

Public-private cooperative study supported by grant in aid from  
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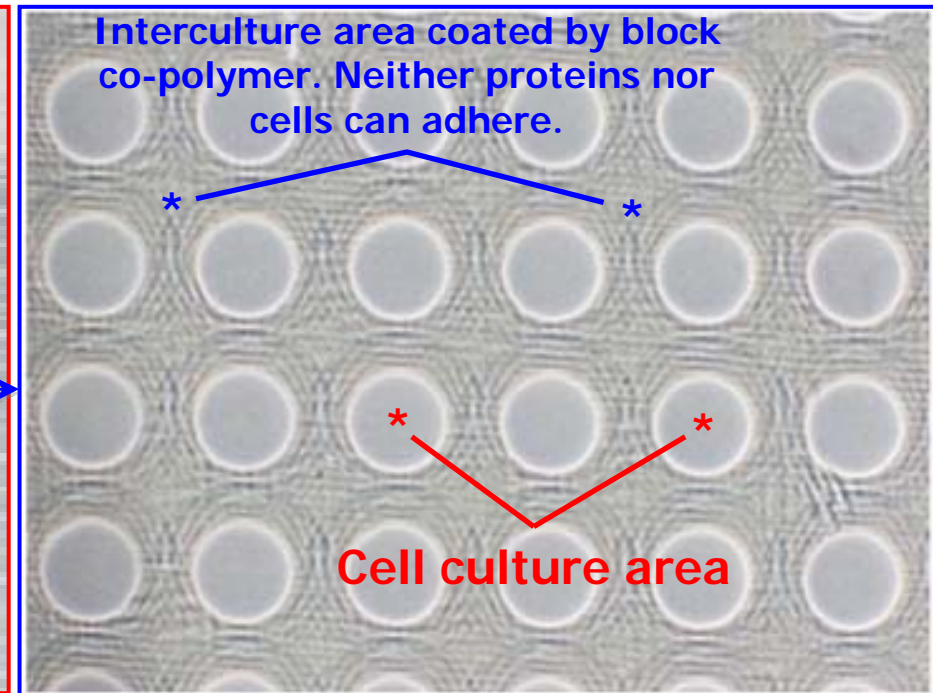
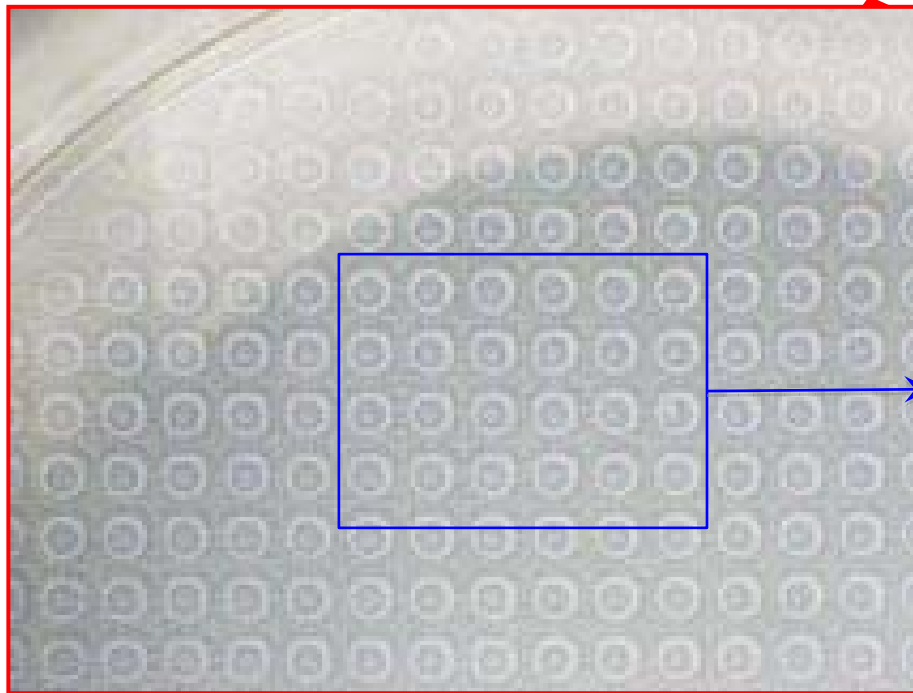
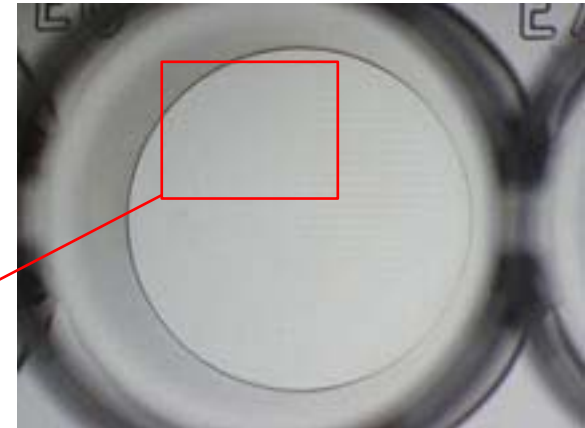
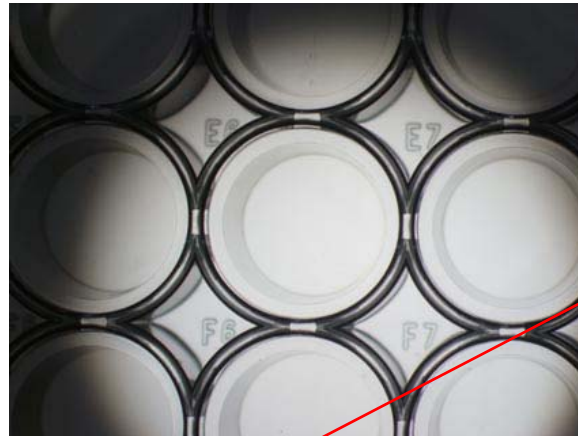
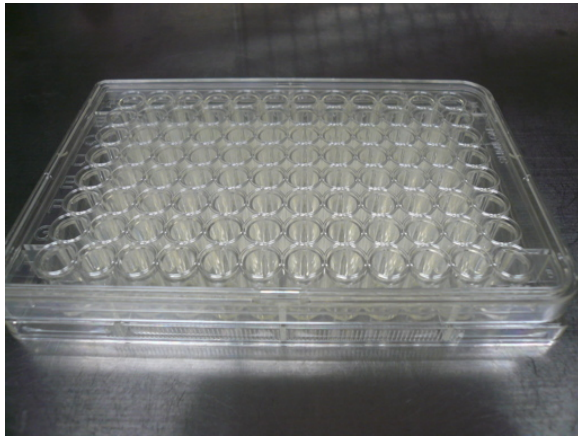
# **HUMAN HEPATOCYTE 3D CULTURE ON CELL-ABLE USING NEWLY OPTIMIZED MEDIUM AND ITS FUNCTIONAL EVALUATION**

