# Introduction to Bio-inspiration & Lab-On-a-Chip

2020 1007 Liver on a chip: 肝臟與肝臟晶片技術簡介

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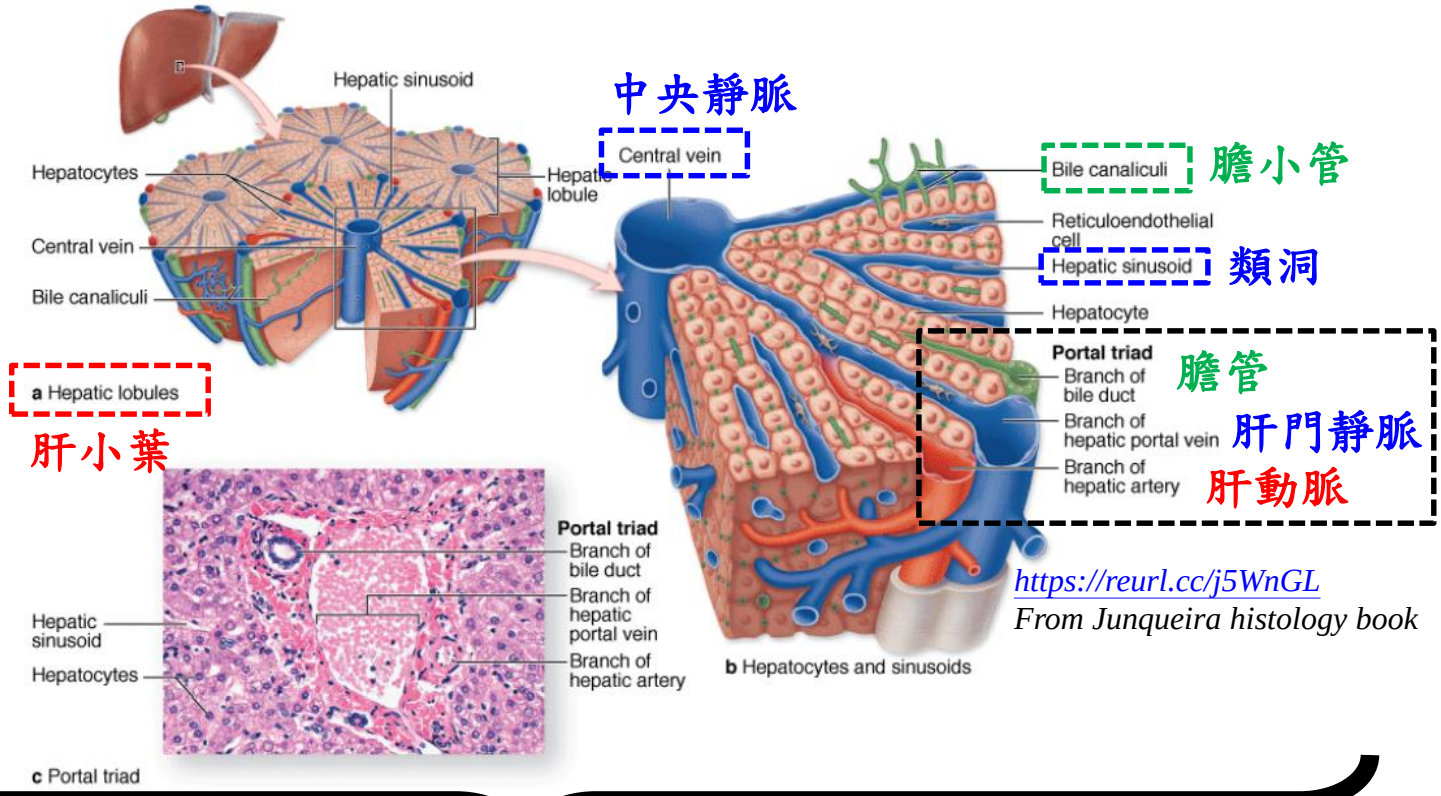
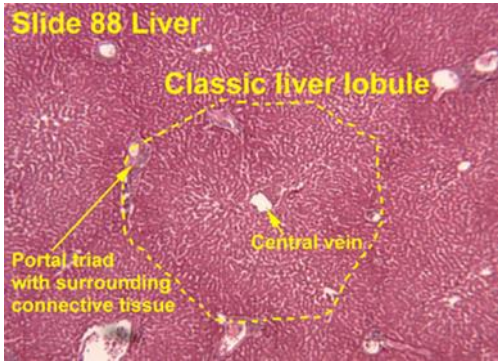
# Liver structure



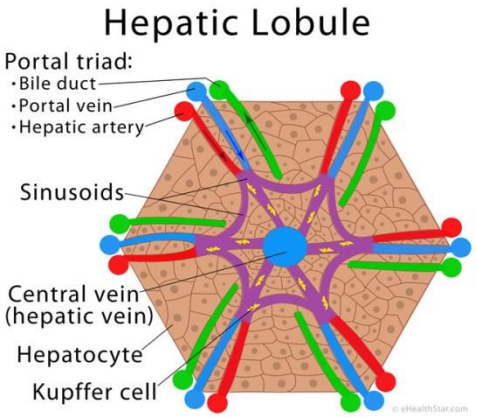
## Hepatocyte optimal microenvironment

Hepatocytes interact with diverse extracellular matrix molecules, non-parenchymal cells, and soluble factors (for ex: hormones, oxygen)




http://www.ouhsc.edu/histology/text%20sections/liver_gall%20bladder_pancreas.html

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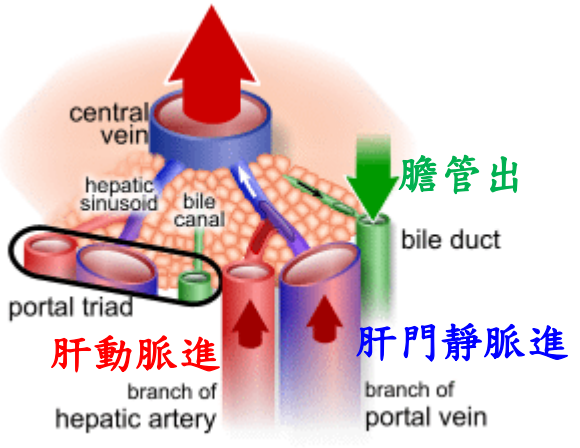
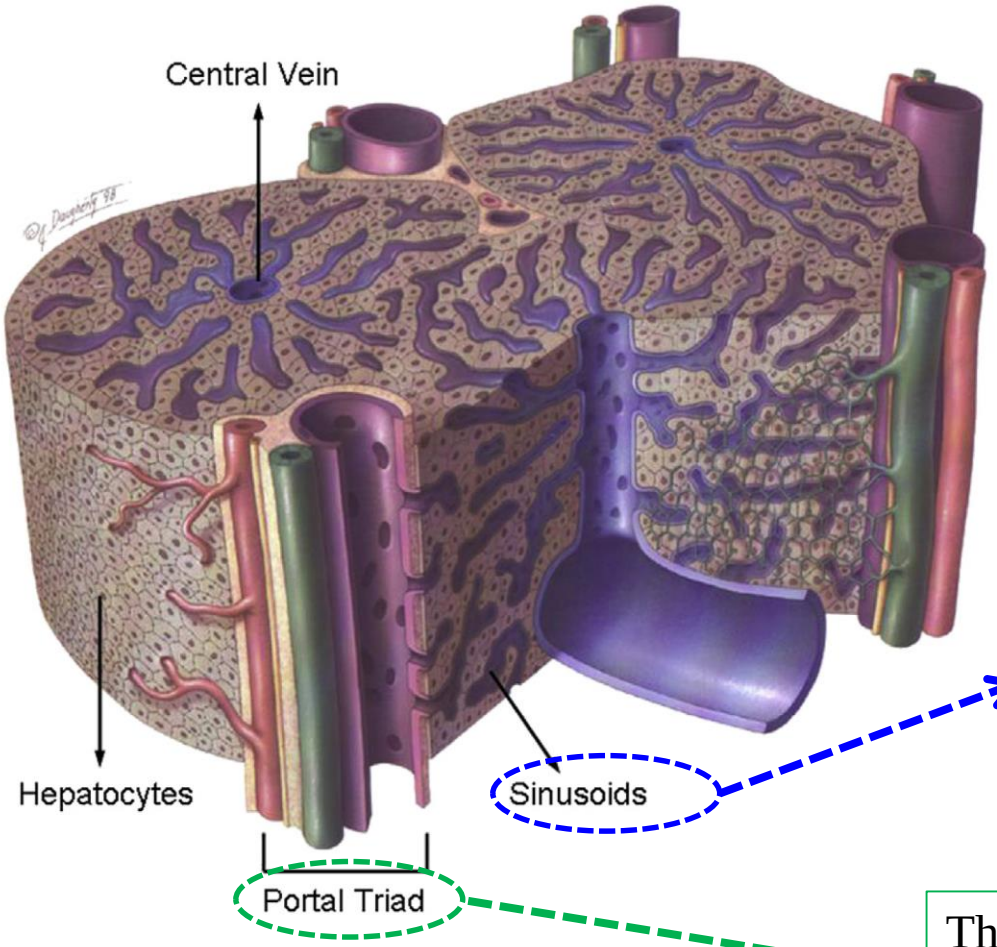


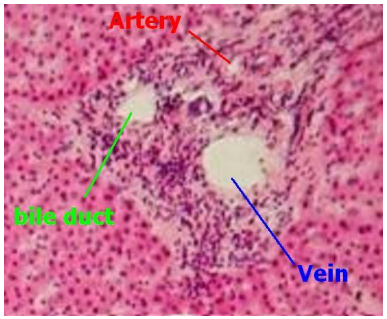

www.blobs.org

# Liver structure (2)

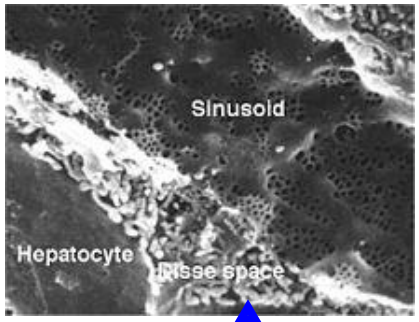





**Portal triad**




**Disse space**




*en.wikipedia.org*

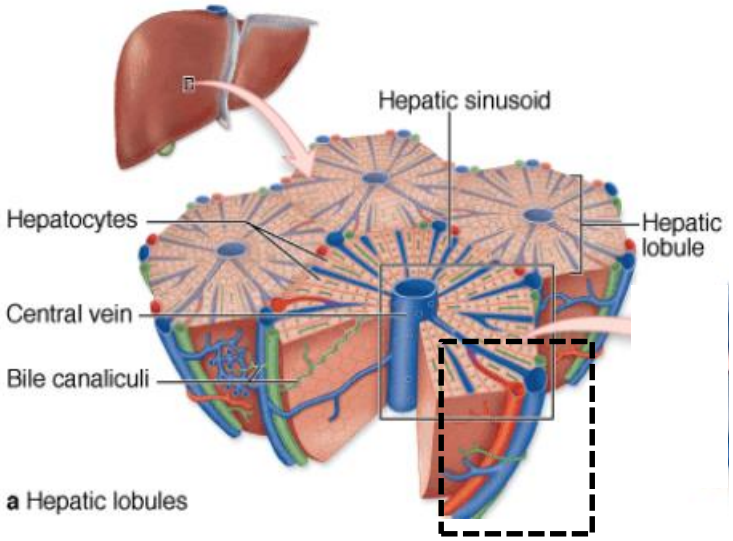
Sinusoids, which are small tortuous vessels lined by a **fenestrated (穿孔)** basement membrane lacking endothelium that is separated from the hepatocyte compartment by a thin ECM region termed the space of Disse

The two lobes of the liver contain repeating functional units called lobules (肝小葉), which are centered on a draining central vein (中央靜脈). Portal triads at each corner of a lobule contain portal venules (肝門小靜脈), arterioles (動脈) and bile ductules (膽管)

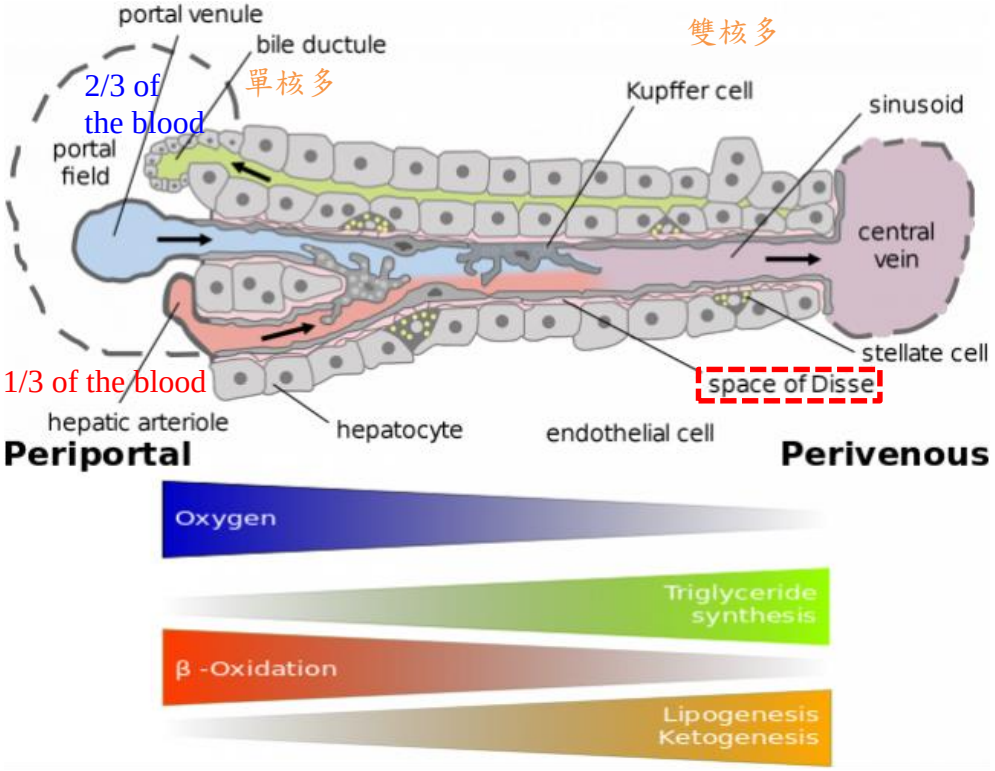
The blood supply to the liver comes from two major blood vessels on its right lobe: the hepatic artery (1/3 of the blood) and the portal vein (2/3 of the blood).

# Liver structure (3)






**Portal triads**




**Disse space** :
The potential extravascular space between the liver sinusoids and liver parenchymal cells

*http://portal-vl.h-its.org/portal-view/liver-function/liver-basics/health/lobule/en/112*

In the space of Disse, hepatocytes are sandwiched between layers of ECM (collagen types I, II, III, IV, laminin, fibronectin, heparan sulfate proteoglycans), the composition of which varies from the portal triad to the central vein (肝細胞在不同地方成分都有所不同)

# Liver zonation



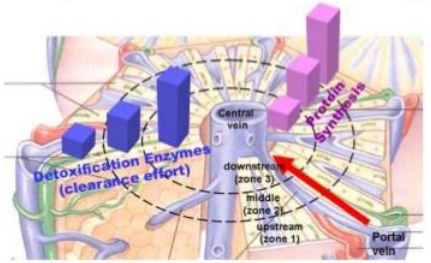
Within the liver lobule, hepatocytes are partitioned into three zones based on morphological and functional variations along the length of the sinusoid (zonation). Zonal differences have been observed in virtually all hepatocyte functions.

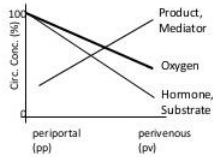
Zonal differences in expression of cytochrome-P450 enzymes have also been implicated in the zonal hepatotoxicity observed with some xenobiotics. Possible modulators of zonation include blood-borne hormones, oxygen tension, pH levels, extracellular matrix composition, and innervation.

A precisely defined microarchitecture allows the liver to carry out its many diverse functions, which can be broadly categorized into protein synthesis (合成) (i.e., albumin, clotting factors), cholesterol metabolism (代謝), bile production, glucose and fatty acid metabolism, and detoxification (解毒) of endogenous (i.e., bilirubin, ammonia) and exogenous (drugs and environmental compounds) substances.

## Liver Zonation

- Hepatic zonation: zone-specific gene expression

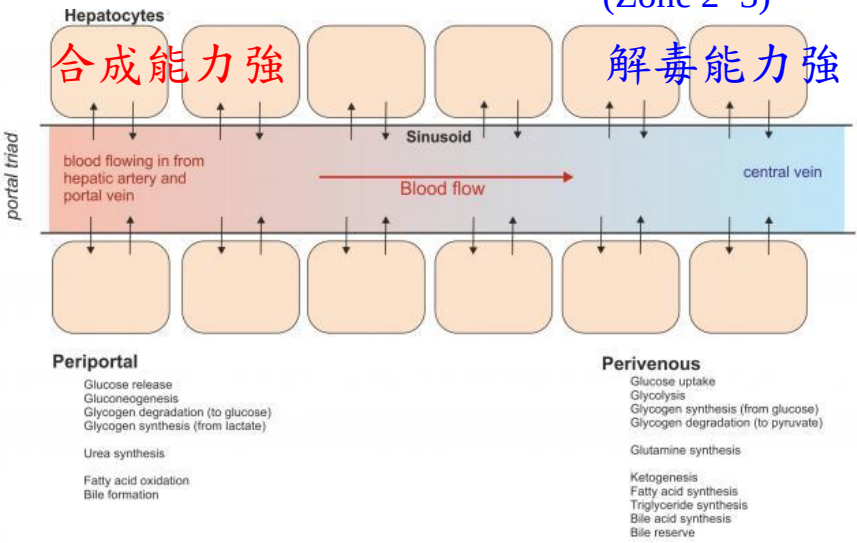



- drug metabolizing enzymes (CYP) mostly expressed in the perivenous region
- CYP enzyme induction expands from PV to PP
- zonal patterns of cell death occur differently for different drugs

*https://reurl.cc/ldQxv6*

單核的肝細胞多　　雙核的肝細胞多 (Zone 2~3)




*http://portal-vl.h-its.org/portal-view-v2/liver-function/liver-basics/health/lobule/en*

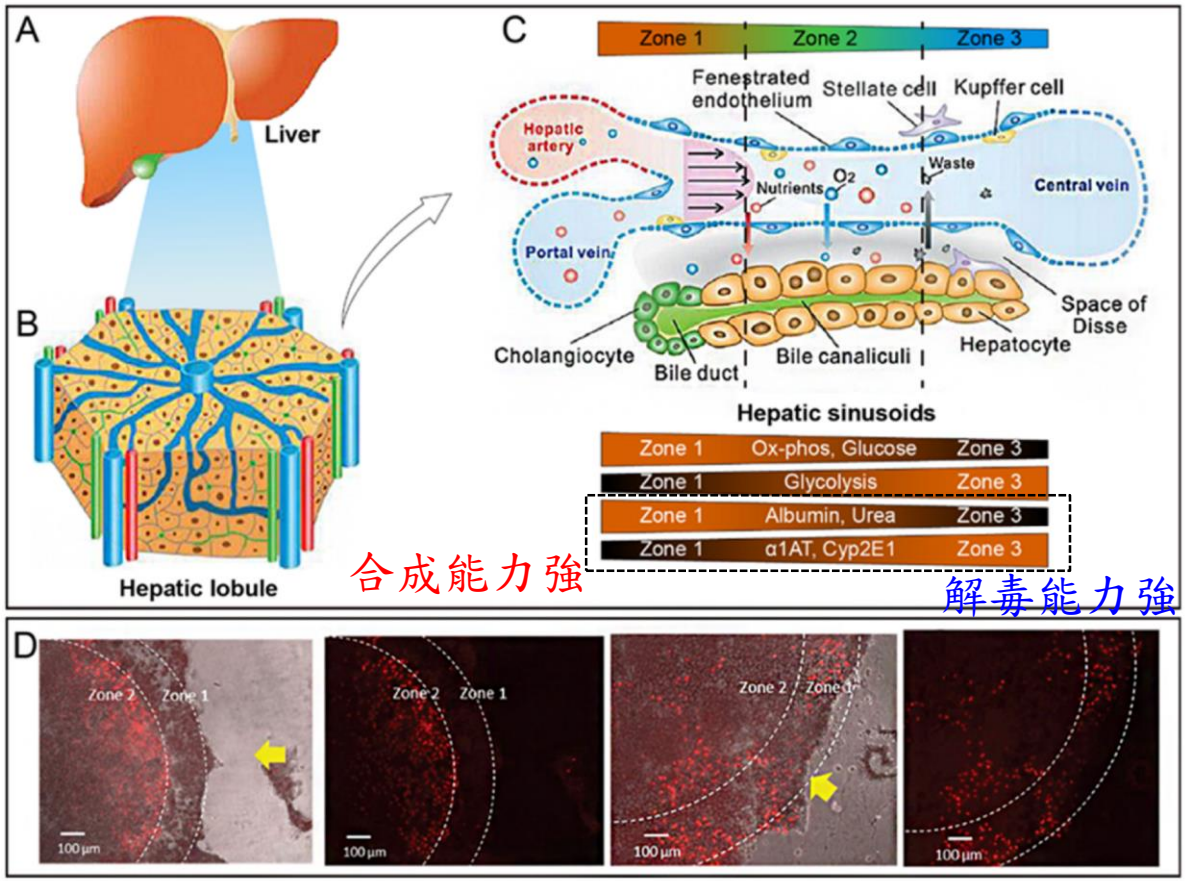


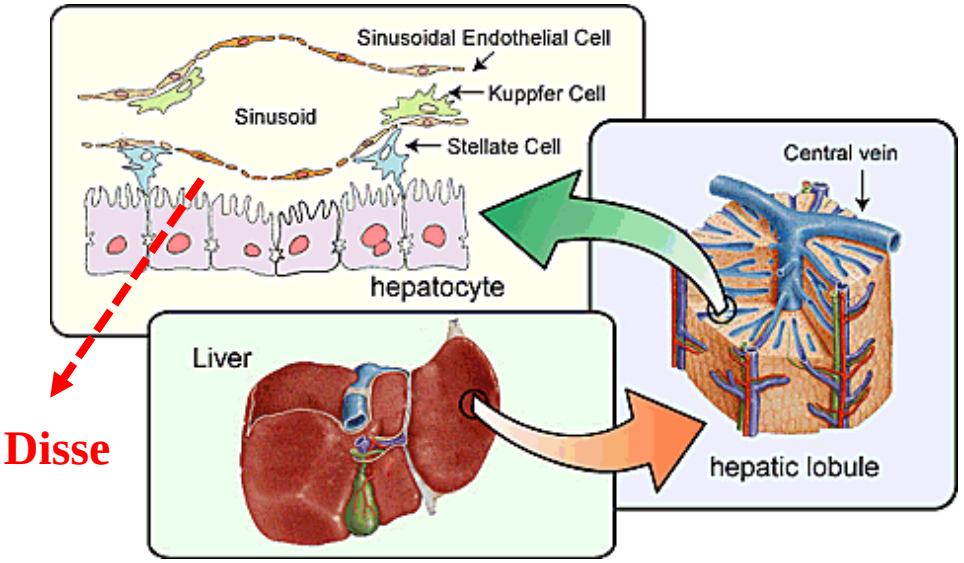

**Figure 1.** Cellular composition and anatomical microstructure of the liver. (**A**) Shape of the liver. It is a red-brown V-shaped organ divided into right and left parts by the hepatic artery, portal vein, hepatic vein, and bile ducts. (**B**) The liver lobule has a hexagonal shape with a diameter of about 1 mm and thickness of about 2 mm. (**C**) Zonation in the lobule. Reproduced with permission from [33]. (**D**) Zonal heterogeneity of acetaminophen-induced hepatotoxicity. The yellow arrow indicates the flow direction. Reproduced with permission from [34].

# Hepatocyte and non-parenchymal cells



## Hepatocyte

Hepatocytes, constituting **~70%** of the liver mass, are arranged in unicellular plates along the sinusoid where they experience homotypic cell interactions




*www.pinterest.com*

## Non-parenchymal cells

Including **stellate cells (ITO cell; 星狀細胞)**, cholangiocytes (biliary ductal cells), **sinusoidal endothelial cells (類洞內皮細胞)**, **kupffer cells (巨噬細胞)** (liver macrophages), natural killer cells, and pit cells (large granular lymphocytes) interact with hepatocytes to modulate their diverse functions

Hepatic stellate cells (here HSC), also known as perisinusoidal cells or Ito cells (earlier *lipocytes* or *fat-storing cells*), are pericytes found in the perisinusoidal space (a small area between the sinusoids and hepatocytes) of the liver also known as the space of Disse. The stellate cell is the major cell type involved in liver fibrosis (肝纖維化), which is the formation of scar tissue in response to liver damage.

*http://en.wikipedia.org/wiki/Hepatic_stellate_cell*

# 肝臟細胞的所有種類

Classical textbooks of histology, such as the 9th edition of Ham's Histology [6], used to give the following list of liver cell types:

1. Hepatocytes
2. Cholangiocytes (biliary epithelial cells, bile duct epithelium)
3. Hepaticmacrophages (Kupffer cells),
4. Fenestrated endothelium of vascular sinusoids, cellular elements of other blood vessels,
5. Ito cells (stellate cells)
6. Stromal fibroblasts
7. Lymphatic vessel cells, lymphocytes and other immune cells
8. Nerve elements.

Later the so-called oval cells were added to the list [7, 8]. Liver hosts a population of stem/progenitor cells, which in rodents includes oval cells [9]. Cell fate experiments suggested that stellate cells can also be the precursors of liver epithelial cells [10, 11].

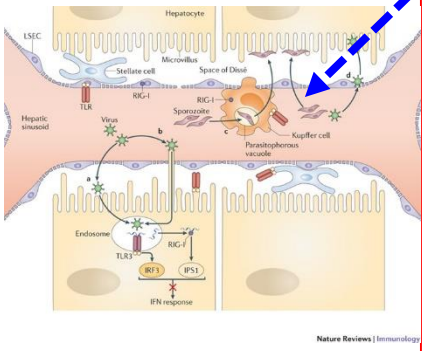
# Basic liver architecture and ECM composition



**Hepatocyte**

- Mesenchyma→ supporting tissue
- Epithelium (parenchyma) → functional cell

**Non-parenchymal cells**



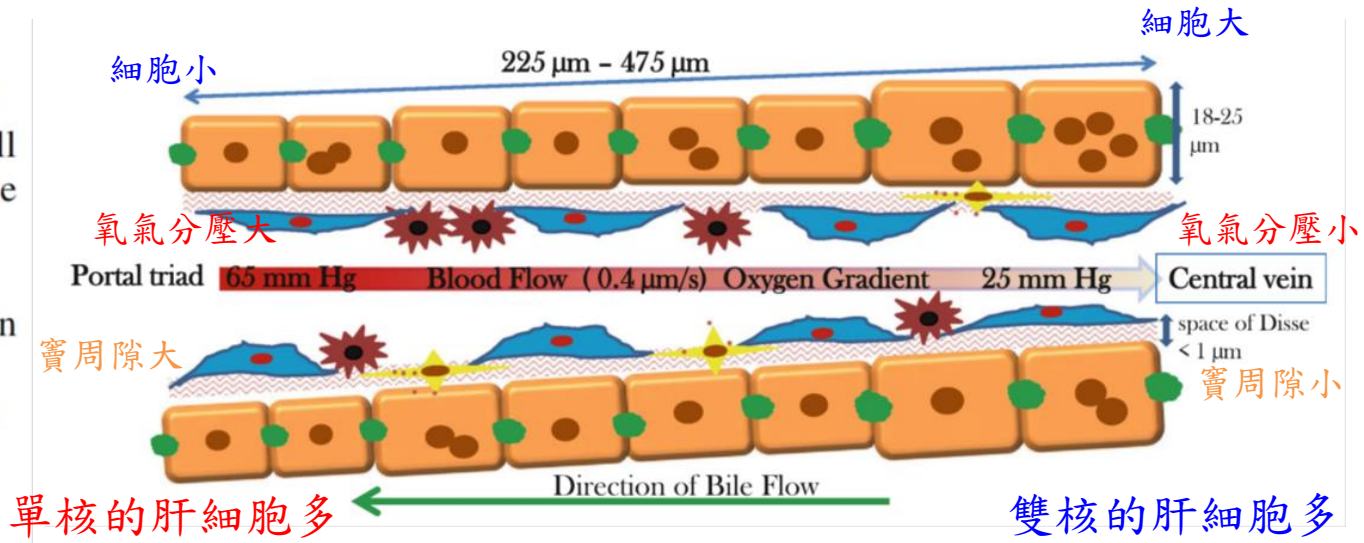

*2012 Living in the liver: hepatic infections*

*2014 Biomaterials for liver tissue engineering*

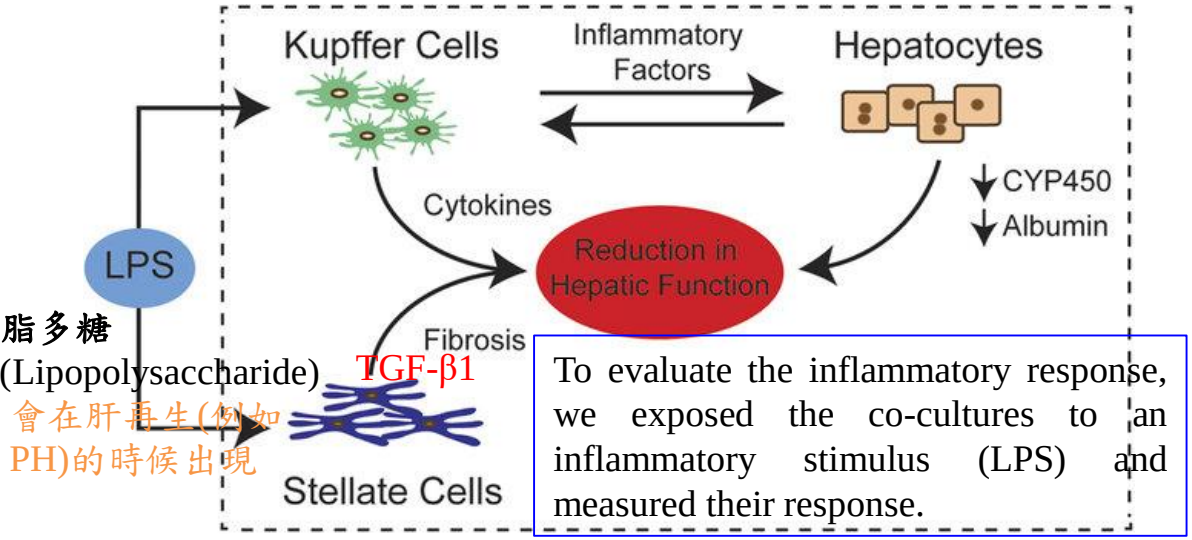
Hepatocytes are **the specialized endothelial cells of liver** that perform a majority of liver functions. Hepatocytes in the vicinity (附近) of sinusoids extend numerous microvilli (微絨毛) into the space of Disse and come into direct contact with blood, facilitating exchange of nutrients.

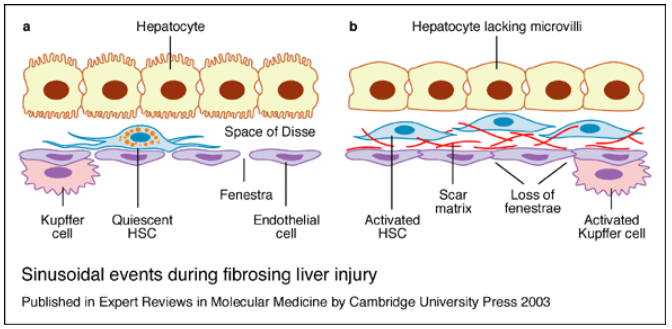
- **LSECs** line the sinusoids of liver, which carry blood from the portal vein to central venule and deliver oxygen to the surrounding parenchyma. Sinusoids are separated from the hepatic parenchyma by a protein-rich interface called the space of Disse. Fenestrae (穿孔), a characteristic feature of LSECs, act like hepatic sieves that provide steric regulation of molecular transport into the parenchyma.
- **Kupffer cells** are specialized macrophages that reside in liver sinusoids. They have a high phagocytic and endocytic activity and secrete several cytokines (IL-1, 6, TNF-α), all of which play a critical role in defense, immunity and liver regeneration.
- **HSCs** are also called fat storage cells or Ito cells. They store vitamin A, produce components of ECM and control sinusoidal contractility. In case of an injury, activated HSCs secrete cytokines and growth factors responsible for liver fibrosis and deposition of ECMs, which contribute to scar formation.
- Other cell types in liver include cholangiocytes (膽管上皮細胞) and hepatic progenitor cells

**Fig. 1** Organization of a liver sinusoid depicting distribution and arrangement of various cell types. The blood flows from the portal triad toward the central vein, creating an oxygen gradient that leads to zonation in liver. The size of hepatocytes, sinusoidal lumen diameter and fenestrae changes from the periportal to perivenous zone




*2014 Biomaterials for liver tissue engineering*



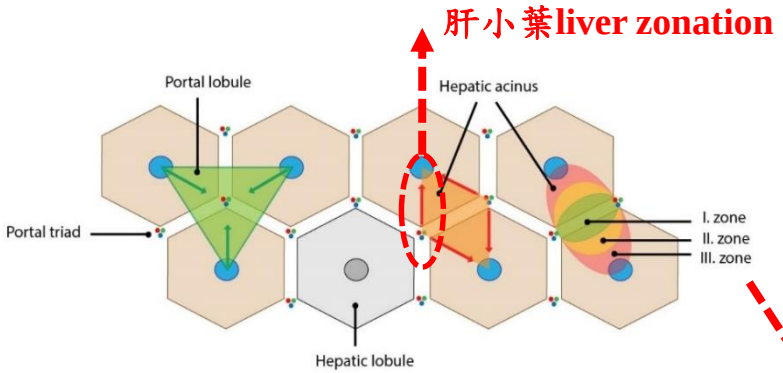

*2003 Sinusoidal events during fibrosing liver injury*

**Figure 5. Interactions between hepatocytes and non-parenchymal cells in co-culture.** External Stimuli and cellular secretions from non-parenchymal cells influence the behavior and overall outcomes. Capturing cellular interactions and evaluating the cumulative responses are essential to create *in vitro* liver mimics.

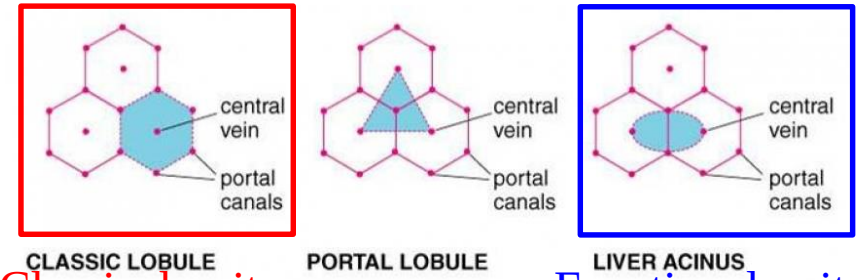
*2016 Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response*

# Liver acinus (肝線泡)





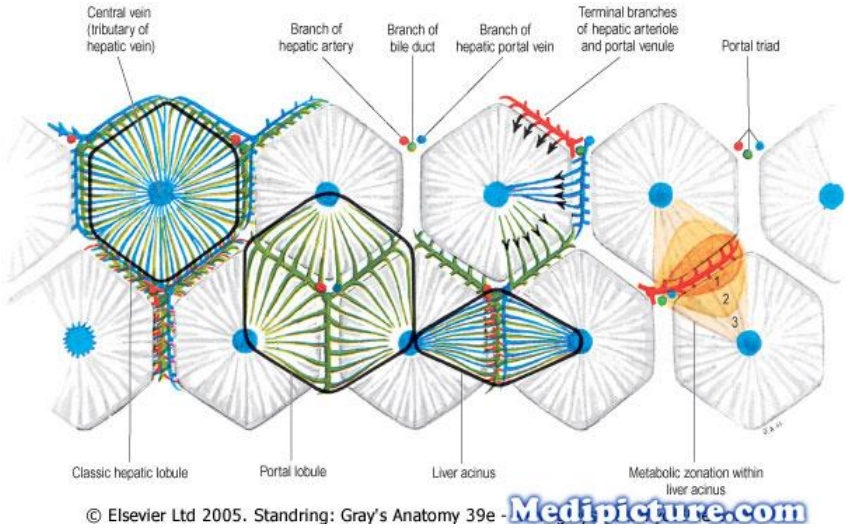

*https://reurl.cc/N6Q7bQ*




Classical unit

Functional unit

*http://www.studydroid.com/printerFriendlyViewPack.php?packId=179082*




*https://reurl.cc/avqWZ7*

The basic functional unit of liver is the acinus (肝線泡). The acinus is constituted of radially transversing strings or plates of parenchymal/hepatocytes and non-parenchymal cells tied between two central veins and centered on a portal triad. It is a miniature model of the typical microenvironment and zonation that exist in liver

*2014 Biomaterials for liver tissue engineering*

The acinus is delineated into three zones: periportal (zone 1), midlobular (zone 2) and centrilobular (zone 3). The zonation occurs as a consequence of the cellular arrangement along the microvasculature and the direction of blood flow. Each of these zones specializes in different metabolic functions as dictated by oxygen tension, presence of the CYP450 enzymes, matrix chemistry, solute gradients and gene expression

# Hepatocyte



- Hepatocytes are polygonal epithelial cells with six or more faces corresponding to their individual position in the overall cell structure.
- The usual life span of hepatocytes is at least 150-200 days. This programmed death of the old hepatocytes is designated apoptosis (細胞凋亡).

**$300 \times 10^{10}$ cells ($3 \times 10^{12}$ cells)**

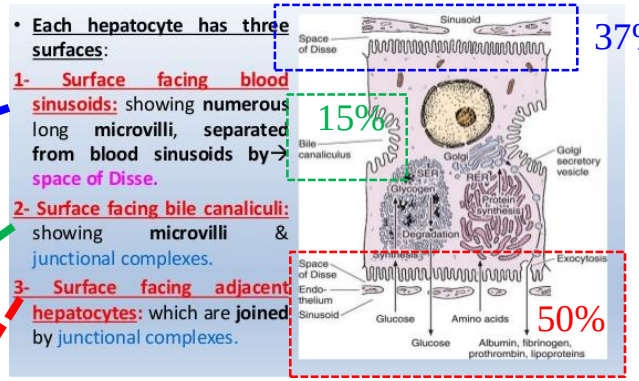
| | |
|---|---|
| Proportion of liver volume | 80% |
| Proportion of total cell number | 60–65% |
| Number of liver cells | 300 billion |
| Number of hepatocytes per g of liver | 171 million |
| Diameter of hepatocytes | 20–40 µm |
| Proportion of hyaloplasm in cell volume | 54,9% |
| Lifespan of hepatocytes | 150 (–200) days |
| Mitosis rate per 10.000–20.000 liver cells | 1 |
| Membrane surface of hepatocytes and organelles (s. p. 26) | 33,000 $\mu m^2$ |

**$171 \times 10^7$ cells ($1.71 \times 10^9$ cells)**

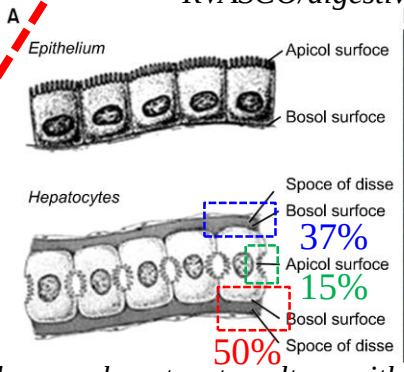
Ex: $1.71 \times 10^9$ cells/g $\times$ 1400 g = $2.4 \times 10^{12}$ cells/body

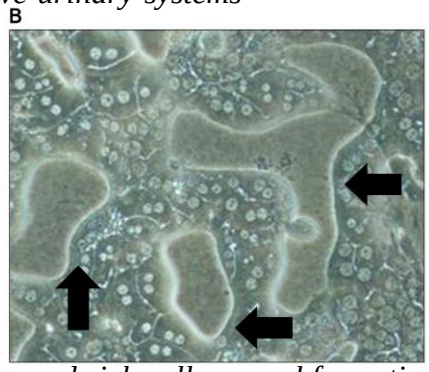
Hepatocytes have a clearly contoured cell membrane which is divided into three compartments defined by morphological and functional **cellular polarization.**

1. About 37% of the external area of the hepatocyte membrane is *sinusoidal surface* (basolateral)
2. About 15% of the outer hepatocyte membrane consist of canaliculi, termed *canalicular surface* (apical).
3. The remaining 50% of the external hepatocyte membrane constitute the smooth *intercellular fissure*, which is connected with Disse's space.




