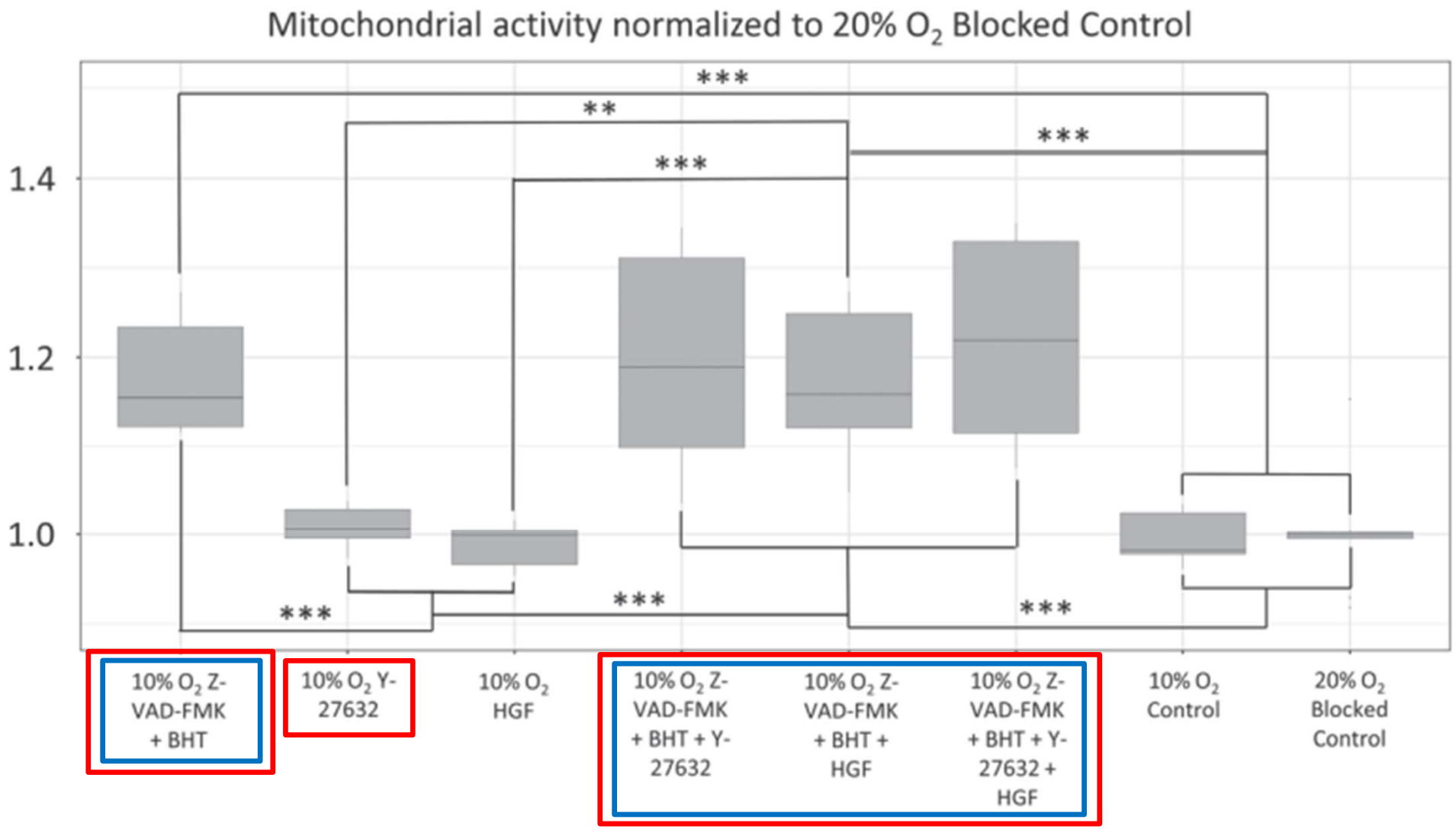




Apoptosis inhibitor + Direct O_2 supply (10%: Artery level)
➡ Complete monolayer

Controlled O_2 supply and Apoptosis inhibitors improved the cell attachment

Effects of reagents and O_2 supply on mitochondrial activity



Concomitant use of anti-oxidants during attachment improved mitochondrial activity

Danoy, *et al., Fundam Toxicol Sci.* 9(4), 135-144, 2022.