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Emiko Ozeki<sup>2</sup>, Takeshi Ikeya<sup>1,2</sup>**

- 1. National Center for Child Health and Development**
- 2. Transparent Inc.**

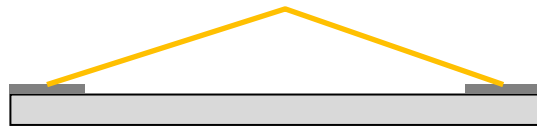
**26th JSSX Annual Meeting, Nov 16 to 18, 2011, Hiroshima**

# Outline of Cell-Able

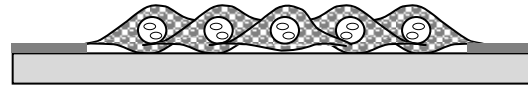


# Standard Protocol of Primary Hepatocyte Culture with Feeder Cells

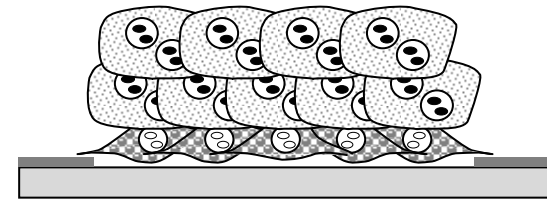
① Plate surface coated with Block co-polymer



② Feeder Cells - Optional



③ Feeder (optional) + Hepatocytes

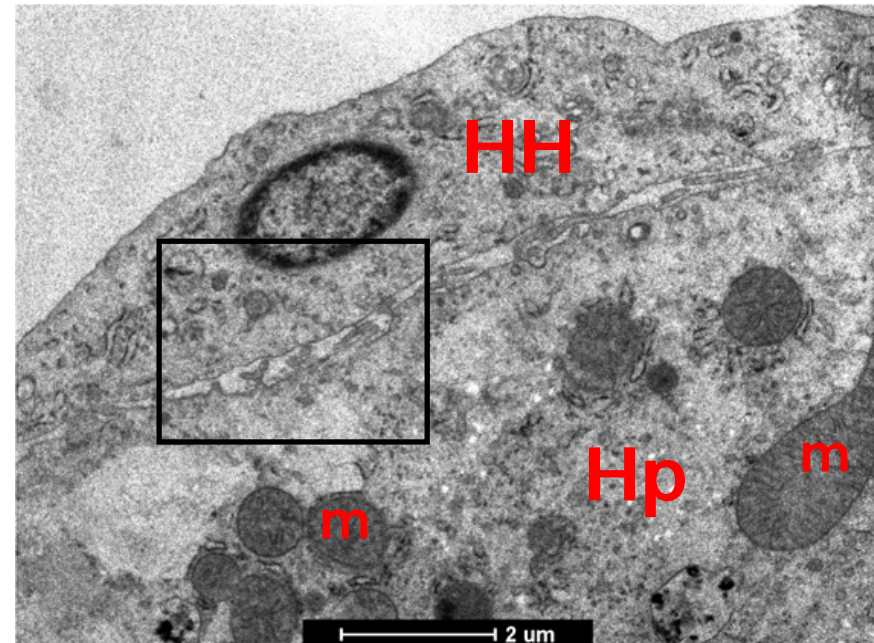
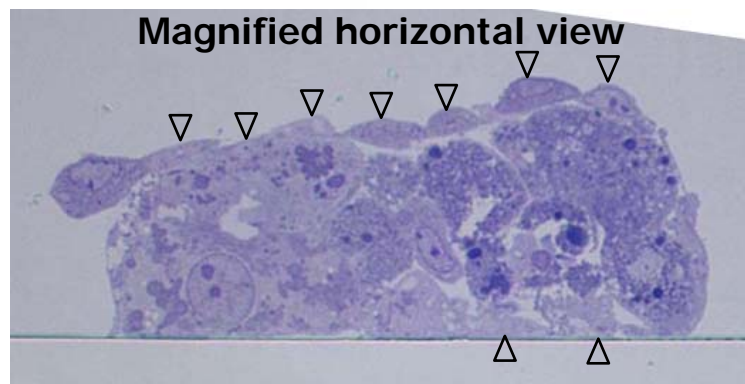
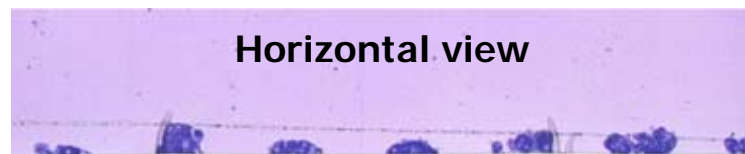
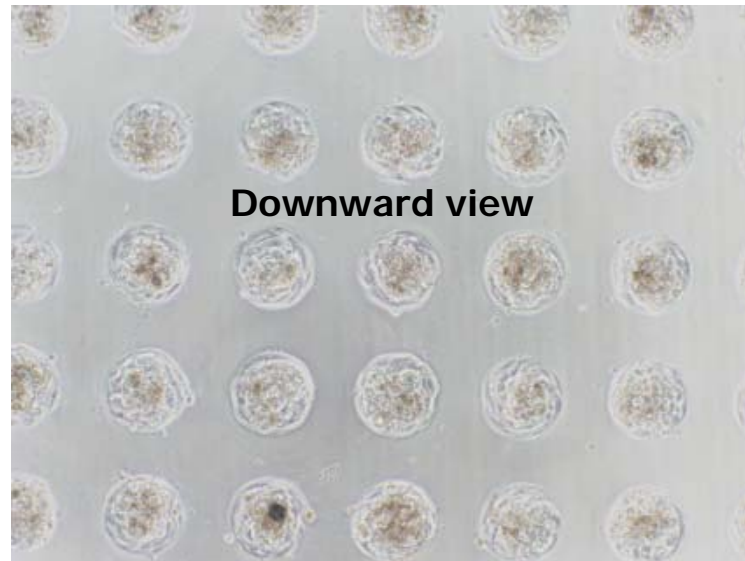