*https://www.slideshare.net/GLADIADORVASCO/digestive-urinary-systems*




*2008 Hepatology Textbook and Atlas 3rd edition*

*2013 Successful mouse hepatocyte culture with sandwich collagen gel formation*

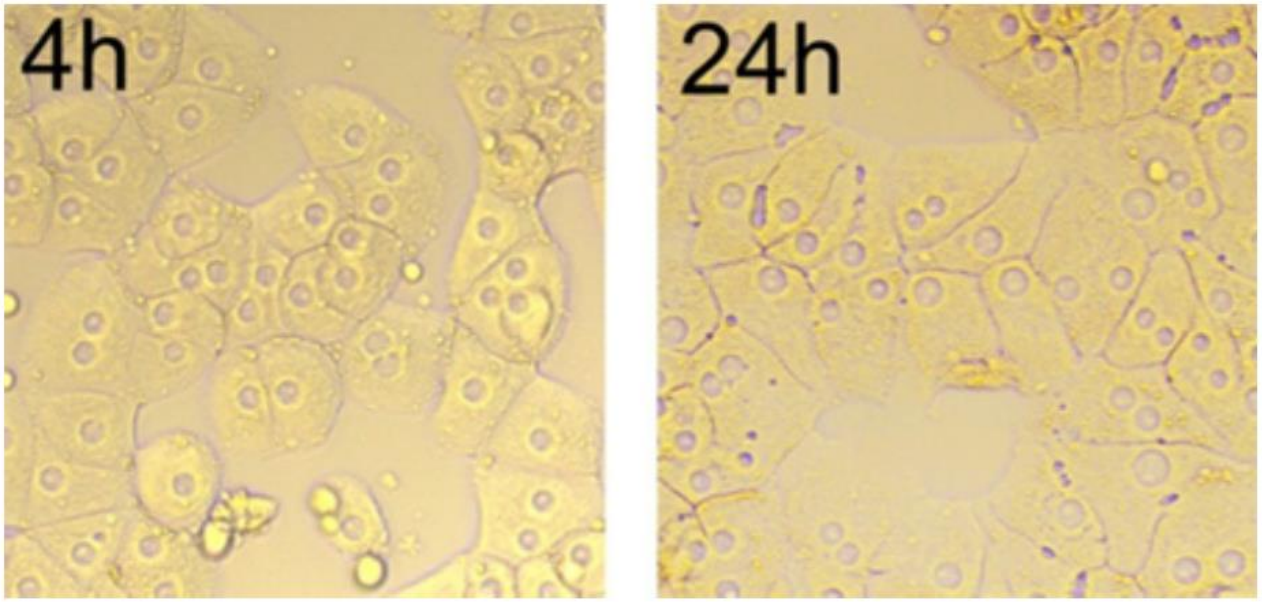



**Figure 2.** Morphology of cultured hepatocytes over time (4 hr to 24 hr), magnification × 200. After cultured for 24 hr, the cells spread in typical monolayer growth, and the junctions between the cells are linear

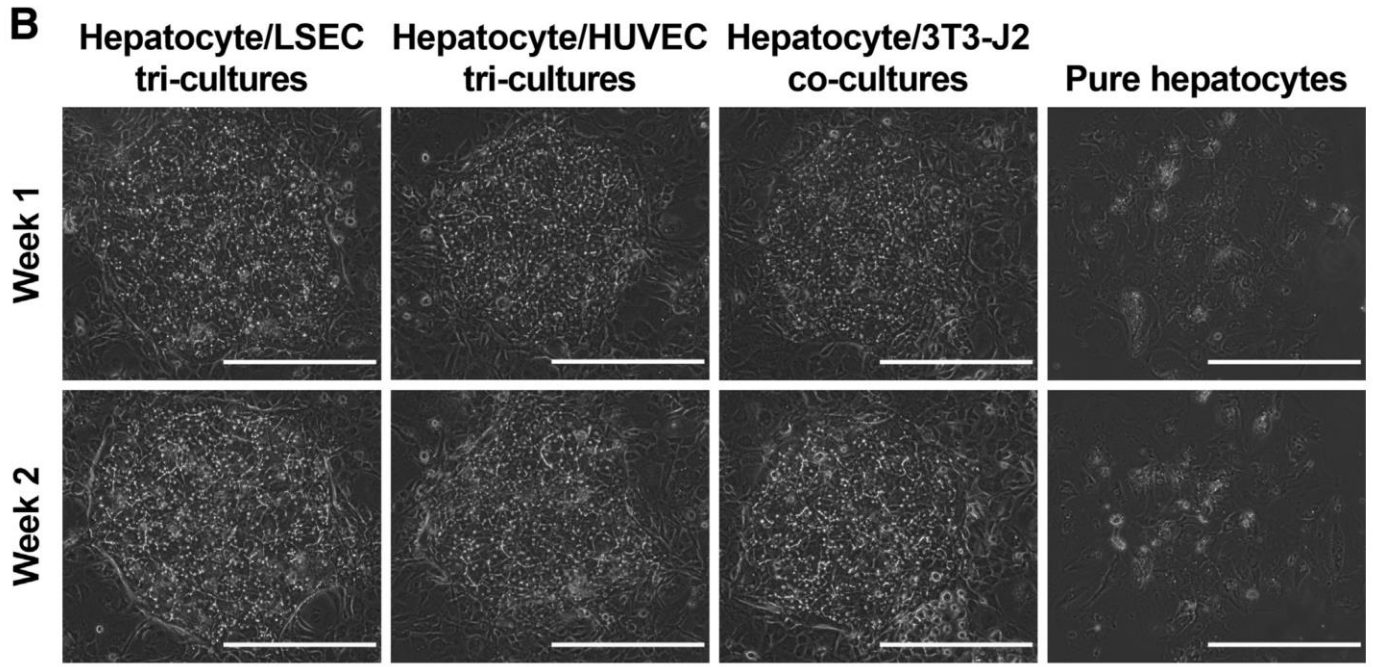



Figure 6. Morphology of PHH/fibroblast/endothelial cell tricultures (containing either LSECs or HUVECs) relative to PHH/3T3-J2 fibroblast control cocultures and PHH monocultures. (A) Schematic depicting the creation of the tricultures; cocultures were created as depicted in Figure 2A. (B) Morphology of tricultures models over the course of 2 weeks in comparison with the PHH/fibroblast cocultures and pure PHH monocultures. Note the prototypical PHH morphology (ie, polygonal shape, multinucleation, and presence of visible bile canaliculi；肝細胞的特徵) in the tricultures/cocultures and spread-out (dedifferentiated) morphology in the PHH monocultures. All scale bars = 400 mm.

# Hepatic functions (1)



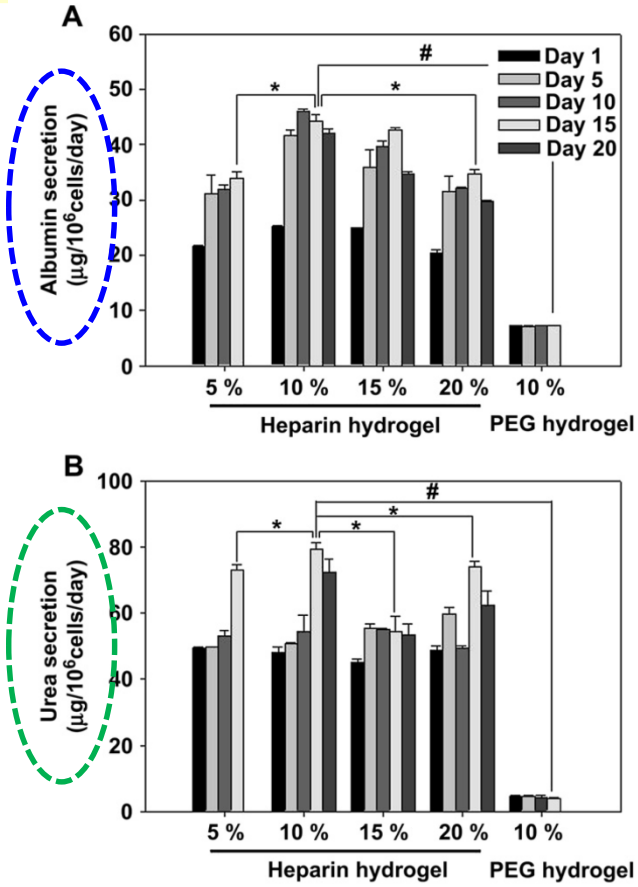
**TABLE 46.2 Hepatic functions**

| Functional classification | Examples |
|---|---|
| Synthetic<br>合成 | Albumin Secretion<br>Alpha-1-antitrypsin Secretion<br>Coagulation Factor Production (II, IX, X)<br>Lipoprotein and apoprotein synthesis<br>Ceruloplasmin production<br>Ferritin production<br>Complement production |
| Metabolic<br>代謝 | Ureagenesis and metabolism<br>Bilirubin Metabolism<br>Steroid Metabolism<br>Gluconeogenesis/Glycogen Production<br>Lipid metabolism |
| Detoxification pluripotent stem cells, direct reprogramming to hepatocytes<br>解毒 | Metabolize, detoxify, and inactivate exogenous and endogenous compounds via cytochrome P450 enzymes, methyltransferases, sulfotransferases, acetyltransferases, UDP-glucuronosyltransferases, and Glutathione S-transferases |
| Bile Production | |

Liver is a vital organ performing protein synthesis, carbohydrate and fat metabolism, detoxification, blood clotting and immune system, hormonal responses, and waste removal in the body

**Human serum albumin** is the most abundant protein in human blood plasma. It is produced in the liver. Albumin constitutes about half of the blood serum protein. It is soluble and monomeric.

**Urea synthesis** was an important index for evaluating liver specific function. The amino acids of cell were broken down in the human body and therefore formed the highly toxic ammonia. The liver could convert the ammonia into urea which was a non-toxic compound and safely transported into the renal system




*2010 Heparin-based hydrogel as a matrix for encapsulation and cultivation of primary hepatocytes*

# Hepatic functions (2)



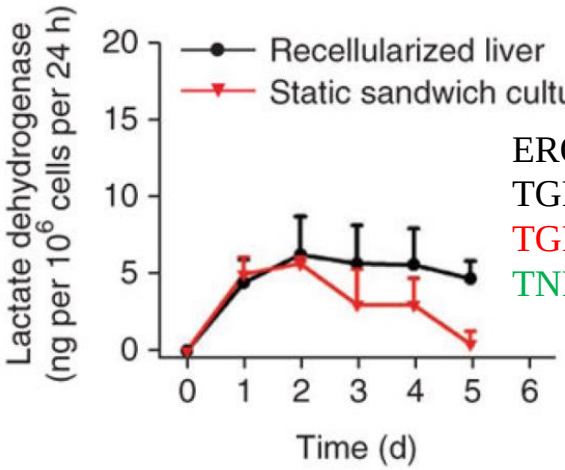
## Other hepatic functions

- Ethoxyresorufin O-dealkylase (EROD)
- TGF-α (Transforming growth factor- α)
- Lactate dehydrogenase (LDH)
- Alcohol dehydrogenase (ADH)
- CYP450

TABLE 2. *Measured hepatocyte functions in cocultures*

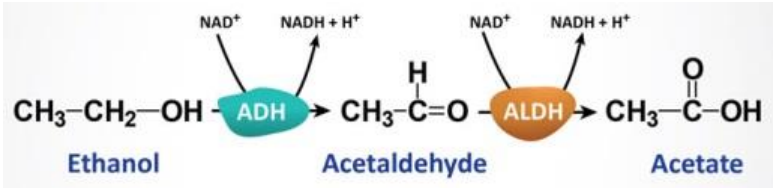
| |
|---|
| Albumin secretion |
| Cytochrome P-450 activity (isoenzymes 1A1, 2B1, 3A1) and inducibility |
| Glutathione S-transferase |
| Tight junctions (detection of ZO-1) |
| Gap junctions (detection of connexin 32, microinjection) |
| Other: pyruvate kinase, transferrin, DNA synthesis, UDP-glucoronyl transferase |

*1999 Effect of cell-cell interactions in preservation of cellular phenotype cocultivation of hepatocytes and nonparenchymal cells*




EROD → CYP1A1 induction
TGF-α → 幫助DNA合成與細胞增殖
TGF-β → 抑制肝細胞生長
TNF-α → 腫瘤壞死因子

*2010 Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix.*

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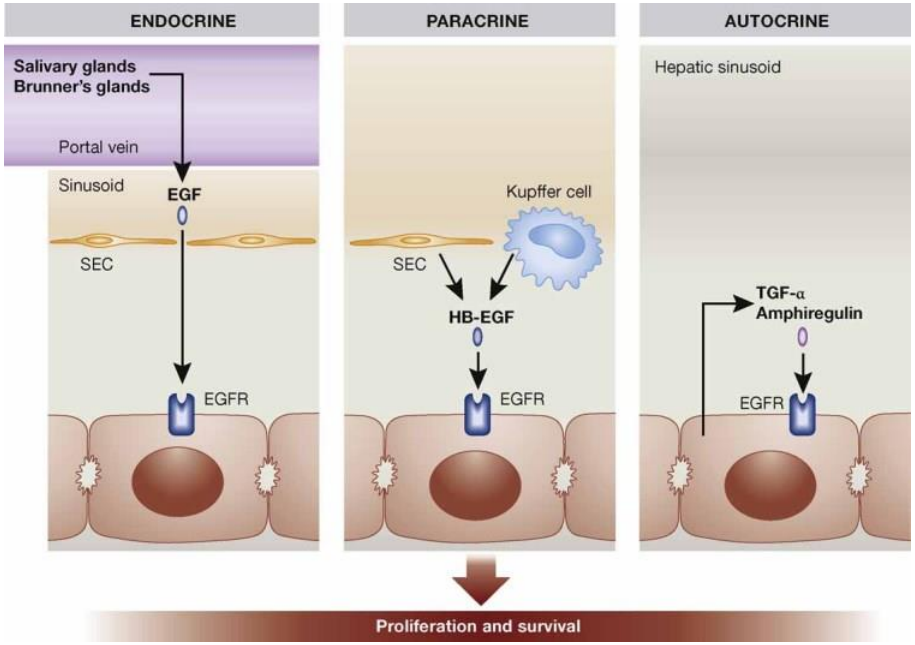


# Hepatic functions (3)

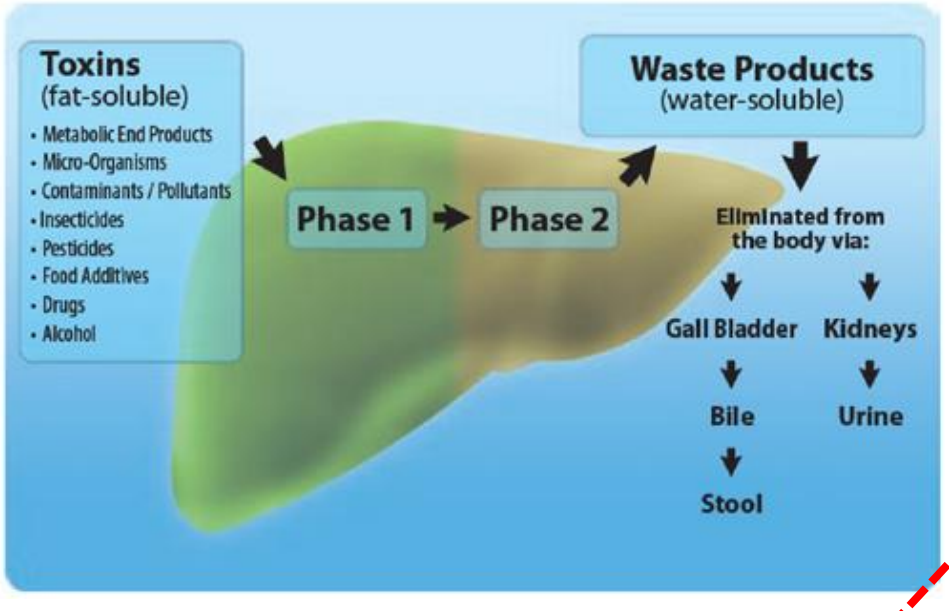


## Drug metabolism in liver

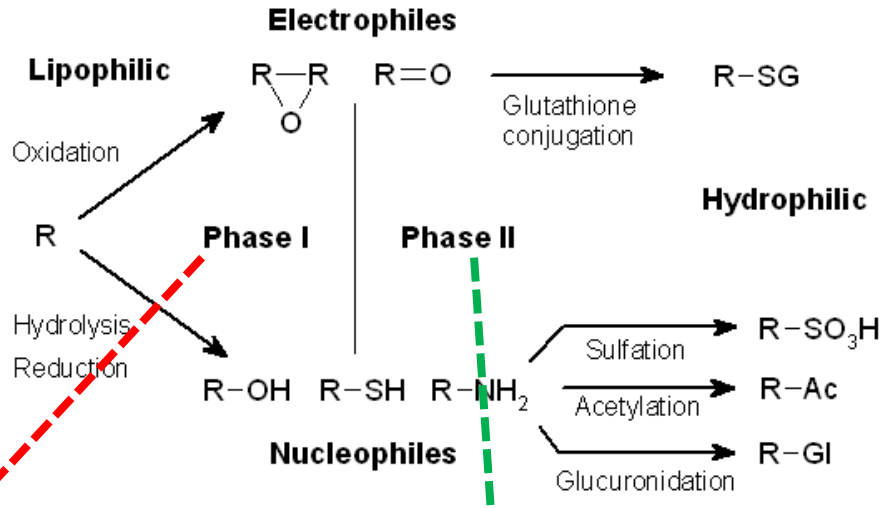
P450 reaction (負責有機受質的氧化作用):

$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$

Figure 1 - Detoxification (Biotransformation) Pathways




*http://en.wikipedia.org/wiki/Drug_metabolism*




脂溶性→極性

**In phase I**, a variety of enzymes act to introduce reactive and polar groups into their substrates. One of the most common modifications is hydroxylation catalysed by the cytochrome P-450-dependent (CYP450) mixed-function **oxidase (氧化酶) system.**

極性→水溶性

**In subsequent phase II** reactions, these activated xenobiotic metabolites are conjugated with charged species such as glutathione (GSH), sulfate, glycine, or glucuronic acid.

# Mechanisms of Liver regeneration



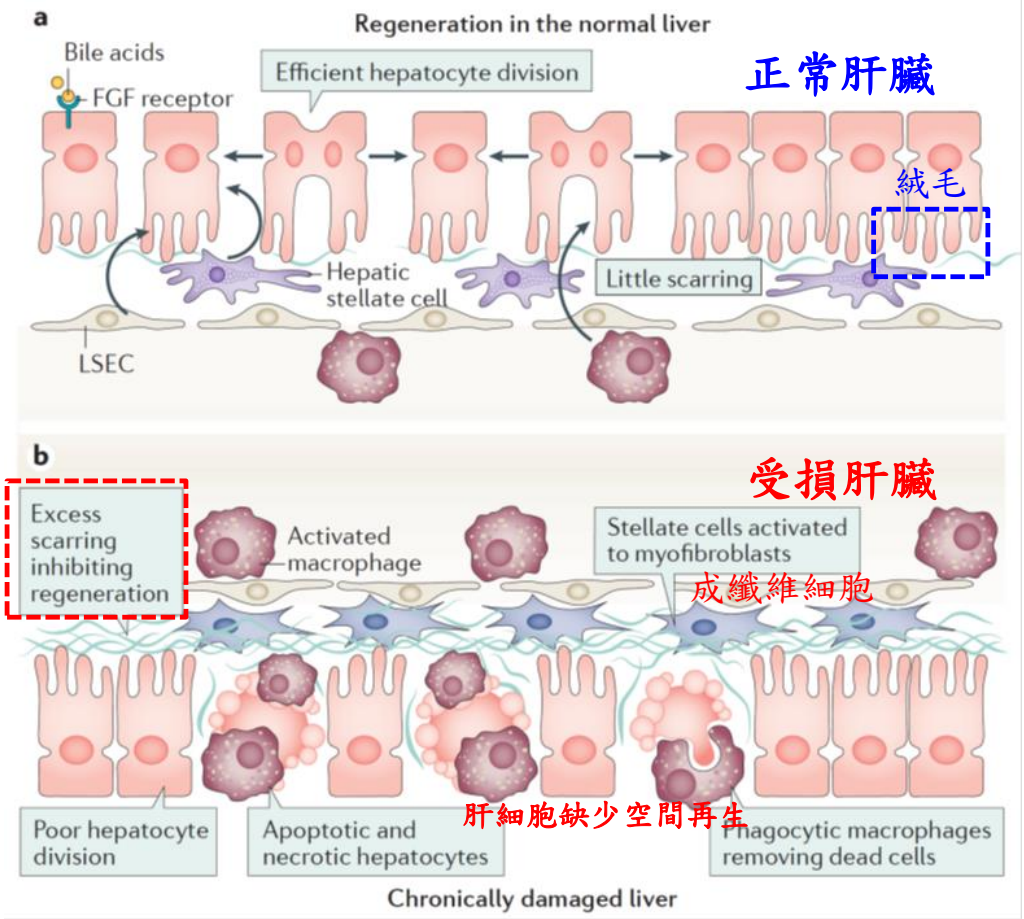
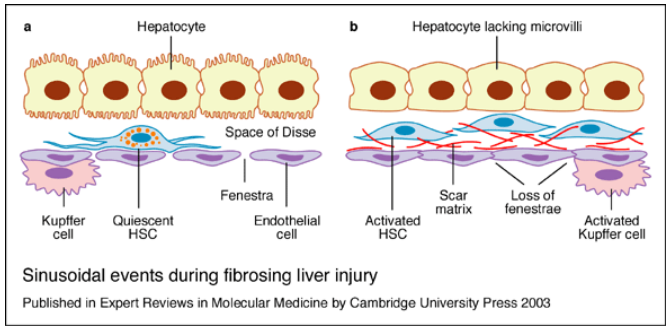



Figure 2 | **Schematic of normal and abnormal liver regeneration. a** | Regeneration in the normal liver follows partial hepatectomy or moderate liver injury. In this setting, bile acids are rapidly upregulated, and serum factors are able to rapidly induce regeneration in the liver. Non-parenchymal cells; macrophages, hepatic stellate cells and liver sinusoidal endothelial cells (LSECs) signal to hepatocytes to leave their mitotically quiescent state and enter mitosis. Hepatic stellate cells are not activated to myofibroblasts and there is little or no scar tissue. **b** | Regeneration in the abnormal, chronically damaged liver is hampered by several factors. Hepatocytes are increasingly senescent and unable to divide efficiently, the hepatic stellate cells are activated to myofibroblasts and excessive scar tissue inhibits regeneration. Excessive cellular debris inhibits efficient liver regeneration. FGF, fibroblast growth factor; LSEC, liver sinusoidal epithelial cells.

*2016 Liver regeneration — mechanisms and models to clinical application*

- In a normal liver, most cells are quiescence and in the G0 phase of cell cycle. Upon PHX or chemical injury, cells enter cell cycle and go through various phases including G1, S (DNA replication occurs here), G2, and mitosis.
- In liver regeneration after PHX, the cell cycle is highly synchronized and cells enter S phase and mitosis together. This discovery led to the use of PHX model as an *in vivo* model of synchronized cell cycle

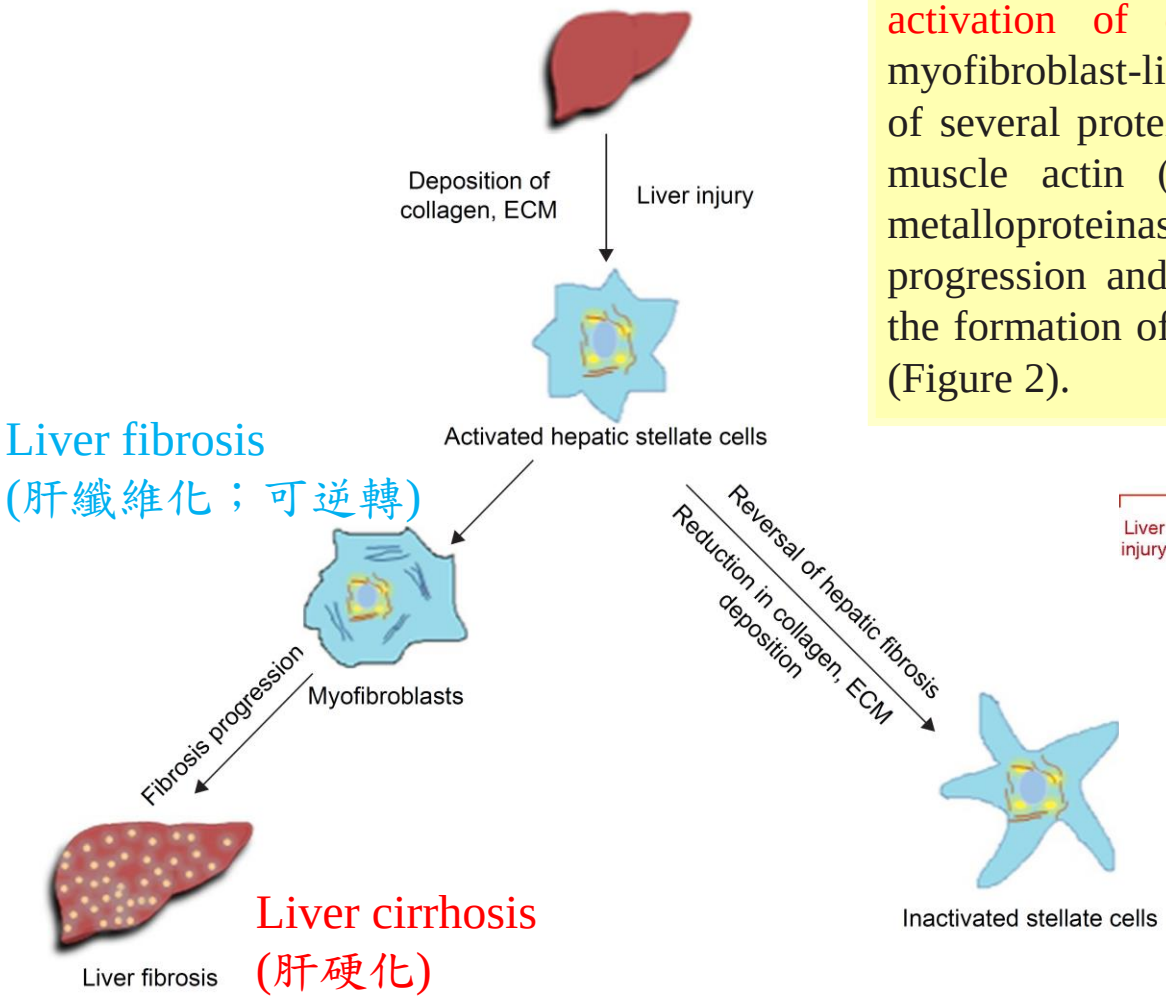
肝纖維化厲害後，這些fenestra會縮小或緊閉，而且space of Disse會增加很多膠原蛋白，於是影響了由sinusoid跟肝細胞的交通

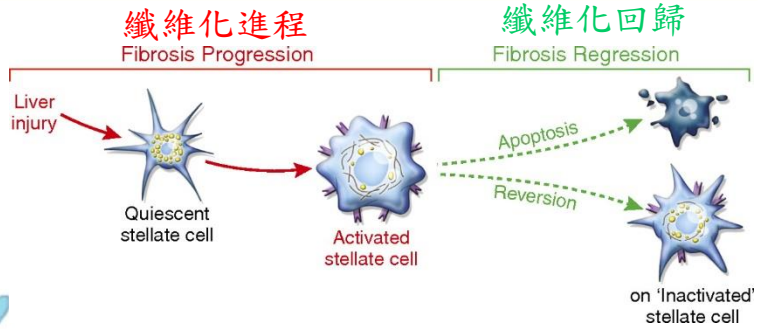



*2003 Sinusoidal events during fibrosing liver injury*

# 肝硬化原理

The pathogenesis of liver fibrosis (肝纖維化) mainly includes the deposition of fibrillar collagen as well as ECM proteins as a result of the wound healing response. The main mechanism behind this is the activation of quiescent (靜止期) HSC in a myofibroblast-like cell with subsequent upregulation of several proteins like interstitial collagen, α-smooth muscle actin (α-SMA), proteoglycans and matrix metalloproteinase (基質金屬蛋白酶；MMPs). The progression and reversal (反轉) of liver fibrosis and the formation of myofibroblast are given subsequently (Figure 2).

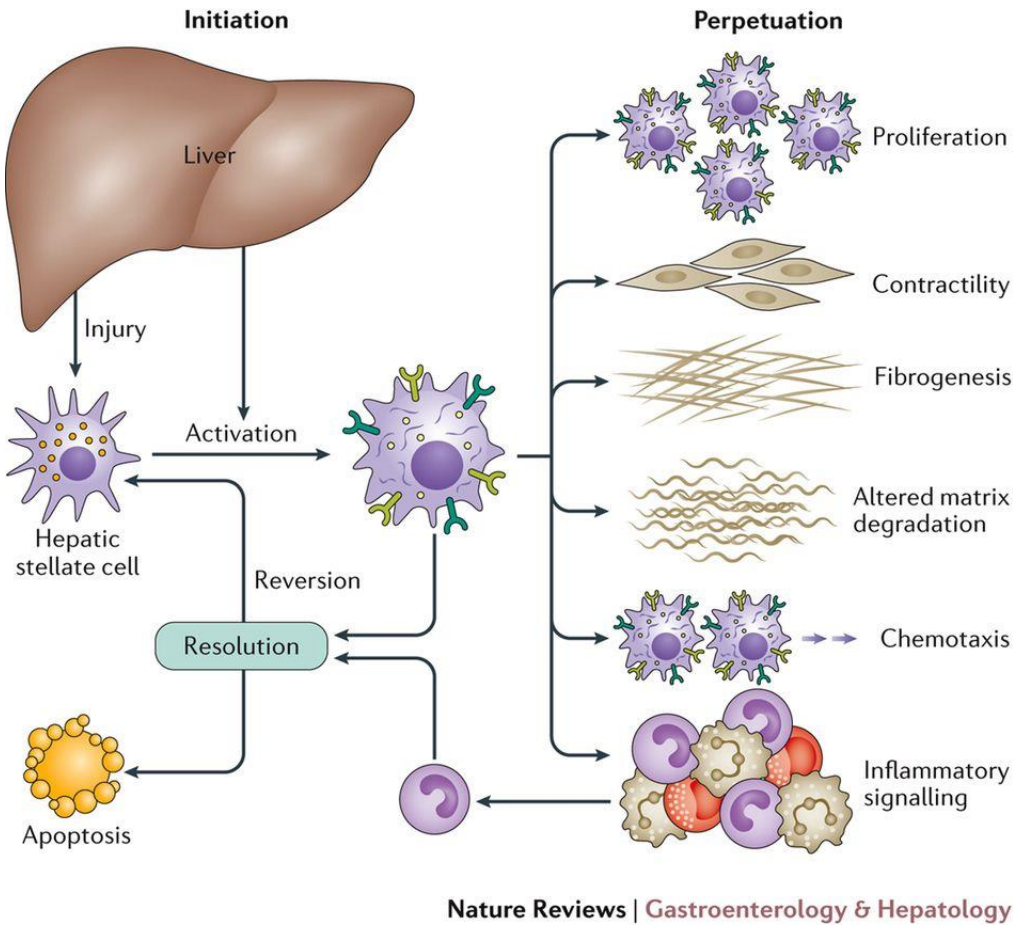



*2012 Fibrogenic cell reversion underlies fibrosis regression in liver*

**Figure 2** Formation of myofibroblasts and progression and reversal of hepatic fibrosis.
**Abbreviation:** ECM, extracellular cell matrix.

*2017 Nanoparticles for the treatment of liver fibrosis*

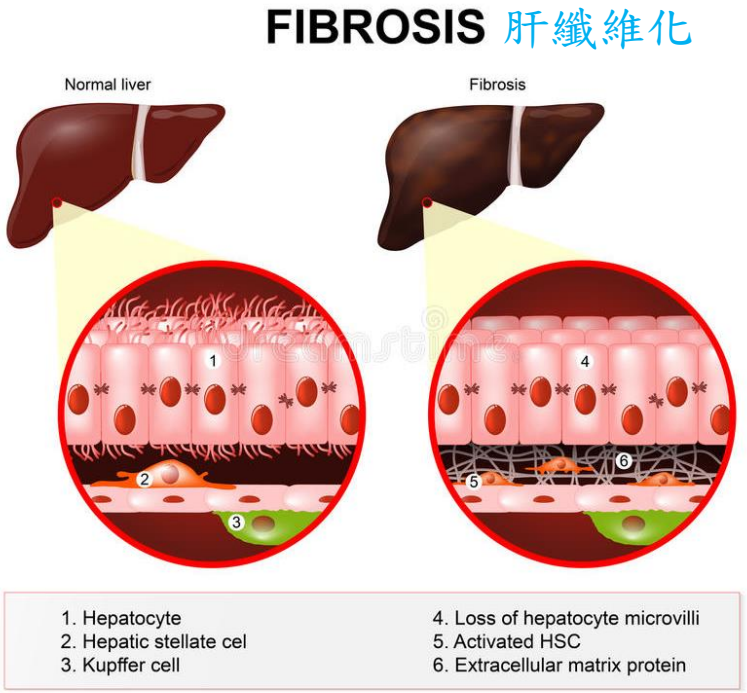



Liver injury initiates the trans-differentiation of quiescent hepatic stellate cells (HSCs) to their activated phenotype. Perpetuation follows, characterized by specific phenotypic changes including proliferation, contractility (收縮), fibrogenesis (纖維化), altered matrix degradation, chemotaxis (趨化性) and inflammatory signalling. During resolution of hepatic fibrosis, activated HSCs can be cleared by apoptosis or reversion to an inactivated phenotype.

*2017 Mechanisms of hepatic stellate cell activation*

# Liver fibrosis *V.S* Liver cirrhosis






*https://reurl.cc/gmGqE*

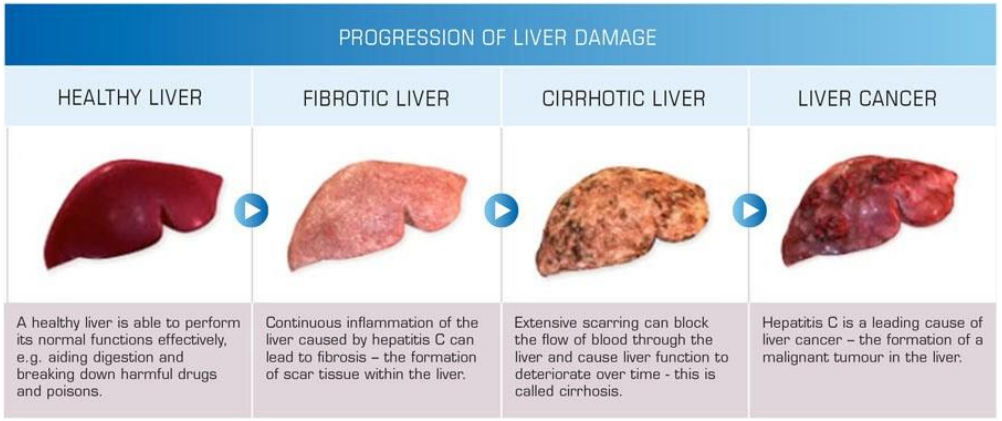
肝硬化


*https://fixhepc.com/blog/item/80-reversal-of-liver-fibrosis-then-and-now.html*

Fibrosis describes **encapsulation** or replacement of **injured tissue** by a collagenous scar.

Cirrhosis is an advanced stage of liver fibrosis that is accompanied by distortion of the hepatic vasculature

*2009 Liver Cirrhosis*




*https://reurl.cc/r897n4*

# Proliferation of differentiated cells

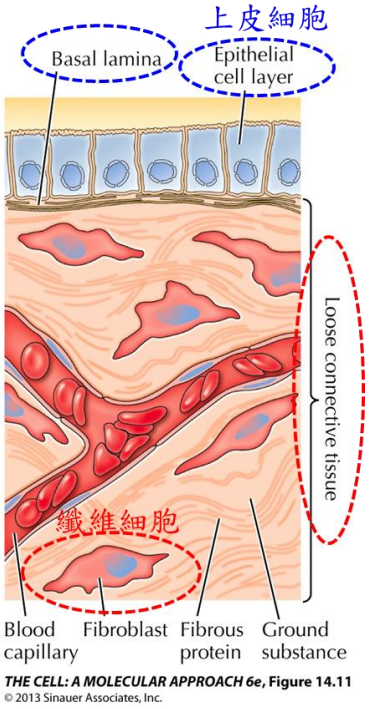


Most types of differentiated cells in adult animals are no longer capable of proliferation. (arrested in $G_0$)

If these cells are lost, they are replaced by the proliferation of less differentiated cells derived from self-renewing stem cells

The reason is:

- In early development, cells proliferate rapidly, then differentiate to form the specialized cells of tissues and organs.
- To maintain a constant number of cells in adult tissues, cell death must be balanced by cell proliferation.




**However….**

- Some differentiated cells retain the ability to proliferate as needed, to repair damaged tissue throughout the life of the organism.
- Fibroblasts in connective tissue can proliferate quickly in response to platelet-derived growth factor (PDGF) released at the site of a wound.

**Conclusion:**

**Endothelial cells** and **fibroblast** are capable of proliferation!!

*via* VEGF *via* PDGF

# Liver regeneration



The endothelial cells of some internal organs are also able to proliferate to replace damaged tissue

舉例來說：

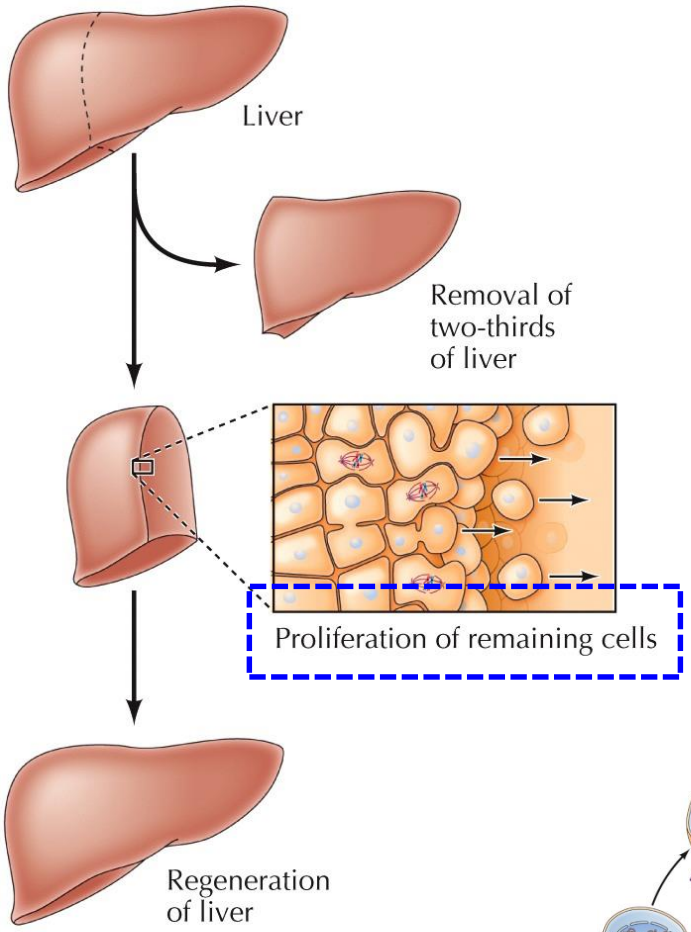
Liver

**Liver cells**

Liver cells are normally arrested in the $G_0$ phase of the cell cycle. However, if large numbers of liver cells are lost, the remaining cells are stimulated to proliferate to replace the missing tissue

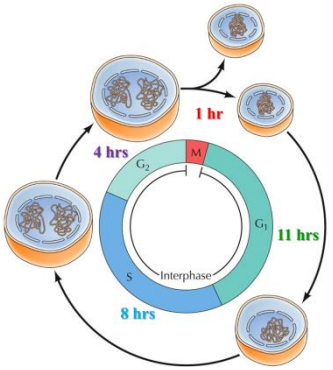
**Liver cell, skin cells:**

These cells exit $G_1$ to enter a quiescent stage of the cycle called $G_0$, where they remain metabolically active but no longer proliferate unless called on to do so by appropriate extracellular signals ($G_0$ 細胞有活性但不增殖)




*THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.15






For a typical proliferating human cell with a total cycle time of 24 hours

Liver sinusoidal endothelial cells (LSECs) have long been noted to contribute to liver regeneration after liver injury. In normal liver, the major cellular source of HGF is the hepatic stellate cell, but after liver injury, HGF expression has been thought to increase markedly in proliferating LSECs. (星狀細胞與類洞內皮細胞都會分泌HGF給hepatocytes)

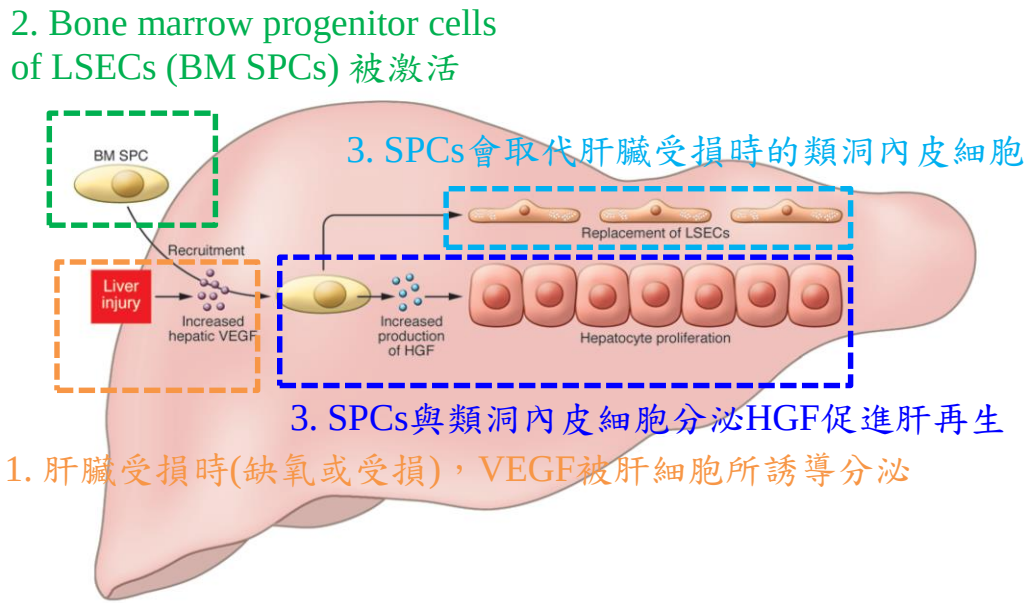



**Figure 1**

BM SPCs and liver regeneration. Schematic depicting the contributions of liver cells and BM SPCs to liver regeneration. Liver injury induces increased hepatic VEGF expression, which drives recruitment of HGF-rich BM SPCs and promotes expression of HGF by resident SPCs and LSECs. HGF, in turn stimulates the proliferation of hepatocytes in liver regeneration. In addition, SPCs replace LSECs that were lost during injury

*2013 Liver sinusoidal endothelial cells and liver regeneration*

# Liver regeneration



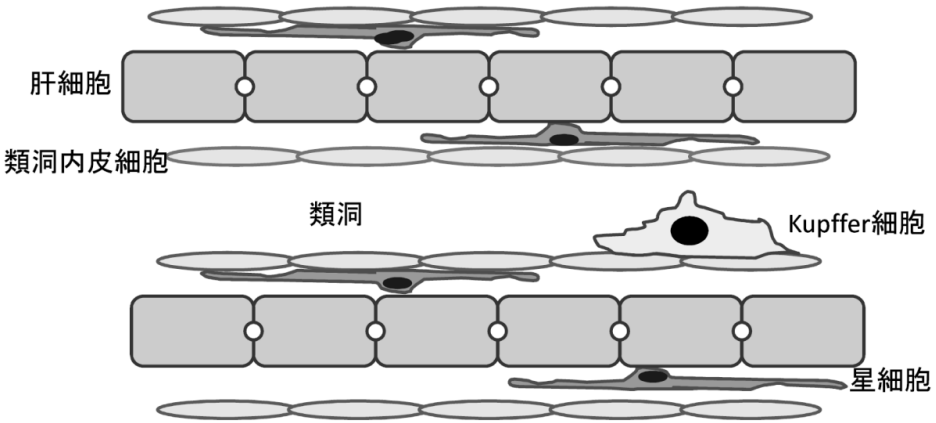
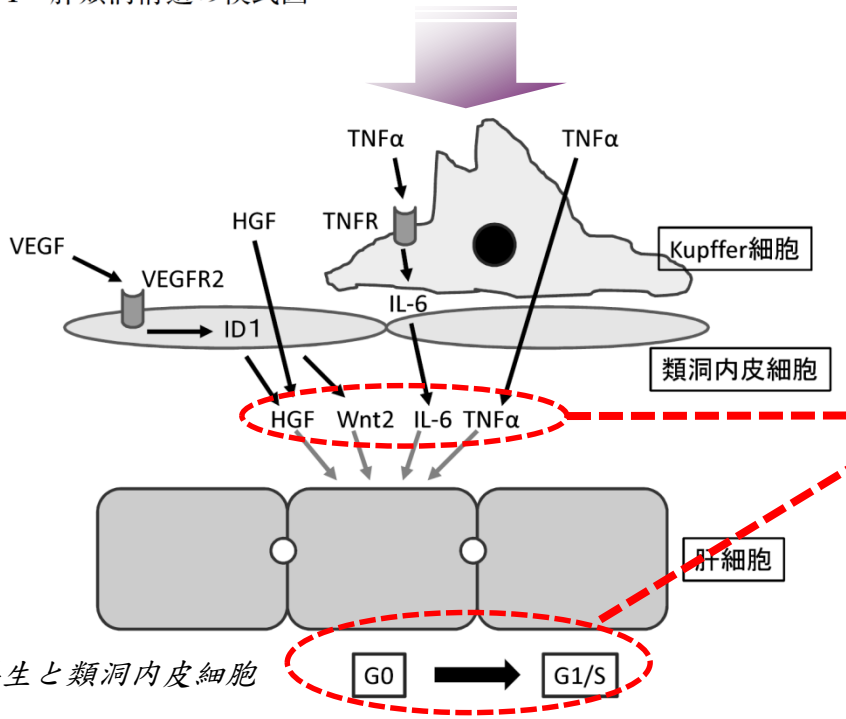



図 1　肝類洞構造の模式図

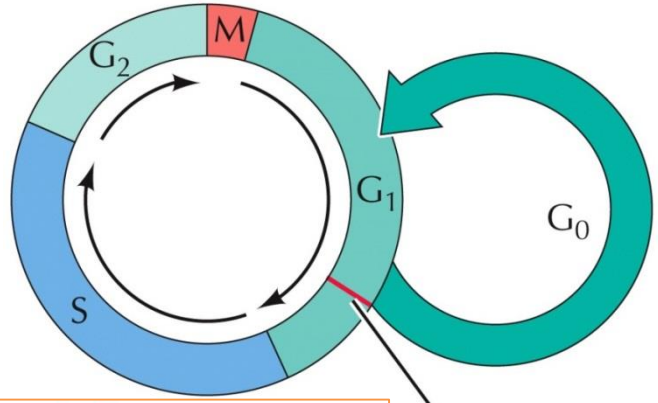
## 肝再生的機制:

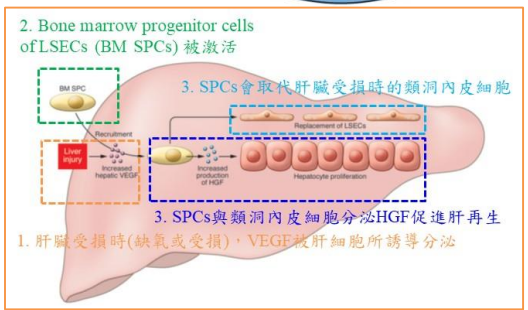
1. 肝的Stem cell 分化成oval cell、小型肝細胞及肝細胞 (較少)
2. 在$G_0$期的肝細胞受到刺激後，自我增值 (主要!!)