Summary so far…

PMP

- High O_2 permeability
- Low sorption characteristics

➡ More reliable tests for hepatocyte metabolism

Nishikawa *et al., Front Toxicol.* Jun 6;4:810478. 2022

➕

Oxidative stress during initial attachment

Human cryopreserved hepatocytes

➡ short time use of anti-oxidants & apoptosis inhibitors
improved cell attachment and function

Danoy, *et al., Fundam Toxicol Sci.* 9(4), 135-144, 2022

⬇

Accurate evaluation of human hepatocyte metabolism

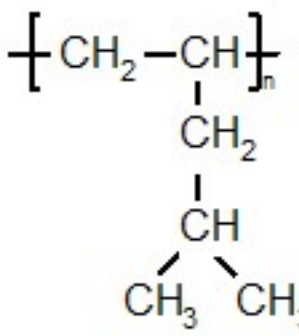
InnoCell

Mitsui Chemicals

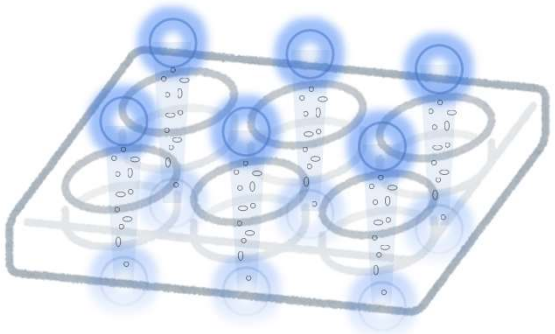
Nishikawa, *et al.*, ***Front Toxicol.,*** 2022

- Excellent cell observability
- High oxygen supply to cell
- Low drug adsorption
- Excellent cell adhesion

PMP



poly(4-methyl-1-pentene)