**Advantage of co-culture with feeder cells**

- Long-lasting CYP activities
- Cryopreserved hepatocyte with low attaching capability can be cultured

**Optimization of culture medium enabled long-lasting CYP activities in feeder-free culture (see below)**

# Microstructure of Hepatocyte-feeder cell heterospheroids formed on the Cell-able



Diesse space-like structure was observed between hepatocyte (Hp) and feeder (HH) cell with microvilli rooted from hepatocyte was observed. m; mitochondria.

Arrow heads indicate HH cells that migrate from the culture plate and enwrap spheroidal hepatocyte mass.

# Materials and Methods

## [Hepatocytes]

**Fresh**; isolated from surgically resected liver in National Center for Child Health and Development (IRB permission No.385, 396)

**Cryopreserved**; Xenotech (799, HC2-6, HC5-7), IVT (TSF, GHA)

## [Feeder cells]

**HH** bovine aortic epithelial cells (JCRB0099), **Mouse 3T3** fibroblasts (ATCC CCL-92, ATCC CCL-163), **Rhesus monkey retinal epithelial cells** (ATCC CRL-1780)

## [Culture medium]

**RM100**; medium for rat hepatocytes (Transparent)

**RM101**; Medium for human hepatocytes (Transparent)

**SE & YY**; Williams E-base Matrigel-containing medium (reported by Enosawa and Yamada in JSSX2009, Kyoto)

**IVT**; InVitroGRO HI Medium

**XENOTECH**; Hepatocyte culture media

**BD**; BD Hepatocyte Culture Medium Kit

## [Culture]

$2 \times 10^4$  human hepatocytes /one well of 96-well type Cell-able

When feeder cells were used,  $8 \times 10^3$  cells / one well of 96-well type Cell-able were seeded two days before hepatocytes inoculation. In addition, cryopreserved feeder cell-seeded plates were also used.

## [CYP activity]

Hepatocytes were incubated with 100 micro-mol/L **testosterone** or **phenacetin** for 3 hrs. Formation of metabolites (6 beta hydroxytestosterone, testosterone glucuronide, acetaminophen) were determined by UPLC (Waters). CYP induction was performed by 72-hr incubation with **rifampicin** (25 micro-mol/L) or **omeprazole** (5 micro-mol/L).