Passage through the restriction point is regulated by extracellular growth factors.



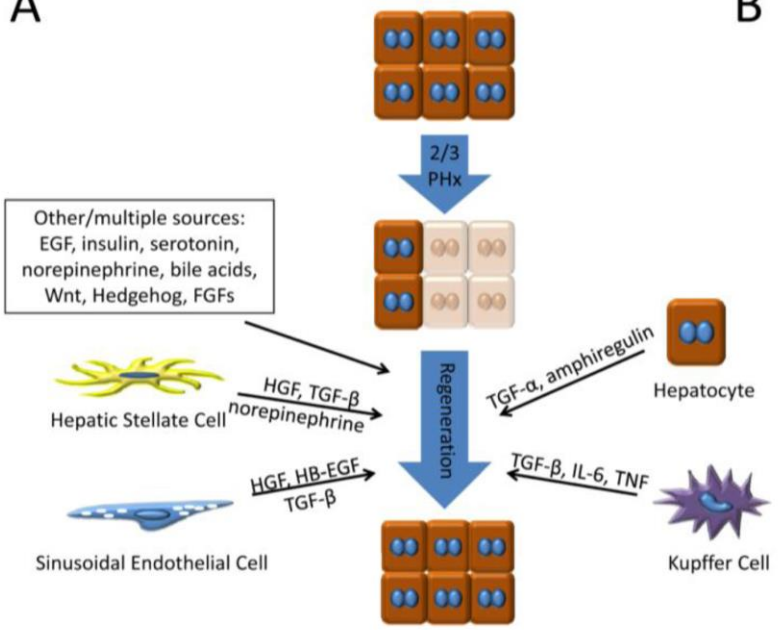

*2012 肝再生と類洞内皮細胞*

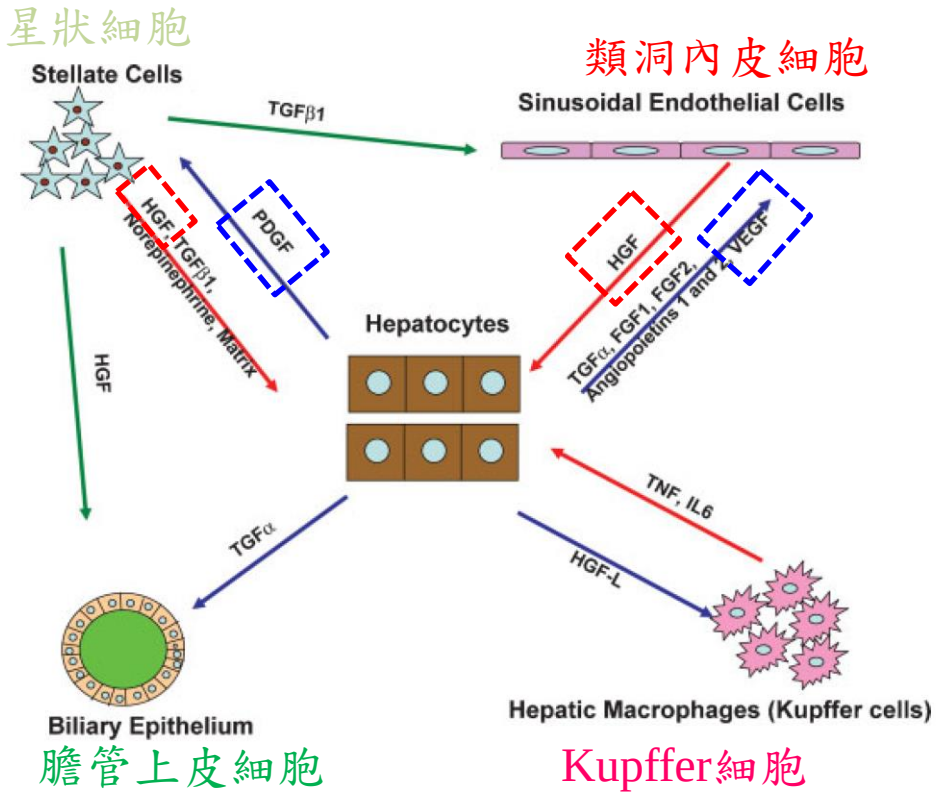


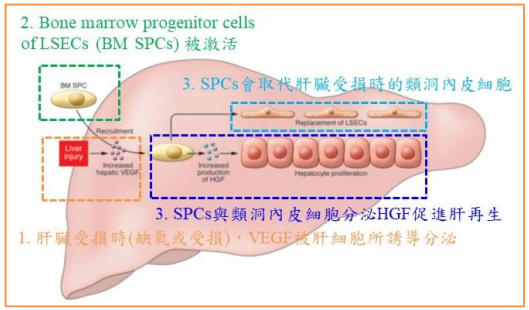
# Liver regeneration



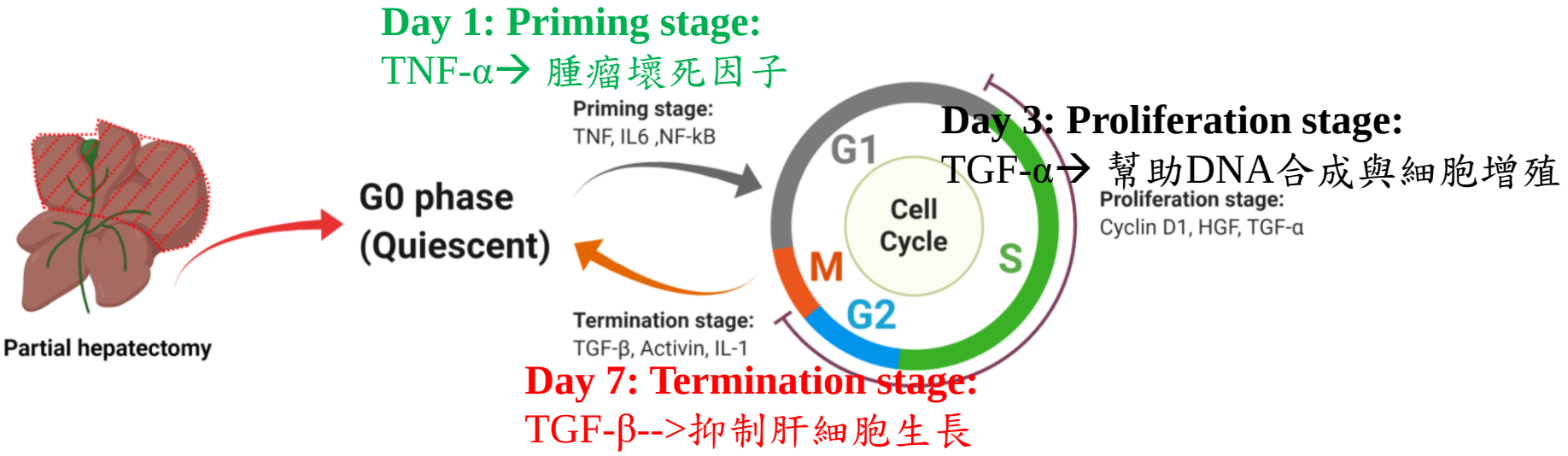



*2012 Signals and Cells Involved in Regulating Liver Regeneration*




*2007 Liver Regeneration*




*2020 The Role of Autophagy for the Regeneration of the Aging Liver*

While adult hepatocytes are normally quiescent, they show phenomenal replicative potential when parenchymal loss occurs. This has made the liver an excellent model for studying organ regeneration[1–4]. The regenerative process is typically divided into the priming, proliferating and termination phases, occurring approximately at first, third and seventh days after resection, respectively[5].

(當肝的實質細胞loss時，肝細胞會展現驚人的複製再生能力；再生過程通常分為啟動，增殖和終止階段，分別在切除後的第一，第三和第七天發生)

*2020 RNA-Seq transcriptome profiling in three liver regeneration models in rats: comparative analysis of partial hepatectomy, ALLPS, and PVL*

# Adult Hepatocytes Are Generated by Self-Duplication Rather than Stem Cell Differentiation

*2014 Adult Hepatocytes Are Generated by Self-Duplication Rather than Stem Cell Differentiation*

ONCLUSION

"Oval cells" or "atypical ductal cells" (ADCs), for regeneration following various types of injury on *in vitro* studies

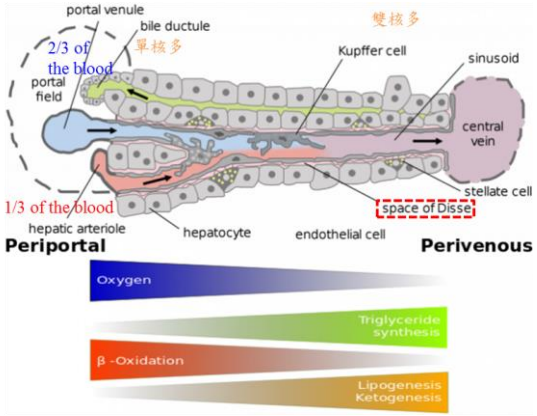
- Contrary to prevailing stem-cell-based models of regeneration, virtually all new hepatocytes come from preexisting hepatocytes.
-

In an adult normal liver, it is generally accepted that hepatocytes are substituted by surrounding hepatocytes when cell death occurs. In the physiological turnover of hepatocytes, it is speculated that liver stem/progenitor cells (LSPCs) barely contribute to the process.

*2016 Transplantation of Thy11 Cells Accelerates Liver Regeneration by Enhancing the Growth of Small Hepatocyte-Like Progenitor Cells via IL17RB Signaling*

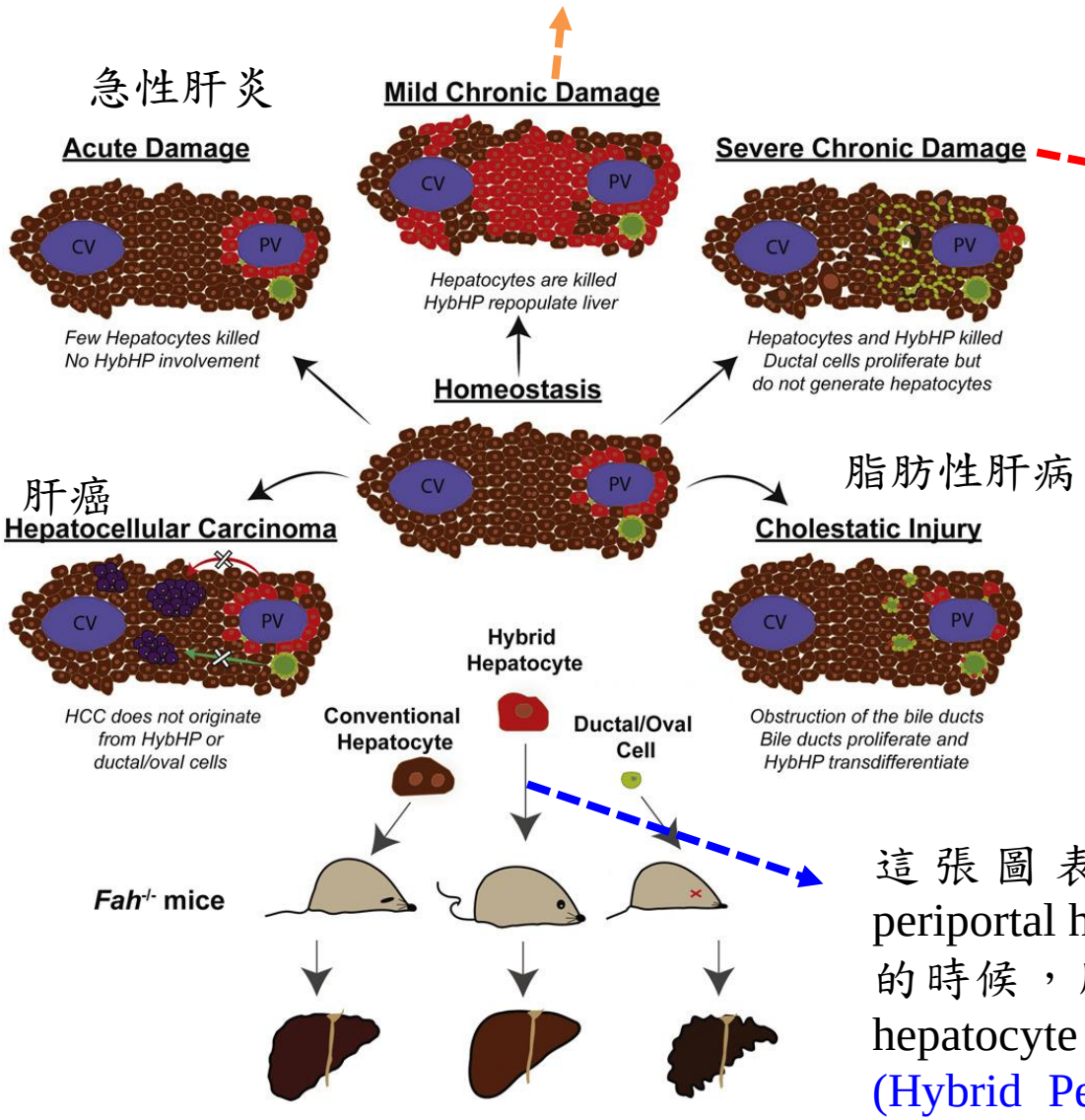
肝再生主要是由週遭回到細胞週期的細胞為主，而非幹細胞繼續分化

所以在liver zonation中，哪裡位置的肝細胞會主要進行肝再生呢?




*http://portal-vl.h-its.org/portal-view/liver-function/liver-basics/health/lobule/en/112*

慢性肝病，受損的肝細胞是由肝門靜脈附近的肝細胞進行再生而來



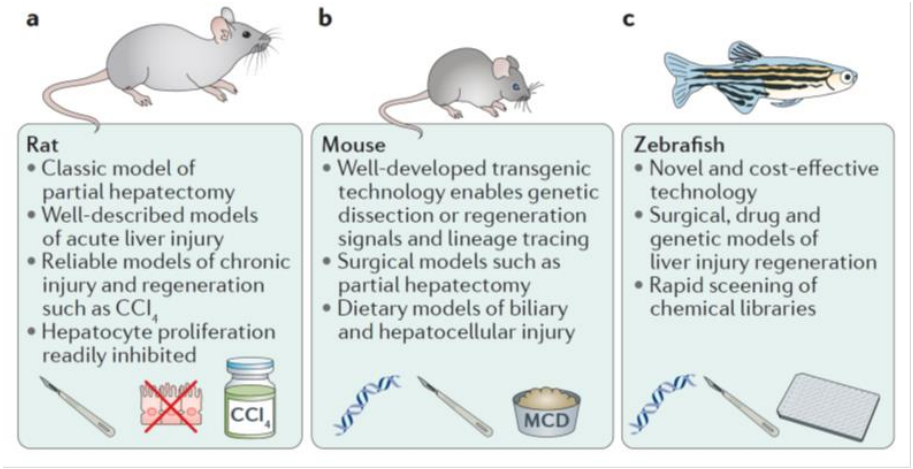

嚴重的慢性肝病，受損的肝細胞來不及由肝門靜脈附近的肝細胞進行再生而來→無法有效再生

這張圖表可說明，只有在移植Hybrid periportal hepatocytes (肝門靜脈旁邊的肝細胞)的時候，肝再生情況才會好，移植primary hepatocyte 或是oval cell效果都不好
(Hybrid Periportal Hepatocytes的分化情況恰巧是介於oval cell 與 primary hepatocyte之間)
→How about small hepatocytes?

*2015 Hybrid Periportal Hepatocytes Regenerate the Injured Liver without Giving Rise to Cancer*

# Animal models of Liver regeneration






The peak of liver regeneration, as measured by the number of hepatocytes in DNA synthetic phase, termed S phase, occurs ~24 h following resection. By 7–10 days after hepatectomy, the rat has largely regrown a normal-sized liver (93%) by hyperplasia of the remnant lobes, and by 20 days following hepatectomy the liver has fully regained its starting volume.

**細胞對肝再生扮演重要角色**

Following such 'normal regeneration' the non-parenchymal cells in the liver, namely the hepatic stellate cells (HSCs), liver sinusoidal endothelial cells (LSECS) and macrophages, act in a coordinated fashion and help to control the epithelial regenerative response.

**生長因子對肝再生扮演重要角色**

IL-6, TNF, hepatocyte growth factor (HGF), epidermal growth factor (EGF) and thyroid hormone have been discovered as humoral factors that control liver regeneration

**造成肝細胞再生過程中失敗的原因:**

The vascular shear stress in the liver's sinusoids caused by the portal blood passing through a small parenchymal volume, which can cause periportal sinusoidal endothelial damage and parenchymal inflammation.

*2016 Liver regeneration — mechanisms and models to clinical application*

# Liver injury models





## Physical injury models

The process of liver regeneration takes approximately 14 days after PHX in rodents

## Chemical injury models

The chemical injury models, though traditionally used, remain relatively less common but have gained some popularity in last decade

Variety of chemicals that are known to induce cell death in the liver resulting in subsequent compensatory liver regeneration have been used including $CCl_4$, thioacetamide (TA), acetaminophen (APAP), chloroform, galactosamine (GalN), allyl alcohol (AA), diethylnitrosamine (DEN), and bromobenzene

Chemical injury models are more complicated than PHX

# Liver failure model



Models of acute liver failure include administration of clinically relevant toxic doses of carbon tetrachloride ($CCl_4$) or acetaminophen, which can induce localized centrilobular necrosis, or surgical resection of two thirds of the liver in a partial hepatectomy model

In these models, animals develop severe liver injury, but if animals can be kept alive for as little as 72 hours following injury, host regeneration can rapidly correct the damage

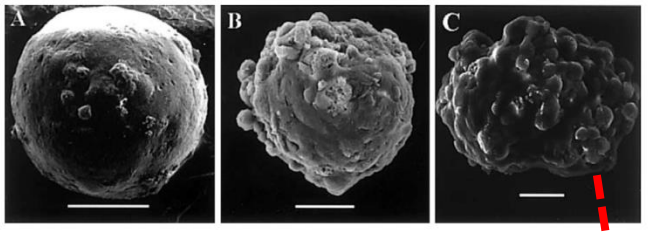
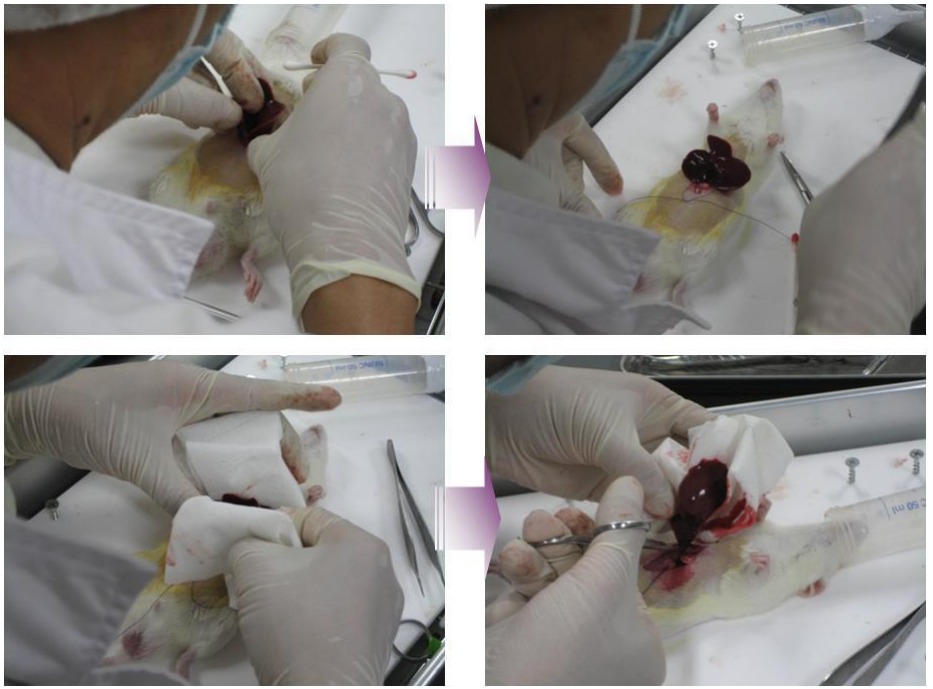



Fig. 4. SEM images of a normal liver spheroid and the spheroids 24 h after exposure to galactosamine and paracetamol. (A) A control liver spheroid with an intact surface. (B) A spheroid with a rough surface after exposure to 40 mM galactosamine (C) A liver spheroid with a disrupted surface after exposure to 50 mM paracetamol. Bars represent 50 μm.

*2003 Characterisation of some cytotoxic endpoints using rat liver and HepG2 spheroids as in vitro models and their application in hepatotoxicity studies. II. Spheroid cell spreading inhibition as a new cytotoxic marker*

Exposure to galactosamine

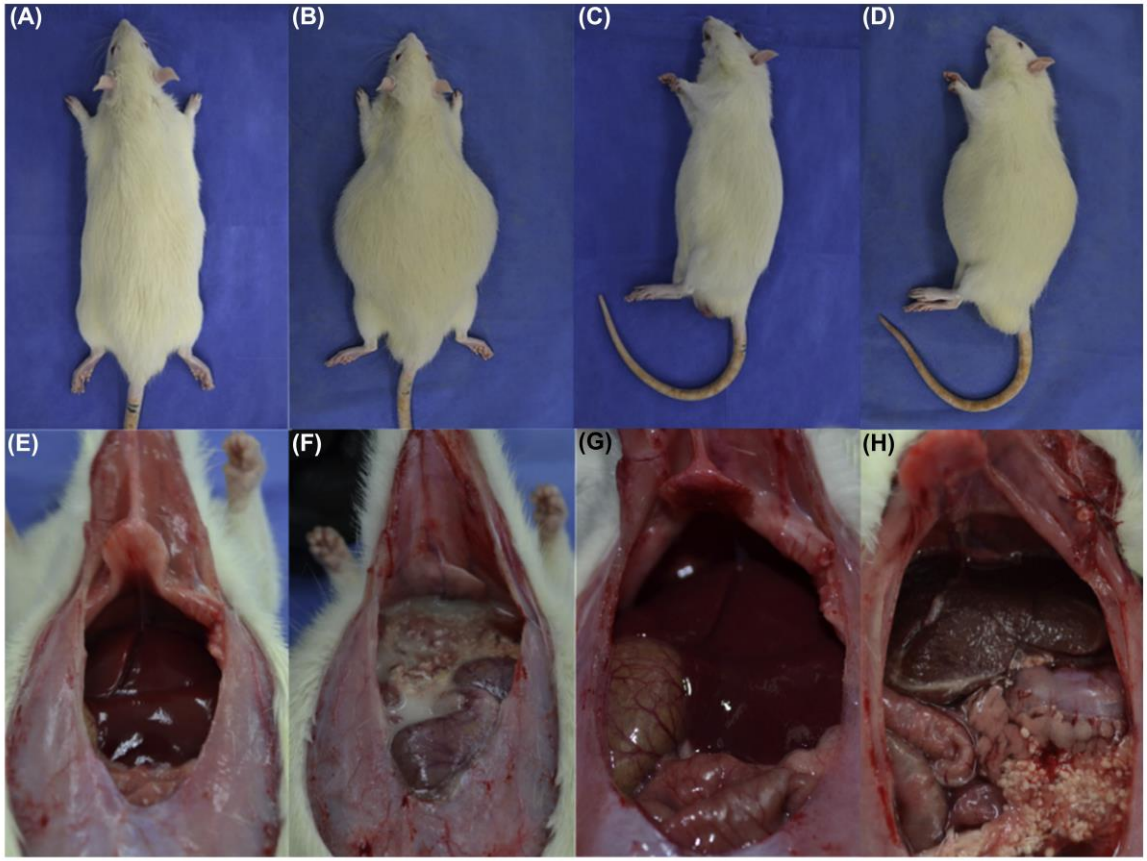


Despite the remarkably high regenerative capacity of the liver,[1] regeneration after extensive hepatectomy presents a significant clinical challenge.[2] Therefore, it provides impetus for the development of new strategies to enhance the potential of tissue regeneration after partial hepatectomy (PH). Liver regeneration is a complex process that is initiated by hepatocyte proliferation and followed by the reconstruction of hepatic lobules. Several growth factors and cytokines have been identified to play crucial roles in this process.[3] During the early phase of liver regeneration, the proliferation of liver sinusoidal endothelial cells (SECs) usually lags that of hepatocytes, which leads to a delayed reestablishment of hepatic sinusoids, decreased blood supply for the regenerating hepatocytes, and thus reduced efficiency of liver regeneration.[4–7] (在肝再生的早期，肝竇內皮細胞（SEC）的增殖通常落後於肝細胞的增殖，這導致肝竇樣物質的重建會延遲。減少了肝細胞再生時的的血液供應，從而降低了肝再生的效率)

These and other evidences demonstrate that insufficient SECs proliferation in the early phase of regeneration following extensive PH is the limiting factor for robust tissue regeneration,[8] and insufficient hepatic growth during the early phase of regeneration will lead to liver failure

## PH、以及SEC對肝再生的影響

*2014 Local Delivery of Vascular Endothelial Growth Factor via Nanofiber Matrix Improves Liver Regeneration After Extensive Hepatectomy in Rats*





會造成Ascitic 腹水

**FIGURE 40.4** Macroscopic differences between normal and carbon tetrachloride ($CCl_4$)-ascitic rats. Panels A and C depict normal rats, whereas panels B and D show ascitic rats. Rats with liver damage (Panel F) present ascites, this means that they accumulate liquid in the abdominal cavity in contrast to a normal rat (Panel E). Panel G depicts a sleek liver with a red uniform color, this liver appertains to a normal rat; however, panel H shows a macronodular liver without a red color; furthermore, this liver looks wrinkled and without brightness and belongs to a $CCl_4$-cirrhotic rat.

**造成腹水的原因：**肝硬化使肝臟變小、fenestra變緊閉、肝內血管壓力增加，加上體內鹽份滯留及血中白蛋白減少下使得腹腔內產生過量體液容積即為腹水

# Liver targeting

There are several strategies to design nanoparticles to access and interact with hepatocytes: (1) sinusoidal intercellular junctions and (2) transcytosis through the sinusoidal endothelial cell lining. Hepatocytes constitute 70–80% of the cells in the liver and are involved in the maintenance of liver functions. A wide variety of nanoparticles have been designed to target these cells [14].

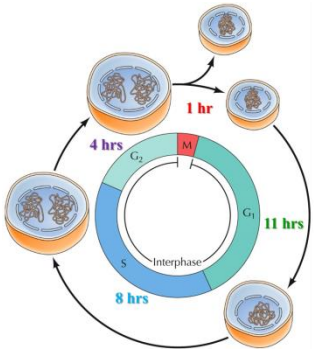
The most commonly targeted receptors used to direct nanoparticles to hepatocytes and hepatocellular carcinoma cells, include: asialoglycoprotein (ASGP) receptor, glycyrrhizin/glycyrrhetinic acid receptor, transferrin (Tf) receptor, low-density lipoprotein (LDL) receptor, high-density lipoprotein (HDL) receptor, hyaluronan receptor for endocytosis (HARE), and immunoglobulin A binding protein [9,13,33].

專一性

- ASGP is the most well characterized hepatocyte-specific receptor system [33]. One of the main advantages of using this ASGP receptor is its innate binding affinity to a wide range of molecules containing galactose and N-acetylgalactosamine residues, such as lactose, lactobionic acid, galactoside, galactosamine and asialofetuin.
- Glycyrrhizin/glycyrrhetinic acid (GL/GA) receptors are expressed on the membrane of hepatocytes as well as other cell types in the kidneys, stomach, and colon to take up glycyrrhizin/glycyrrhetinic acid via receptor-mediated endocytosis [34–37]. While GL/GA receptors are not as specific to hepatocytes as ASGP receptors, their corresponding ligands GL and GA have anti-hepatitis and anti-hepatotoxic functionality [37], marking them useful for actively targeting nanoparticles to hepatic diseases. The interaction between polymeric chitosan nanoparticles surface-modified with glycyrrhizin (CS-GL nanoparticles) and hepatocytes was studied using flow cytometry and confocal laser microscopy [38]. The result showed that CS-GL nanoparticles preferred to be taken up by hepatocytes and the uptake amount was almost five times higher than hepatic nonparenchymal cells. In vivo studies show that both doxorubicin (DOX) loaded and glycyrrhetinic acid modified recombinant human serum albumin nanoparticles (DOX/GA-rHSA nanoparticles) [35] and DOX loaded chitosan/poly(ethylene glycol)-glycyrrhetinic acid (CTS/PEGGA) nanoparticles [36] can effectively inhibit tumor growth in H22 tumor-bearing mice.
- Other clinically relevant hepatocyte and hepatocellular carcinoma ligand-receptor systems include Tf and LDL systems due to their efficient receptor recycling, enabling more rounds of endocytosis before receptor desensitization or down-regulation [33].

# Assays used to assess liver regeneration






(Late) M
Cytokinesis in progress

G2 nearly M
Blue nucleus and light brown cytoplasm. Chromosome condensation not complete (cannot see individual chromosomes)

S
Dark brown to very dark brown nuclear staining

**S**
- Dark brown nuclear
- Blue cytoplasm

**$G_2$**
- Brown nuclear
- Brown cytoplasm

**M**
- Blue chromosomes
- Light brown cytoplasm

M
Chromosomes blue, cytoplasm brown throughout (with obvious mitotic figures)

G2
Light brown nucleus and cytoplasm. No evidence of daughter cell. Nuclear envelope disappearing, but mitosis not yet started

**$G_1$**
- Light brown nuclear
- Blue cytoplasm

G1
(w.G0)
Light brown nuclear staining

G0
No brown staining (nucleus or cytoplasm)

**$G_o$**
- Blue nuclear
- Blue cytoplasm

FIGURE 2.4 PCNA cell-cycle stage determination. Each phase of the cell cycle is listed in this image with a description of the visual characteristics of each phase. In brief, G0, or quiescent cells, lacks, any brown staining. G1 cells exhibit light brown nuclear staining, while S-phase cells exhibit dark brown to nearly black nuclear staining; G1 and S-phase cells have blue cytoplasm. Cells in G2 have brown cytoplasm and brown nuclei, while cells in mitosis have light brown cytoplasm but bright blue chromosomes. Please refer to the insets above for additional information.

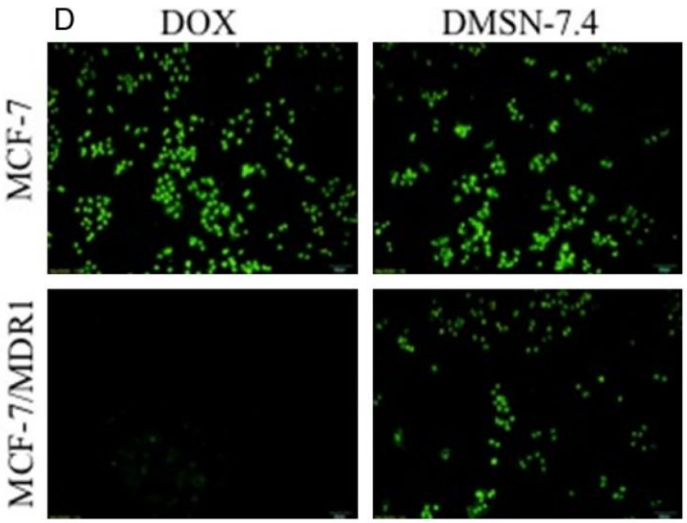
**PCNA Immunolocalization**
PCNA expression is induced in late G1, peaks in S-phase, and is reduced thereafter.

*2016 Liver regenaration Basic Mechanisms, Relevant Models and Clinical Applications Chapter .2. MODELS TO STUDY LIVER REGENERATION*

## TUNEL assay

The *in vitro* cytotoxicity of DMSNs was tested via terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay and cell viability by MTT assay. **TUNEL assay was also used to detect apoptosis** [7]. Cell apoptosis after drug treatment was determined by TUNEL assay using the in situ cell death detection kit-POD (Roche, Burgess Hill, UK) according to the manufacturer's instructions. Photographs of apoptosis of the two cell lines were recorded under a X fluorescence microscope at ×100 magnification.

TUNEL assays can efficiently detect cell apoptosis by labeling the terminal ends of nucleic acids of DNA fragments. As shown in Figure 4D, DMSN-7.4 was able to induce not only MCF-7 cell apoptosis, but also MCF-7/MDR cell apoptosis, whereas equivalent free DOX showed no obvious **apoptosis fluorescence** after 24 h incubation.




D. TUNEL assay showing apoptosis by free DOX and DMSN-7.4 in MCF-7 cells and MCF-7/MDR cells after 24 h incubation.

*2014 Increasing the cytotoxicity of doxorubicin in breast cancer MCF-7 cells with multidrug resistance using a mesoporous silica nanoparticle drug delivery system*

# Liver failure and current treatments



**Liver failure can be generally separated into two major categories:**

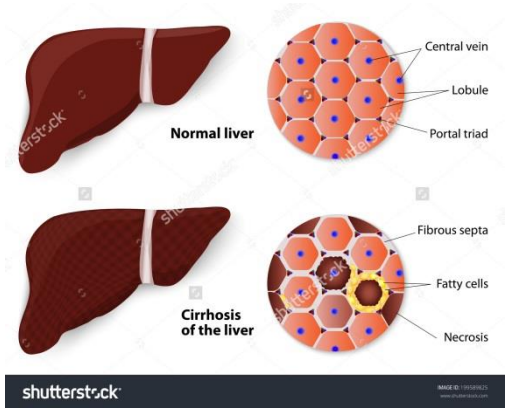
**Fulminant hepatic failure**

1. Rare, but exhibits a high mortality rate (28%)
2. Include acetaminophen overdose, idiosyncratic drug reactions and viral hepatitis A and B

**Chronic hepatic failure**

1. Much more common than fulminant failure
2. Include hepatitis B and C virus, alcohol-induced and non-alcoholic fatty liver disease (NAFLD)

Cirrhosis (肝硬化) initiated by hepatitis C infection is the most frequent cause for liver transplantation




*https://reurl.cc/py3GXb*

**Liver transplantation is currently the only therapy shown to directly alter mortality**

# Major causes of chronic liver diseases



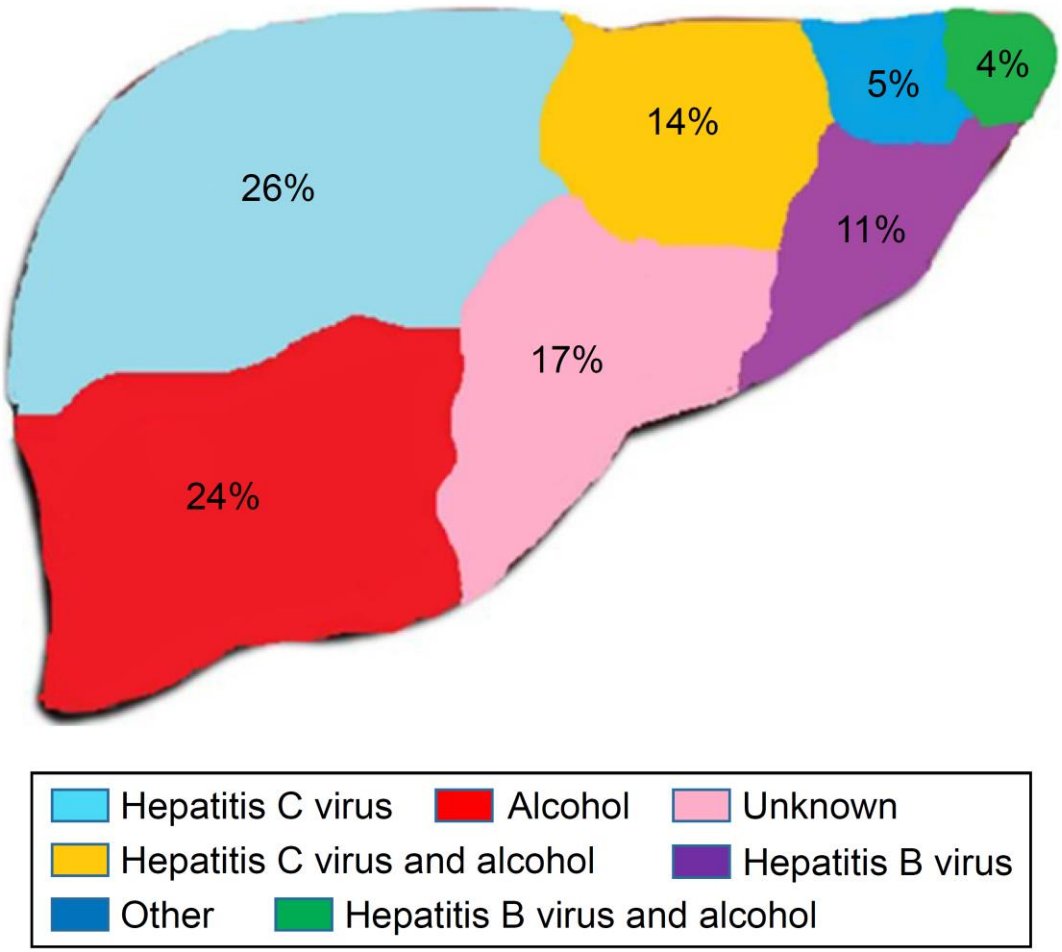


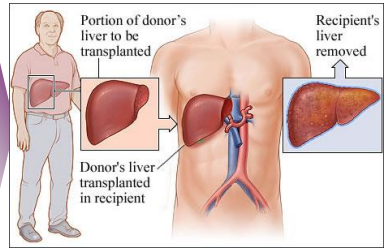

**Figure 1** Major causes of chronic liver diseases

# Various methods for curing the liver disease



**Way 1: (biological extracorporeal support)**


*www.plurk.com*




*http://medifitbiologicals.com/live-transplant/*

**Drug**

1. The use of non-heart-beating donors
2. Split liver transplants from cadaveric or living donors

**Transplantation**

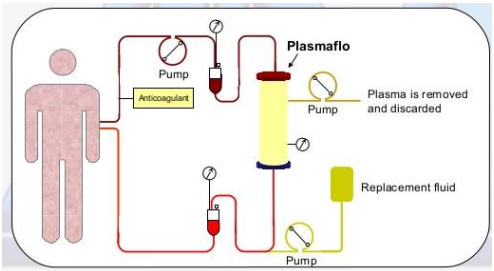
- Partial hepatectomy (PH) or chemical injury induces the proliferation of the existing mature cell populations.
- However, biliary and vascular complications are major concerns in these procedures

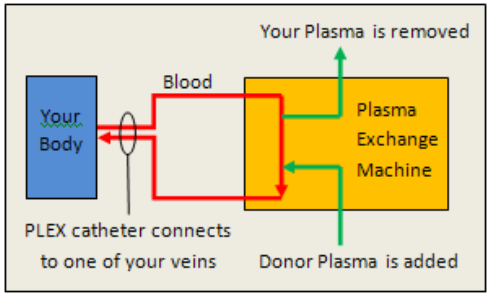
**Donor shortage is still the problem**

**Way 2: (non-biological extracorporeal support)**

1. Plasma exchange
2. Plasmapheresis (血漿置換)
3. Hemodialysis
4. Molecular adsorbents recirculation system
5. Hemoperfusion over charcoal or various resins

**Plasma exchange**




*https://www.slideshare.net/WanMed1/plasma-therapy-training-presentation*

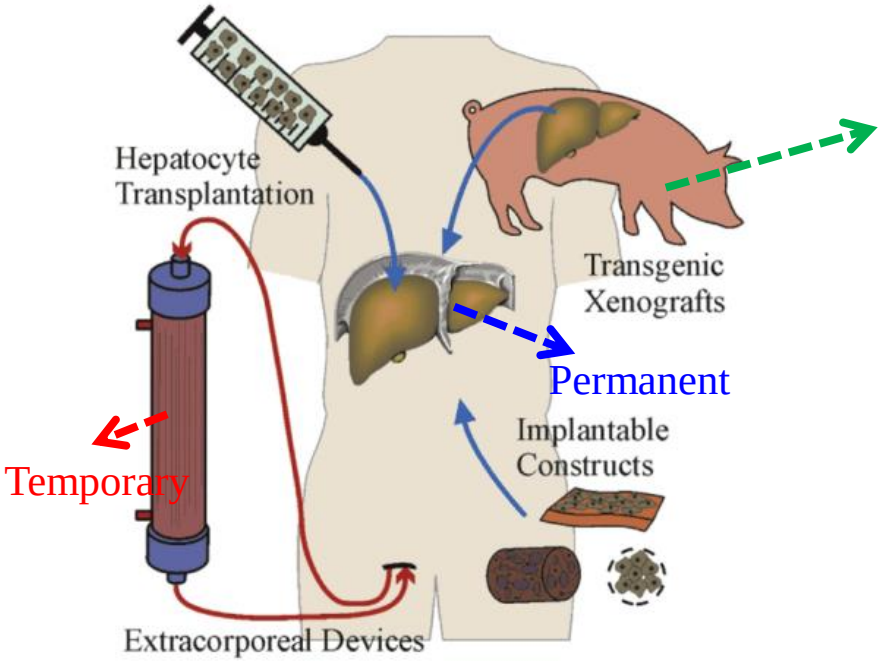
However, limited in narrow range of functions (detoxification, synthetic and metabolic process)

**Cell-based therapies for liver disease may be better**

# Cell-based therapies for liver disease



**Cellular therapies**




Primary porcine hepatocytes have been utilized in a range of BAL device configurations with some encouraging results. However, the utility of xenogeneic porcine cells for human liver therapies is restricted by immunogenicity and the potential for xenozoonotic transmission of infectious agents such as porcine endogenous retrovirus (PERV).

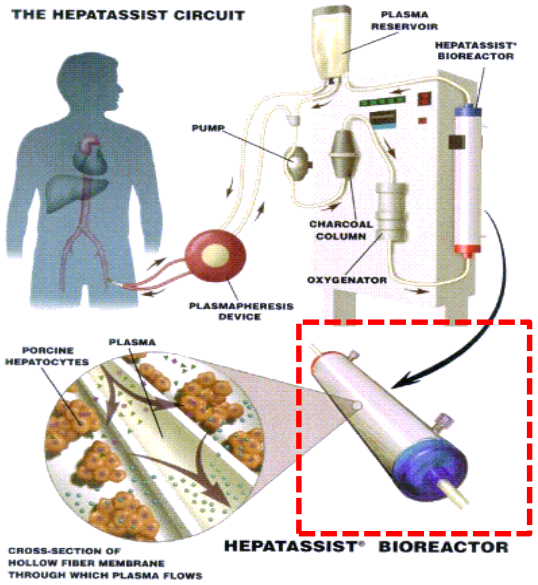
Recognizing these concerns, recent efforts have led to the development of PERV-free pigs as well as genetically modified pigs that are transgenic for human proteins, thereby decreasing their immunogenicity.

