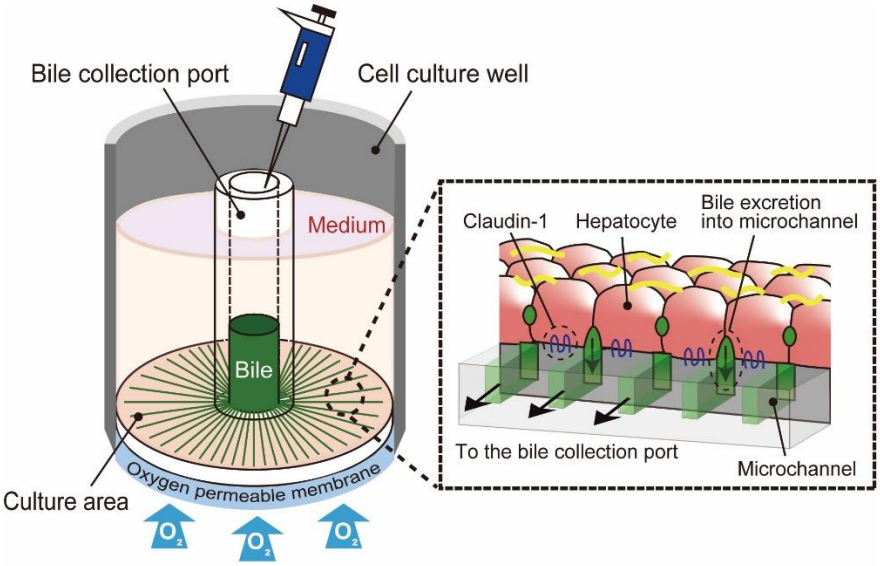
April 1, 2026

Graduate School of Engineering, The University of Tokyo
Nagoya City University
Kanazawa University
Mitsui Chemicals, Inc.

A Novel Hepatocyte Culture Device Enabling Continuous Bile Excretion In Vitro

Key points of the presentation

- Using microfabrication technology and cell polarity control technology, we have developed a novel hepatocyte culture device that enables continuous excretion of bile components secreted by cultured hepatocytes into the extracellular space and the non-invasive recovery of such components.
- Using this method, we succeeded in recovering biliary metabolites at a concentration 13.7 times higher than that of conventional non-continuous and invasive bile recovery methods using hepatocyte culture.
- The continuous excretion of bile from cultured hepatocytes is a highly significant breakthrough toward the reproduction of liver physiological function *in vitro*, and is expected to be applied to drug discovery and liver disease research.



Novel Hepatocyte Culture Device Enables Continuous Excretion of Bile Components Secreted from Cultured Hepatocytes

Overview

A research group consisting of Professor Yasuyuki Sakai, Associate Professor Masaki Nishikawa, and Project Researcher Fumiya Tokito from the Graduate School of Engineering of the University of Tokyo, Professor Hiroyuki Kusuhara from the Graduate School of Pharmaceutical Sciences of the University of Tokyo, Professor Hiroshi Arakawa from the Graduate School of Pharmaceutical Sciences of Nagoya City University, Professor Yukio Kato from Faculty of Pharmacy in Kanazawa University, and Director Satoshi Yamasaki of the Cell Culture Solution Department of the New Business Development Center of Mitsui Chemicals, Inc., has succeeded for the first time in generating a system that continuously excretes bile components secreted from cultured hepatocytes into microfluidic channels[*1] and recovers the bile components noninvasively.

In this research, by making full use of microfabrication techniques[*2] and technologies to control hepatocyte polarity using tight junction proteins, continuous extracellular bile excretion and noninvasive recovery were achieved, which is normally difficult with conventional culture methods. Furthermore, with this method biliary excreted metabolites were recovered at a concentration 13.7 times higher than that of conventional discontinuous and invasive bile recovery methods using hepatocyte culture.

Achieving continuous bile excretion from cultured hepatocytes in this way is a major breakthrough toward reproducing liver physiological function *in vitro*[*3] and is expected to make a significant contribution to drug discovery and liver disease research.