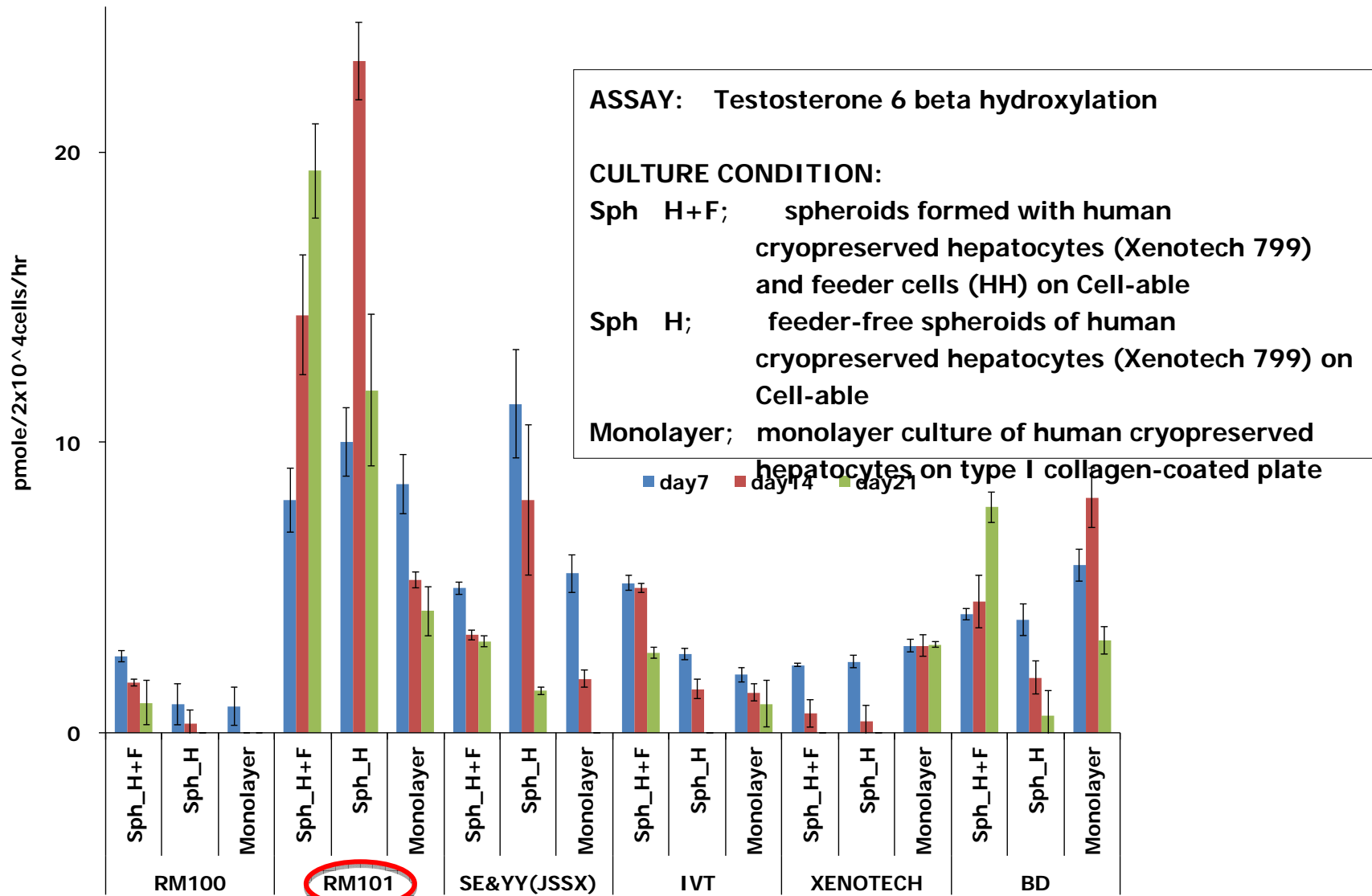
## [Transporter activities]

**Influx 1**; tritiated ([ $^3\text{H}$ (G)]) **taurocholate** (1 micro-mol/L) as a substrate and Rifamycin SV (100 micro-mol/L) as an inhibitor.

**Influx 2**; tritiated ([ $^6,7\text{-}^3\text{H}$ (N)]-**estrone** sulfate (1 micro-mol/L) as a substrate and taurocholate (100 micro-mol/L) as an inhibitor.

**Efflux**; carboxy-dichlorofluorescein diacetate (**CDF-DA**) (10 micro-mol/L)

# Optimum Culture Medium



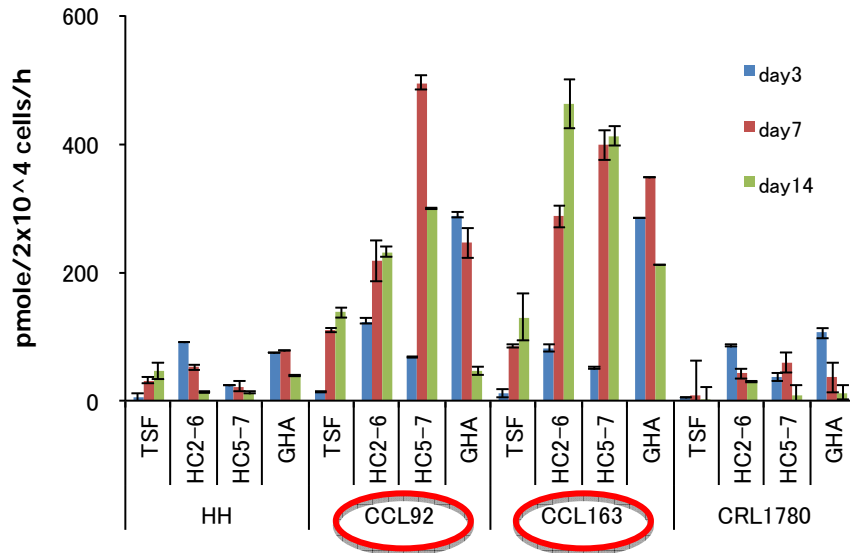
**RECOMMENDED**

RM101 (Transparent) showed Excellent CYP activities even with feeder-free culture