Limitation:

Inability of hepatocytes from the *in vivo* hepatic microenvironment to maintain hepatocyte-specific phenotype and function *in vitro* is the problem

# Bioartificial liver device (For temporary use)






*https://reurl.cc/Q3RMOO*

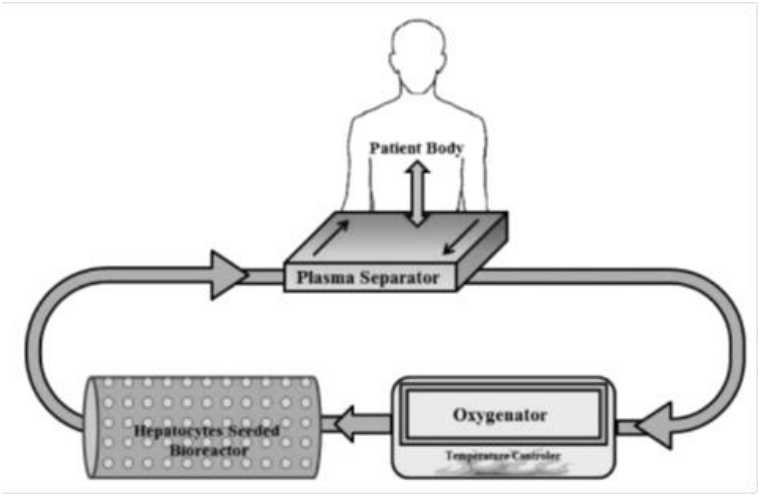



**Figure 1.** A proposed set up of bio-artificial liver (BAL) system. The system comprises the filtration of patient venous blood passes through plasma separator unit connected to oxygenator unit under a controlled temperature. Furthermore, the plasma is then passed through hepatocytes activated bioreactor and return back to the patient body along with the blood cells.

*2011 Extracorporeal Bioartificial Liver for Treating Acute Liver Diseases*

The first bioartificial liver device was developed by Dr. Kenneth Matsumara and was named an invention of the year by Time magazine in 2001. Animal liver cells are suspended in a solution and a patient's blood is processed by a semipermeable membrane that allow toxins and blood proteins to pass but restricts an immunological response.

Cell sources inside BAL now include :

1. Primary porcine hepatocytes
2. Primary human hepatocytes
3. Human hepatoblastoma (C3A)
4. Immortalized human cell lines and stem cells

*https://en.wikipedia.org/wiki/Bioartificial_liver_device*

# Bioartificial liver device (2)



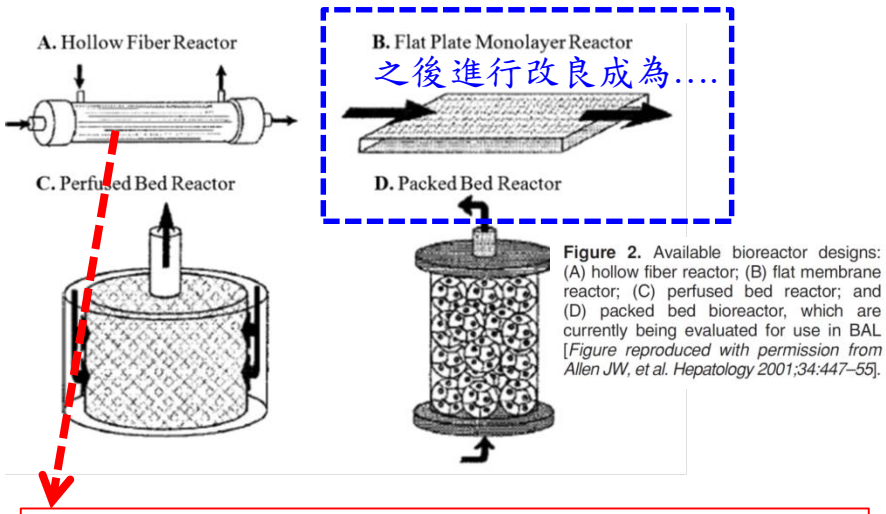



**Figure 2.** Available bioreactor designs: (A) hollow fiber reactor; (B) flat membrane reactor; (C) perfused bed reactor; and (D) packed bed bioreactor, which are currently being evaluated for use in BAL [*Figure reproduced with permission from Allen JW, et al. Hepatology 2001;34:447–55*].

Hepatocytes are suspended in a gel solution, such as collagen, which is injected into a series of hollow fibers. Nutrient media is circulated through the fibers to sustain the cells. During use, plasma is removed from the patients blood. The patient's plasma is fed into the space surrounding the fibers. The fibers, which are composed of a semi-permeable membrane, facilitate transfer of toxins, nutrients and other chemicals between the blood and the suspended cells.

*https://en.wikipedia.org/wiki/Bioartificial_liver_device*

**Compared to liver dialysis (人工肝臟的優勢):** The advantages of using a BAL, over other dialysis-type devices (e.g. liver dialysis), is that metabolic functions (such as lipid and plasma lipoprotein synthesis, regulation of carbohydrate homeostasis, production of serum albumin and clotting factors, etc.), in addition to detoxification, can be replicated without the use of multiple devices.

**Table 1.** Comparison of the available bioartificial liver approaches.

| BAL Designs | Features | Cell Source | Cell Growth Time | Merit/Demerits | Success |
|---|---|---|---|---|---|
| Flat membrane | Sandwiched cultivation of cell in flat configuration on membranes | Pig<br>Rat<br>Humans | <1 month | *Advantages:*<br>1. Uniform cell distribution<br>2. Ease of scale up<br>3. In vivo like microenvironment<br>*Disadvantages:*<br>1. Large dead volume<br>2. Low surface area/ volume ratio<br>3. Susceptible to viral infection | Preclinical trials going on in rats and pigs |
| Hollow fiber system | Cell cultivation inside or outside the tubes in tube shell configuration | Rat<br>Pig | <25 days | *Advantages:*<br>1. large surface area/ volume ratio<br>2. Efficient exchange of nutrients and waste material<br>3. Porous system for cell support<br>4. High product recovery<br>5. Immunoisolation<br>6. Ease of scale up<br>7. In vivo like microenvironment<br>*Disadvantages:*<br>1. Expensive<br>2. Nonuniform cell distribution<br>3. Ineffective convective mass transfer | Clinical trials conducting on ELAD, HepatAssist LSS, BLSS, RFB-BAL, AMC-BAL |
| Packed bed | Beads used to entrap cells and then packing them in a column | Rat<br>Pig | <16 days | *Advantages:*<br>1. High surface area/volume<br>2. In vivo-like microenvironment<br>3. Ease of scale up<br>4. Immunoisolation<br>5. High capacity for cell mass<br>*Disadvantages:*<br>1. Risk of release of cells from beads | Preclinical trials of UCLA-BAL |
| Spheroids culture technology | Culturing of cell aggregates called spheroids on substratum | Rat<br>Pig | <21 days | *Advantages:*<br>1. Cell-to-cell contact<br>2. Ease of scale up<br>*Disadvantages:*<br>1. Limited oxygen and nutrient supply<br>2. Chances of viral infiltration | Preclinical trials on PUF-HALSS |

ELAD, extracorporeal liver assist device; LSS, liver support system; BLSS, bioartificial liver support system; RFB, radial flow bioreactor; AMC-BAL, Academic Medical Center bioartificial liver.

細胞存活時間都很短!

*2011 Extracorporeal Bioartificial Liver for Treating Acute Liver Diseases*

# Cell sources for liver cell-based therapies



Due to the paucity (缺乏) of human liver tissue as a cell source, **alternative cell sources** have been explored with inherent strengths and drawbacks. However, the criteria to characterize these alternative cell sources as a hepatocyte or hepatocyte-like cells have not been standardized and vary greatly among different studies. No single test has been demonstrated to be sufficient to determine whether a particular cell type truly recapitulates hepatocyte function; as a result, several tests must be carried out to query various domains of hepatocyte function, including bile production, detoxification, metabolic, and synthetic functions.

**TABLE 46.1 Cell sources for liver therapies**

| Cell source | Critical issues |
|---|---|
| Primary hepatocytes<br>Human adult and fetal, xenogeneic | Sourcing, expansion, phenotypic instability, immunogenicity, safety (xenozoonotic) |
| Immortalized hepatocyte lines<br>Tumor-derived, SV40, telomerase, spontaneously immortalized | Range of functions, genomic instability, safety (tumorigenicity) |
| Stem Cells<br>Embryonic, liver progenitors (hepatoblasts, oval cells), other lineages (HSC, MAPC), Induced pluripotent stem cells, direct reprogramming to hepatocytes | Sourcing, differentiation efficiency, phenotypic instability, immunogenicity, safety (tumorigenicity) |

**TABLE 46.2 Hepatic functions**

| Functional classification | Examples |
|---|---|
| Synthetic | Albumin Secretion<br>Alpha-1-antitrypsin Secretion<br>Coagulation Factor Production (II, IX, X)<br>Lipoprotein and apoprotein synthesis<br>Ceruloplasmin production<br>Ferritin production<br>Complement production |
| Metabolic | Ureagenesis and metabolism<br>Bilirubin Metabolism<br>Steroid Metabolism<br>Gluconeogenesis/Glycogen Production<br>Lipid metabolism |
| Detoxification pluripotent stem cells, direct reprogramming to hepatocytes | Metabolize, detoxify, and inactivate exogenous and endogenous compounds via cytochrome P450 enzymes, methyltransferases, sulfotransferases, acetyltransferases, UDP-glucuronosyltransferases, and Glutathione S-transferases |
| Bile Production | |

# Cell sources for liver cell-based therapies (*cell line*)



Immortalized hepatocyte cell lines such as:

1. HepG2 (human hepatoblastoma)
2. HepG2 derived line C3A
3. HepLiu (SV40 immortalized)
4. Immortalized fetal human hepatocytes

However, these cell lines lack the full functional capacity of primary adult hepatocytes, and for clinical applications there is a risk that oncogenic factors or transformed cells could be transmitted to the patient

**Table 1.** Cell choices for liver tissue engineering

| Cell | Source | Application | Advantages | Disadvantages |
|---|---|---|---|---|
| Human hepatocytes | Discarded human liver tissue | Bio-artificial liver.<br>Implantable devices.<br>Cell transplantation. | High host compatibility. | Reduced availability.<br>Poor *in vitro* proliferation. |
| Porcine hepatocytes | Porcine liver | Bio-artificial liver.<br>Implantable devices. | Enhanced availability compared with human hepatocytes. | Potential zoonotic disease transmission.<br>Protein–protein incompatibility.<br>Possible immune response. |
| Tumour derived human hepatocyte cell lines | Cell lines derived from human hepatic carcinoma. | Bio-artificial liver.<br>Implantable devices. | Easy storage, maintenance and *in vitro* proliferation. | Tumorigenicity.<br>Reduced functional performance. |
| Immortalized human hepatocyte cell lines | Human hepatocytes immortalized by gene transfection. | Bio-artificial liver.<br>Implantable devices. | Easy *in vitro* proliferation, storage and maintenance.<br>Reduced tumorigenicity compared with tumour derived cell lines. | Potential tumorigenicity.<br>Long-term safety concerns.<br>Reduced functional performance. |
| Adult stem cells (MSCs) | Adult tissues | Cell transplantation.<br>Implantable devices. | Unlimited availability.<br>Less safety concerns. | Transdifferentiation of MSCs to myofibroblasts.<br>Lower efficiency of the available hepatic differentiation protocols.<br>Longer duration of hepatic differentiation. |
| Oval cells | Human fetal liver | Cell transplantation. | Native liver stem cells.<br>Can differentiate to both hepatocyte and biliary cells. | Cell isolation is difficult.<br>Limited availability.<br>Tumorigenic potential. |
| Human fetal hepatocytes | Human fetal liver | Bio-artificial liver.<br>Implantable devices. Cell transplantation. | Easy cell isolation.<br>Can undergo few cell divisions *in vitro*. | Lower functional efficiency.<br>Reduced availability.<br>Ethical concerns.<br>Possible tumorigenicity. |
| Hepatoblast | Human fetal liver at early stage of gestation | Cell transplantation. | Extensive *in vitro* proliferation. | Ethical issues.<br>Limited availability. |
| Embryonic/ induced pluripotent stem cells | Pluripotent cell lines derived from discarded human embryo (embryonic) or genetically modified adult cells (induced pluripotent) | Bio-artificial liver.<br>Implantable devices. | Unlimited availability and indefinite proliferation.<br>Hepatic differentiation capability. | Ethical issues.<br>Possible teratoma formation. |
| Induced hepatocyte like cells (iHep) | Genetically modified adult cells to express hepatic genes. | | Easy method for generating hepatocytes within a limited time period. | Functional stability and safety. |

*2013 Liver tissue engineering and cell sources issues and challenges*

# HepaRG human hepatocytes



The most human hepatocyte cell lines (HepG2, Hep3B, PLC/PRFs Huh7, HBG) are derived from liver tumors

But these cells have gradually lost most of the metabolic properties of normal liver

近來研究

Human hepatoma HepaRG cell line has the following advantages:

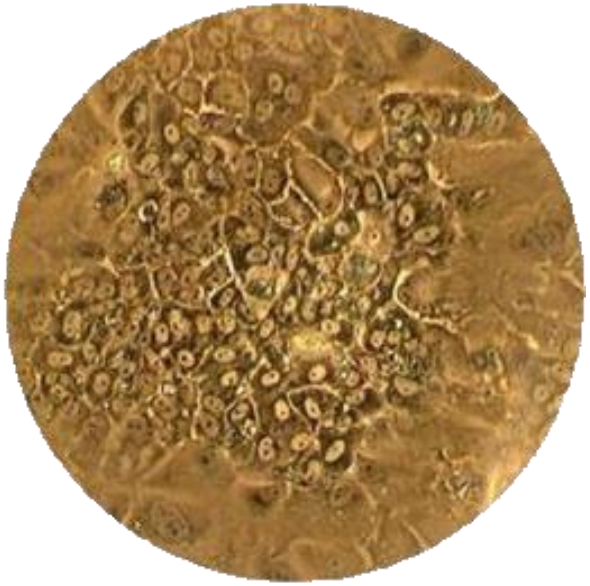
1. Retained the expression of both liver-specific plasma proteins and liver-specific glycolytic enzymes,
2. High expression and inducibility of the major phase I and phase II detoxification enzymes

Human hepatoma HepaRG cell line

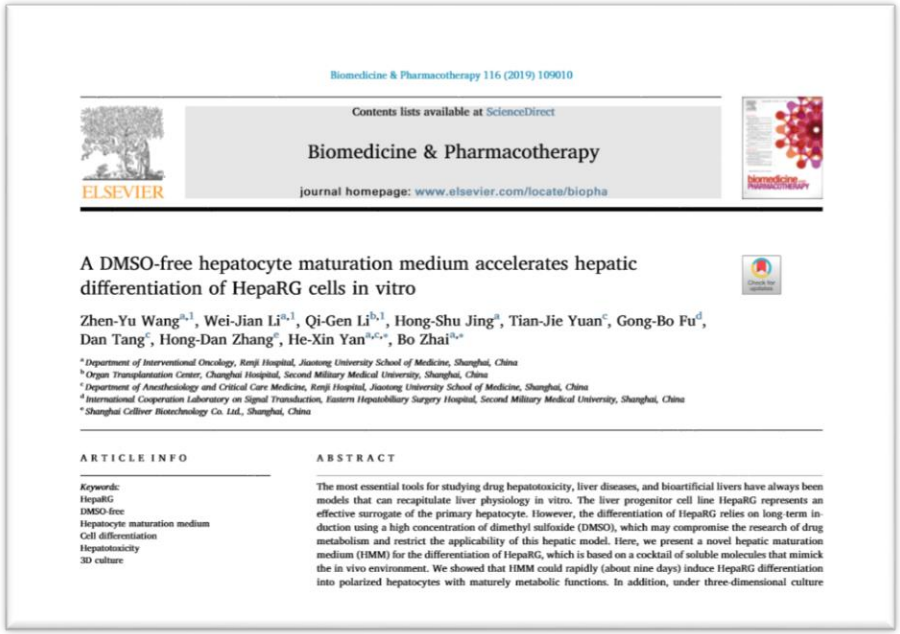
**Advantage of HepaRG**

- **HepaRG** is the most innovative and useful hepatic cell line
- A unique and well established hepatic cell system able to produce early hepatic progenitor cells as well as completely mature human hepatocytes.

*http://www.heparg.com/*



*http://www.bnpands.com/biopredic.html*



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# A DMSO-free hepatocyte maturation medium accelerates hepatic differentiation of HepaRG cells in vitro

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Keywords:
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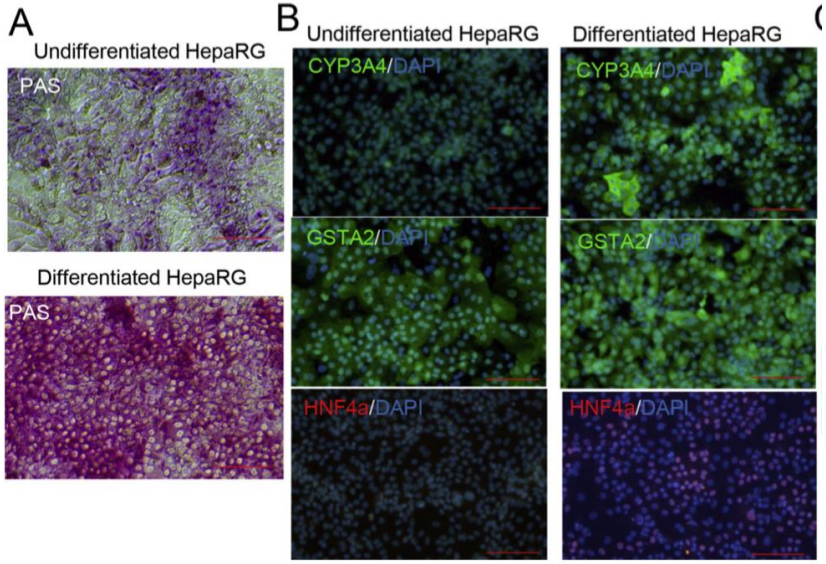
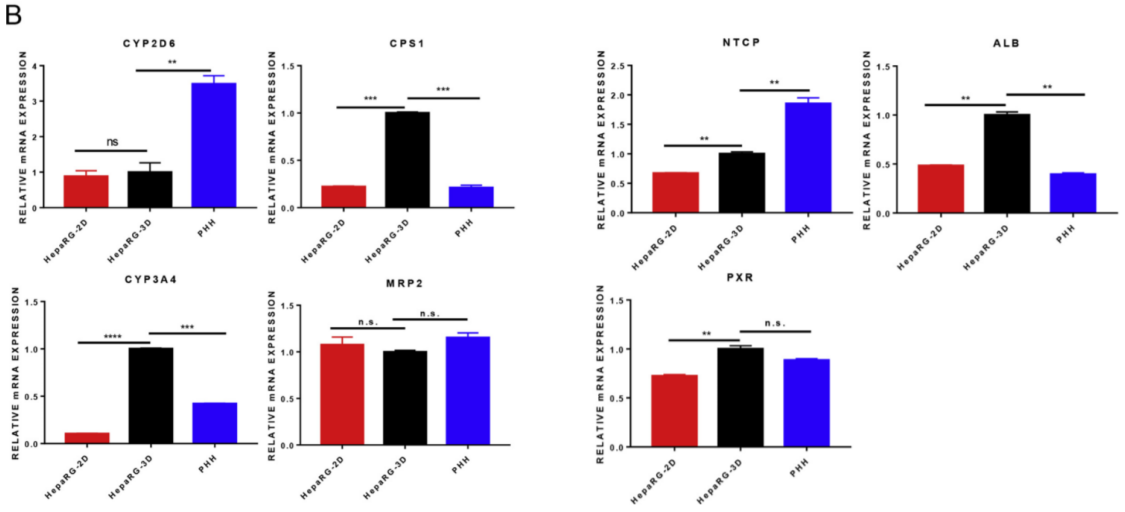
Hepatocyte maturation medium
Cell differentiation
Hepatotoxicity
3D culture

ABSTRACT

The most essential tools for studying drug hepatotoxicity, liver diseases, and bioartificial livers have always been models that can recapitulate liver physiology in vitro. The liver progenitor cell line HepaRG represents an effective surrogate of the primary hepatocyte. However, the differentiation of HepaRG relies on long-term induction using a high concentration of dimethyl sulfoxide (DMSO), which may compromise the research of drug metabolism and restrict the applicability of this hepatic model. Here, we present a novel hepatic maturation medium (HMM) for the differentiation of HepaRG, which is based on a cocktail of soluble molecules that mimick the in vivo environment. We showed that HMM could rapidly (about nine days) induce HepaRG differentiation into polarized hepatocytes with maturely metabolic functions. In addition, under three-dimensional culture




Glycogen and immunofluorescence staining of differentiated and undifferentiated HepaRG cells. (A) Periodic Acid-Schiff staining of HepaRG before (top) and after (bottom)the 9-day differentiation of HMM. (B) Detection of hepatic proteins (CYP3A4, GSTA2, and HNF4α) of undifferentiated and differentiated HepaRG after the 9-day cultures by immunofluorescence staining.




QPCR analyses of the expression levels of 7 hepatocyte function genes in HepaRG-3D, HepaRG-2D, and freshly isolated primary hepatocytes. Error bars represent s.d.; n=3; **P < 0.01, ***P < 0.001, ****P < 0.0001, n.s., no significant.

# Cell sources for liver cell-based therapies (*Primary cells*)



**Primary human hepatocytes** are the ideal cell type for cell-based therapies,

However, culture *in vivo*

1. Loss of liver-specific functions
2. Significant proliferative capacity during regenerative responses is limited

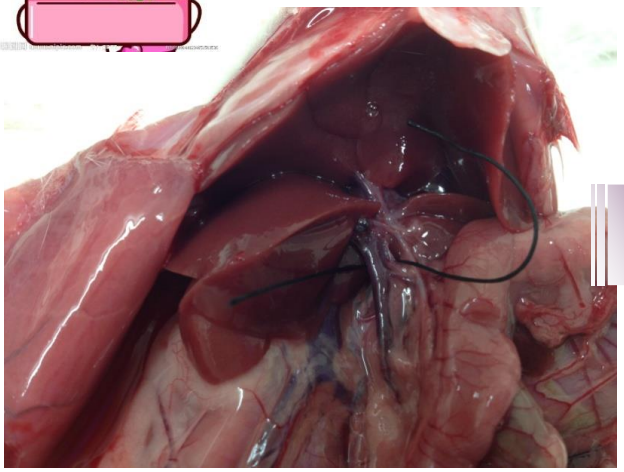
Primary human hepatocytes are considered as the most pertinent *in vitro* model. Unfortunately, their unpredictability, scarce availability, and inter-donor basal variability greatly hamper their use

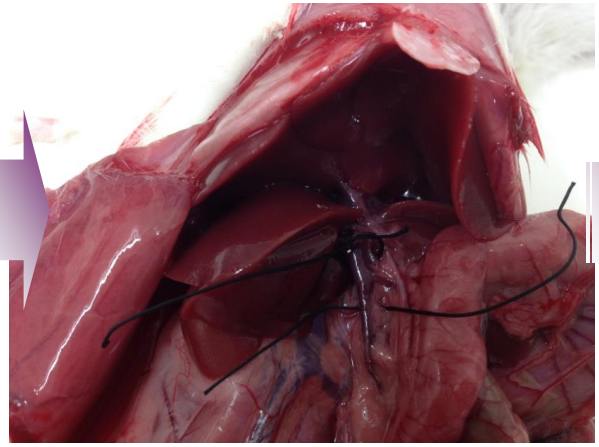
改善方法

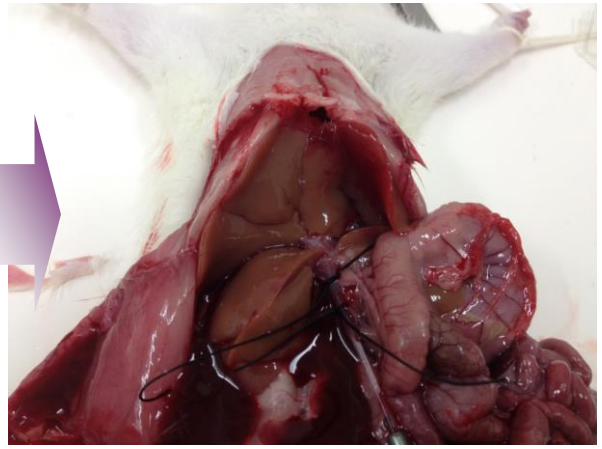
Due to the limitations in mature hepatocyte expansion *in vitro*, alternative cell sources are being pursued. These include various stem cell and progenitor populations, which can self-renew *in vitro* and exhibit multi-potency or pluripotency and thereby serve as a possible source of hepatocytes, as well as other non-parenchymal liver cells.

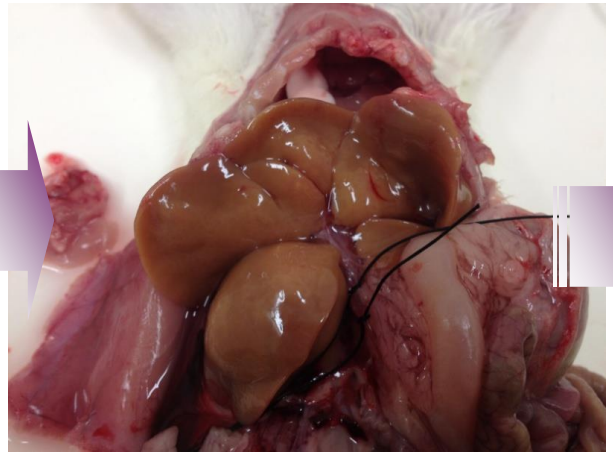
# Collagenase perfusion

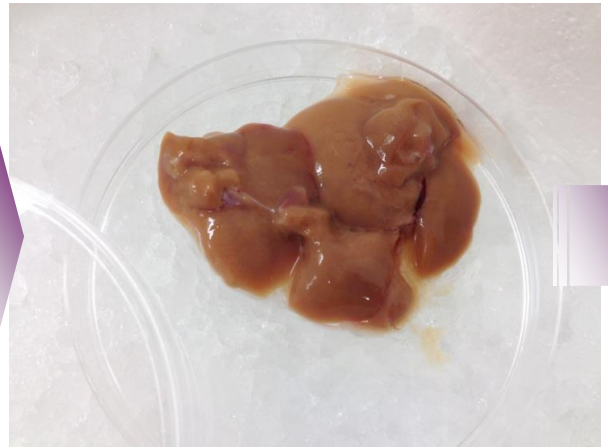


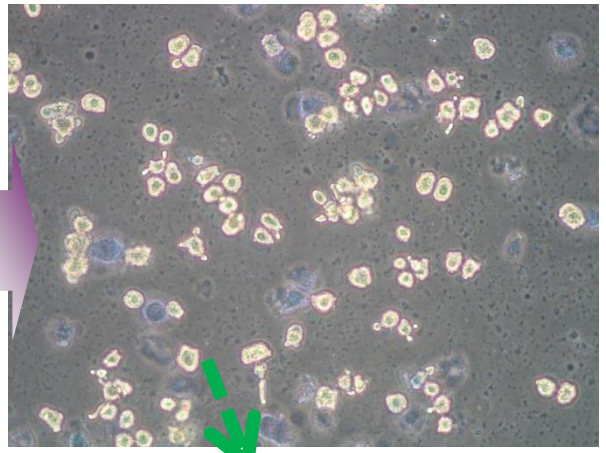













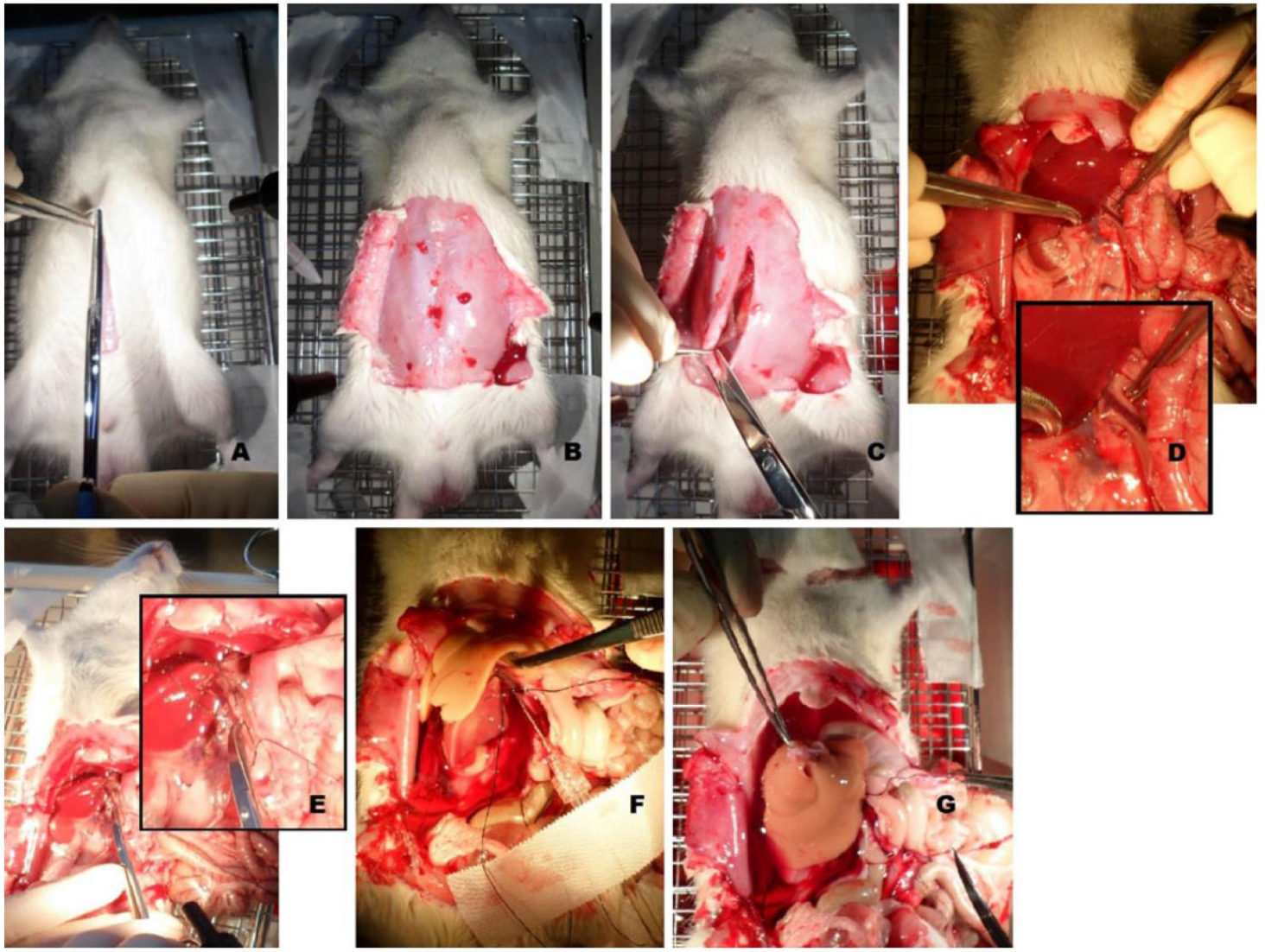
Normally nonviable cells are removed at the first change of medium

**Video: Primary mouse hepatocyte isolation**

*https://www.youtube.com/watch?v=oOHUXk-KBVs*

# Collagenase perfusion





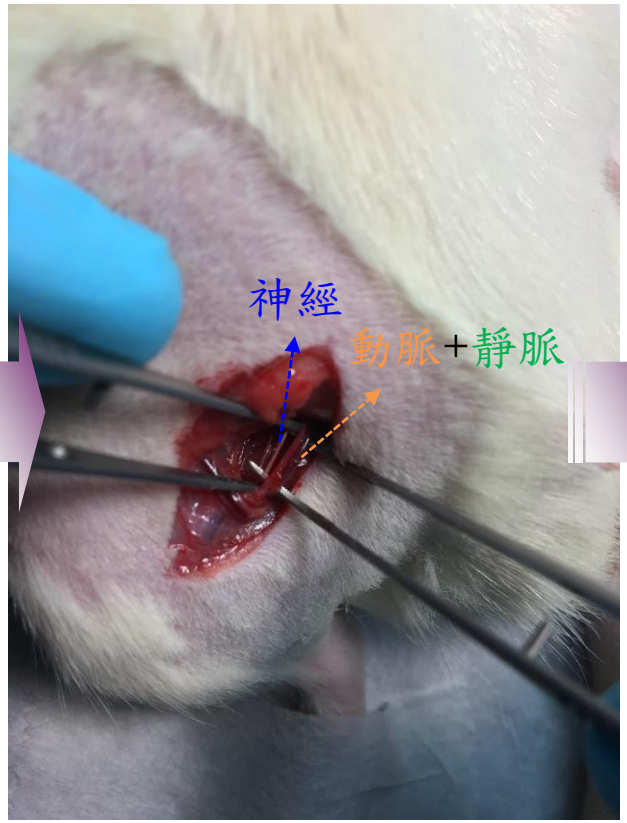

*2013 Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME*

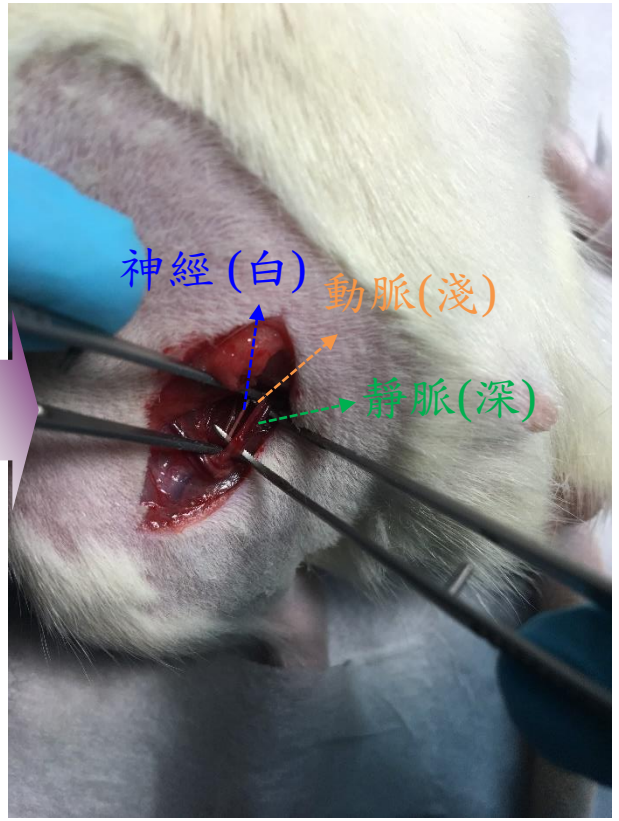
# 股靜脈注射(Intravenous injection; IV)

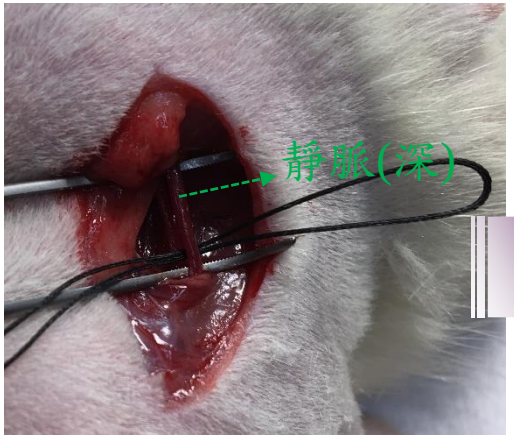


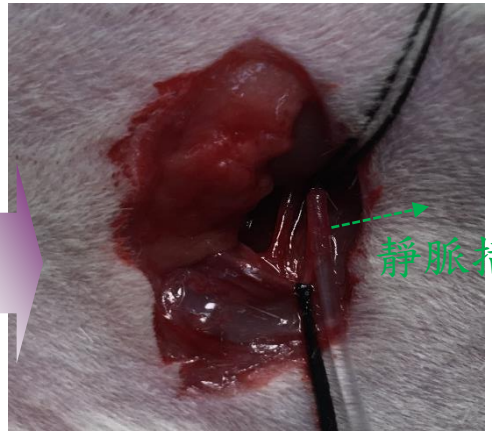






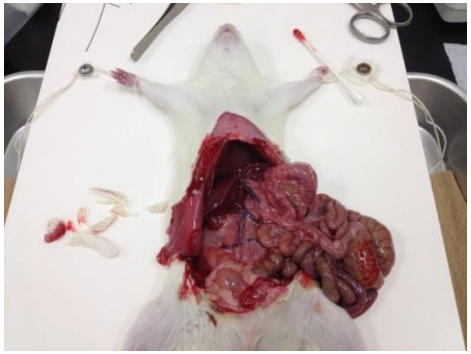




# Animal experiments

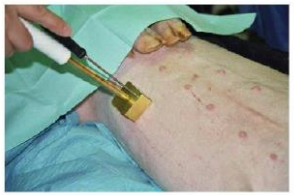


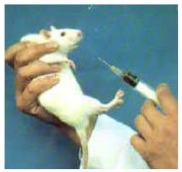
動物保護法(民國 100 年 06 月 29日修訂)

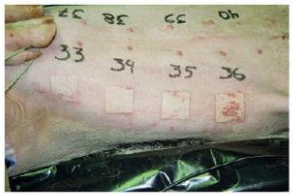
第15條 使用動物進行科學應用，應儘量避免使用活體動物，有使用之必要時，應 以最少數目為之，並以使動物產生最少痛苦及傷害之方式為之。 中央主管機關得依動物之種類，訂定實驗動物之來源、適用範圍及管理辦法。



## 動物會不會疼痛、恐懼？







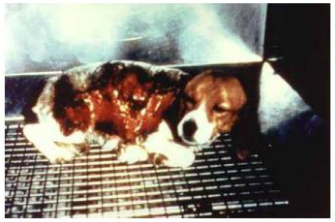
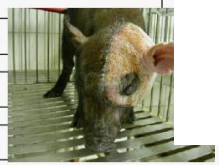


Table 2: Post Procedural Pain Potential [a, b] 疼痛等級

| Minimal to Mild [c] | Mild to Moderate [d] | Moderate to Severe [e] |
|---|---|---|
| Catheter implantation | Minor laparotomy incisions 剖腹手術 | Major laparotomy/organ incision |
| Tail clipping | Thyroidectomy 切除甲狀腺 | Thoracotomy 開胸手術 |
| Ear notching | Orchidectomy 切除睪丸 | Heterotopic organ transplantation |
| Subcutaneous transponder placement | C-section | Vertebral procedures |
| Superficial tumor implantation | Hypophysectomy 切除腦下垂體 | Burn procedures 燒燙傷 |
| Orbital sinus venotomy | Thymectomy | Trauma models |
| Rodent embryo transfer | Embryo transfer in non-rodents | Orthopedic procedures 整形手術 |
| Multiple injections | Bone marrow collection | |
| Non-corneal ocular procedures | Corneal procedures | |
| Intracerebral electrode implantation | | |
| Vasectomy | | |
| Vascular access port implantation | | |
| Craniotomy (periosteal pain) | | |
| Superficial lymphadenectomy | | |

[a] Table adapted from "Guidelines for the Assessment and Management of Pain in Rodents and Rabbits". 2006, American College of Laboratory Animal Medicine



動物疼痛與緊迫表徵

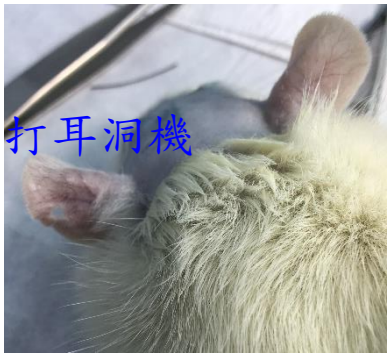
| | Mice | Rats | Rabbits |
|---|---|---|---|
| Decreased Food and Water Consumption | X | X | X |
| Weight loss | X | X | X |
| Self-Imposed Isolation/hiding | X | X | X |
| Self-mutilation, gnawing at limbs 自殘 | X | X | X |
| Rapid Breathing | X | X | X |
| Opened-Mouth Breathing | X | X | X |
| Abdominal Breathing | X | X | X |
| Grinding Teeth 磨牙 | | X | X |
| Biting/Growling/Aggression | | X | X |
| Increased/Decreased Movement | X | X | X |
| Unkempt Appearance (Erected, Matted, or Dull Haircoat) | X | X | X |
| Abnormal Posture/Positioning (e.g., Head-pressing, Hunched Back) | X | X | X |
| Restless Sleep | | | X |
| Tearing (Including Porphyrin), Lack of Blinking Reflex 流淚 | | X | X |
| Dilated Pupils | | | X |
| Muscle Rigidity, Lack of Muscle Tone | X | X | X |
| Dehydration/Skin Tenting/Sunken Eyes | X | X | X |
| Twitching, trembling, tremor 顫抖 | X | X | X |
| Vocalization (Rare) | X | X | X |
| Redness or Swelling Around Surgical Site | X | X | X |
| Increased Salivation | | | 24X |

**Replacement**

**Reduction**

**Refinement**

- Animal Ethics Committee (AEC)
- Animal Research Committee (ARC)
- Institutional Animal Care and Use Committee (IACUC)



# 人體試驗倫理委員會
# (*Institutional Review Board*，簡稱*IRB*)

一零四台實動訓字第 19 號

## 研習證明

## *Animal User Orientation Certification*

學員 許乙雨 於中華民國 104 年 3 月 20 日參加本委員會舉辦之實驗動物使用者訓練課程共 4 小時，成績合格特此證明

*Xu-Yi Yu has participated the Humane Animal Care and Use Training Lecture (4 hours) of Institutional Animal Care and Use Committee (IACUC) of National Taiwan University in March, 2015.*

國立臺灣大學實驗動物照護及使用委員會 召集委員

*Institutional Animal Care and Use Committee (IACUC)*
*National Taiwan University*

Since 1895
國立臺灣大學醫學院附設醫院
NATIONAL TAIWAN UNIVERSITY HOSPITAL

## 證 明 書

## Certificate of Completion

茲證明 侯詠德 完成臺大醫院臨床試驗中心主辦『醫學研究之利益衝突與規範』教育訓練並通過測驗發給 1 小時課程證明

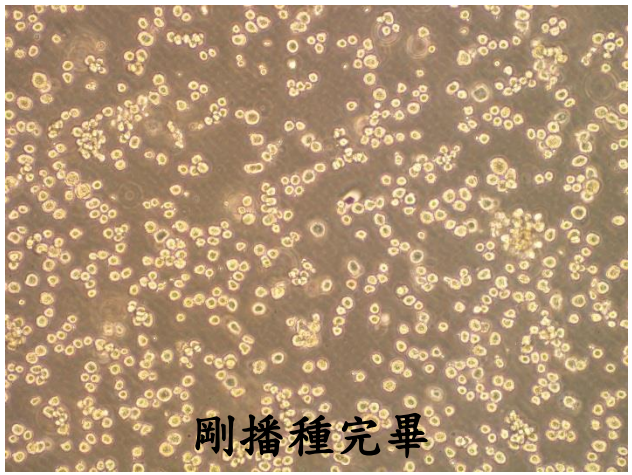
This certificate is awarded to **Yung-Te Hou** for completing 1 hours of GCP training and for passing the certification test in ***Guidelines for Conflict of Interest Issues in Medical Research***.