Contents of the presentation

Biliary excretion is a liver function essential for maintaining body homeostasis. Bile produced by hepatocytes plays an important role in lipid digestion, cholesterol metabolism, and excretion of drugs and metabolites. Generally, evaluation of bile excretion in humans is invasive and difficult. Therefore, an *in vitro* human liver culture model that can faithfully reproduce the bile excretion process is expected to be a highly useful tool for pharmacokinetic and liver disease studies. However, in conventional hepatocyte culture methods, bile only accumulates in bile canaliculi[*4] or duct-like tissues because there is no excretory outlet for secreted bile, and it is difficult for bile components to be continuously excreted out of cells and tissues as in liver tissue in vivo.

The present research group has developed a new hepatocyte culture device that can continuously excrete bile components excreted by cultured hepatocytes into a microchannel and recover the bile components by utilizing microfabrication technology (Fig. 1). The microchannel (width: 3 μm, height: 10 μm) is densely patterned on the surface of the culture part of the device, and bile components secreted by hepatocytes are excreted into the microchannel and transported to a central bile collection port. In addition, placing the device on an oxygen-permeable culture plate (InnoCell^{TM}) manufactured by Mitsui

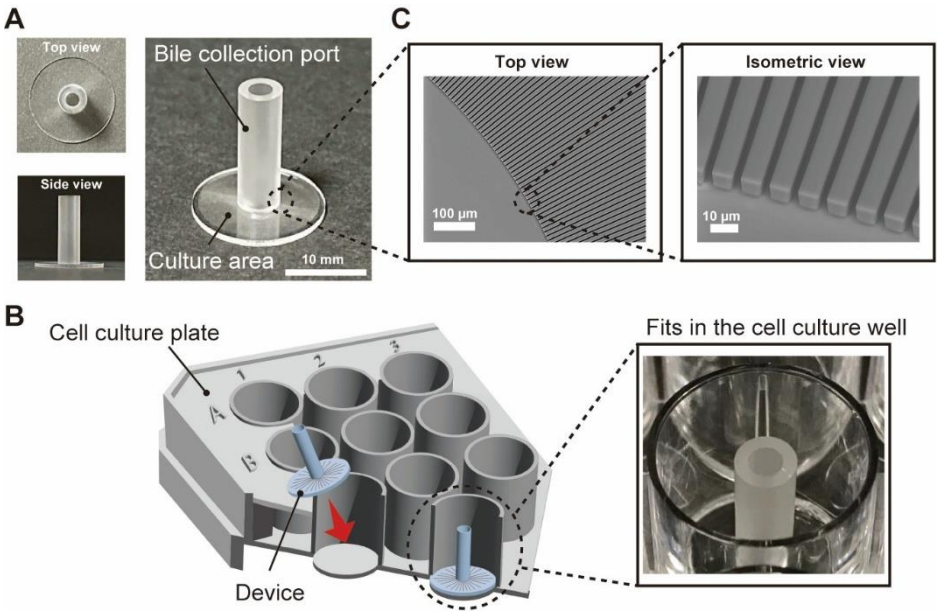


Figure 1: Hepatocyte culture device fabricated in this study

(A) External view of the culture device. (B) Installation of the culture device on a commercial cell culture plate. (C) Scanning electron microscope (SEM) image of the microchannel formed on the device culture surface.

Chemicals, Inc. enables sufficient oxygen supply to hepatocytes, which promotes the formation of bile canaliculi.

When primary rat hepatocytes and human hepatocytes (PXB-cells® and HepaSH®) were cultured on the device and exposed to fluorescently labeled bile acids, continuous excretion of the bile acids into the microchannel was confirmed (Fig. 2A), and the bile acids were successfully recovered from the bile collection port. In addition, endogenous bile acids secreted by rat and human hepatocytes were successfully recovered, and species differences in bile acid composition were observed (Fig. 2B). In addition, by coating the culture surface of the device with the hepatic tight junction protein claudin-1[*5], bile duct lumina, which are normally closed between adjacent hepatocytes, successfully formed between the hepatocytes and the culture surface (Fig. 3A). This resulted in a 1.8-fold increase in the excretion of bile metabolites into the microchannel. Conventional bile recovery methods have the problem of diluting bile excreted metabolites with a large volume of recovery fluid, but with the use of this device, bile excreted metabolites were recovered at greatly increased concentration of 13.7 fold increase(Fig. 3B).