# Optimum feeder cell

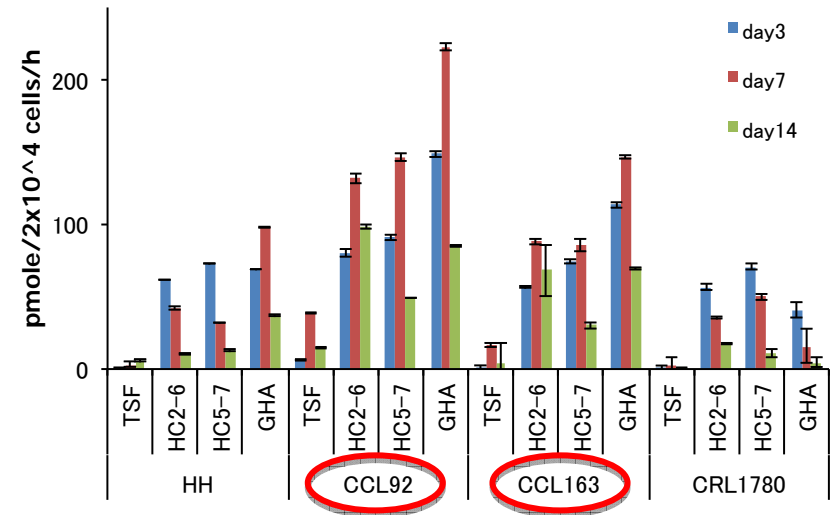
Hepatocytes; TSF, HC2-6, HC5-7, GHA

Comparison of feeder cells (basal activity)  
Testosterone → 6βHydroxytestosterone



**RECOMMENDED**

Comparison of feeder cells (basal activity)  
Testosterone → Testosterone glucuronide



**RECOMMENDED**

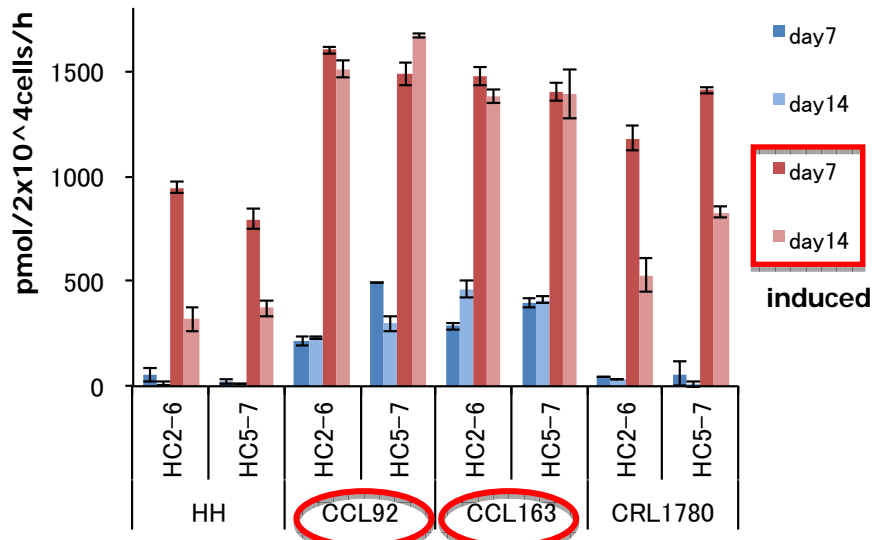
Cell lines examined		
Designation	Code No.	Origin
HH	JCRB0099	Bovine aortic epithelium
3T3-Swiss albino	ATCC CCL-92 (JCRB9019*)	Mouse fibroblast
BALB/3T3 clone A31	ATCC CCL-163	Mouse fibroblast
RF/6A	ATCC CRL-1780	Rhesus monkey retinal epithelium