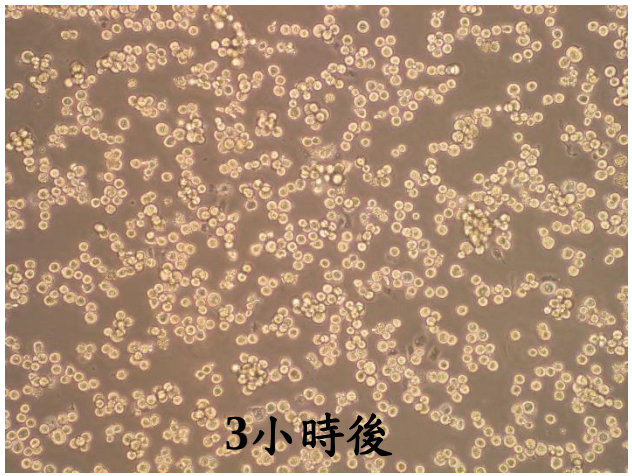
發證日期/Date：2016-10-17

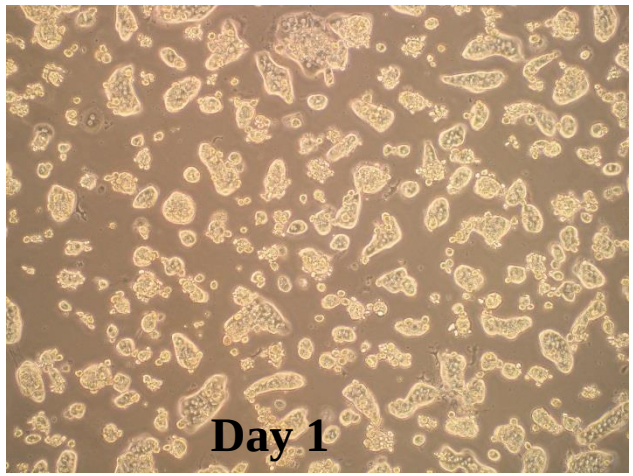
證書編號/No. ：201610171872

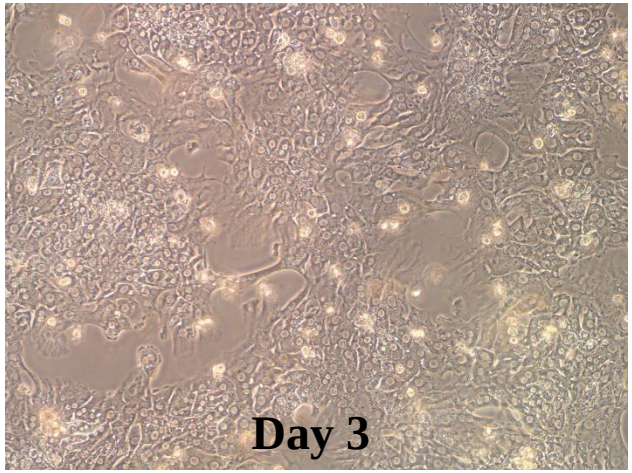
臨床試驗中心主任 陳建煒
K. Arnold Chan, MD, ScD, FISPE
Director, Clinical Trial Center

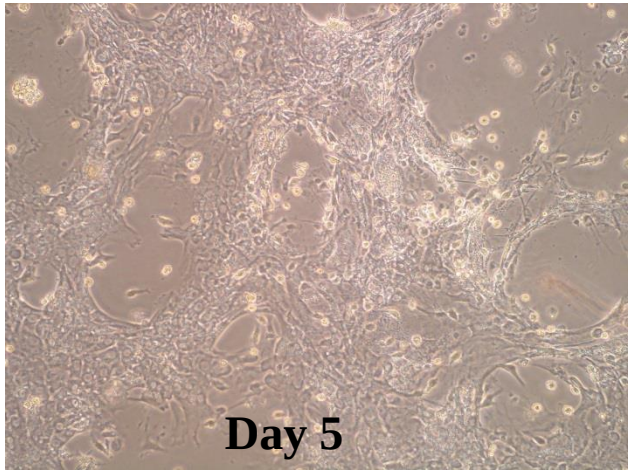
# Primary hepatocyte culture; density=$10^5$ cells/mL

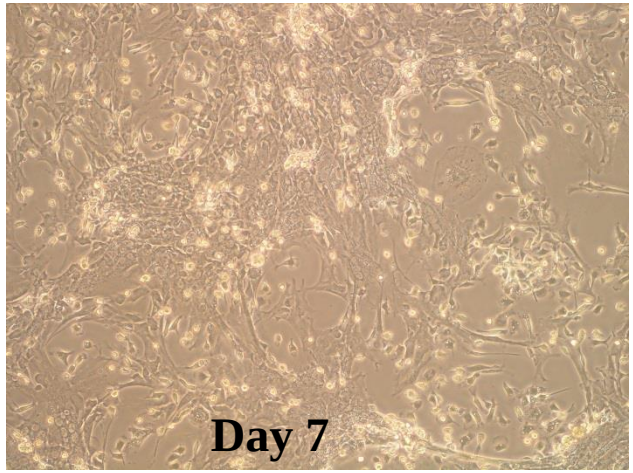


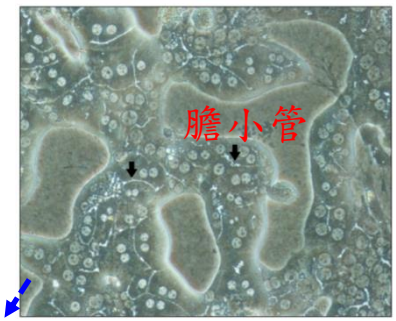



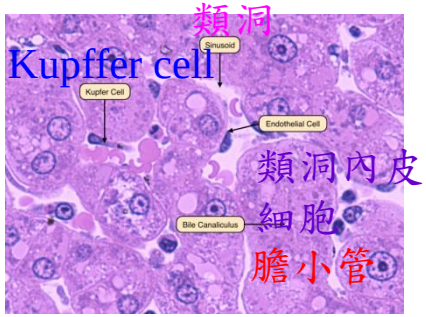




Clear cytoplasmic margin

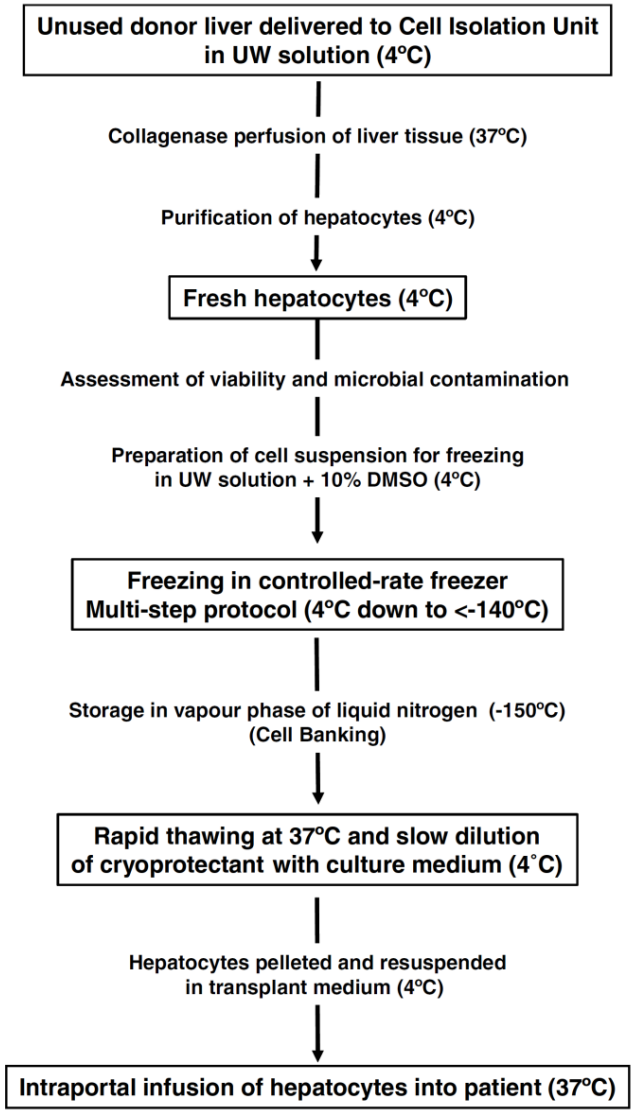



Hepatocytes exhibit desirable morphology with polarized bile canaliculi (膽小管)

*https://reurl.cc/MdOegp*

# Cryopreservation






*2006 Cryopreservation of isolated human hepatocytes for transplantation: State of the art.*

- A variety of techniques have been developed to enable the cryopreservation of human hepatocytes.
- This enables the large number of hepatocytes that are prepared from a single liver to be stored and thawed with reproducible cellular function. This option has opened the door to a variety of in vitro pharmacologic and infectious disease studies.

# Cell sources for liver cell-based therapies (*Fetal and adult progenitor cells*)

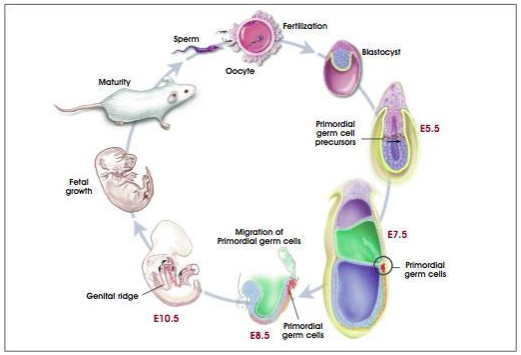


Fetal hepatoblasts (肝母細胞) are liver precursor cells present during development that exhibit a bipotential differentiation capacity, defined by the capability to generate both hepatocytes and bile duct epithelial cells. Sourcing problems associated with fetal cells have led researchers to search for resident cells that have progenitor properties in the adult (胎兒細胞取得是個問題→肝前驅細胞 maybe the solution).

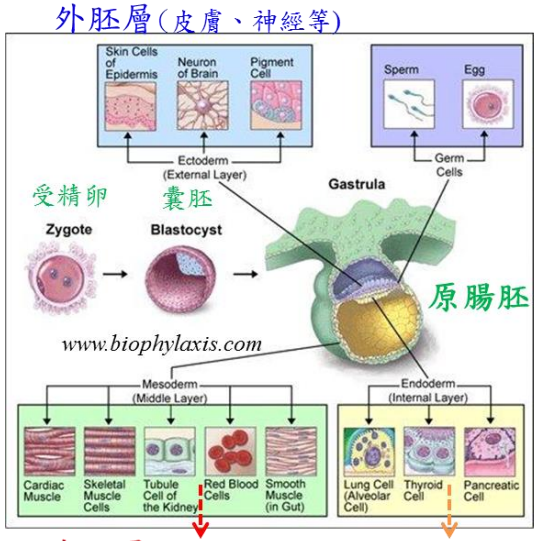
- Along similar lines, Weiss and colleagues have demonstrated the development of **bipotential** mouse embryonic liver (BMEL) cell lines derived from mouse E14 embryos that exhibit characteristics comparable to **fetal hepatoblasts** and **oval cells**. These BMEL cells are proliferative, can be induced to be **hepatocyte-like** or **bile duct epithelial-like** ***in*** *vitro*, and can home to the liver to undergo bipotential differentiation *in vivo* within a regenerative environment.
- More recently, biopotential human embryonic liver cells have been isolated and, similar to mouse BMEL cells, are proliferative and capable of bipotential differentiation

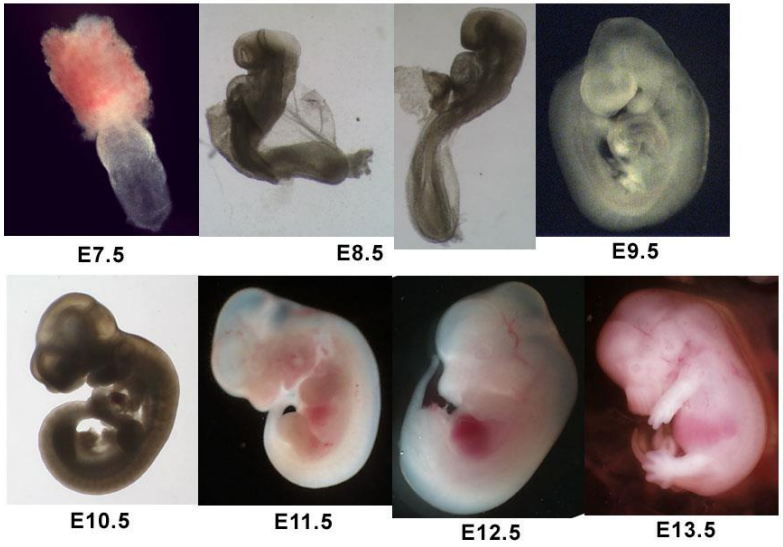
# Mouse embyro






*stemcells.nih.gov*




*www.swarthmore.edu*

*www.biophylaxis.com*

1. Full term is about 19-21 days
2. The optimal age for preparing cultures from a whole disaggregated embryo is around 13 days

**E13:** The embryo is relatively large but still contains a high proportion of undifferentiated mesenchyme, which is the main source of the culture

Most individual organs begin to form (Except brain and heart) at **day 9**

Difficult to isolate until about **day 11**

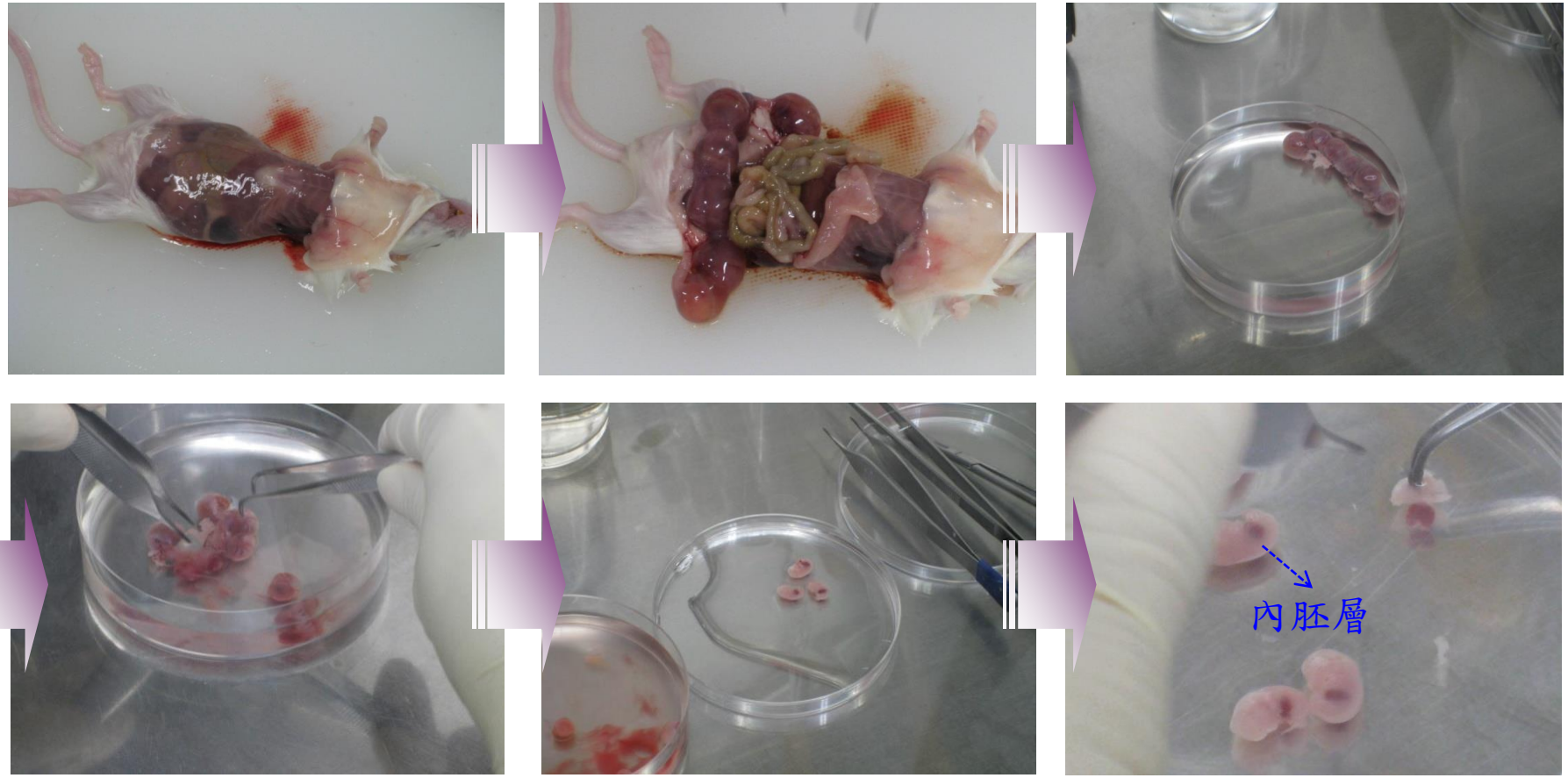
Dissection of individual organs is easier at **day 13-14**

Most of the organs are completely formed by the **day 18**

**Gestation** **Day 9** **Day 11** **Day 13-14** **Day 18**

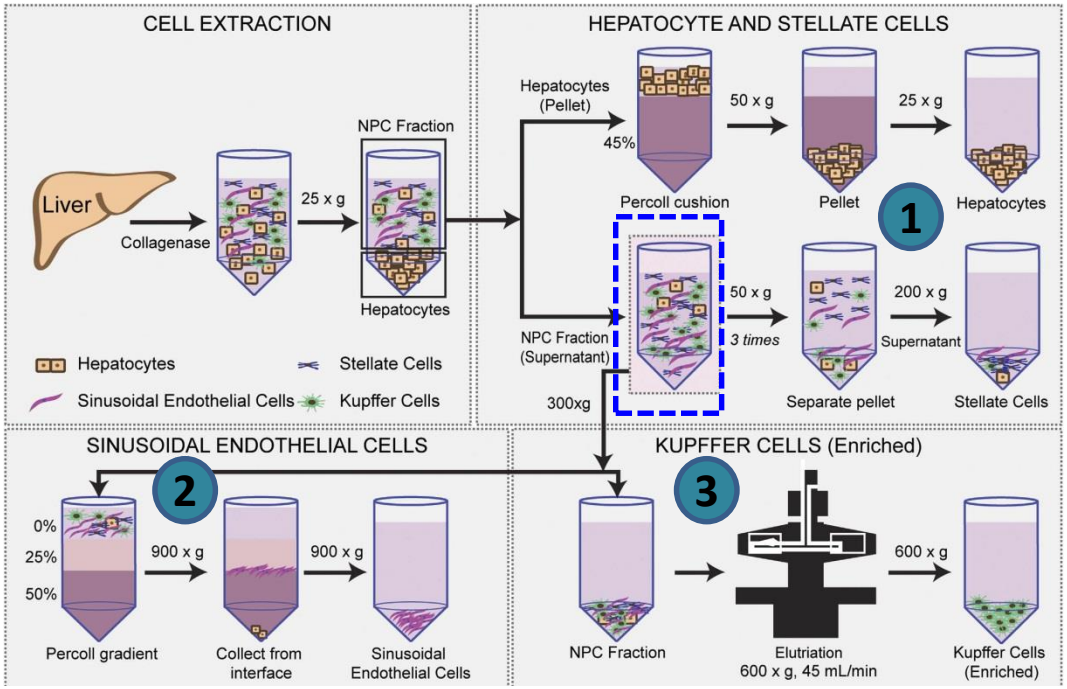
# Fetal hepatocyte isolation



# Isolation of primary hepatocytes and non-parenchymal cells from rat liver





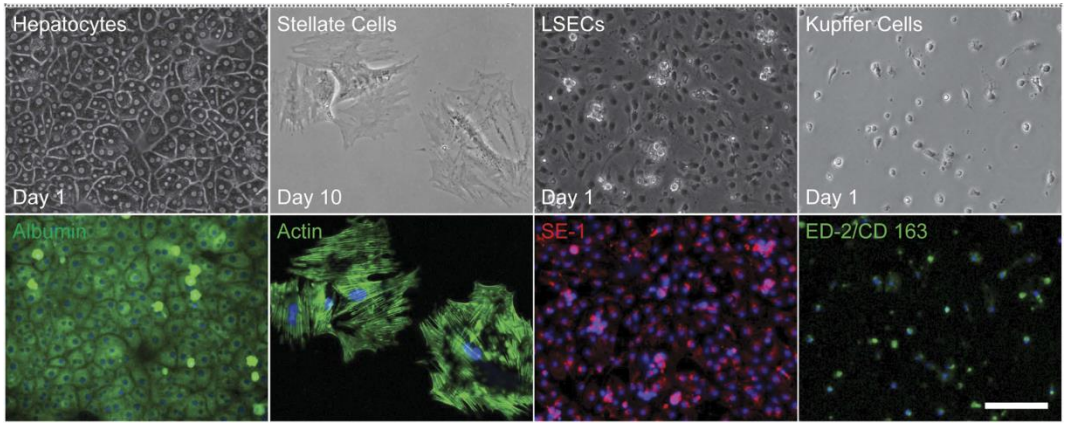




**Figure 1. Isolation and characterization of primary hepatocytes and non-parenchymal cell fractions from rat livers.** Rat livers were extracted from rats and digested using collagenase and cells obtained as a suspension. Hepatocytes were pelleted and enriched using a percoll cushion (45%), and pelleted. The non-parenchymal fraction is obtained as the supernatant from hepatocyte isolation.

- Stellate cells were isolated using centrifugation (pelleted at 200 × g) and cultured for 7–10 days with 1 passage before use.
- Sinusoidal Endothelial cells were isolated using a percoll gradient (interface between 50% and 25%).
- Kupffer cells were isolated using centrifugal elutriation (at 600 × g, 45 mL/min flow rate) and obtained as a pellet.

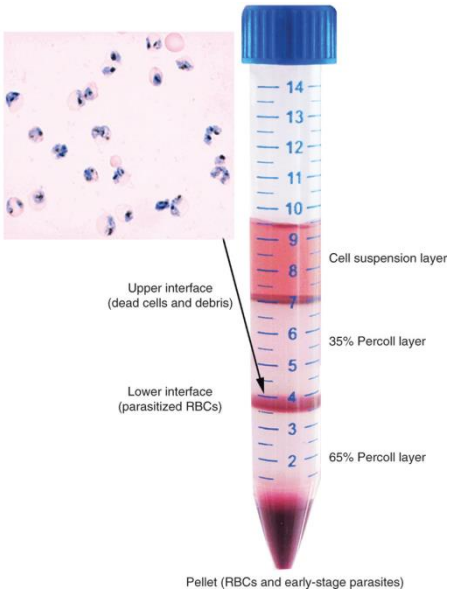
Isolated cells were stained for markers hepatocytes (albumin), stellate (actin), sinusoidal endothelial cells (SE-1) and Kupffer cells (ED-2/CD 163) respectively. Scale bar = 100 μm.

*2016 Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response*

# Cell separation by density

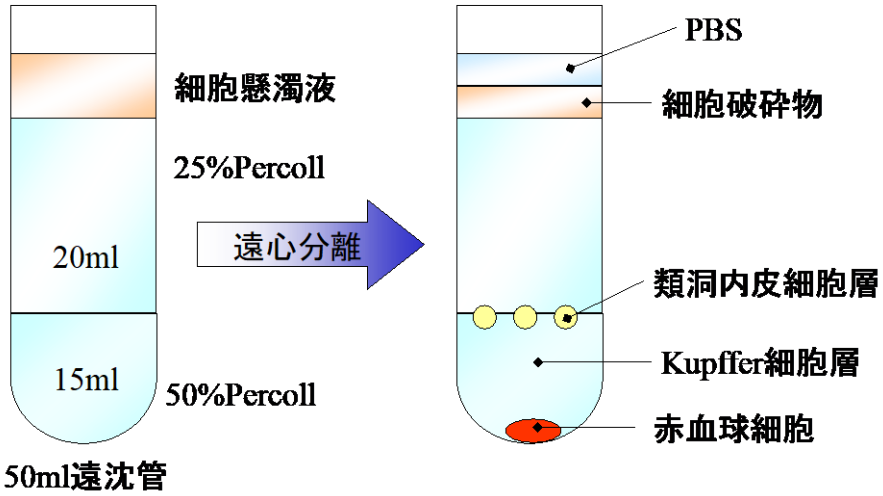


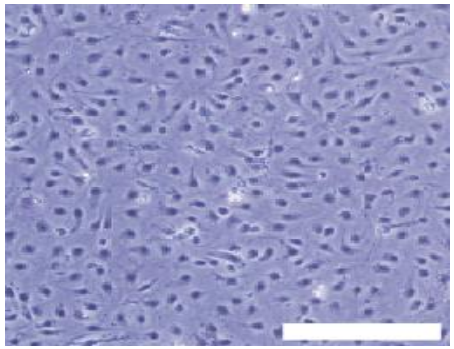
Spinning the cells at such a high g force may damage them. In addition, Percoll may be taken up by cells, so it is preferable to layer cells on top of a preformed gradient



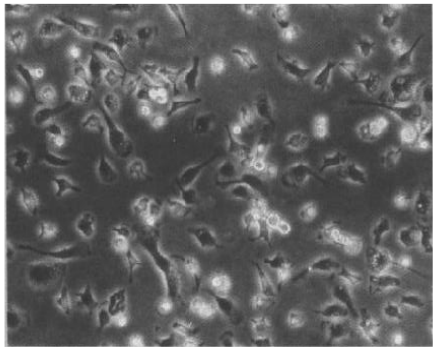

*2011 Rapid isolation of single malaria parasite-infected red blood cells by cell sorting*


*https://reurl.cc/odRjoj*



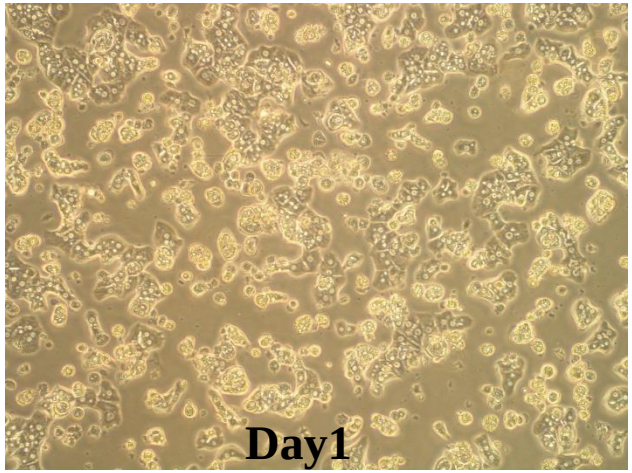



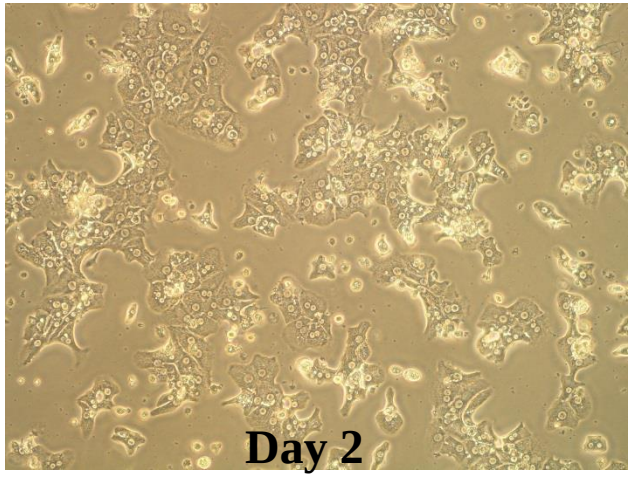
Sinusoidal endothelial cells

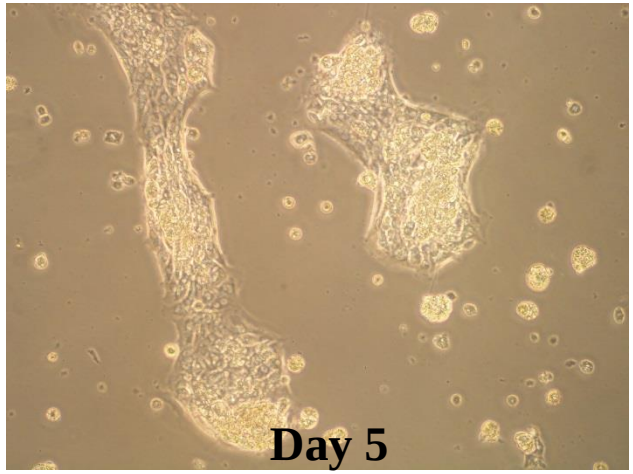


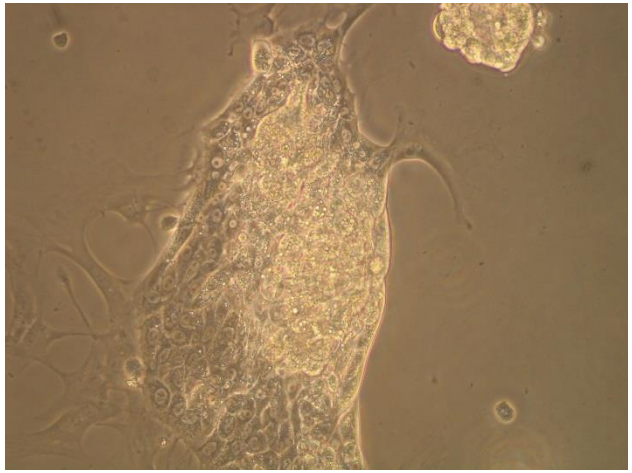
Kupffer cells

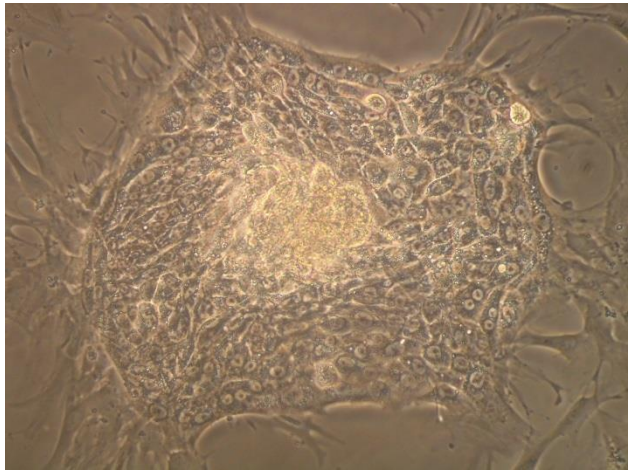
# Small hepatocyte culture; density=$10^5$ cells/mL

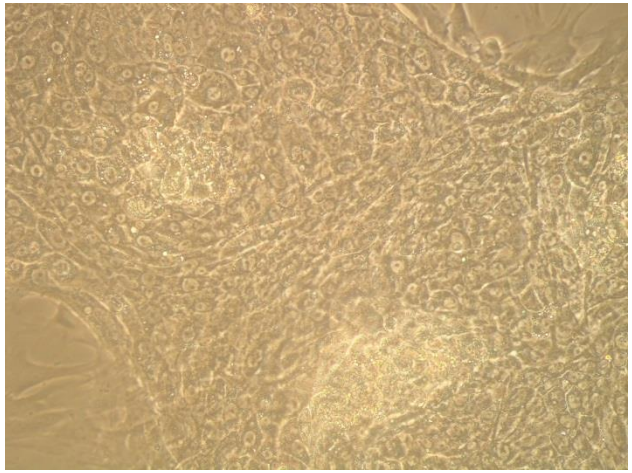


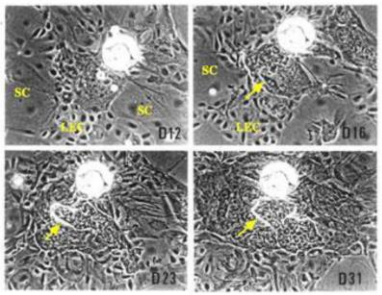



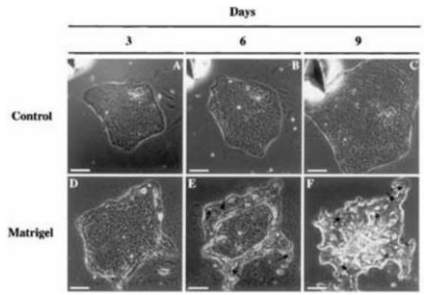



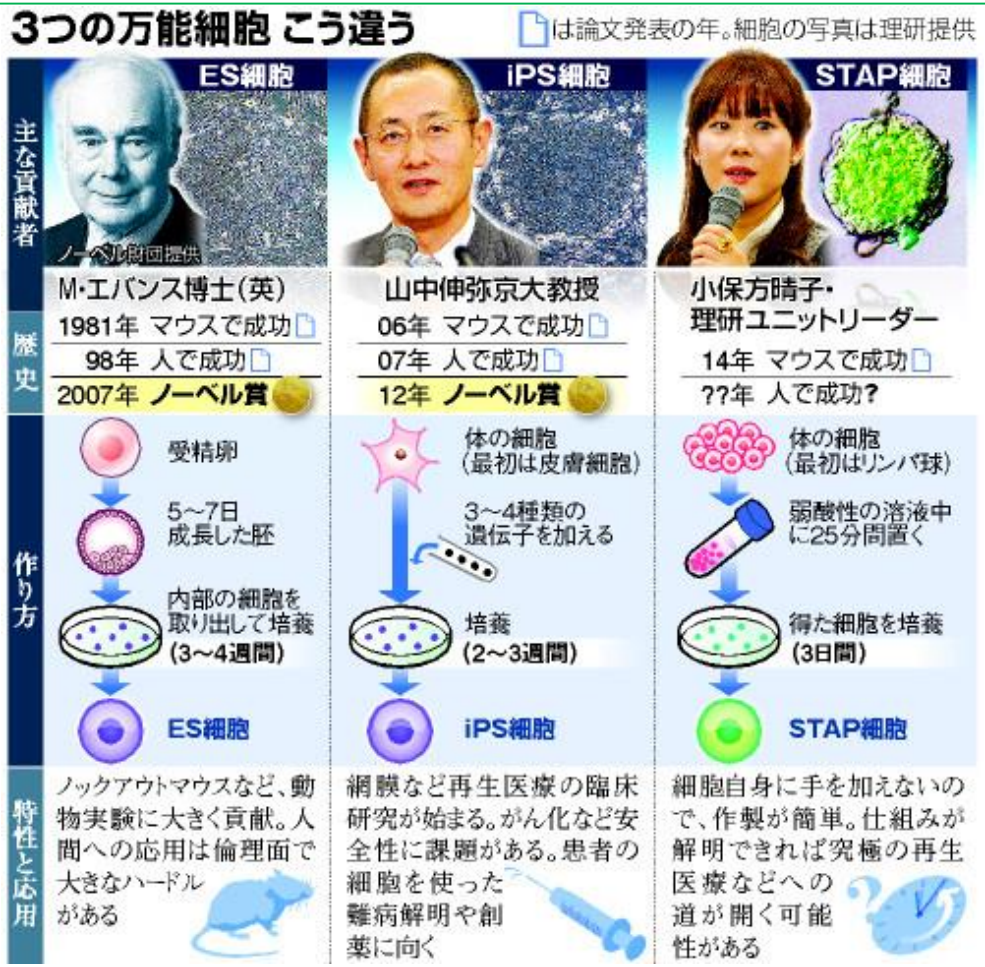




*2005 肝細胞培養と小型肝細胞について*

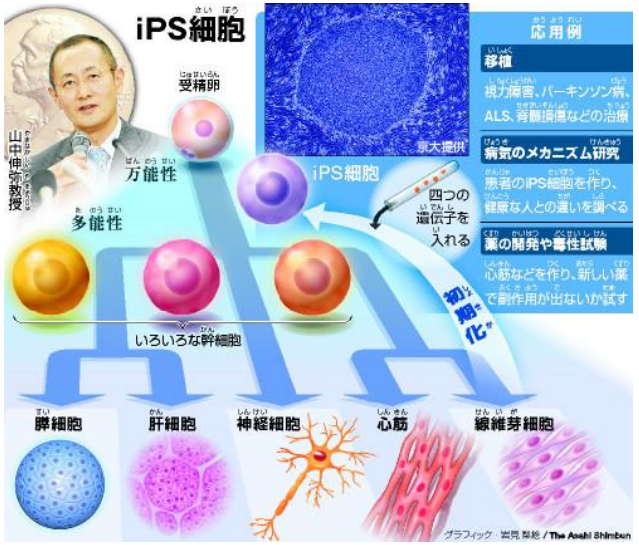
# Cell sources for liver cell-based therapies (*Pluripotent stem cells*)



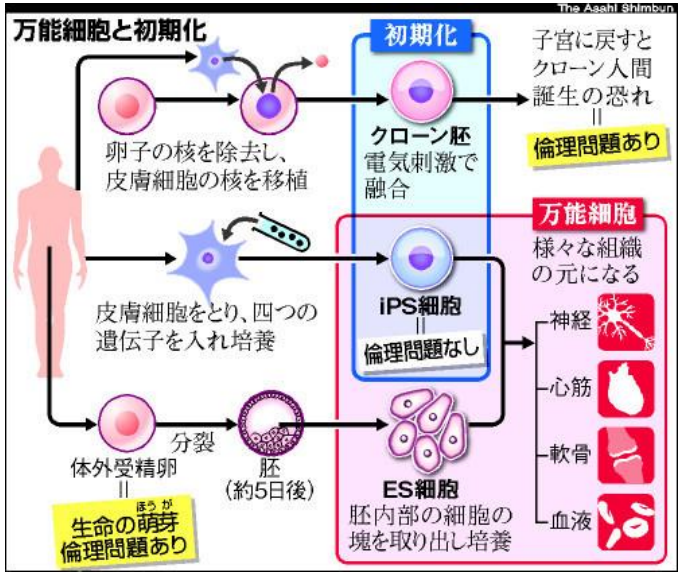
Mature cells from a differentiated compartment, such as fibroblasts from the dermis, may be reprogrammed to produce what have been called induced pluripotent stem cells (iPS cells) that are capable of differentiating into several different lineages




*www.asahi.com*




*ssassassak-news.blog.so-net.ne.jp*




*www.asahi.com*

# NEWS

- ヒトの肝臓の細胞に2種類の特殊な化合物を加えることで、肝臓の細胞の元になる「肝前駆細胞」に変化させることに成功したということです。


**Video (2018 0313)**

*http://www.nicovideo.jp/watch/sm32877472*

We identify **polyvinyl alcohol (PVA)** as a functionally superior replacement for serum albumin that is compatible with good manufacturing practice. These conditions afford between 236- and 899-fold expansions of functional HSCs over 1 month

成功培養出細胞的膠水「Arabic Yamato」=攝於5月29日(小窗山亮磨)

## 2019 0530 用膠水成分大量培養出造血幹細胞 專家稱令人跌破眼鏡

- 有關治療白血病時的重要細胞，東京大學與美國史丹佛大學等團隊已在老鼠身上大量培養成功。
- 以往即使運用高價的培養液，也幾乎無法讓細胞增生，而這次卻使用了市售膠水的成分培養成功。這可能有助於針對白血病等研發出劃時代的治療法，專家驚訝地表示：「（這項研究成果）就好比是哥倫布立蛋一樣。」
- 可轉換為白血球與紅血球的造血幹細胞，即使運用0.5公升要價數萬日圓（1萬日圓約合新台幣2880元）的培養液，也很難成功增生。
- 東京大學的特任副教授山崎聰，曾針對培養液的成分等毫無遺漏地一一進行研究。結果在他運用其中之一的聚乙烯醇(PVA)培養後，便成功增加了數百倍的幹細胞。
- PVA為衣服上漿劑和膠水的主要成分。山崎在實際嘗試後，確認便利商店賣的膠水也可成功培養。身為共同著作人、在理化學研究所經辦細胞銀行的室長中村幸夫表示：「驚訝到令人忍不住懷疑起結果。眾家研究者應該都跌破眼鏡了吧。」

*2019 Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation*

# Application of iPS cell in Japan



## 1. iPS細胞の解説

*http://www.youtube.com/watch?v=9b1j2mBK7Es*

## 2. 世界初「T細胞」量産に成功　がん治療などに光明（2013/01/04）

*http://www.youtube.com/watch?v=PBu3lsWdDF0*

## 3. 世界初！京大がヒトiPS細胞から腎細胞生成に成功（2013/01/23）

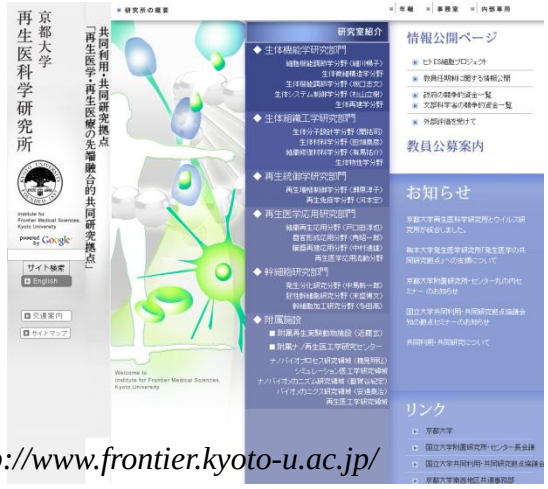
*http://www.youtube.com/watch?v=gcxnpc7qNQ4*

## 4. iPS細胞からヒト肝臓作製　横浜市立大が発（2013/07/04）

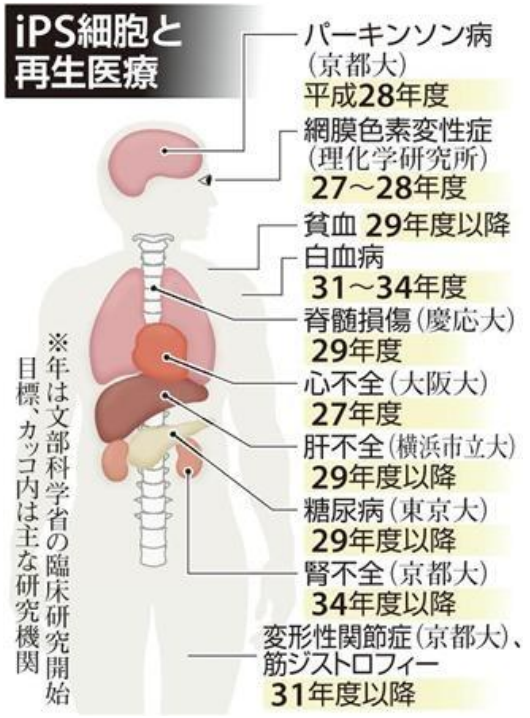
*http://www.youtube.com/watch?v=ze_oQDpakrs*

## 5. 世界初iPS細胞使った手術実施（2014 0912）

*http://www3.nhk.or.jp/news/html/20140912/t10014562731000.html*




*http://www.frontier.kyoto-u.ac.jp/*




*http://news.goo.ne.jp/picture/sankei/nation/snk20140913081.html*

# Pluripotent stem cell derived hepatocyte



**iPS細胞:横浜市大が肝臓のもと作製 マウス体内で機能 (2013/7/4)**

- さまざまな種類の細胞になりうるヒトの人工多能性幹細胞（iPS細胞）から、肝臓のもととなる「小さな肝臓」を作り、マウスの体内で機能させることに世界で初めて成功したと、横浜市立大の谷口英樹教授（再生医学）の研究チームが発表した。谷口教授らは、ヒトのiPS細胞を肝臓の細胞になる直前の「**内胚葉**（ないはいよう）細胞」に成長させ、血管を作る細胞や細胞同士をつなぐ細胞と一緒に培養した。
- その結果、培養皿の中で細胞が自然に**5ミリ**ほどの球状に集まり、血管がある小さな肝臓ができたという。この肝臓をマウスの体内に移植したところ、ヒトの肝臓でしか作られないたんぱく質などがマウスの血液から確認された。さらに、薬剤で肝不全にしたマウスに移植した結果、30日後の生存率は、移植しない場合の約30%から90%以上に高まったという。

*http://mainichi.jp/select/news/20130704k0000m040136000c.html*

**横浜国立大、iPS使い人工肝臓作製 血管細胞と一緒に培養 (2015/1/12)**

- 横浜国立大学の福田淳二准教授らは血管の細胞とiPS細胞を一緒に培養し、血管のような微小な構造を備えた人工肝臓を開発した。肝臓特有のたんぱく質をつくり、代謝という作用もあった。肝機能を調べると実物の肝臓の10分の１程度だった。移植手術を待つ間に使う用途を考えており、10年以内をめどに応用したい考えだ。
- 手のひら大の装置に入れた材料の中では、血管の細胞がいくつも集まって血管に似た構造が9本できた。