This is the first culture system in the world in which bile components can be continuously excreted from cultured hepatocytes, and represents a major breakthrough in the reproduction of liver physiology *in vitro*. These results are expected to contribute significantly to future drug discovery and liver disease research.

This study was approved by the Office for Life Science Research Ethics and Safety, The University of Tokyo.

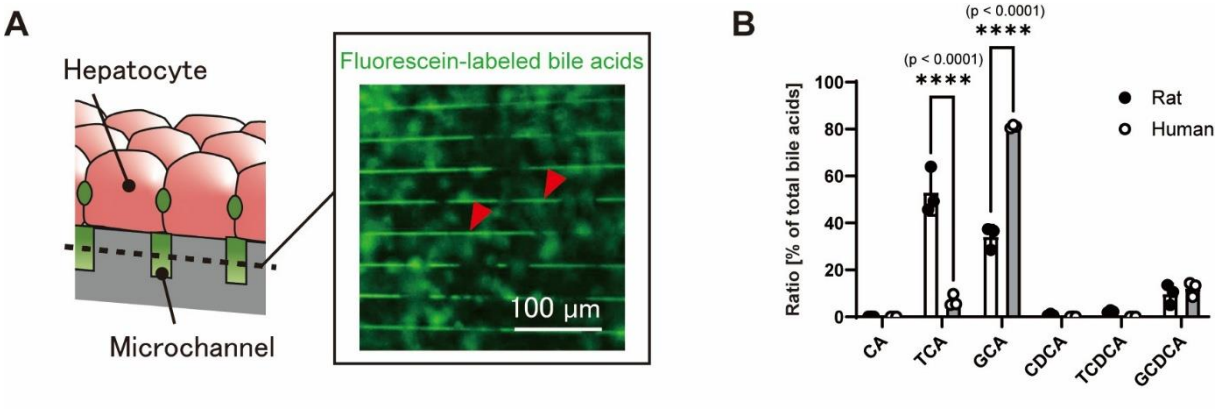


Figure 2: Bile excretion into the microchannel and species differences in recovered bile acids between humans and rats

(A) Excretion of fluorescently labeled bile acids into the microchannel. Red arrows indicate fluorescently labeled bile acids excreted into the microchannel.

(B) Bile acid composition in the bile collection port of human and rat hepatocyte cultures using the device. CA: cholic acid, TCA: taurocholic acid, GCA: glycocholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid.

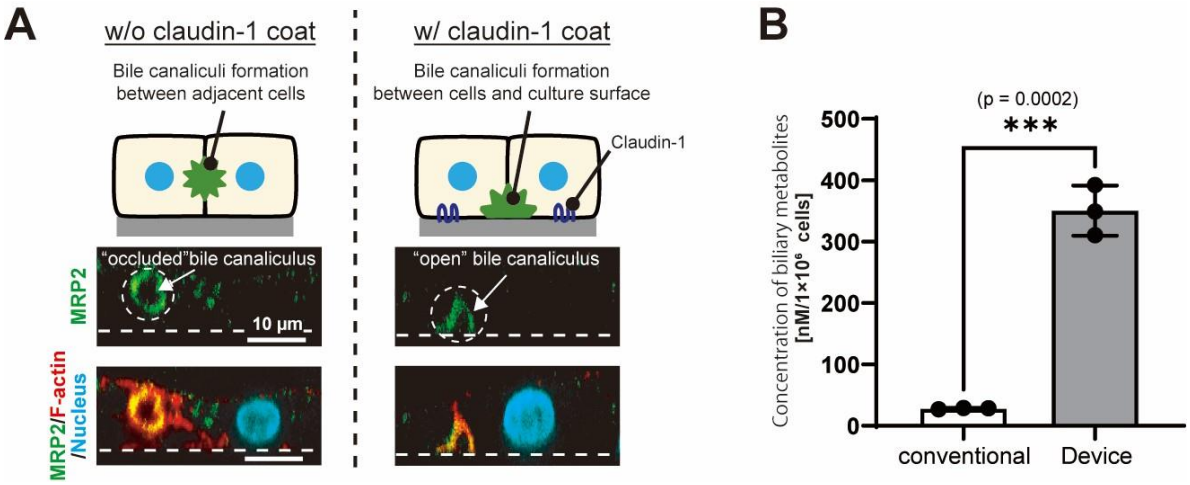


Figure 3: Control of hepatocyte polarity by claudin-1 and high recovery of biliary excretion metabolites

(A) Observation of "open " bile canaliculus formation by claudin-1 coating on the device culture surface. (B) Comparison of the recovery concentration of bile excretion metabolites between conventional invasive bile collection methods and bile collection methods using this device.