\*ATCC CCL-92 is also distributed by JCRB as JCRB9019 in Japan

# Optimum feeder cell (Induction study)

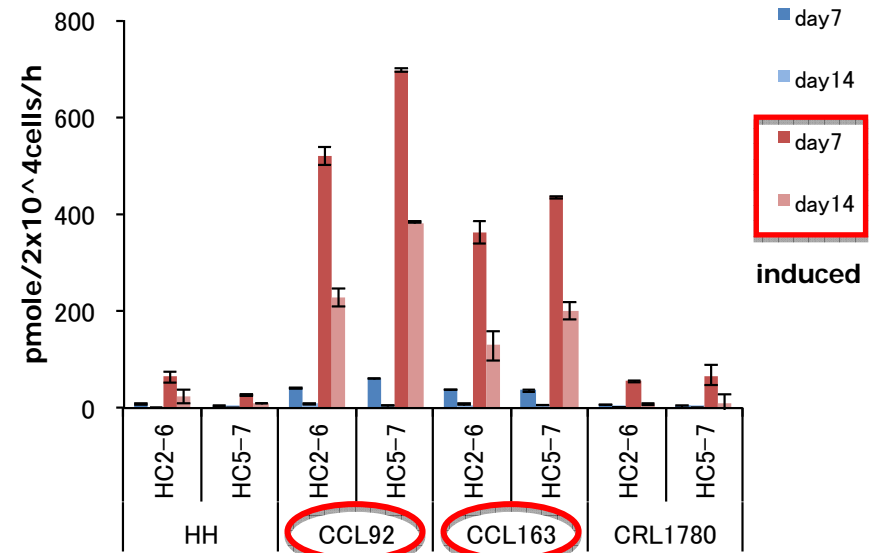
Hepatocytes; Xenotech HC2-6

Induction study (Rifampicin)  
testosterone → 6βhydroxy testosterone

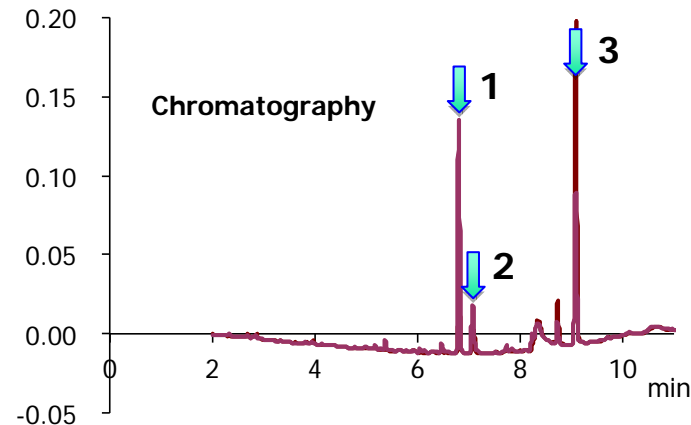


**RECOMMENDED**

Induction study (Omeprazole)  
Phenacetin → Acetaminophen



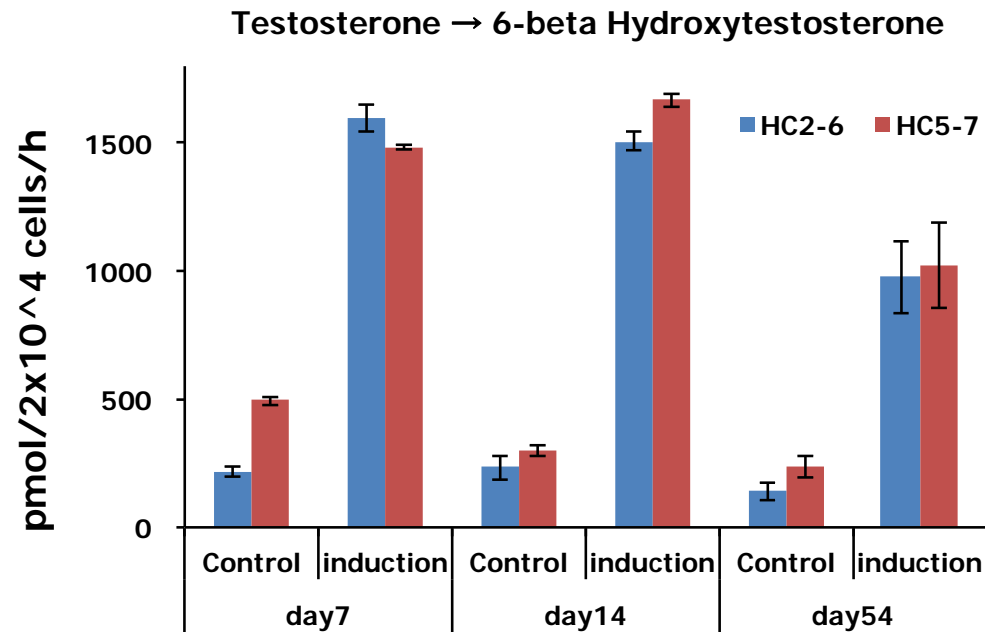
**RECOMMENDED**





# Long-lasting CYP Activity of Cryopreserved Human Hepatocytes Cultured on Cell-able

Hepatocytes; Xenotech HC2-6 and HC5-7  
Feeder cells; ATCC CCL-92



The initial activity of each lot was 549.6 and 214.8 pmol/2x10<sup>4</sup>/h, respectively.

# Influx Transporter Activity (1)

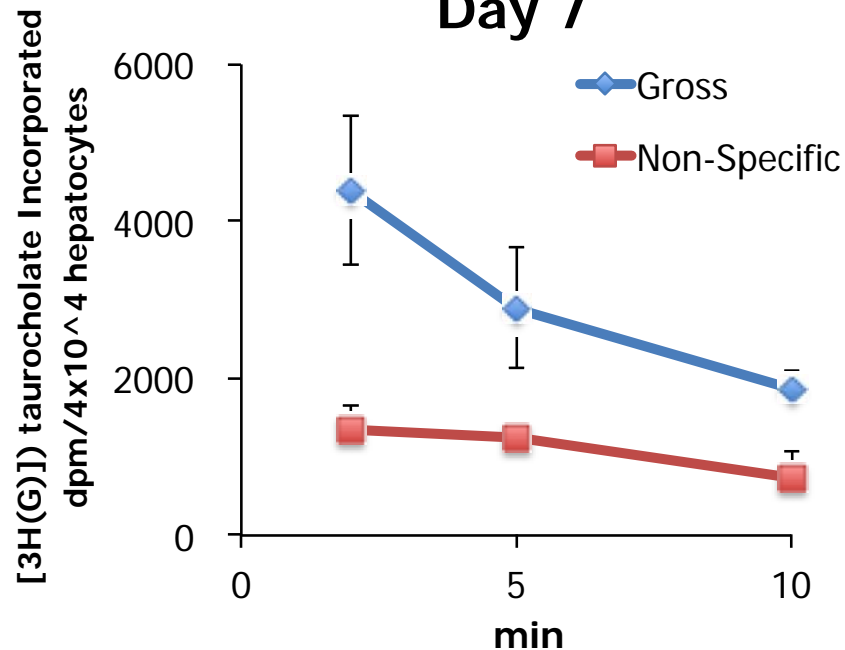
Hepatocytes; freshly isolated human hepatocytes