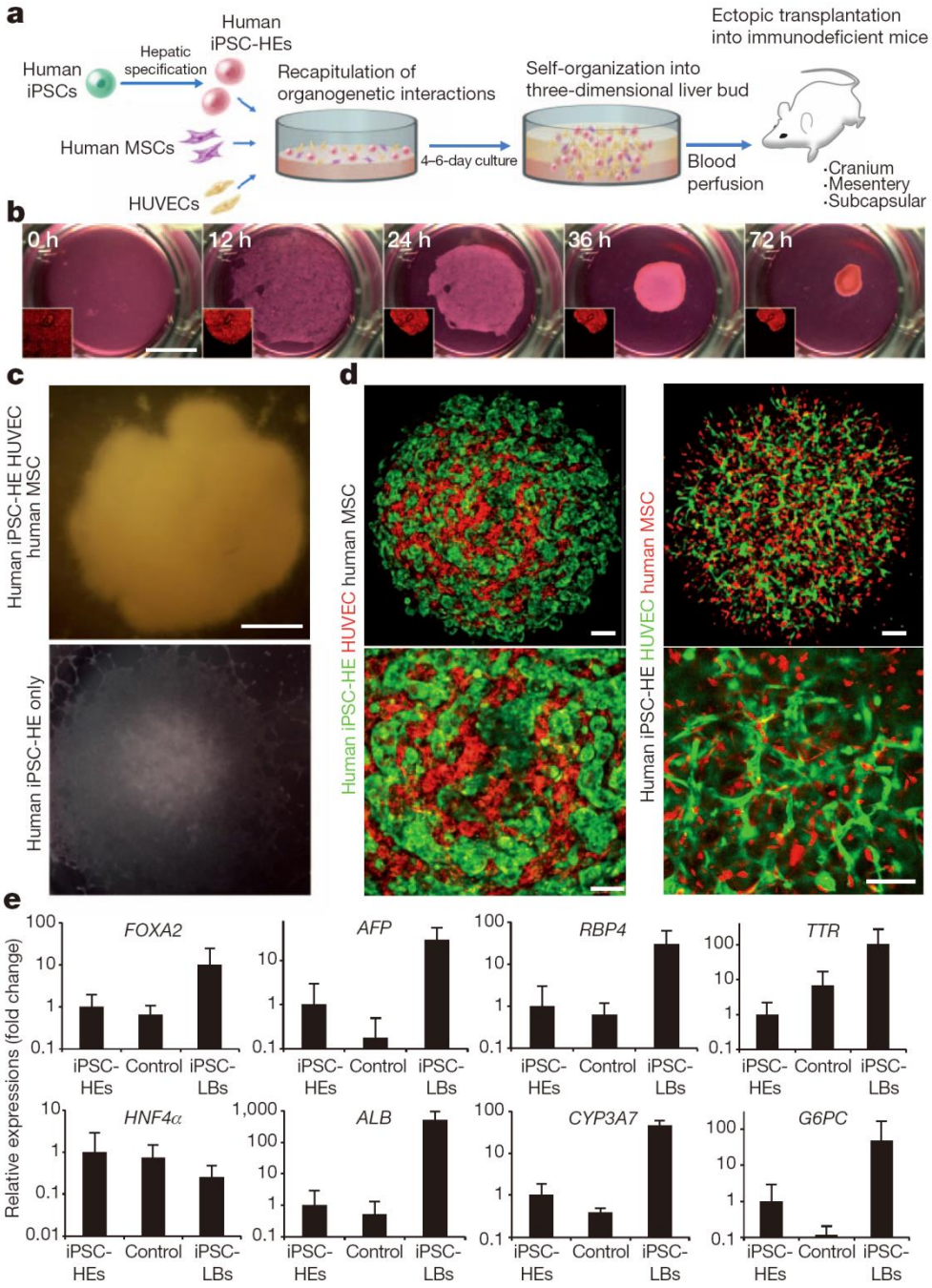
*https://reurl.cc/XkGz8j*

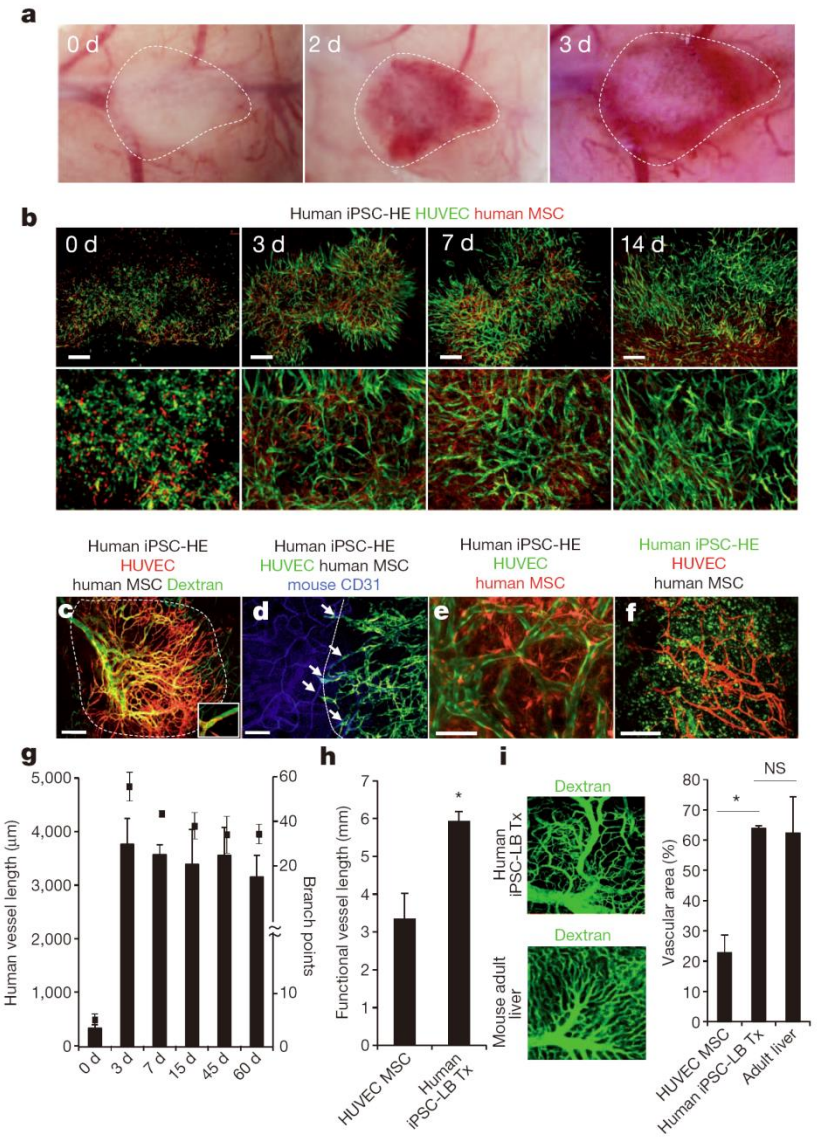
## To date clinic application

To date, **only one example** of *in vivo* engraftment of pluripotent stem cell-derived hepatocyte-like cells has been reported

BUT!! The following need to address safety concerns:

1. The potential for pluripotent cell-derived teratoma（畸胎瘤）formation
2. The oncogenic（致癌）risks associated with integrating vectors used to generate some iPS lines




*2013 Vascularized and functional human liver from an iPSC-derived organ bud transplant*

# ラット使い人工肝臓作製　九大、iPS細胞で (2020 0605)

- 九州大病院の武石一樹助教らのグループは5日までに、ヒトのiPS細胞を使って人工肝臓を作製し、移植したラットの体内で機能させることに成功したと発表した。ヒトへの移植に応用できれば、肝不全の患者の治療に役立つ可能性がある。
- 研究成果は3日、米科学誌電子版に掲載。武石助教によると、iPS細胞由来の人工肝臓の作製は世界初という。
- 研究では、ヒトのiPS細胞から肝臓に必要な肝細胞や胆管細胞、血管内皮細胞を分化させ、ラットの肝臓から細胞を抜き取って作った鋳型に分化した細胞を注入し、ラットに移植できるミニ人工肝臓を作製した。
- 人工肝臓にはラットの血管、胆管の構造が残っていて、別のラットに移植したところ、肝臓で生成されるタンパク質「アルブミン」ができるなど肝臓の機能が認められたという。
- ヒトへの応用には、iPS由来の肝細胞を多く培養し、ブタなど大きな動物を使ってよりスケールの大きい人工肝臓を作る必要がある。武石助教は「肝不全の根治治療は肝移植しかないが、現在はドナー不足が深刻だ。治療に応用できるよう研究を進めたい」と話した。〔共同〕

*https://r.nikkei.com/article/DGXMZO60010500V00C20A6910E00?s=5&fbclid=IwAR2t9GqSh5-fJt_kkBmHP-k_Vrf3blnzBCFvhnRq_Xt3AGsCZQR6gUZWUnk*

# *In vitro* hepatic culture models (2D culture)



Primary hepatocyte survival and liver-specific functions *in vitro* through modifications in microenvironmental signals including:

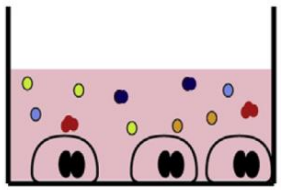
1. Soluble factors (medium composition)
2. Cell-matrix interactions
3. Heterotypic cell-cell interactions with non-parenchymal cells.

The supplementation of culture media with:

1. **Physiological factors** such as hormones, corticosteroids, growth factors, vitamins, amino acids or trace elements
2. **Non-physiological small molecules** such as phenobarbital and dimethyl-sulfoxide (DMSO)

**→Can modulate hepatocyte function**

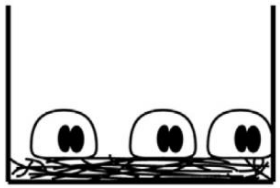
## Certain mitogenic factors



Soluble Factors

EGF and HGF can induce limited proliferation in rat hepatocytes *in vitro*, but these mitogens result in negligible (微不足道) proliferation of human hepatocytes *in vitro* (生長因子對於肝細胞的體外增殖影響有限)

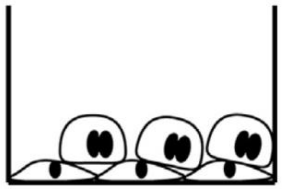
## Heterotypic cell-cell interaction



Extracellular Matrix

Wide variety of non-parenchymal cells from both within and outside the liver are capable of supporting hepatocyte function for several weeks in co-culture contexts *in vitro*, even across species barriers, suggesting that the mechanisms responsible for non-parenchymal cell-mediated stabilization of hepatocyte phenotype may be conserved

## A variety of ECM coatings

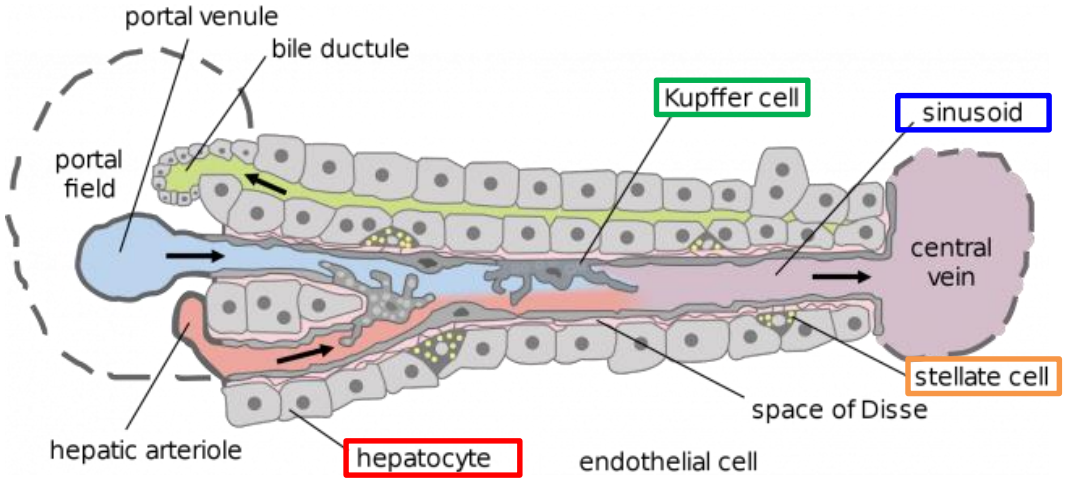


Co-Cultivation

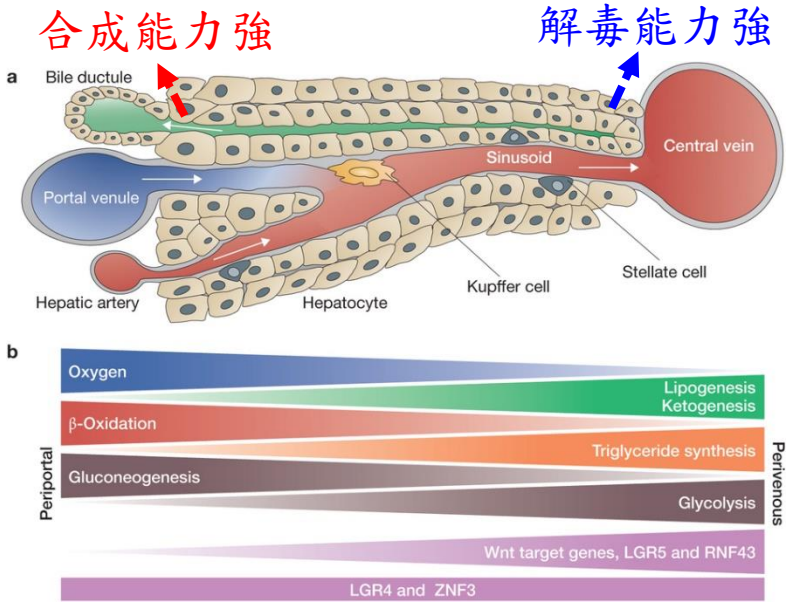
A variety of ECM coatings (most commonly collagen type I) have been shown to enhance hepatocyte attachment to the substrate, but this usually occurs concomitantly with hepatocyte spreading and a subsequent loss of hepatocyte function (Coating ECM會讓細胞增殖而非增加機能)

# *In vitro* hepatic culture models (2D culture)






*http://portal-vl.h-its.org/portal-view-v2/liver-function/liver-basics/health/cell/en/*




*2016 Orchestrating Wnt signalling for metabolic liver zonation*

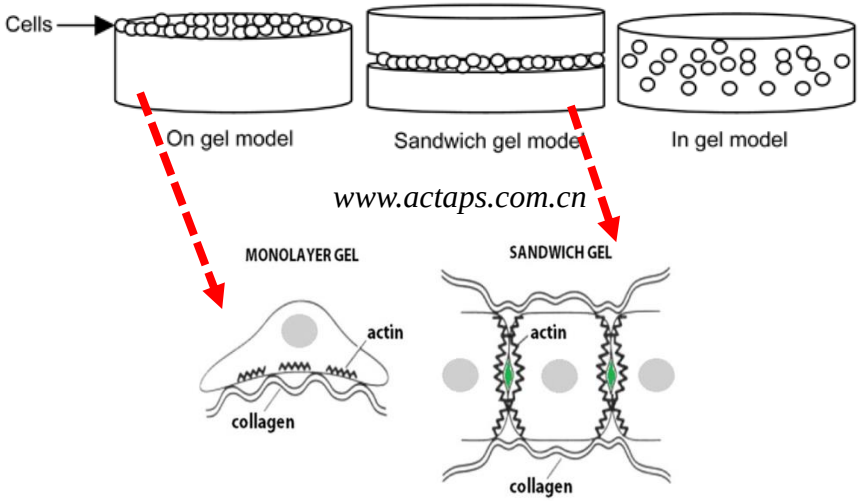
- Co-cultures of hepatocytes and Kupffer cells have been used to examine mechanisms of hepatocellular damage,
- Co-cultures of hepatocytes and liver sinusoidal endothelial cells (which are also phenotypically unstable in monoculture upon isolation from the liver) have highlighted the importance of hepatocyte-endothelial cell interactions in the bidirectional stabilization of these cell types .

# *In vitro* hepatic culture models (3D culture)

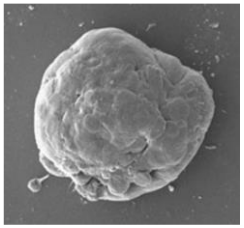


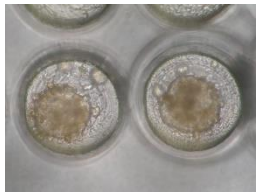
## 3D gel culture

The gel (collagen) or sponges (gelatin) are used which provides the matrix for the morphogenesis and cell growth. The cells penetrate these gels and sponges while growing.




## 3D spheroid culture





*2013 Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME*

- Under spheroidal culture conditions, hepatocyte survival and functions are improved over standard monolayers on collagen.
- Potential mechanisms underlying these effects include an increased extent of homotypic cell-cell contacts between hepatocytes, the retention of a three-dimensional cyto-architecture, and the three-dimensional presentation of ECM molecules around the spheroids. Some limitations of conventional spheroidal culture include a tendency for secondary aggregation of spheroids and the resultant development of a necrotic core (核心壞死) in the larger aggregates due to diffusion-limited transport of nutrients and waste, and the lack of control over the cell numbers within each spheroid.

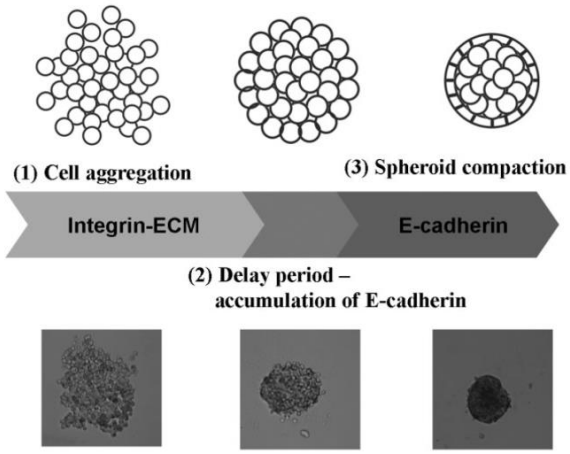
# Histotypic culture: Spheroid



**Spheroid Applications:**

1. Study the penetration of cytotoxic drugs, antibodies, or other molecules used in targeted therapy
2. Useful in the study of cell killing by biological targeted radionuclides
3. Spheroid cultures have also been used in confrontation experiments to assess the invasiveness of spheroids derived from malignant cell populations that are grown in close proximity to normal cell cultures

A variety of methods are being developed to prevent secondary aggregation of initially-formed spheroids and control cell-cell interactions, including microfabricated scaffolds, bioreactor systems, encapsulation techniques and synthetic linkers.



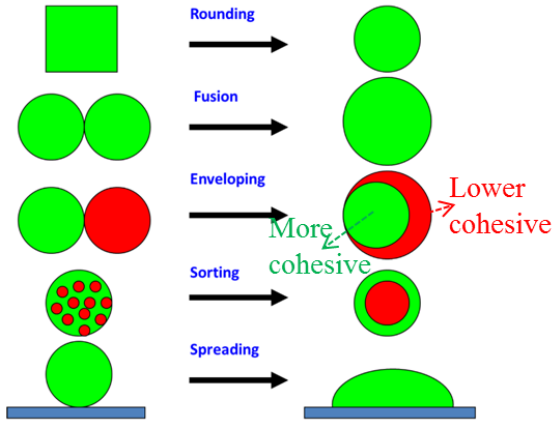




Fig. 5.3. Evidence of fluidity of tissue spheroids.

*2008 Recent advances in three-dimensional multicellular spheroid culture for biomedical research*

**Video: 3D spheroid formation in InSphero GravityPLUS system**

*http://www.youtube.com/watch?v=t4TjUeRhhkY*

# Histotypic culture: Spheroid (2)



**Spheroid Preparation method**

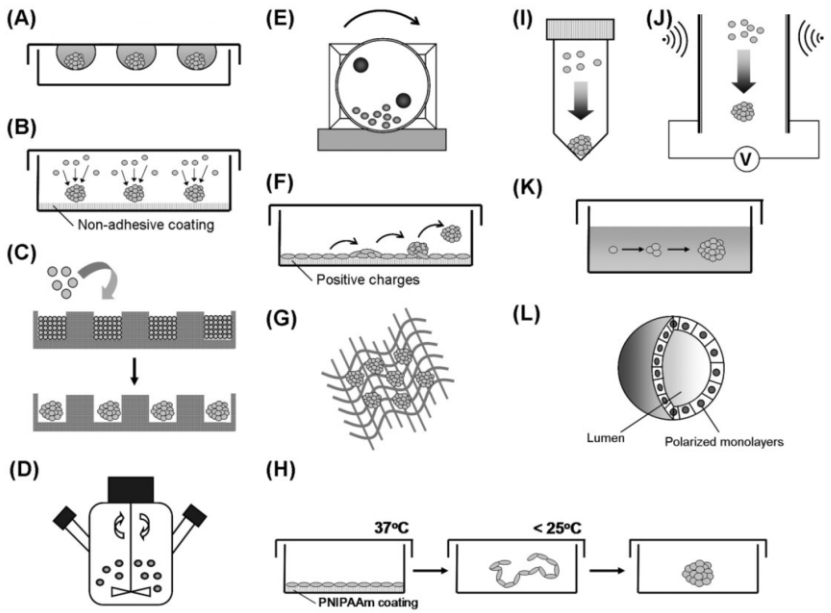
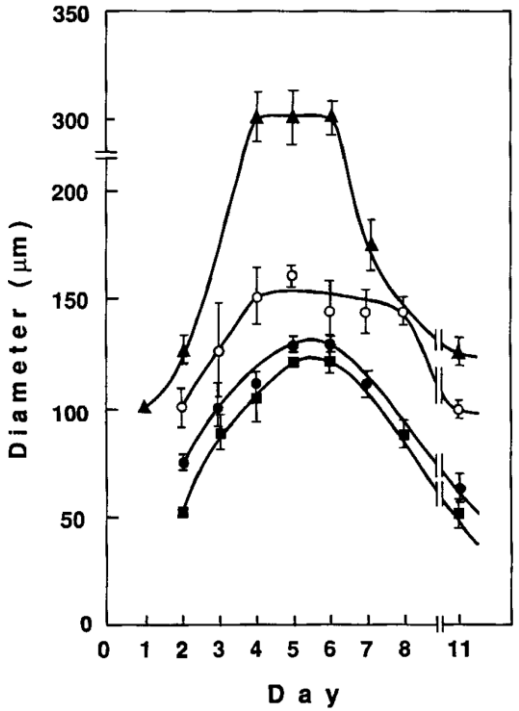
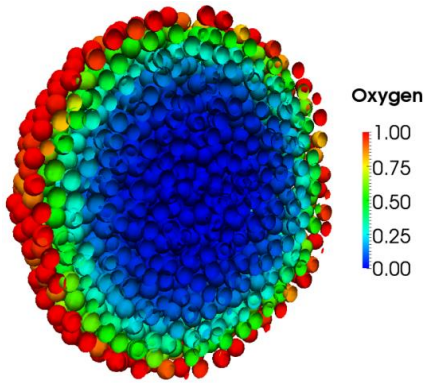
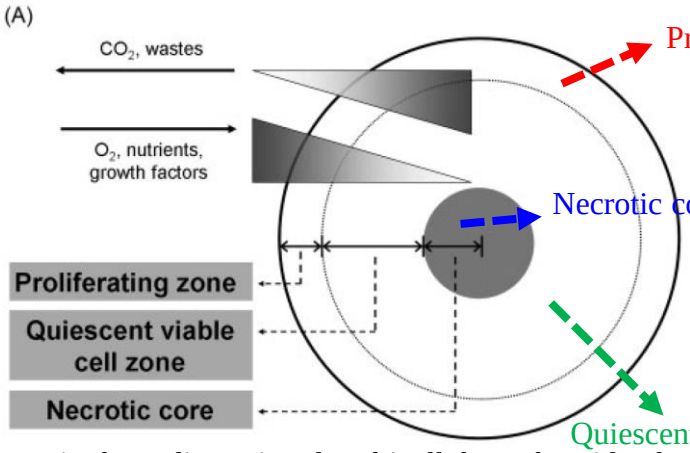



Figure 3. Methods for MCS generation. (A) Hanging-drop culture. (B) Single cell culture on nonadhesive surface. (C) Micromolding techniques. (D) Spinner flask culture. (E) Rotary cell culture systems. (F) Hepatocyte self assembly on Primaria dishes. (G) Porous 3-D scaffolds. (H) The use of PNIPAAm-based cell sheets. (I) Centrifugation pellet culture. (J) Electric, magnetic or acoustic force cell aggregation enhancement. (K) Monoclonal growth of tumor spheroids. (L) Polarized epithelial cysts.

Spheroid size




*1993 Importance of cell aggregation for expression of liver functions and regeneration demonstrated with primary cultured hepatocytes*

*2008 Recent advances in three-dimensional multicellular spheroid culture for biomedical research*

# Histotypic culture: Spheroid (3)



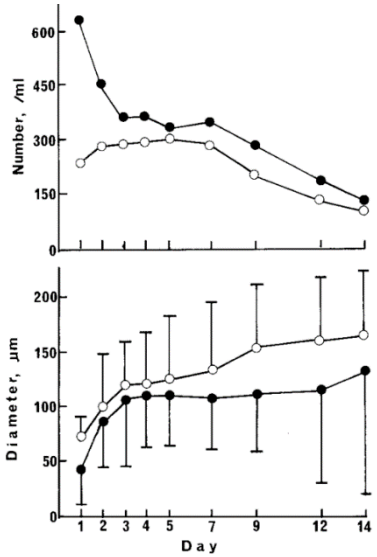



**FIG. 2.** Changes in number (top) and diameter (bottom) of multicellular spheroids during 2 weeks of culture. (○) An uncoated Primaria dish; (●) Falcon 3001 dish coated with proteoglycan fraction. Each point represents the mean of triplicate cultures with standard deviation indicated as a vertical line.

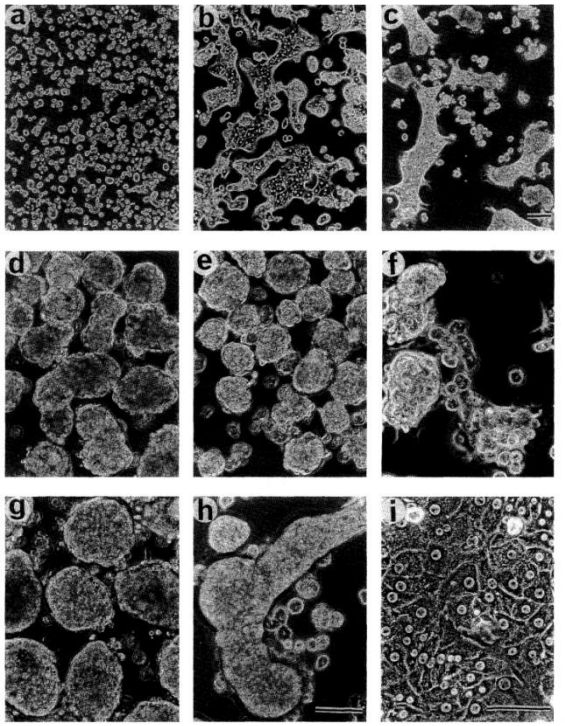



**FIG. 1.** Phase-contrast microscopic features of multicellular spheroids of adult rat hepatocytes. (a–d) Uncoated Primaria dish at 0 h (a); 24 h (b); 52 h (c) 4 days (d) after seeding; (e) Falcon 3001 dish coated with proteoglycan fraction at Day 4; (f) Falcon 1008 dish at Day 4; (g) Falcon 3001 dish coated with albumin at Day 4; (h) Falcon 3001 dish coated with 2-hydroxymethyl methacrylate at Day 4; (i) Falcon 3001 dish coated with type I collagen at Day 4. Bars, 100 µm.

**TABLE 1**

Solid-State Regulation of Cell Assembling of Adult Rat Hepatocytes in Primary Culture

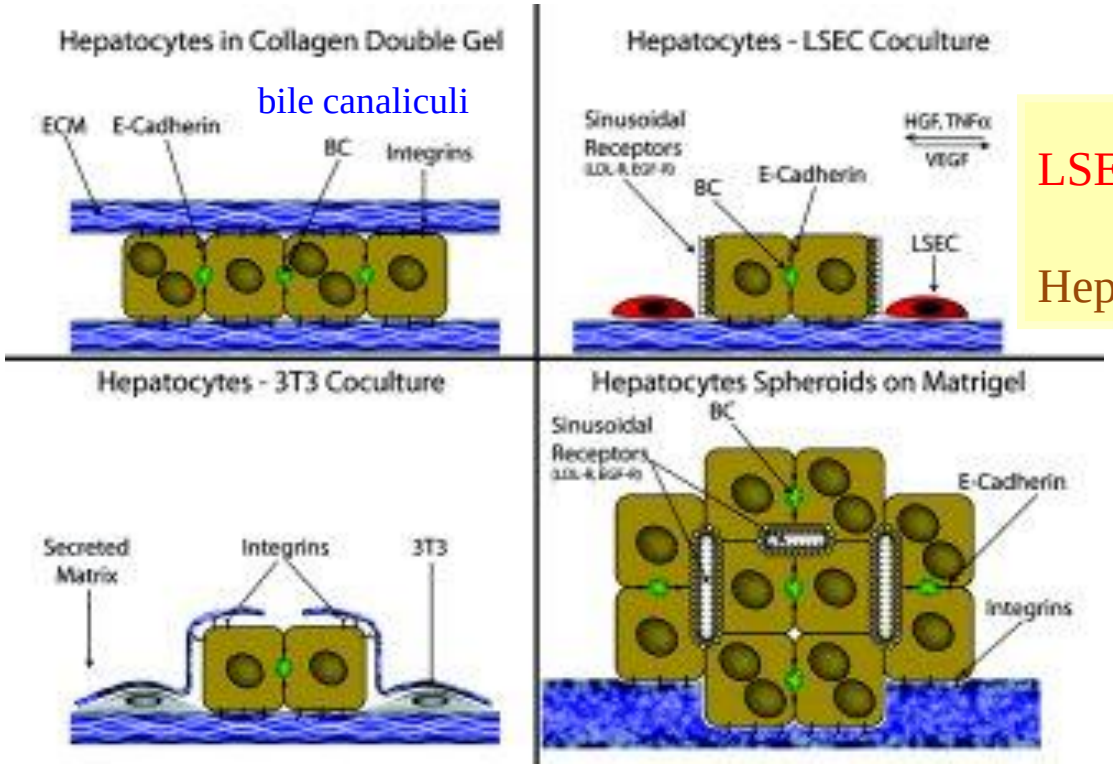
| Coated substances | Positively charged polystyrene | Hydrophobic polystyrene | Negatively charged polystyrene |
|---|---|---|---|
| None | Spheroid | Island monolayer, hemispheroid | Sheet monolayer |
| Proteoglycan fraction[a] | Spheroid | Spheroid | Spheroid |
| Dermatan sulfate | Spheroid | Hemispheroid | Hemispheroid |
| Heparan sulfate | Spheroid | Multilayer | Multilayer |
| Heparin | Spheroid | Multilayer | Multilayer |
| Collagen fraction[a] | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Type I collagen | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Type III collagen | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Type IV collagen | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Glycoprotein fraction[a] | Multilayer | Multilayer | Multilayer |
| Fibronectin | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Laminin | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Fetal calf serum | Sheet monolayer | Sheet monolayer | Sheet monolayer |
| Bovine serum albumin | Spheroid | Spheroid | Spheroid |
| Methacrylate | Hemispheroid | Hemispheroid | Hemispheroid |

*Note.* Typical form of cell assembling at Day 4 was described.
[a] Material was isolated from rat liver reticulin fibers.

*1990. Formation of multicellular spheroids of adult rat hepatocytes in dishes with positively charged surfaces and under other nonadherent environments*

# *In vitro* hepatic culture models (Co-culture)






**HGF, TNF-α**
LSEC→Hepatocyte

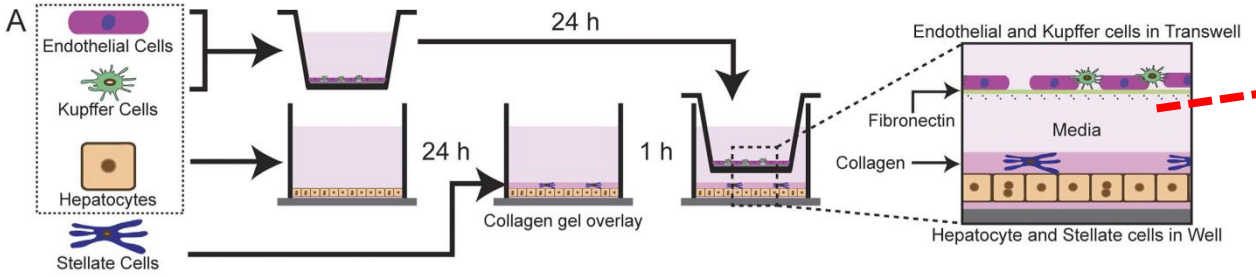
**VEGF**
Hepatocyte→LSEC

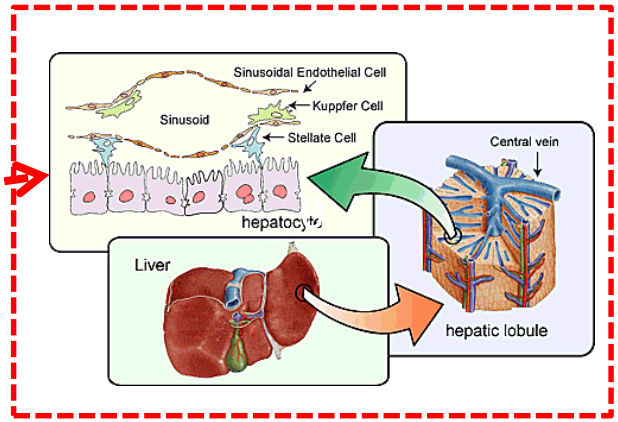
Figure 8: Schematic of a hypothetical model showing the polarization of hepatocytes in different culture configurations. ECM, extracellular matrix; BC, bile canaliculi; LSEC, liver sinusoidal endothelial cells; LDL-R, low-density lipoprotein receptor; EGF-R, epidermal growth factor receptor; HGF, hepatocyte growth factor; TNF-α tumor necrosis factor α; VEGF, vascular endothelial growth factor.

*2006 Liver endothelial cells promote LDL-R expression and the uptake of HCV-like particles in primary rat and human hepatocytes*

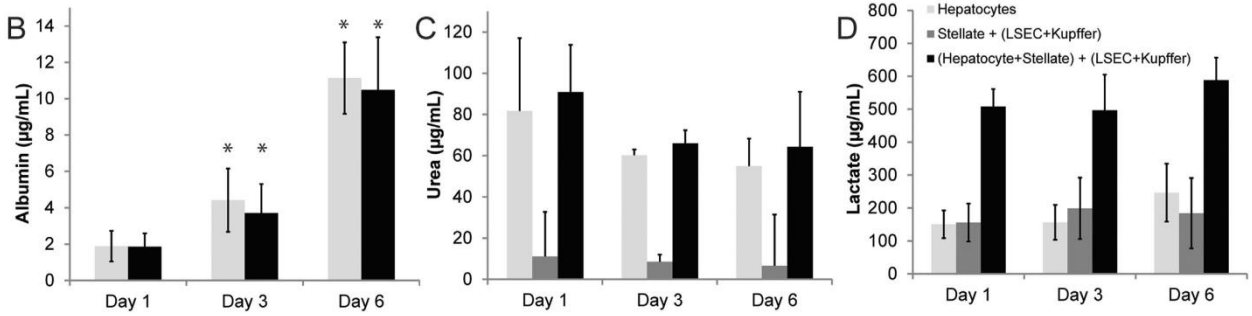
# *In vitro* hepatic culture models (Co-culture)

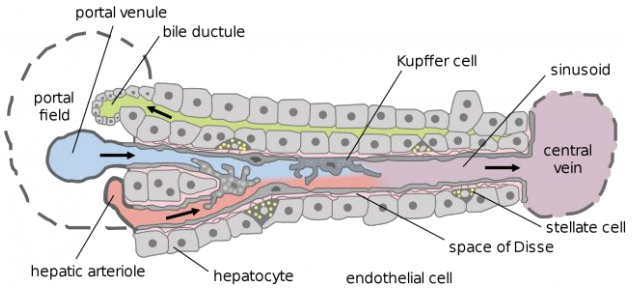





*www.pinterest.com*




Transwell (by PET)

*https://goo.gl/4ycF6J*

**Figure 2. Co-culture setup, and functional activity of hepatocyte monocultures, NPCs and hepatocyte-NPC formats.** (**A**) Hepatocytes, LSECs and KCs were isolated from a rat liver and seeded on collagen coated well and fibronectin coated transwell respectively and cultured overnight. SCs are overlaid on top of hepatocytes in a collagen-pre mix and allowed to gel for 1 h. The transwell with LSEC and Kupffer cells was then added to the well to create the co-culture. (**B**) Albumin, (**C**) Urea and (**D**) Lactate production of hepatocyte monocultures, NPC and hepatocyte-NPC cultures. (*$p < 0.05$, values compared to Day 1).

*2016 Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response*

# Liver models



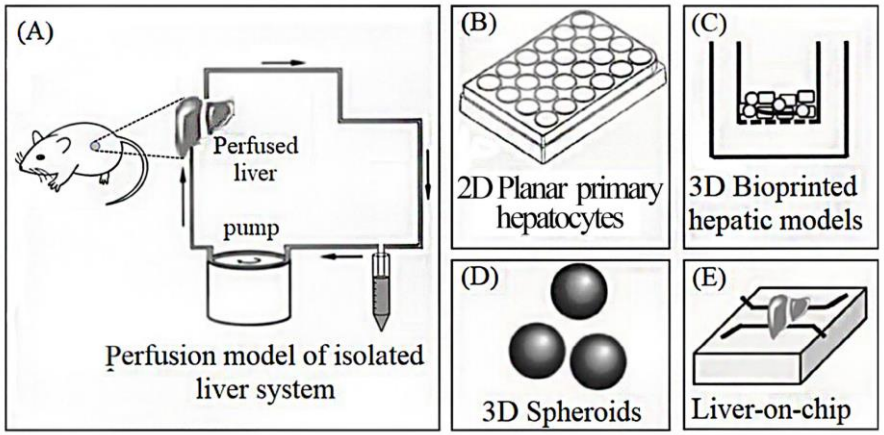



**Figure 2.** Liver models used commonly in vitro. (**A**) Perfusion model of an isolated liver system; (**B**) 2D planar primary rat hepatocytes; (**C**) 3D-printed liver tissue; (**D**) 3D spheroids; (**E**) liver-on-chip.

**Table 2.** Advantages and limitations of in vitro liver models (note: these methods may have crossovers).

| In Vitro Approaches | References | Advantages | Limitations |
|---|---|---|---|
| Monolayer | [8,9] | Easily manipulated, low-cost, good repeatability. | Cannot recapitulate in-vivo like cellular morphology and 3D microenvironment, loss of cell polarity, poor function. |
| Co-culture | [44–46] | Multi-cellular environment, cell-cell interaction, improve functions and longevity, cellular polarity. | Difficult isolation of NPCs, variations of NPCs, differentiation status and viability are varied depending on culture conditions. |
| 3D culture | [11,47–50] | Recapitulation of 3D microenvironment and ECM properties, improve gene and protein expression, improve functions and longevity, cellular polarity. | Complicated methods of culture. Necrotic regions within 3D cellular models caused by oxygen diffusion. |
| Spheroids | [41,46,51] | In vivo-like microenvironment, cellular interaction, maintain liver-specific functionality over long term culture, enhanced CYP 450 and transporter expression, formation of secondary structure (e.g., bile canalicular-like structure). | Spheroid size limitation (~200 µm) and variations, necrotic cores, Oxygen and nutrient diffusion through cellular aggregates. |
| Liver-on-a-chip | [35,52–58] | Dynamic microenvironment, suitable for co-culture, 3D culture, and spheroid, improve liver-specific, functionality, enhanced CYP 450 and transporter expression formation of secondary structure, pattern cells spatially, high through put and low cost. | Complicated methods of operate chip and culture cell in the chip, required perfusion systems, non-specific binding of drugs to chip materials, may wash away molecules in the chamber under perfusion, no standard yet. |

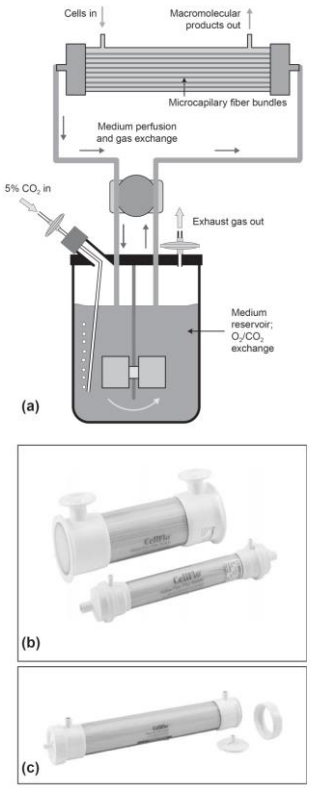
*2019 Engineered Liver-On-A-Chip Platform to Mimic Liver Functions and Its Biomedical Applications A Review*

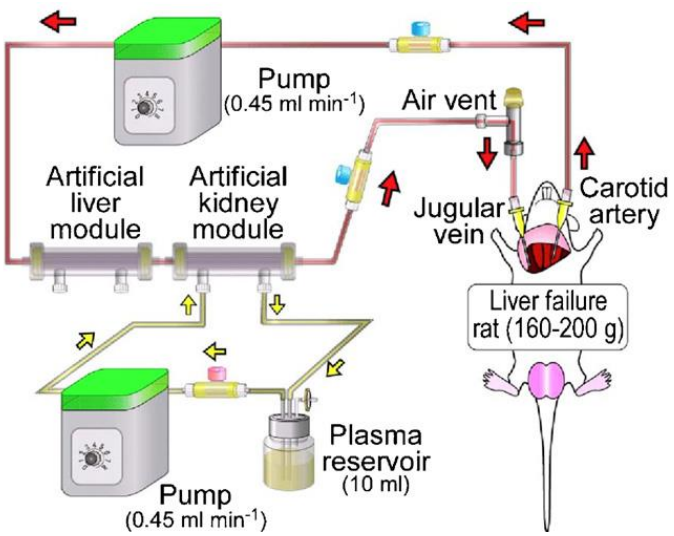
# *In vitro* hepatic culture models (Bioreactor)



**Hollow fibers** are used which helps in more efficient nutrient and gas exchange. In recent years, perfusion chambers with a bed of plastic capillary fibers have been developed to be used for histotypic type of cultures. The cells get attached to capillary fibers and increase in cell density to form tissue like structures.

**Bioreactor:** culture vessel for large-scale production of cells, either anchored to a substrate or propagated in suspension. Can also be used for smaller scale three-dimensional culture of constructs for tissue engineering




## Future work

Perfusion systems containing hepatocellular aggregates exhibit desirable cell morphology and liver-specific functions for several weeks in culture

Incorporation of multiple reactors in parallel has been explored as an approach for high-thoughtput drug screening studies

# *In vitro* hepatic culture models (Microfludic system)



Why using microfluidic system?