High O_2 permeability

6 well

24 well

96 well

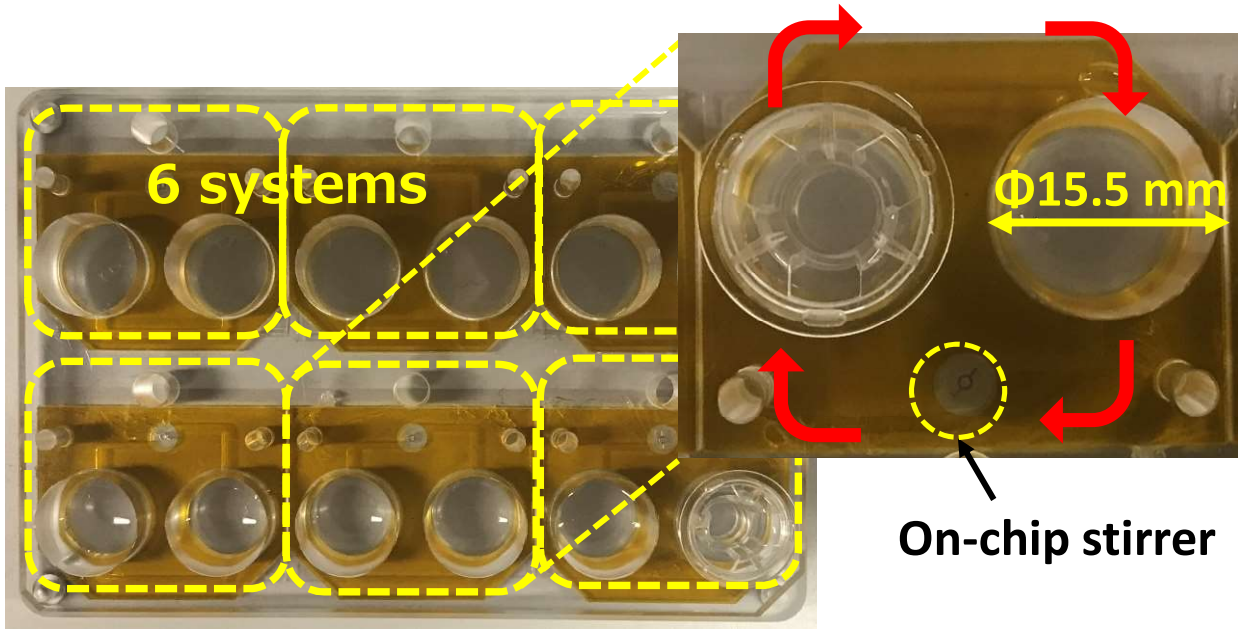
INCP0001

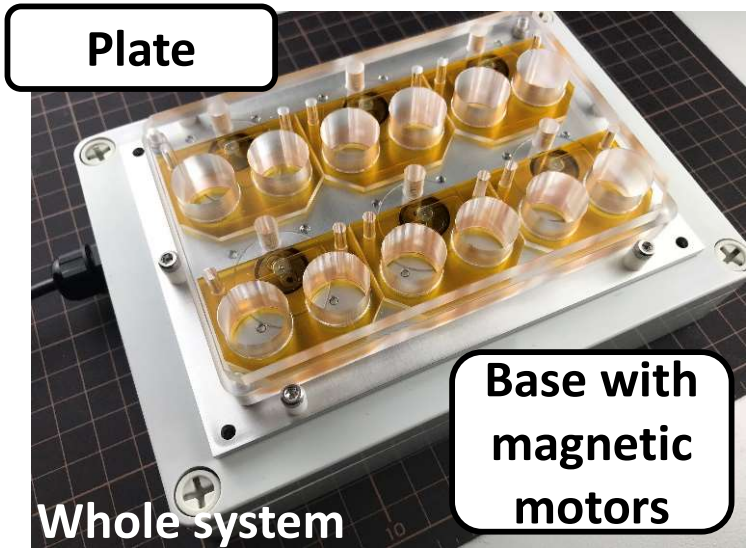
PMP + on-chip perfusion

Kinetic Pump Integrated Microfluidic (KIM) Plate

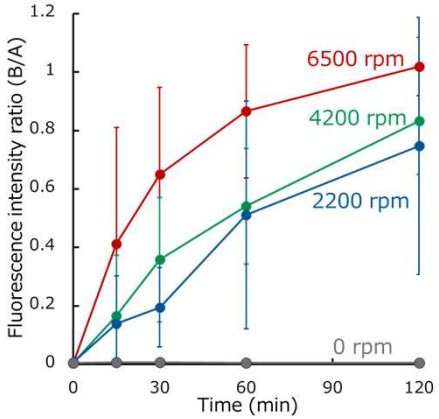
Shinha K et al, Micromachines 12, 1007 (2021)

※Patent# 2020-065197





On-chip-stirrer perfusion



Commercialization by Sumitomo Bakelite Co.

- **Open access**
  - ➡ easy handling
- **SBS standards**
- **Compatible with other commercially available cell culture wares**
- **On-chip stirrer**
  - ➡ no connection tubes
- **O2 supply through PMP**
  - ➡ High cell density

Kinetic Pump Integrated Microfluidic (KIM) Plate

SUMITOMO BAKELITE CO., LTD.

Shinha K et al, Micromachines 12, 1007 (2021)

Kimura Lab @Tokai Univ.

Oxygen supply is a fundamental requirement

Oxygen

Interaction

Simulate *in vivo* human responses

Function

ATP

Production **Consumption**

GLC

O_2

LAC

ATP

ATP

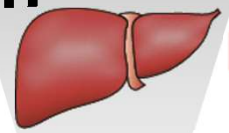
O_2

Anaerobic
2ATP

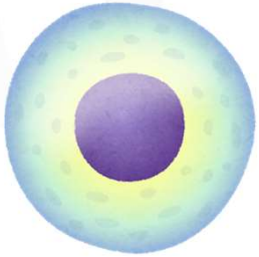
Aerobic
36ATP

Body

Organ



Cell



Energy Metabolism

Summary

PMP

- High O_2 permeability
- Low sorption characteristics

➡ More reliable tests for hepatocyte metabolism

Nishikawa *et al., Front Toxicol.* Jun 6;4:810478. 2022

Cryopreserved cells

Human cryopreserved hepatocytes

➡ short time use of anti-oxidants & apoptosis inhibitors

Danoy, *et al., Fundam Toxicol Sci.* 9(4), 135-144, 2022

Accurate evaluation of human hepatocyte metabolism

Sufficient oxygen supply is a fundamental requirement to ensure physiological responses in vitro

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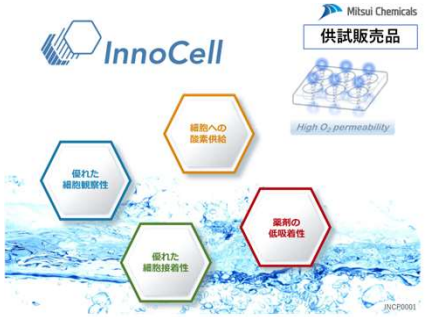
- Mistui Chem,
- Meiji, etc

AMED



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