Related information:

Comparative analysis of bile canaliculi formation in fresh and flask-delivered human hepatocytes from humanized mouse livers under sufficient oxygen supply, F. Tokito, Y. Gong, D. A. Kurniawan, S. Kaneko, H. Shioda, S. Lee, A. Kushima, M. Inamatsu, C. Tateno, H. Choi, M. Nishikawa, Y. Sakai, *Fundamental Toxicological Sciences,* 11(1), 17-25 (2024)

Induction of open-form bile canaliculus formation by hepatocytes for evaluation of biliary drug excretion, H. Arakawa, Y. Nakazono, N. Matsuoka, M. Hayashi, Y. Shirasaka, A. Hirao, I. Tamai, *Communications Biology,* 6(1):866 (2023)

Presenter and Researcher Information

The University of Tokyo
Graduate School of Engineering
Professor Yasuyuki Sakai
Associate Professor Masaki Nishikawa
Project Researcher Fumiya Tokito
Professor Taichi Ito
Associate Professor Natsuko F. Inagaki
Graduate School of Pharmaceutical Sciences
Professor Hiroyuki Kusuhara

Graduate School of Pharmaceutical Sciences, Nagoya City University
Professor Hiroshi Arakawa

Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University

Professor Ikumi Tamai [*currently Emeritus Professor]
Professor Yukio Kato
Associate Professor Yusuke Masuo

Mitsui Chemicals, Inc.
Cell Culture Solutions Office, New Business Development Center
Director Satoshi Yamasaki
Manager Jingjing Yang
Advanced Materials and Solutions Laboratory, Research Headquarters
Manager Katsuhiro Esashika

Article Information

Journal: Cell Reports Physical Science
Title: Continuous Bile Excretion from Polarized Hepatocytes on a Microchannel-Patterned Device
Authors: Fumiya Tokito, Hiroshi Arakawa, Yuya Nakazono, Mikito Kiyofuji, Yang Liang, Fukino Kono, Mathieu Danoy, Hyunjin Choi, Ya Gong, Katsuhiro Esashika, Jingjing Yang, Satoshi Yamasaki, Natsuko F. Inagaki, Taichi Ito, Kenpei Tai, Hiroyuki Kusuhara, Yusuke Masuo, Yukio Kato, Ikumi Tamai, Takeshi Katsuda, Masaki Nishikawa, and Yasuyuki Sakai
DOI: 10.1016/j.xcrp.2026.103229
URL: https://www.cell.com/cell-reports-physical-science/fulltext/S2666-3864(26)00135-9

Research grants

This study was supported by the Japanese Agency for Medical Research and Development under Grant Nos. 22be1004201h0001 and 25bk0104189h0001; JSPS KAKENHI under Grant No. JP 23KJ0541; JST A-STEP under Grant No. JPMJTR24UC; and the Research Support Project for Life Science and Drug Discovery (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from the AMED under Grant No. JP25ama121051.

Glossary

(* 1) Microchannel: A microchannel fabricated on a plastic or rubber substrate using microfabrication technology. It is used to precisely flow liquids and perform chemical and biological operations such as mixing, reaction, and separation on a very small scale.

(* 2) Microfabrication technology: A technology that forms a micrometer-sized (1/1000 of 1 mm) precise shape or structure on the surface or inside of a material.

(* 3) *In vitro*: In vitro. It refers to an artificial environment such as in a test tube or an incubator.

(* 4) Bile canaliculus: A duct formed between adjacent hepatocytes that carries bile secreted by hepatocytes.

(* 5) Claudin-1: A type of membrane protein that tightly adheres cells to each other and seals gaps between epithelial cells, including hepatocytes.