In order to promote oxygen delivery while protecting hepatocytes from deleterious shear effects, gas-permeable membranes with endothelial-like physical parameters, grooved substrates and microfluidic microchannel networks have been integrated into several bioreactor designs

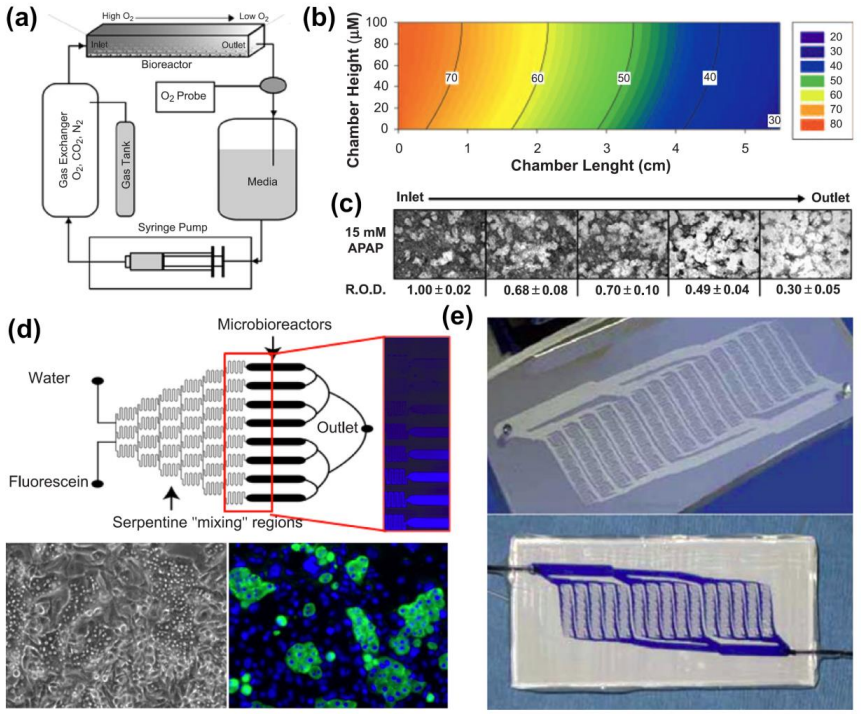



**FIGURE 46.4**

**Bioreactors for *in vitro* liver applications.** (a) Zonation and toxicity in a hepatocyte bioreactor. Co-cultures of hepatocytes and non-parenchymal cells are created on collagen-coated glass slides and placed in a bioreactor circuit where the oxygen concentration at the inlet is held at a constant value. Depletion of oxygen by cells creates a gradient of oxygen tensions along the length of the chamber, similar to that observed *in vivo*. (b) Two-dimensional contour plot of the medial cross section of the reactor depicting the cell surface oxygen gradient formed with inlet pO2 of 76 mmHg and flow rate of 0.3 mL/min.
(c) Bright-field images of perfused cultures treated with 15 mM acetaminophen (APAP, a hepatotoxin) and stained with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as a measure of cell viability from five regions along the length of the bioreactor. The intensity of MTT staining is reported as relative optical density (R.O.D) values. The zonal pattern of APAP toxicity seen here is consistent with that observed *in vivo* [83]. (d) A zonal microbioreactor array that incorporates serpentine mixing regions and two sources is able to create a gradient of fluorescein (shown in blue on right) in an array of microbioreactors containing random co-cultures of hepatocytes and non-parenchymal cells (bottom). (e) A bilayer microfluidics device designed with a microchannel network that mimics liver vasculature so as to support the large metabolic needs of hepatocytes contained within an adjacent chamber. *(Figure panels reproduced with permission from [82].)*

# Organ on a chip



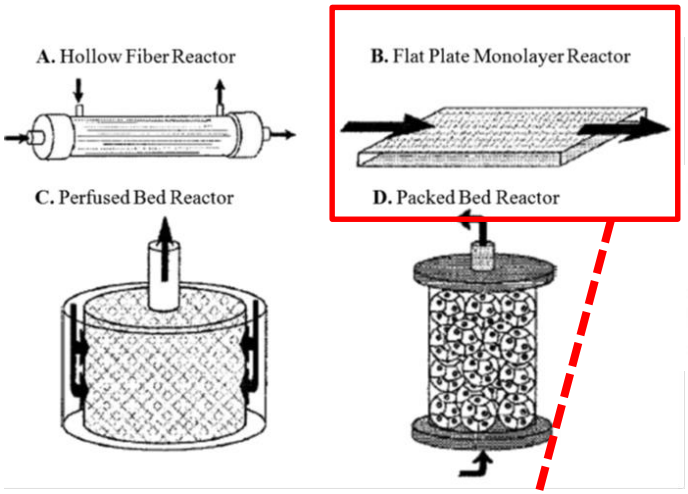
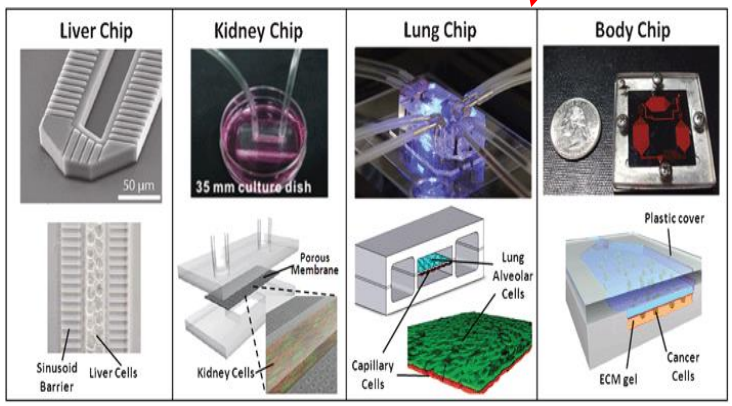
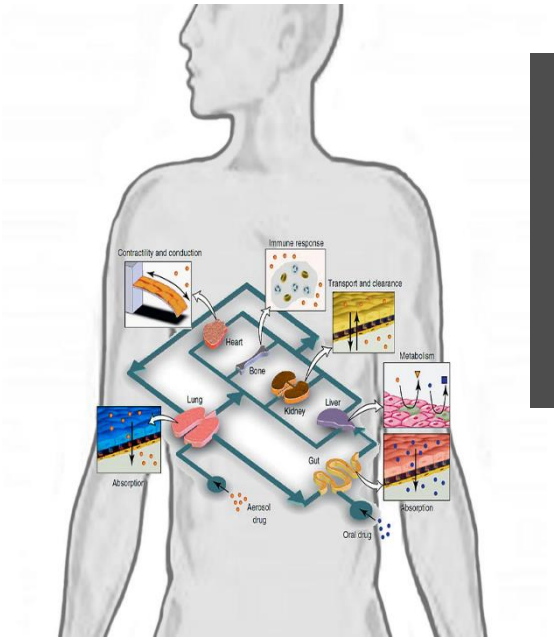


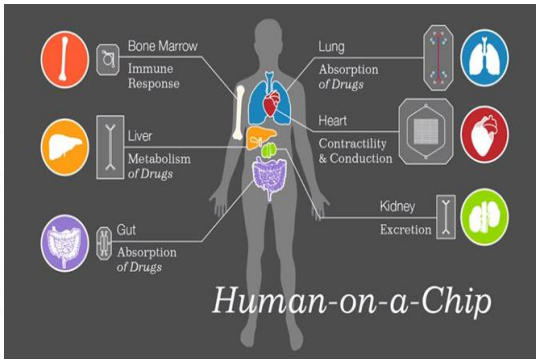

**Figure 2.** Available bioreactor designs: (A) hollow fiber reactor; (B) flat membrane reactor; (C) perfused bed reactor; and (D) packed bed bioreactor, which are currently being evaluated for use in BAL [*Figure reproduced with permission from Allen JW, et al. Hepatology 2001;34:447–55*].

*2011 Extracorporeal Bioartificial Liver for Treating Acute Liver Diseases*




*2012 Microengineered physiological biomimicry: Organs-on-Chips*



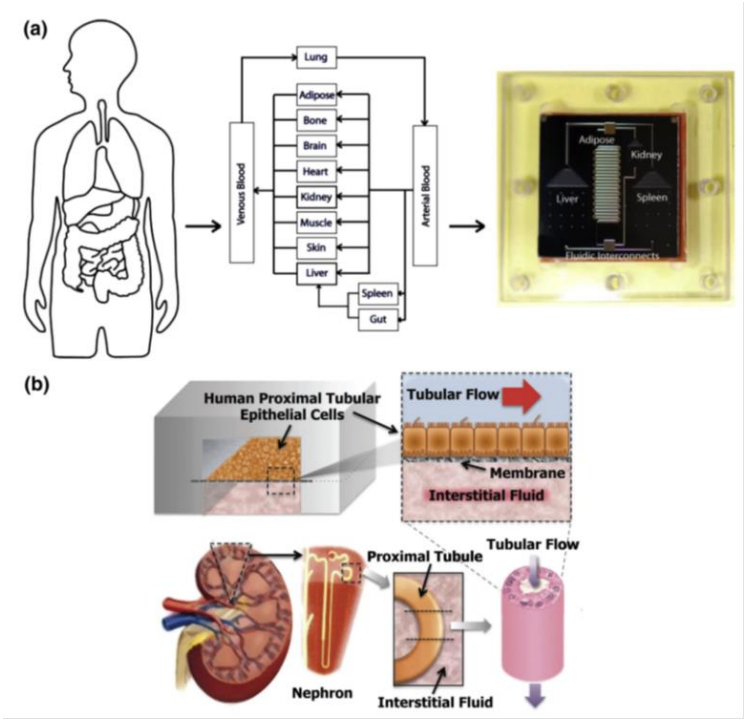




*2011 From 3D cell culture to organs-on-chips, Trends in Cell Biology*

# Organ-on-a-chip (2)



The two most well-recognized advantages of organ-on-a-chip devices are their ability to provide (1) physiologically reasonable and (2) spatiotemporally controllable microenvironments. Together, these benefits make the organ-on-a-chip concept a powerful tool for biological studies.




## Organ on a chip主要用途

- First advantage is greatly favorable when applied as a drug-testing platform. Conventional drug-testing techniques, such as *in vitro* culture and animal tests, often do not provide environments that are physiologically relevant to humans, whereas organ-on-a-chip does.
- The second advantage is greatly useful when it is applied to *in situ* mechanistic studies such as real-time cell migration research

**FIGURE 20.1** Two types of organ-on-a-chip devices.
(a) Schematic of a body-on-a-chip device consisting of interconnected organ compartments. The flow directions and rates were designed to mimic physiologic communication between organs. *(Figure reproduced from Shuler and Esch (2010).)* (b) Schematic of proximal tubule-on-a-chip device that mimics a unique organ microenvironment. The ECM-coated porous membrane and flow-induced shear stress provide cells with a more in vivo-like environment that contributes to better cell function. *(Figure reproduced from Jang et al. (2013).)*

# The importance of Liver-on-a chip



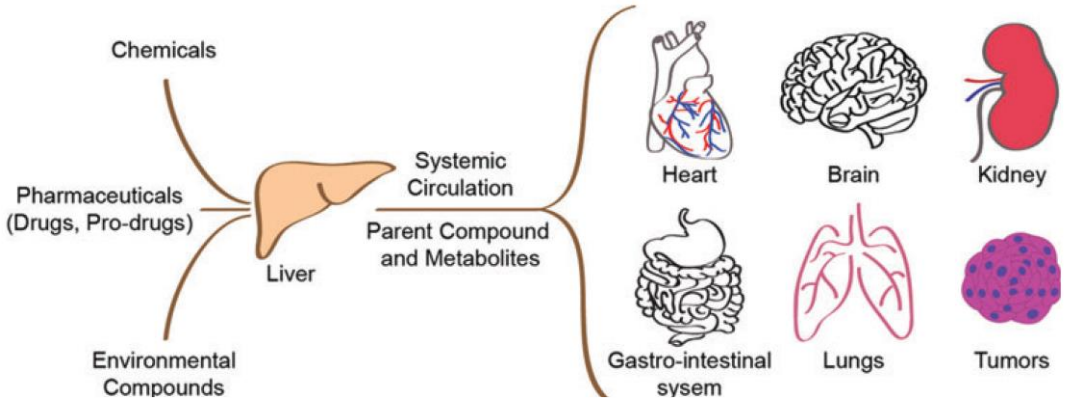



**FIG. 1.** Liver is the central metabolizing organ in the human body. Pharmaceuticals, chemicals, and environmental compounds interact with liver and other organs in the body directly or in metabolized form.

Apart from hepatic toxicity, liver-generated metabolites are transported through systemic circulation to other tissues resulting in interaction with other organs, including kidney, heart, brain, gut, and lungs, and targets such as tumors (Fig. 1). The drug–metabolite interaction of liver with other organ(s) can be exploited for the desired therapeutic action of the drug as exemplified in the case of chemotherapy prodrug tegafur, where liver biotransformation generates toxic metabolite 5-fluorouracil (5-FU), which is responsible for the antitumor efficacy of the drug.[3,4]

# Liver-on-a chip model



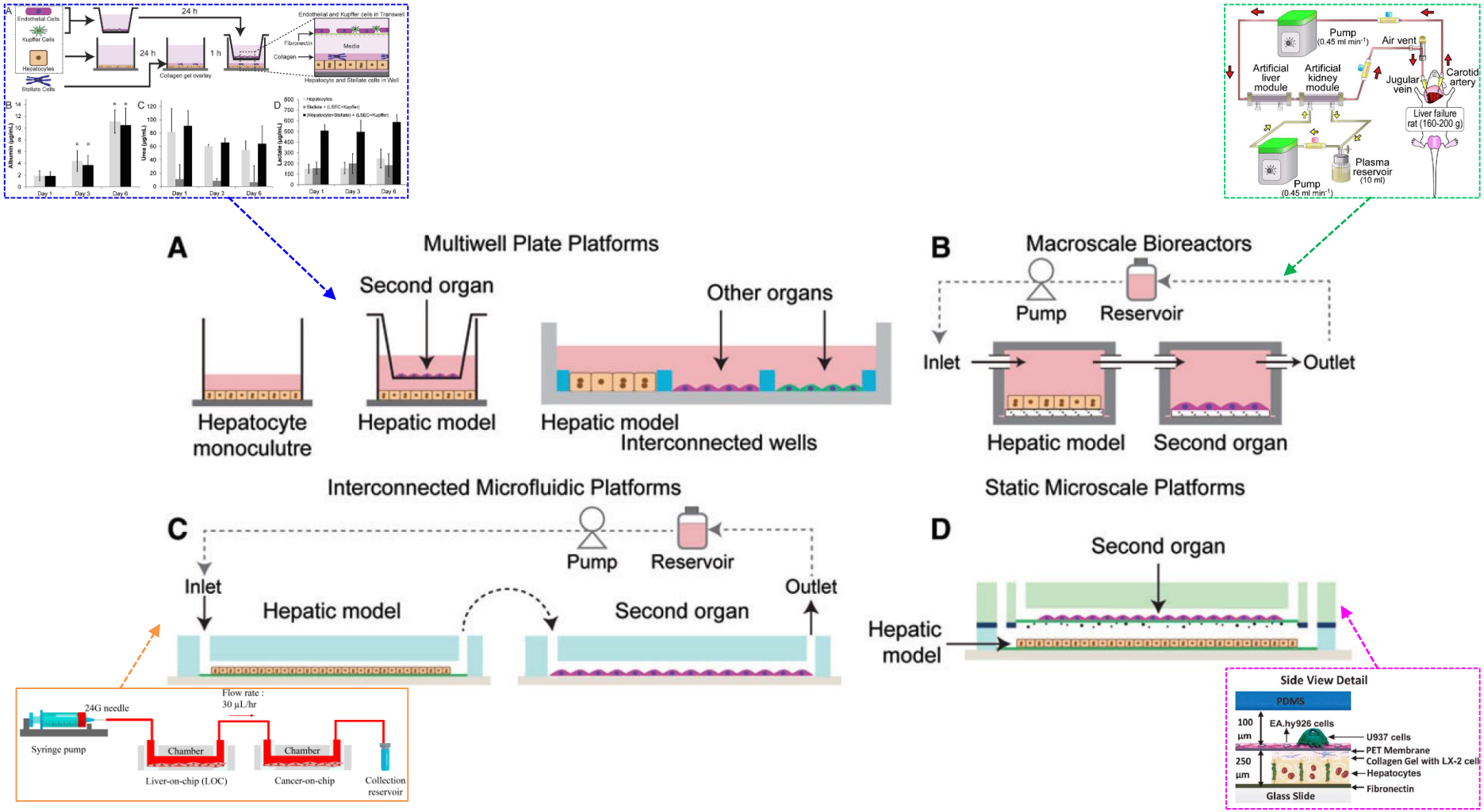



**FIG. 3.** Broad classification of cell-culture systems used for developing liver-based multiorgan models. (A) Multiwell plate (monoculture, transwell, and interconnected culture), (B) Macroscale bioreactor, (C) Interconnected microfluidic platforms and (D) Static microscale platforms.

*2016 Emerging In Vitro Liver Technologies for Drug Metabolism and Inter-Organ Interactions*

# Liver-Organ system



Table 1. Systems Conducive to Interfacing Liver Models to Other Organ Models

| *Organ systems* | *Drug (metabolite)* | *Observations* |
|---|---|---|
| | **Multiwell transwell coculture** | |
| Liver–gastrointestinal (PHH)—(Caco-2)[48] | Bretrylium, Chlorothiazide, Diazepam, Theophylline, Acyclovir, Terbutaline, Lorazepam, Oxazepam, Ganciclovir, Caffeine, Pirenzepine, Metoclopramide, Cyclosporine, Sildenafil, Timolol, Midazolam, Nifedipine, Imipramine, Chlorpromazine, Fluoxetine, Desipramine, Propranolol, Nicardipine, and Verapamil | Reasonable correlation ($r^2 = 0.86$) between the model prediction and the oral bioavailability |
| Liver–gastrointestinal (PHH)-(Caco-2)[49] | PEG-4000, Doxorubicin, Mannitol, Acyclovir, Nadolol, Terbutaline, Ranitidine, Furosemide, Atenolol, Cimetidine, Etoposide, Metolazone, Sulfasalazine, Hydrochlorothiazide, Methylprednisolone, Propranolol, Hydrocortisone, Alprenolol, Metoprolol, Antipyrine, Caffeine, Carbamazepine, Ketoprofen, Naproxen, Theophylline, Verapamil, Corticosterone, and Dexamethasone | Reasonable correlation ($r^2 = 0.73$) between the model prediction and the oral bioavailability |
| | **Interconnected multiwell plate** | |
| Liver–Kidney–Lung–CNS–Blood vessels–Cancers (primary hepatocytes)—(proximal tubule epithelial cells)—(small airway epithelial cells)—(astrocytes)—(aortic endothelial cells)—(MCF-7)[50] | Tamoxifen | ↓ Viability of MCF-7 cells<br>Relative cytotoxicity to other organs |
| Liver–Kidney–Lung (PHH)–(renal proximal tubule cells)—(small airway epithelial cells)[52] | Aflatoxin B1 (*(AFB1)-8,9-epoxide*) | ↓ Viability of hepatocytes<br>Relative cytotoxicity to other organs |
| Liver–fibroblasts (PHH)—(3T3)[53] | Aflatoxin B1 (*(AFB1)-8,9-epoxide*)<br>cyclophosphamide (*4-hydroxycyclophosphamide and phosphoramide mustard*) tamoxifen | ↓ Viability of 3T3 cells<br>↓ Viability of hepatocytes |
| | **Macroscale bioreactor platform** | |
| Liver–Vascular (HepG2)—(HUVEC)[55] | | ↑Albumin production<br>↑Urea production<br>↑Viability<br>↑Nitric oxide production |
| Liver–fat–vascular (HepG2)—(omental adipose tissue)—(HUVEC)[56] | | Network of interactions between various systems in fasting, postabsorptive states and postprandial states in type 1 and type 2 diabetes. |
| Liver–vascular (HepG2/C3a)—(HUVEC)[57] | | Numeric relationship between cells in cell number scaling mode and metabolic and surface scaling models. |
| Liver–gastrointestinal (Liver slices from male Wistar rats)—(Jejunum/Ileum slices from male Wistar rats)[58] | 7-ethoxyxoumarin<br>7-hydroxycoumarin<br>Lidocaine (*MEG-X*) | Short-term culture in connected system<br>↓CYP7A1 Expression |
| Liver–gastrointestinal (HepG2)—(Caco-2)[59] | benzo[a]pyrene | ↑Permeability of Caco-2 in flow model |
| | **Microfluidic interconnected system** | |
| Liver–lung–fat (HepG2/C3A)—(L2)—(3T3-L1)[60] | Naphthalene (*Naphthoquinone*) | Reduced naphthalene and naphthoquinone-induced glutathione (GSH) depletion |
| Liver–bone marrow–tumor-resistant tumor) (HepG2/C3A)—(MEG-01)—(MES-SA)—(MES-SA/DX-5)[61] | Doxorubicin (*Doxorubicinol*), Nicardipine, Cyclosporine A | Synergistic effect of drugs on cell proliferation |
| Liver–GI–Fat–Kidney–Bone Marrow (HepG2/C3A)—(Caco-2/HT29-MTX)–Empty compartments[62] | Acetaminophen | Dose-dependent hepatotoxicity |
| Liver–lung–kidney–fat (HepG2/C3A)—(A549)—(HK-2)—(Primary human preadipocyte, HPA)[63] | | ↑A549 PROD enzyme activity<br>↑HPA adiponectin secretion<br>↓C3A albumin secretion<br>↓HK-2 GGT enzyme activity |
| Liver–colon tumor–bone marrow (HepG2/C3A)—(HCT-116)—(Kasumi-1)[64] | 5-Fluorouracil | ↓ Viability of the cells |
| Liver–GI–intestine–cancer (HepG2)—(Caco-2)—(MCF-7)[65] | Cyclophosphamide (*phosphoramide mustard*), Tegafur (*5-Fluorouracil*) | ↓MCF-7 viability |
| Liver–tumor (HepG2/C3A)—(GBM-M059K)[66] | Temozolomide (*MTIC*), Ifosfamide (*IPM*) | ↓GBM Viability |
| Liver–skin (HepaRG, primary human hepatic stellate cells)—(Skin Biopsy)[67] | Troglitazone | Long-term tissue culture |
| Liver–kidney (HepG2/C3A and HepaRG)—(MDCK)[15] | Ifosfamide (*Chloroacetaldehyde*) | ↑CYP3A4, CYP3A5, CYP2B6<br>↓MDCK Proliferation |
| Liver–kidney (HepaRG)—(MDCK)[68] | Ifosfamide (*Chloroacetaldehyde*) | Pharmacokinetic model coupling ROS and glutathione (GSH) |
| | **Static microscale platform** | |
| Liver–cancer[46](hepatocytes)-(MCF-7) | Tegafur–uracil (*5-Fluorouracil*) | ↓MCF-7 viability |

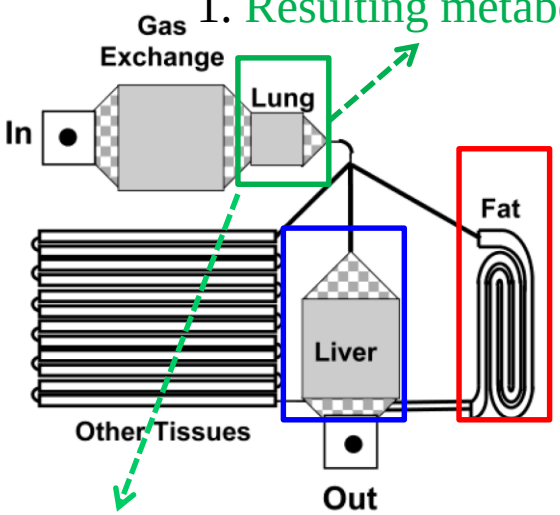
↓, decrease; ↑, increase; ↔, no change; GMB, glioblastoma multiforme; PHH, primary human hepatocytes; ROS, reactive oxygen species.

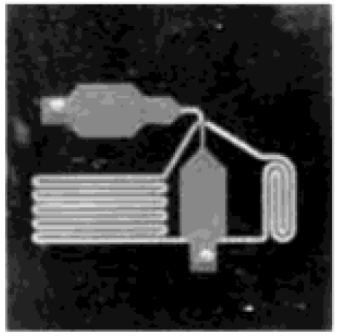
*2016 Emerging In Vitro Liver Technologies for Drug Metabolism and Inter-Organ Interactions*

# Shuler and colleagues' animal-on-a-chip (μCCA)



1. Resulting metabolites (1,2-環氧萘) circulated to the lung compartment (Toxic)




Naphthalene is fat soluble

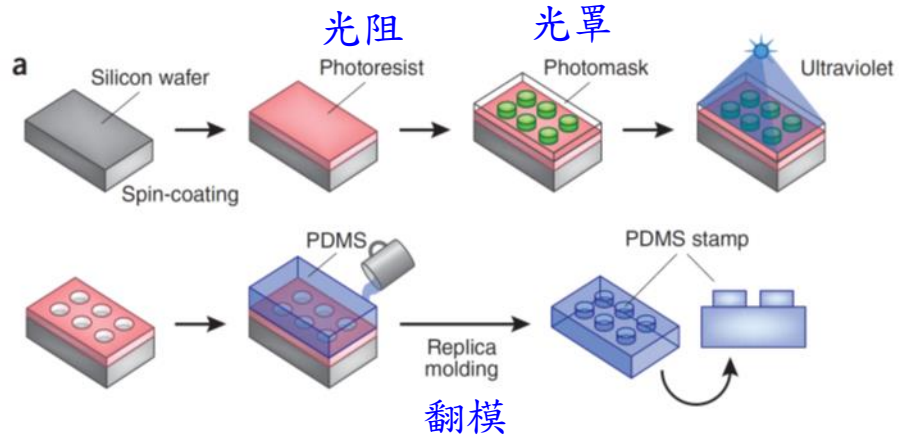
Among the four compartments, naphthalene (萘) was administered to the μCCA and flow was recirculated. Over a span of 6h, the lung compartment showed substantial glutathione (穀胱甘肽)depletion—a marked difference from control experiments with naphthalene but lacking a functioning liver compartment

2. 1,2-環氧萘會與glutathione (GSH; 抗氧化劑) 結合排出

- One outstanding example of this concept is Shuler and colleagues' animal-on-a-chip (2010, 2011) device that reproduced Naphthalene's liver-mediated lung toxicity. In this study, a four-chamber μCCA device (lung, liver, fat, and other tissues compartments) was designed to reproduce the pharmacokinetics of Naphthalene in the human body.
- Naphthalene (萘) was first metabolized by the liver compartment, and then, the resulting metabolites circulated to the lung compartment where cytotoxicity occurred. Since the drug toxicity occurs in organs other than the organ of interest, these side effects could never have been detected in vitro if there were no multiple compartments or no network connecting the discrete organ analogs.

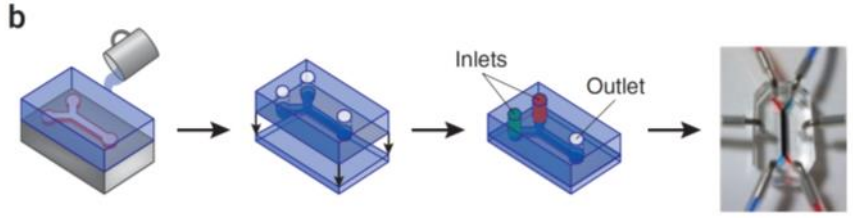
# Soft lithography






This is done by pouring a liquid polymer, such as poly-dimethylsiloxane (PDMS), on an etched silicon substrate and allowing it to polymerize into an optically clear, rubber-like material, essentially creating a rubber stamp (**Fig. 1a**).

正光阻→經過曝光後會溶解
負光阻→經過曝光後不會溶解



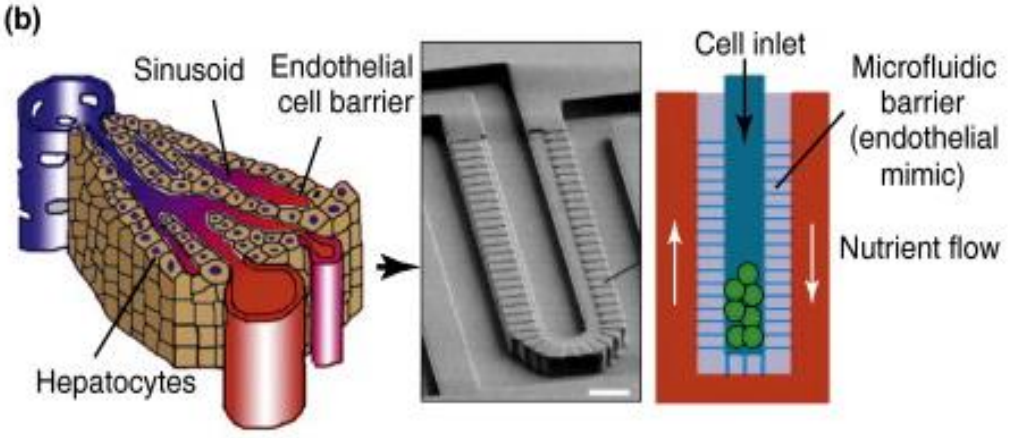

This approach was modified by inverting the PDMS mold and conformally sealing it to a flat smooth substrate, such as glass, to create open cavities in the form of small (cross section < 1 mm × 1 mm), linear, hollow chambers, or 'microfluidic channels,' with openings at both ends of the polymer block for perfusion of fluids (**Fig. 1b**).

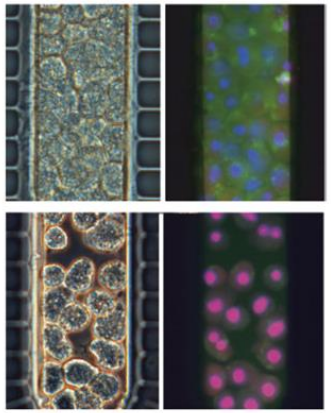
A key feature of PDMS culture systems is that they are optically clear, which allows real-time, high-resolution optical imaging of cellular responses to environmental cues

*2014 Microfluidic organs-on-chips*

# Some examples of Liver on a chip



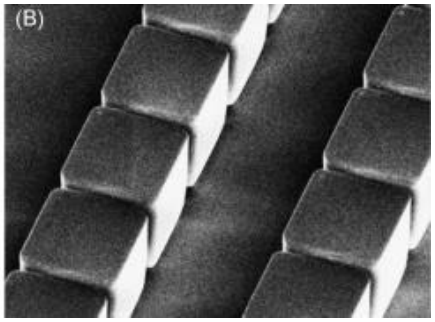


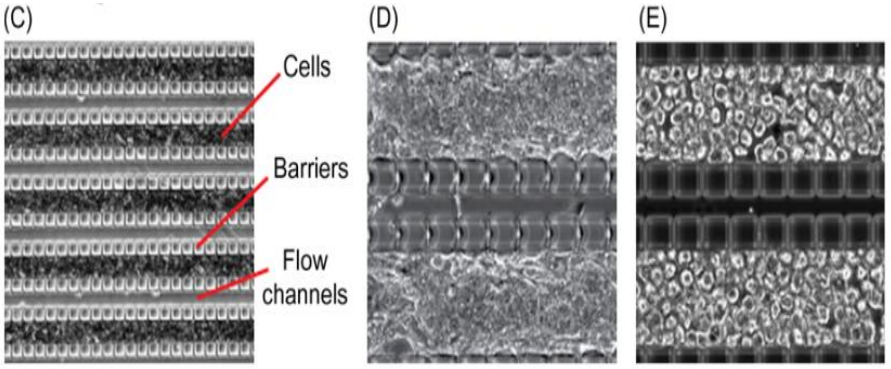



- Scale bar : 50μm
- **Chamber大約50μm**
- Barrier 的間隙2μm
- 特點：
- 間隙特別小，
- 模擬肝臟in vivo環境

*2013 Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME*

*From 3D cell culture to organs-on-chips,2011*



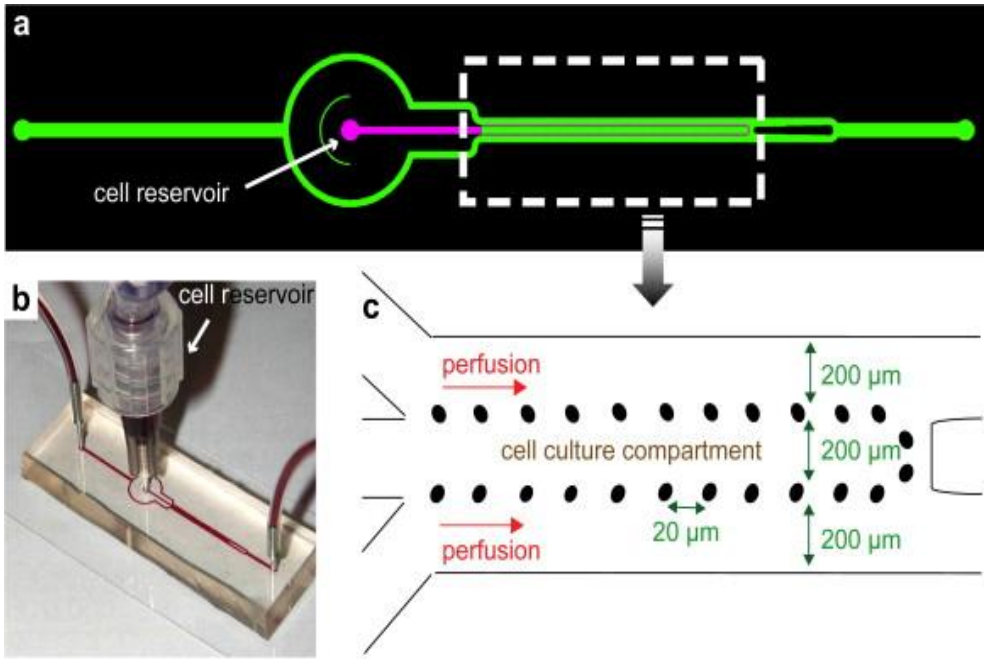

*2013 Microfluidic Cell Culture Systems Chapter 14 – Microfluidic Hepatotoxicity Platform,2013*

- **Channel 寬 50 μm**
- barrier為邊長55μm立方體
- 之間間隙為3μm
- Medium perfusion rate 100μl/day

# Some examples of Liver on a chip

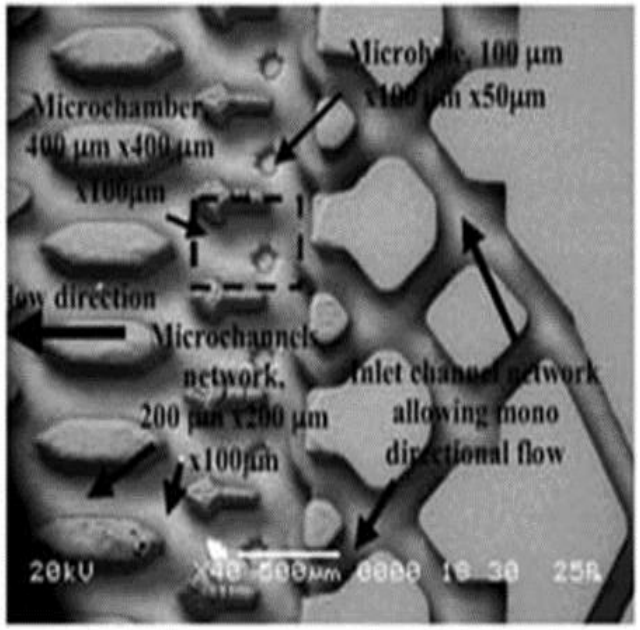


- 1 cm (length) × 600 μm (width) × 100 μm (height)
- 放入已成球體狀的 spheroid 細胞
- Chamber 寬 200μm
- barrier 間隙20μm




*2008 A gel-free 3D microfluidic cell culture system*

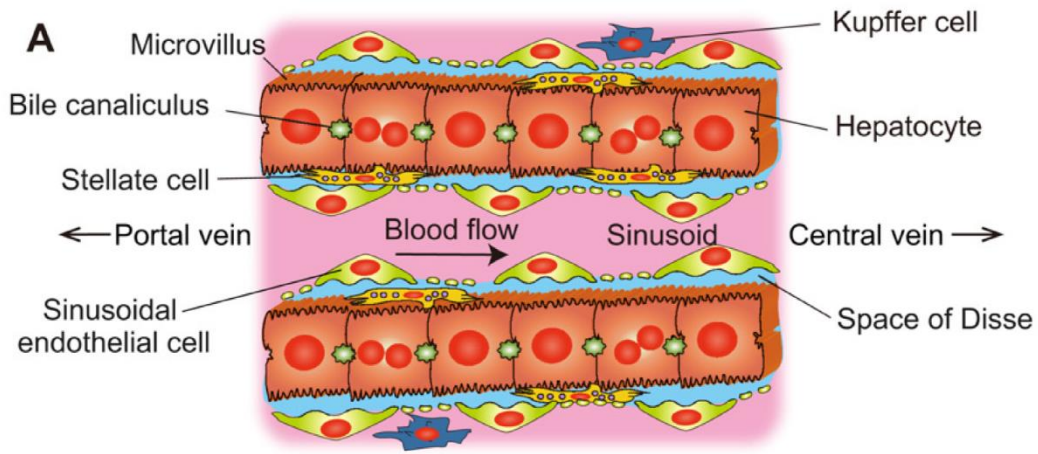
- microholes (100 μm × 100 μm × 50 μm)
- microchambers (400 μm × 400 μm × 100 μm)
- Two PDMS layers are of 600 μm height
- 放入primary初代細胞
- 很多小巧思的游泳池迷宮
- 特點：有特別設置 holes，有另外的管路提供氧氣



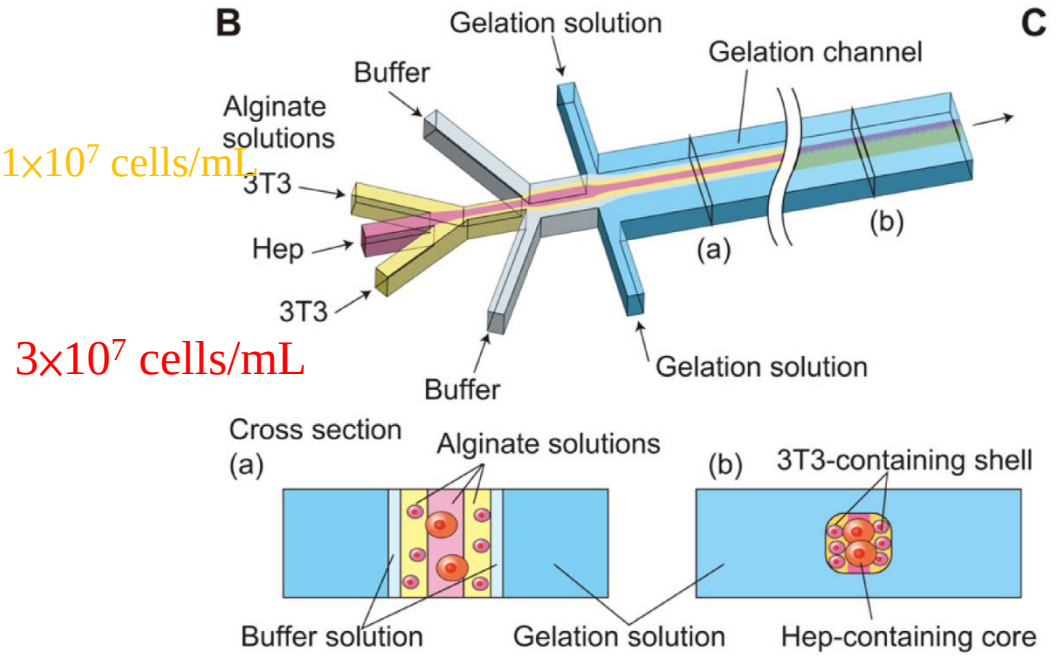

*2008 Microfluidic PDMS (Polydimethylsiloxane) Bioreactor for Large-Scale Culture of Hepatocytes*

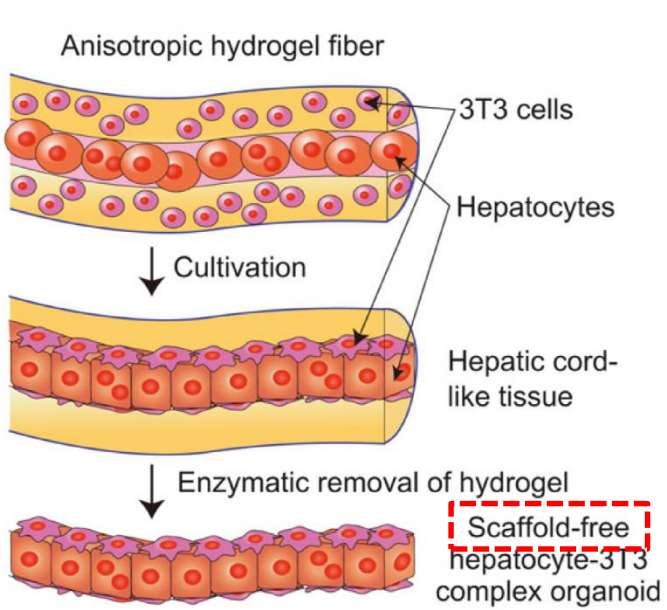
# Some examples of Liver on a chip






- Width: 400 µm
- Length: 50 mm
- Depth: 160 µm

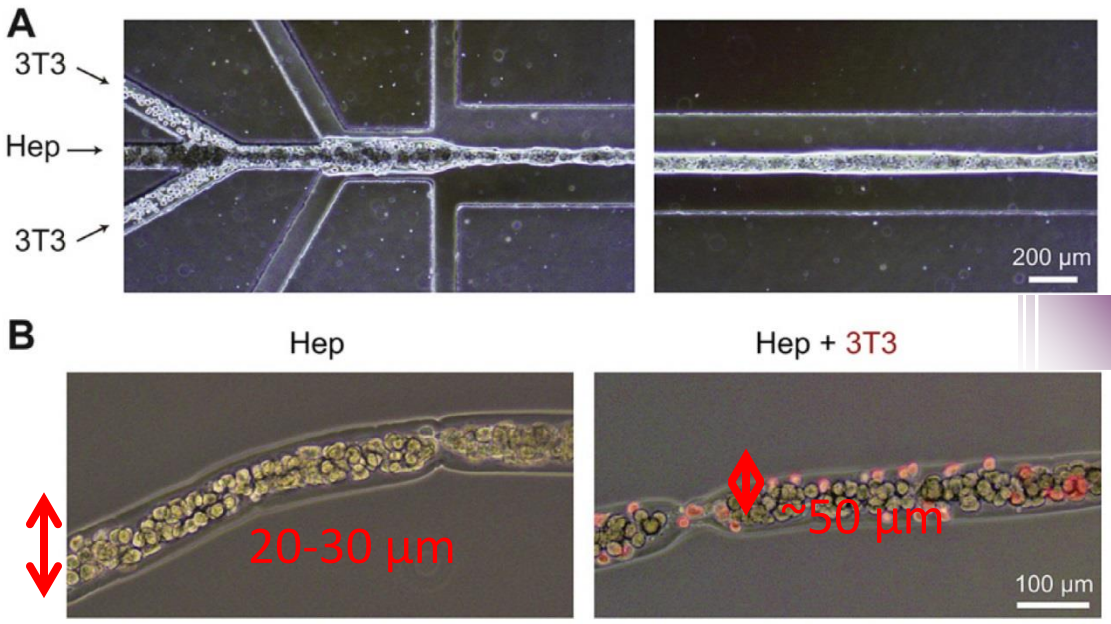


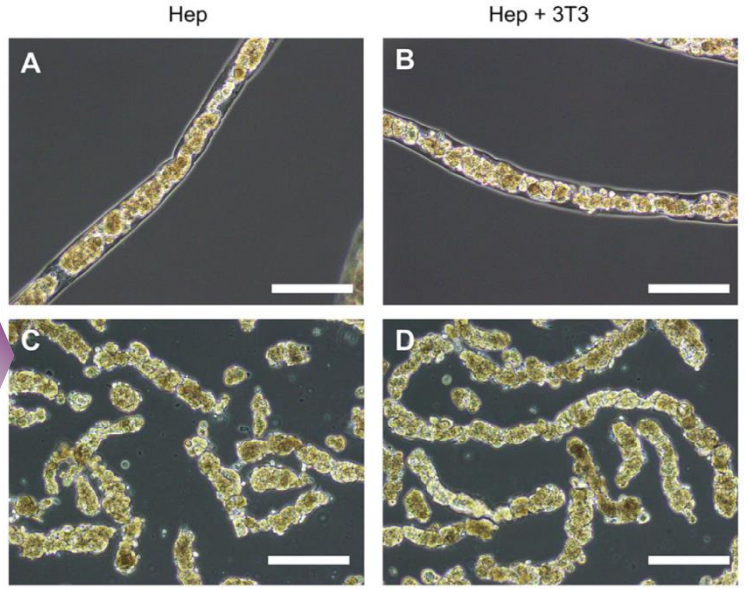




*2012 Controlled Formation Of Heterotypic Hepatic Micro-organoids In Anisotropic Hydrogel Microfibers For Long-term Preservation Of Liver-specific Functions*