Feeder; ATCC CCL-163

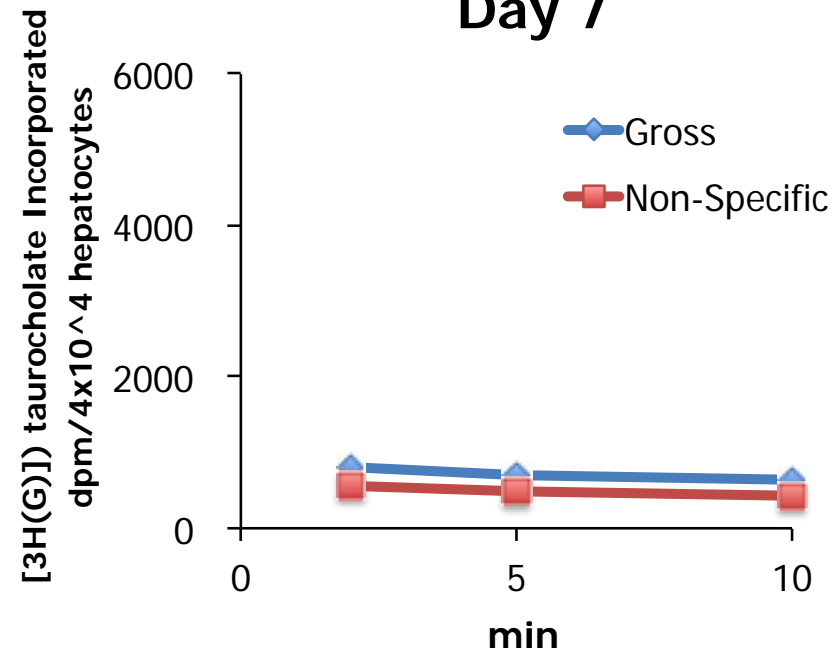
Tracer; [3H(G)]-taurocholate

inhibitor; Rifamycin SV

## Spheroid culture by Cell-able Day 7



## Monolayer culture by collagen-coated plate Day 7



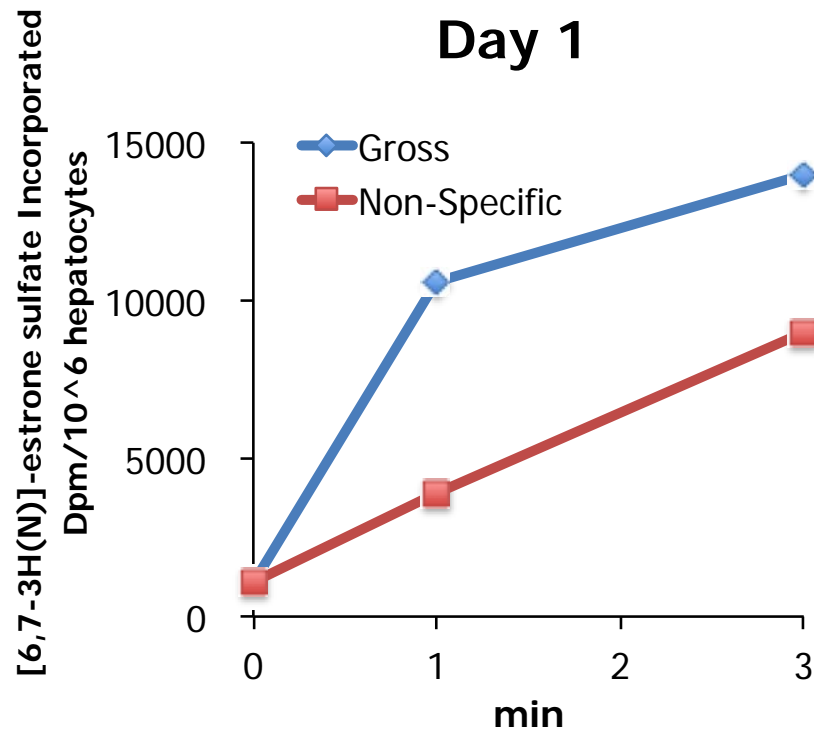
Hepatocyte spheroids showed good influx transporter activity.

Non-specific incorporation was determined under the existence of inhibitor (Rifamycin SV).

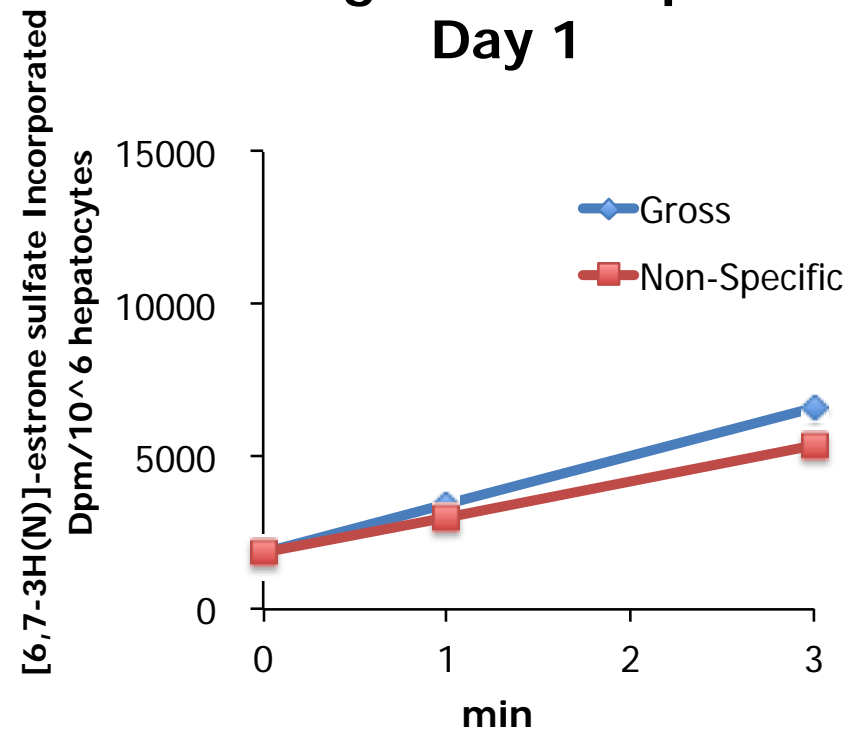
# Influx Transporter Activity (2)

Hepatocytes; cryopreserved human hepatocytes from surgically resected liver  
Feeder; ATCC CCL-163  
Tracer; [6,7-3H(N)]-estrone sulfate  
inhibitor; taurocholate

## Spheroid culture by Cell-able Day 1



## Monolayer culture by collagen-coated plate Day 1



Hepatocyte spheroids showed good influx transporter activity.

Non-specific incorporation was determined under the existence of inhibitor (taurocholate).

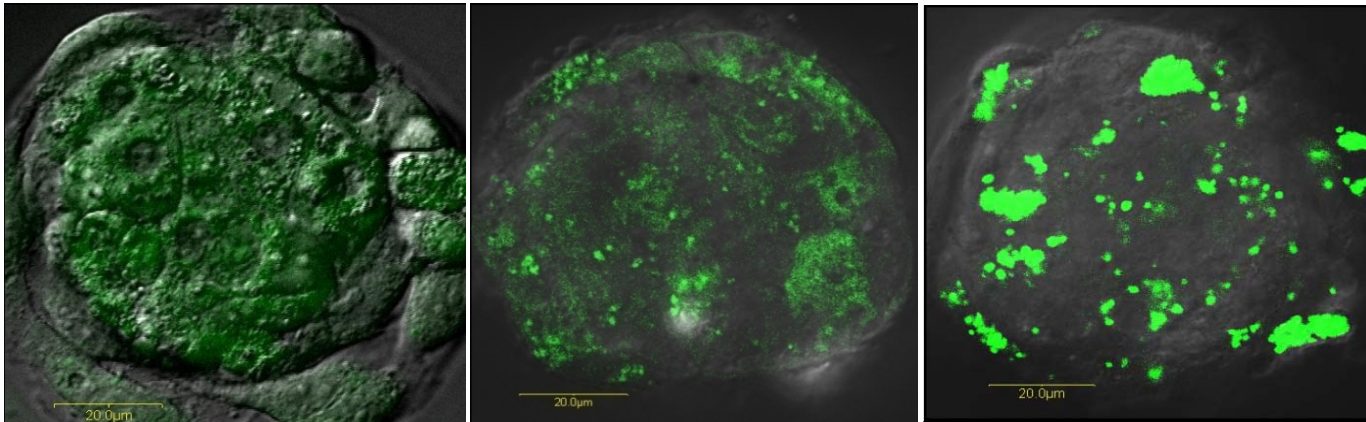
# Hepatocyte spheroid showing efflux transporter activities examined by CDF-DA exclusion into intercellular bile pools

hepatocytes; freshly isolated human hepatocytes  
feeder cells; ATCC CCL-92

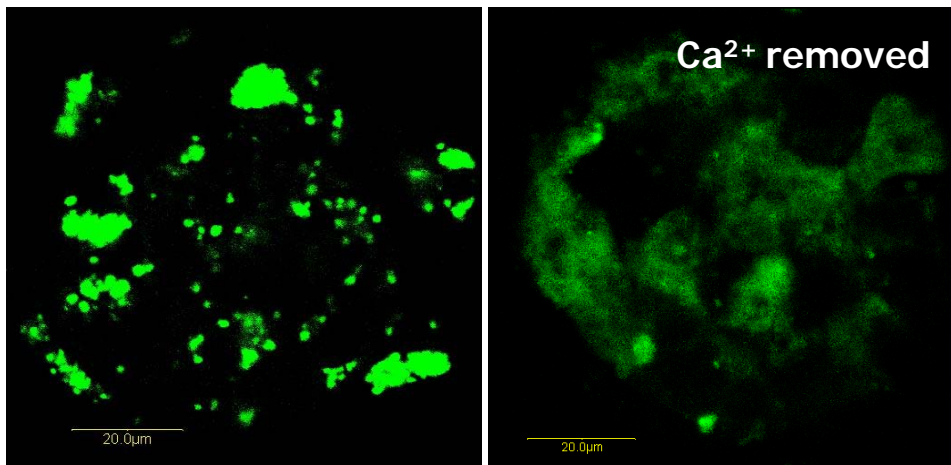
Culture day 2

day 4

day 7



Bile pool formation and CDF exclusion were becoming marked with the increase of culture days or maturation of spheroid. (Above)



Bile pools almost disappeared by removal of  $\text{Ca}^{2+}$  ions. (Left)

# Acknowledgment

Authors would like to express sincere gratitude to people who donate hepatocytes for scientific research.

# Conclusion

- ✧ The best medium for human hepatocyte spheroid culture on Cell-able is RM101 by Transparent. [Slide No.6]
- ✧ The RM101 medium can maintain CYP activities without feeder cells on Cell-able. [Slide No.6]
- ✧ Mouse 3T3 fibroblasts are more effective on hepatocytes culture as feeder cells than bovine endothelial or monkey epithelial cells on Cell-able. [Slide No.7, 8, 9]
- ✧ The human hepatocyte spheroids formed on Cell-able showed influx and efflux transporter activities. [Slide No.10, 11, 12]