# Some examples of Liver on a chip





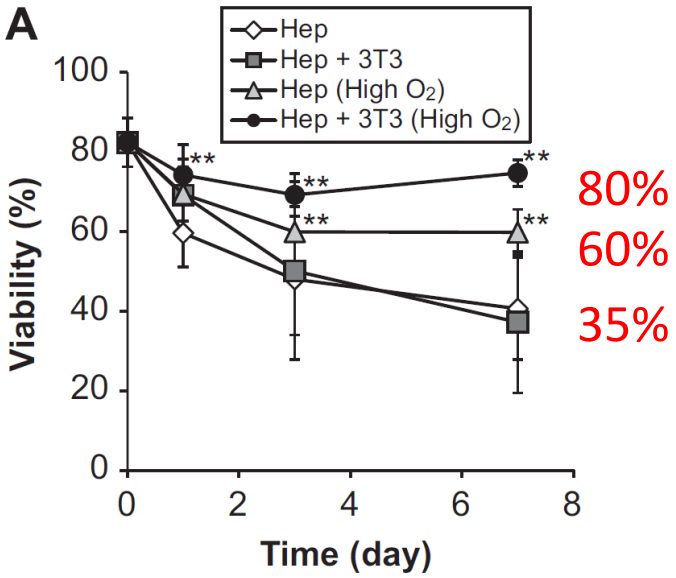



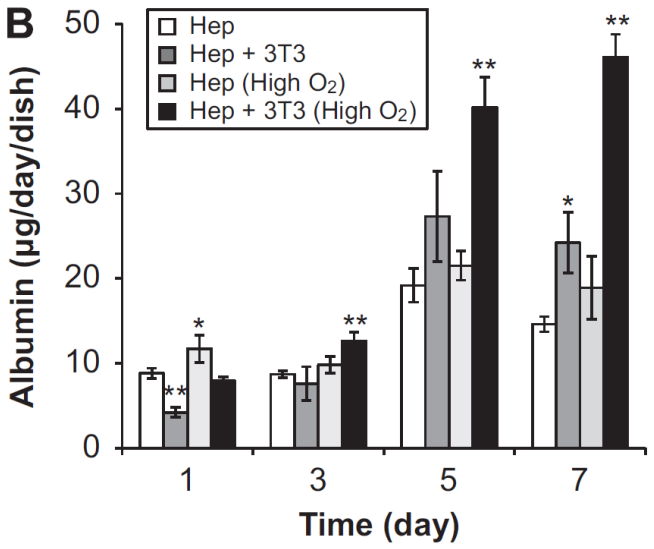

201±102 μm

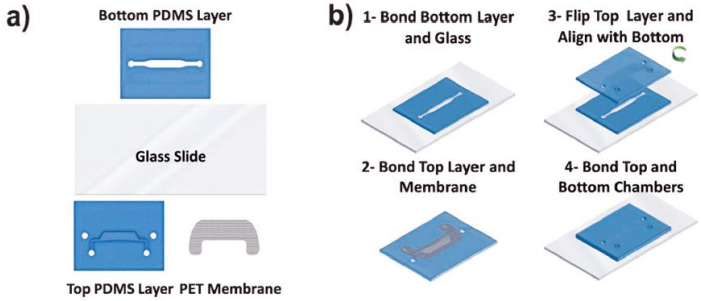
viability : 50%

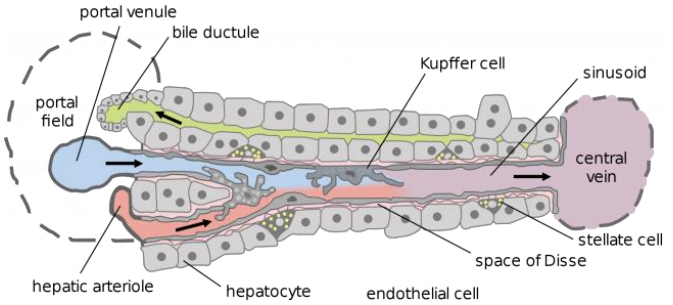
179±102 μm

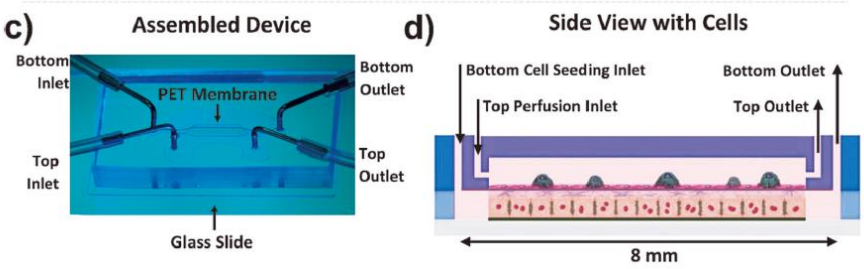
viability : 80%

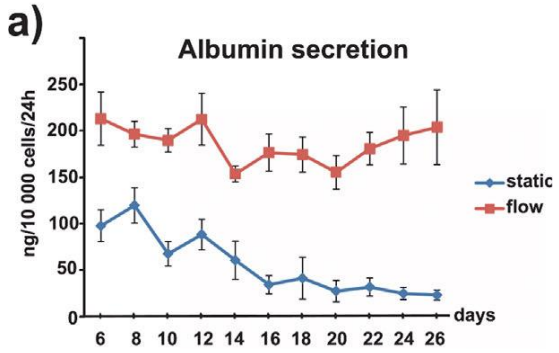


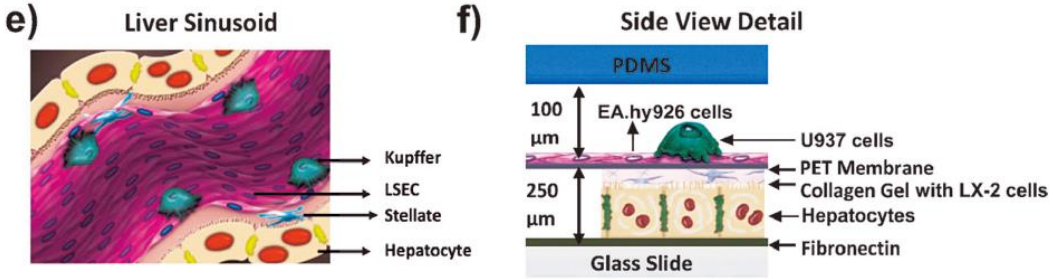



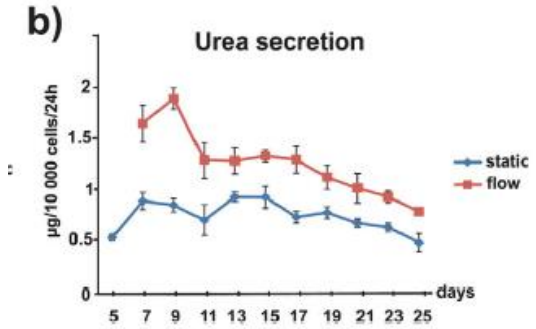


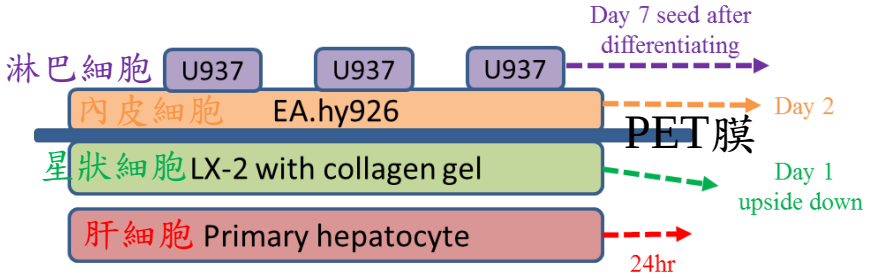



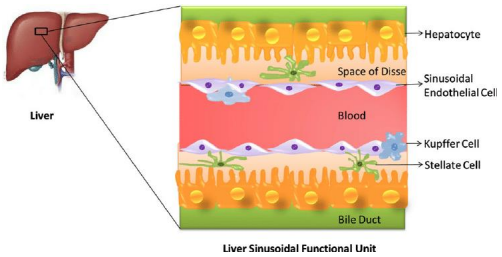




**U937:** lymphocyte in lung
**EA.hy926 :** umbilical vein endothelial cells
**LX-2** : Human Hepatic Stellate Cell

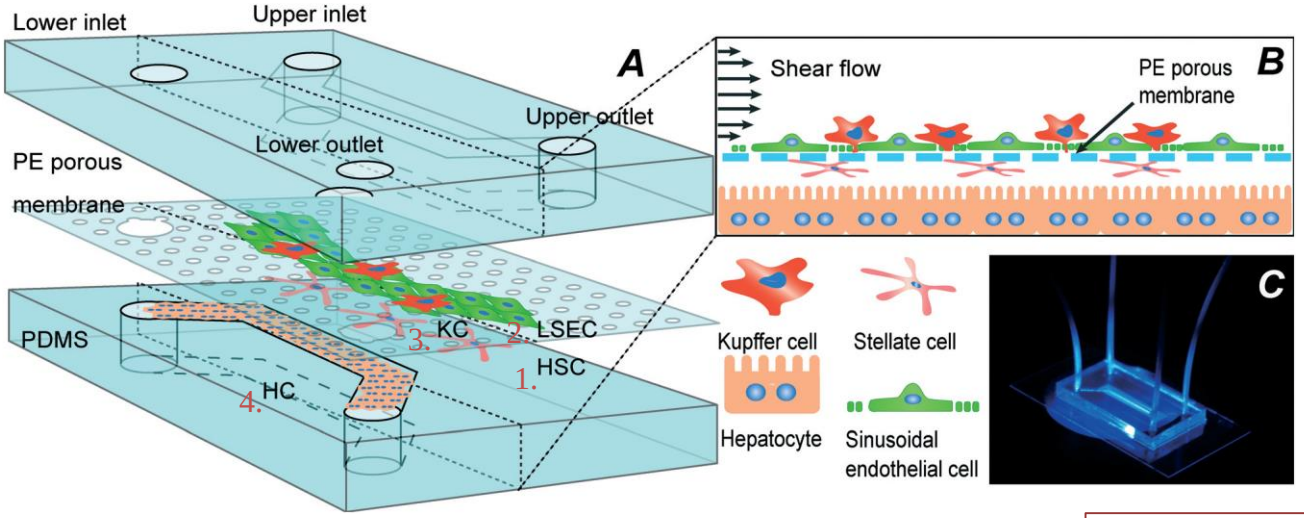
*2016 Long-Term Maintenance of a Microfluidic 3D Human Liver Sinusoid*

*Young Bok Kang.et al. Layered Hepatocytes and Endothelial Cells on a Transwell Membrane: Toward Engineering the Liver Sinusoid. (2013).*




- **Reconstruction of an organ-specific liver sinusoid chip**

Sinusoidal sieve plate Disse space




Cultured steps:
1. HSCs
2. LSECs
3. KCs
4. HCs

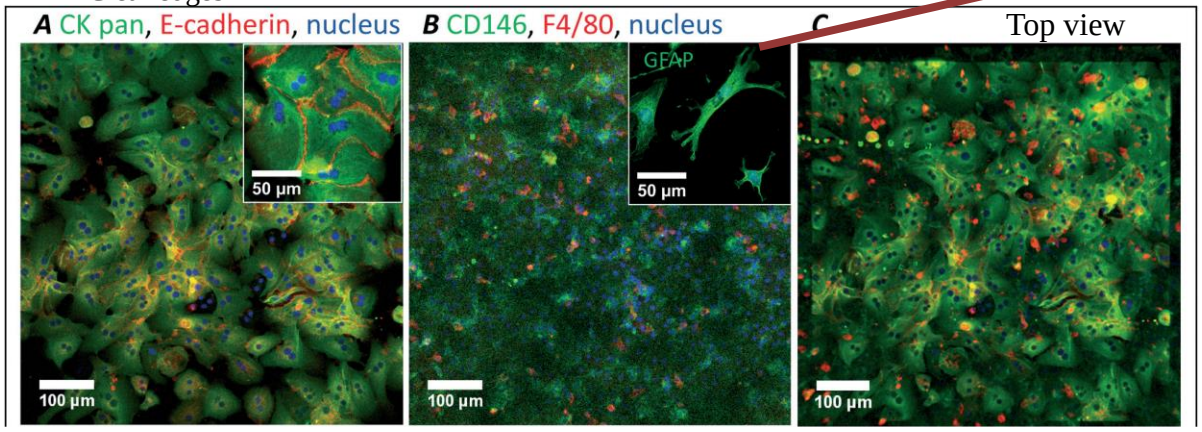
- **Biological identification of hepatic cells inside the liver chip**

HC
Polygonal shape
Double nucleus
Clear edges

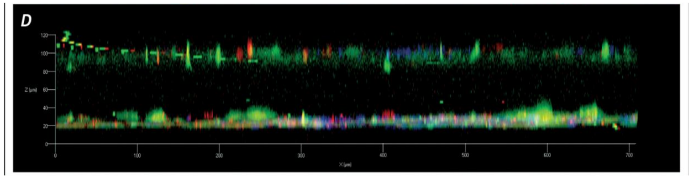
LSEC
CD146
Monolayer
Cobblestone shape

KC
F4/80
Polymorphic shape

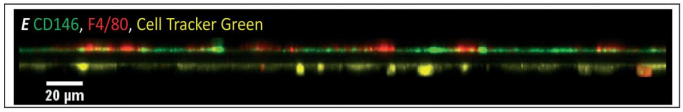
HSC
GFAP
Stellate-like shape




Side view




Lateral view→
well separated by 10$\mu$m membrane height.

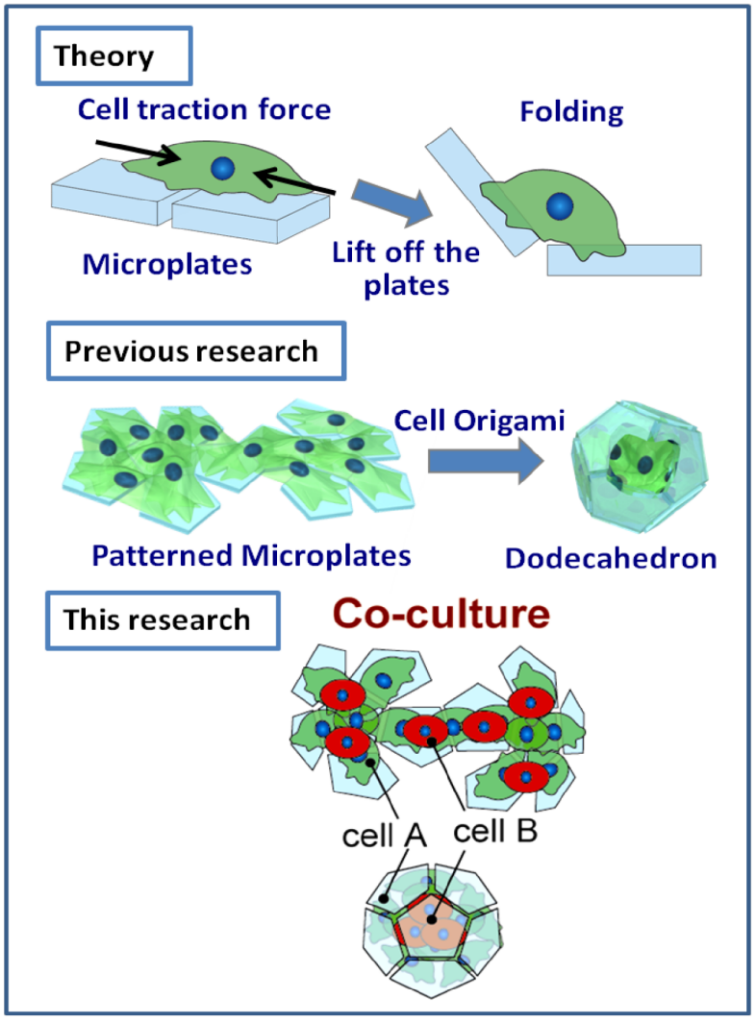


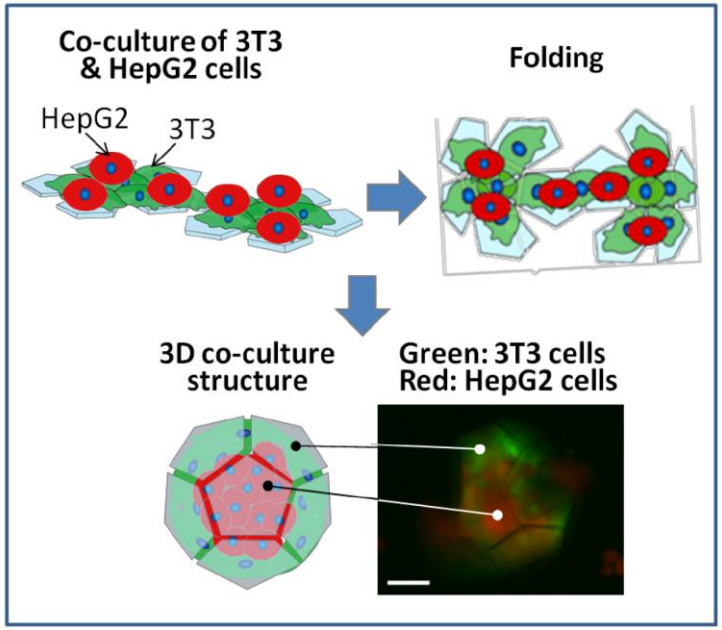

KC LSEC HSC

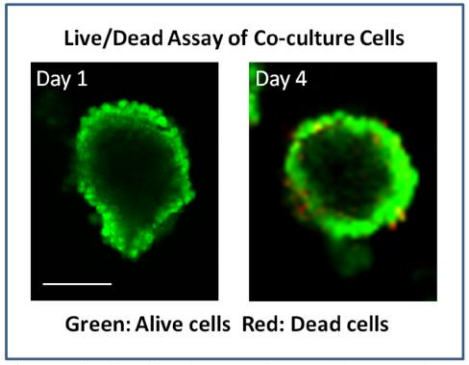
*2017 Mimicking liver sinusoidal structures and functions using a 3D-configured microfluidic chip*

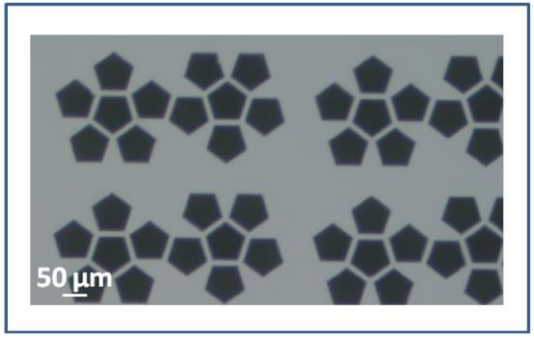
# Some examples of Liver on a chip






*2016 SIMPLE AND RAPID FORMATION OF 3D CO-CULTURE CELL LADEN MICROSTRUCTURES BY USING CELL ORIGAMI TECHNIQUE, MicroTAS conference*

# Origami technique

聚對二甲苯（Poly-*p*-xylylene）

- 利用 Cell traction force將其摺疊(Microplate 之材質為Parylene C) 。
- 利用 Alginate 使細胞不在非Microplate之位置上生長。

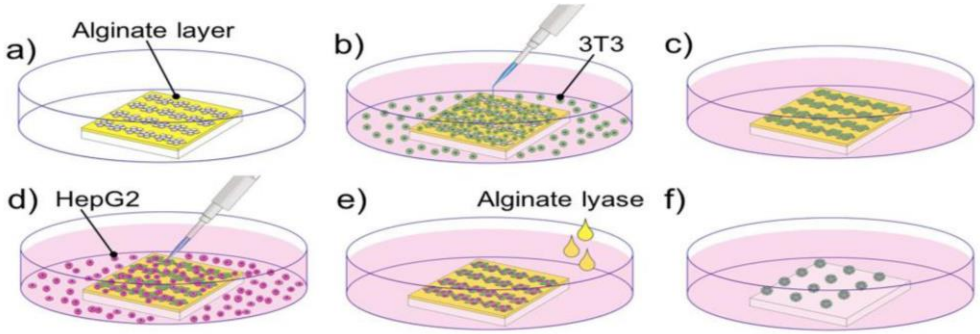
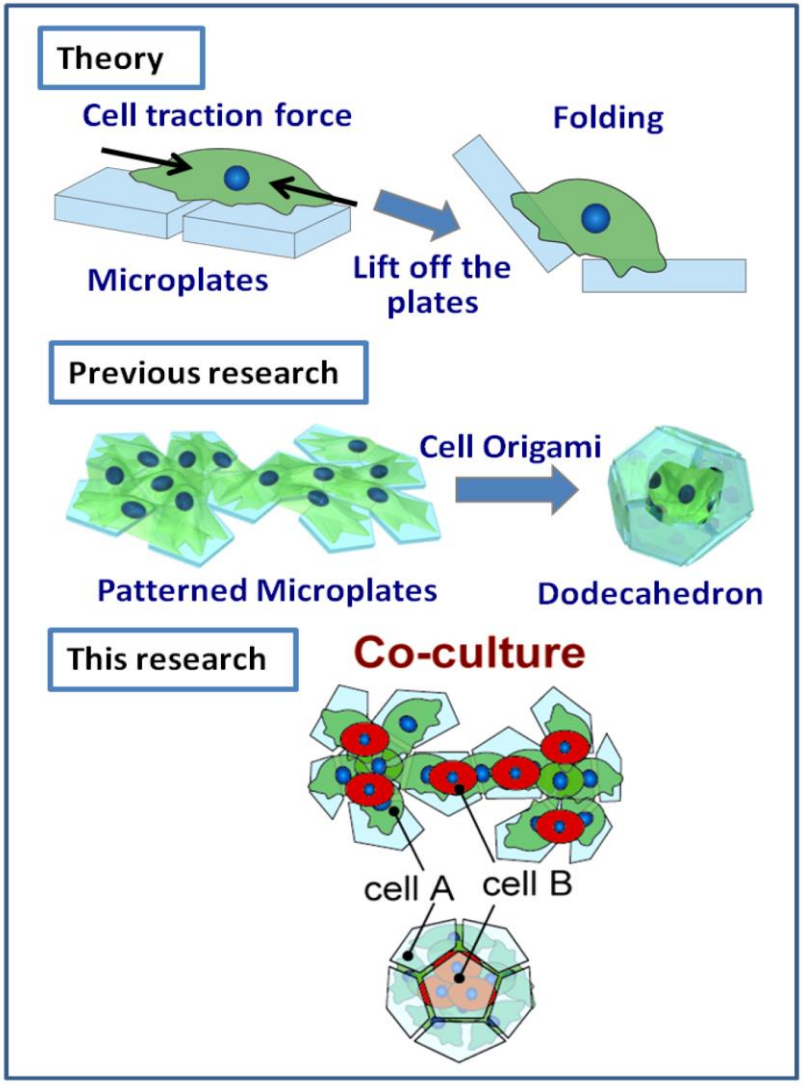



**Figure .1.** Processes of seeding culturing cells on the microplates.

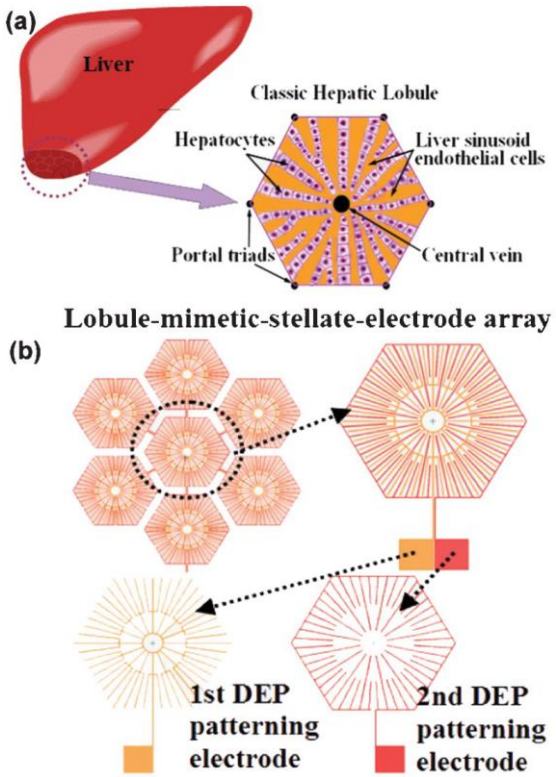



*2018 Origami-based self-folding of co-cultured NIH/3T3 and HepG2 cells into 3D microstructures*

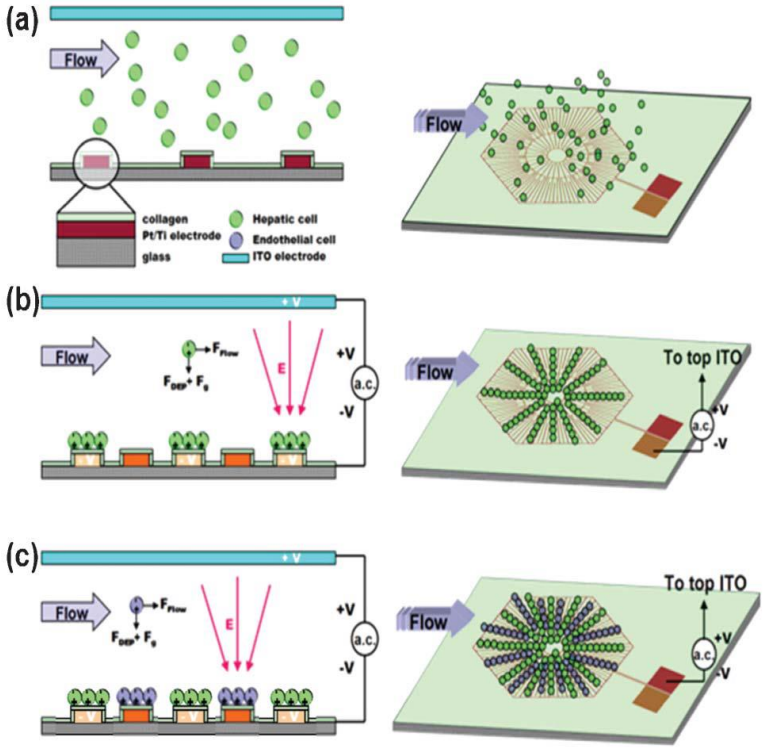
# 介電泳式的細胞分離



- 利用 Dielectrophoresis, (DEP) ，使其順電場方向來排列。
- 流道材質為 ITO 玻璃→可導電。
- DEP-manipulating sugar medium (8.5% sucrose, .3% dextrose and 10 mM HEPES in dd$H_2O$; conductivity: 80 mS $cm^{-1}$)




**介電泳**是電介質在非均勻電場中受力的現象。這一力的存在不需要物體本身帶電。所有粒子在電場環境裡都存在介電泳現象




- 如果外加電場的空間分佈是不均勻的，那麼這些被 (電偶) 極化了的微粒就會受到一份淨力 (以下稱之為「介電泳動力(dielectrophoretic force)」)，進而造成不同程度的漂移運動。
- 因此在 Medium 調整使其導電 → 電場可在 medium 分佈。

# Some examples of Liver on a chip



**Diameter= 169 ± 6 µm**

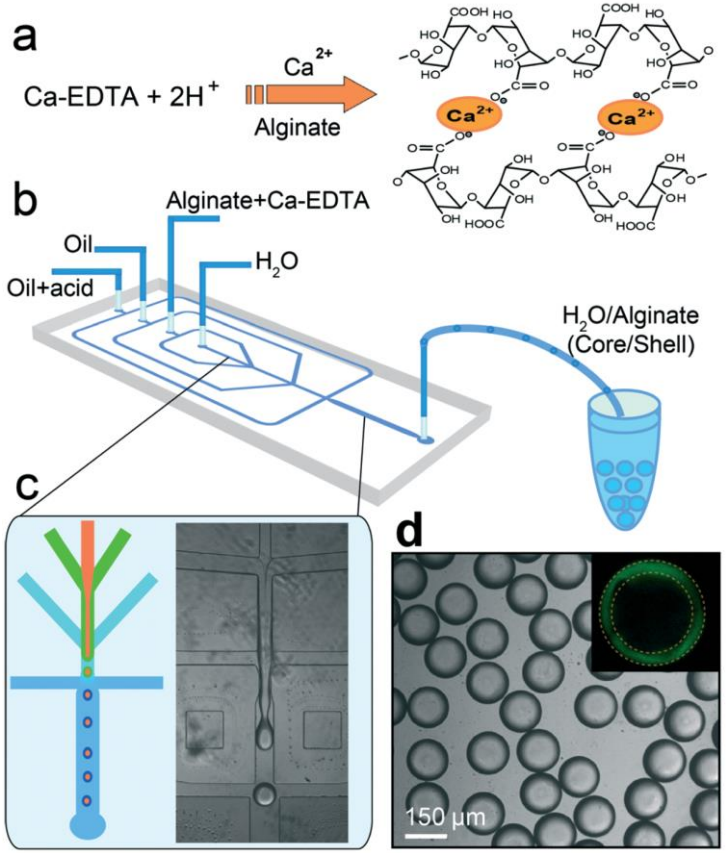



Fig. 1 Construction of the 3D scaffold in a drop consisting of an aqueous core and a hydrogel shell. a) Crosslink of the alginate network by triggered release of $Ca^{2+}$ from the Ca-EDTA complex. b) Schematic diagram of the PDMS device. c) Fabrication of core–shell droplets using w/w/o double emulsions as templates. Alginate in the shell is crosslinked by *in situ* triggered release of $Ca^{2+}$. d) Monodisperse core–shell droplets generated using the droplet-based microfluidics. The shell of alginate hydrogel is clearly identified under a confocal microscope when alginate is labeled with fluorescein, as shown in the inset.

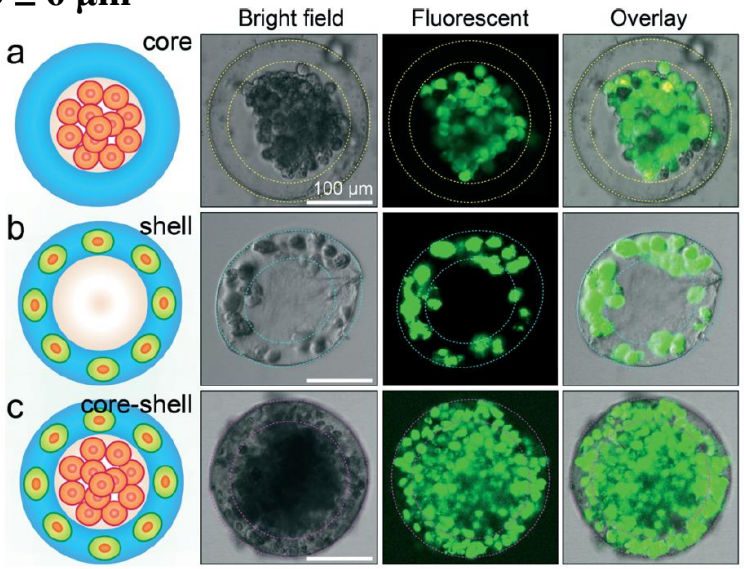
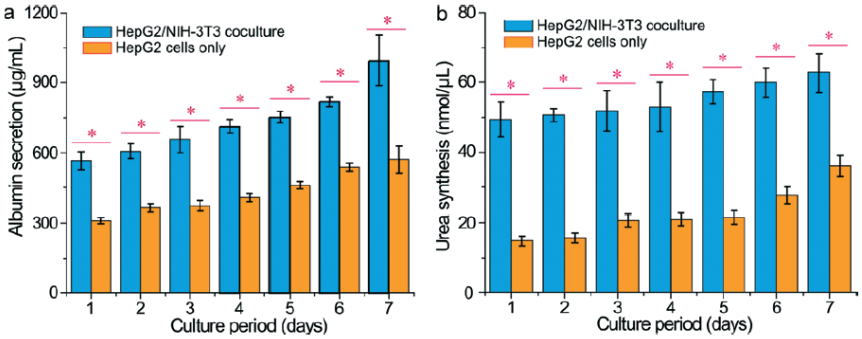



Fig. 2 Spatial assembly of different cells in the 3D core–shell scaffold. a) HepG2 cells confined in the core by the hydrogel shell. b) NIH-3T3 fibroblasts immobilized by the crosslinked alginate network in the shell. c) Simultaneous assembly of hepatocytes in the core and fibroblasts in the shell, forming an artificial liver in a drop. Cell viability is characterized by the calcein AM/EthD-1 staining kit. The scale bars are 100 µm.




*2016 Controlled assembly of heterotypic cells in a core–shell scaffold: organ in a droplet*

# Some examples of Liver on a chip



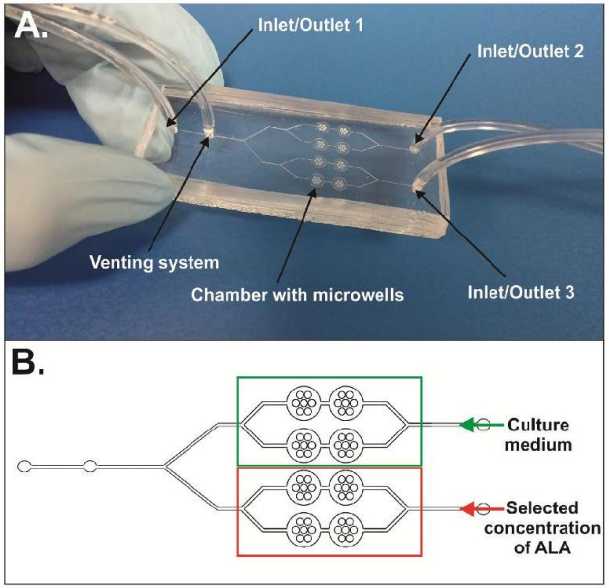
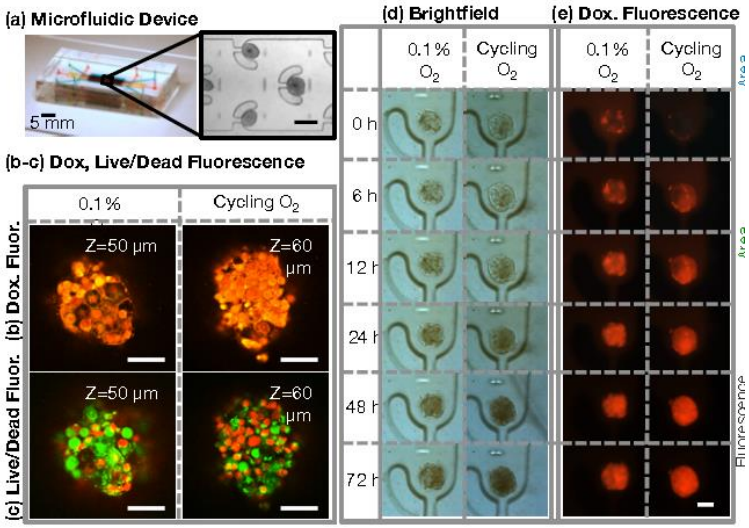
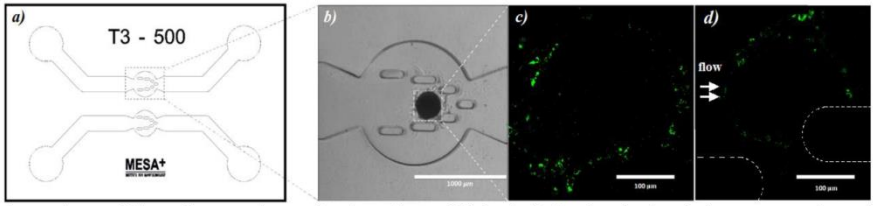



*Figure 1: (A) The microfluidic system (B) Scheme of microchannels and microchambers network. Distribution of control and selected ALA concentrations in the microsystem.*




dynamic conditions by creating a laminar flow (24µl.min$^{-1}$) in the device led to an accelerated penetration of NPs deeper in the tissue, and longer exposure led to even deeper penetration.

*Figure 2. a) Microfluidic design for tissue trapping with size up to 500 µm. b) Single 4T1 spheroid (300 µm diam.) trapped in a microchip. c-d) Confocal Imaging of one 4T1 tissue in contact with green fluorescent silica nanoparticles (100 nm), after incubation for 24 h in static conditions (c) and 12h in dynamic conditions (d).*

# Some examples of Liver on a chip



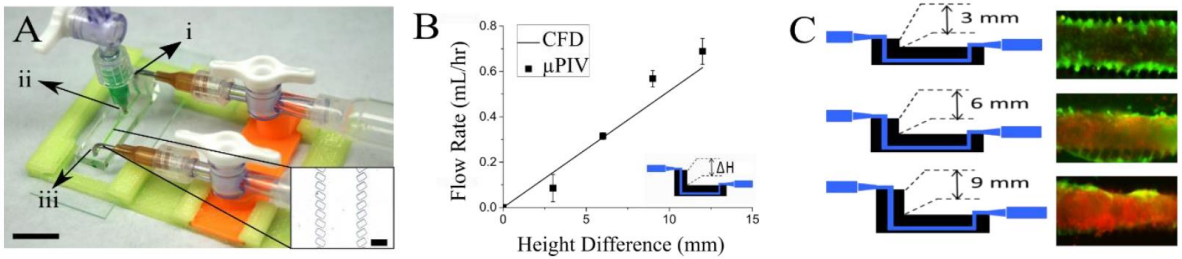



**Figure 1:** (A) Pump free microfluidic culture device setup. (i) Media inlet. (ii) Seeding inlet. (iii) Media outlet. (B) Live-dead staining of microfluidic culture primary rat hepatocyte with different flow rate. (C) Simulated flow rate by CFD and micro-particle imaging velocimetry of the pump free microfluidic culture device. Data are averages of 5 individual sets with S.E.M. Scale bar = 100 µm

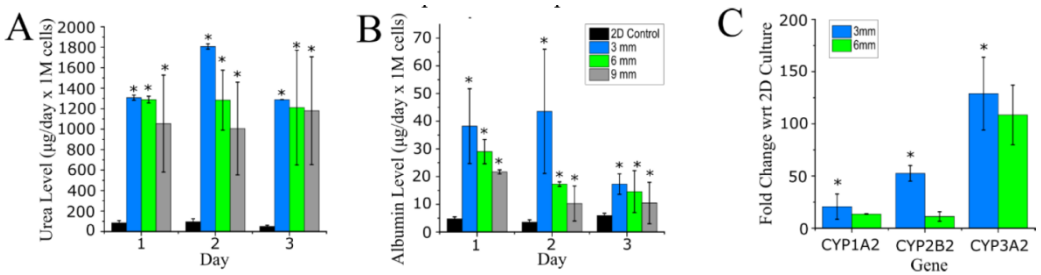



**Figure 2: (A)** Urea synthesis of primary rat hepatocytes in microfluidic cultures and in 2D static cultures. **(B)** Albumin synthesis of primary rat hepatocytes in microfluidic cultures and 2D static cultures. **(C)** Cytochrome P450 metabolic functions of primary rat hepatocytes under perfusion cultures. Fold change is measured with respect to 2D static cultures. Data are averages of 5 individual sets with S.E.M.

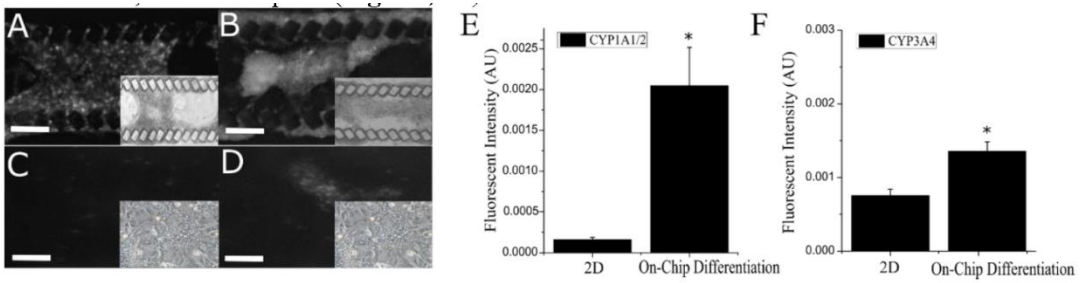



**Figure 3:** CYP1A2 activities of HepaRG-hepatocytes after 14 days differentiation in **(A)** 3D microfluidic device and **(B)** 2D static culture as indicated by ethoxyresorufin-0-deethylase (EROD) fluorescent enzymatic assay. **(C-D)** CYP3A4 activities of HepaRG-hepatocytes after 14 days differentiation in **(C)** 3D microfluidic device and **(D)** 2D static culture, as indicated by Vivid® CYP3A4 Blue fluorescent enzymatic assay. Inserts are phase contrast images of respective cultures. **(E)** Quantification of the CYP1A2 activities. **(D)** Quantification of the CYP3A4 activities. Scale bar = 100 µm. Data are averages of 5 individual sets with S.E.M.

*2016 A PUMP-FREE 3D MICROFLUIDIC PLATFORM FOR LONG TERM DIFFERENTIATION OF HUMAN LIVER PROGENITOR CELLS. MicroTAS conference*

µTas 2018
Kaohsiung, Taiwan • 11-15 November 2018
The 22nd International Conference on Miniaturized Systems for Chemistry and Life Sciences

*https://cbmsociety.org/conferences/microtas2018/*

27-31 October 2019
Basel+Switzerland
µTAS 2019
The 23rd International Conference on Miniaturized Systems for Chemistry and Life Sciences

*https://microtas2019.org/*

4-9 October 2020
Online
µTAS 2020
THE 24TH INTERNATIONAL CONFERENCE ON MINIATURIZED SYSTEMS FOR CHEMISTRY AND LIFE SCIENCES

*https://microtas2020.org/*

# Harvard-MIT liver chip narrows gap between *in vitro* and *in vivo* drug tests

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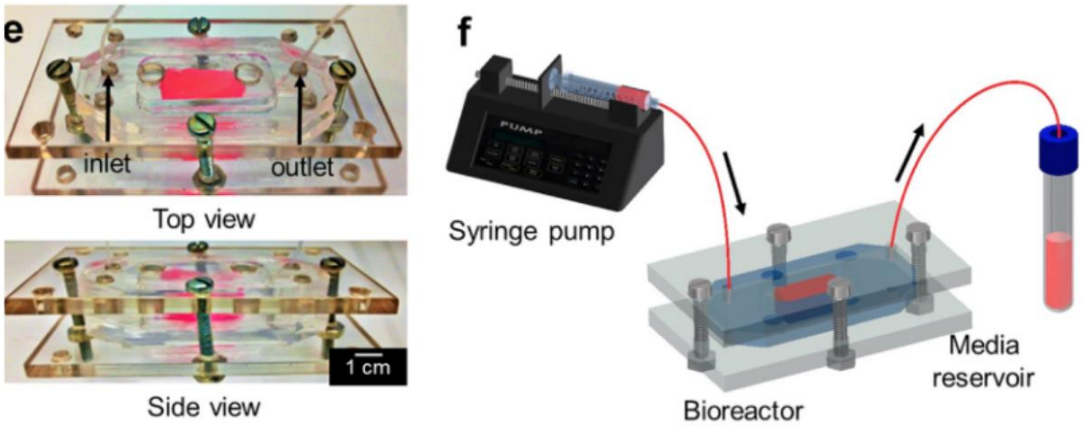


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## Harvard-MIT liver chip narrows gap between in vitro and in vivo drug tests

August 15, 2017 / 0 Comments / in Non-Animal Alternatives News /

A team of scientists from seven institutes across the globe combine the most recent liver tissue research to create am advanced 3D liver tissue model that can be used to test the effects of drugs on humans. The integration of a bioprinted vascularized construct with a bioreactor into the original organ-on-a-chip design help to create a more realistic liver model which better replicates the functions and environments of human livers. With these new advancements scientists further narrow the gap between in vitro and in vivo drug tests bringing science closer to eliminating the use of animal models for drug tests.

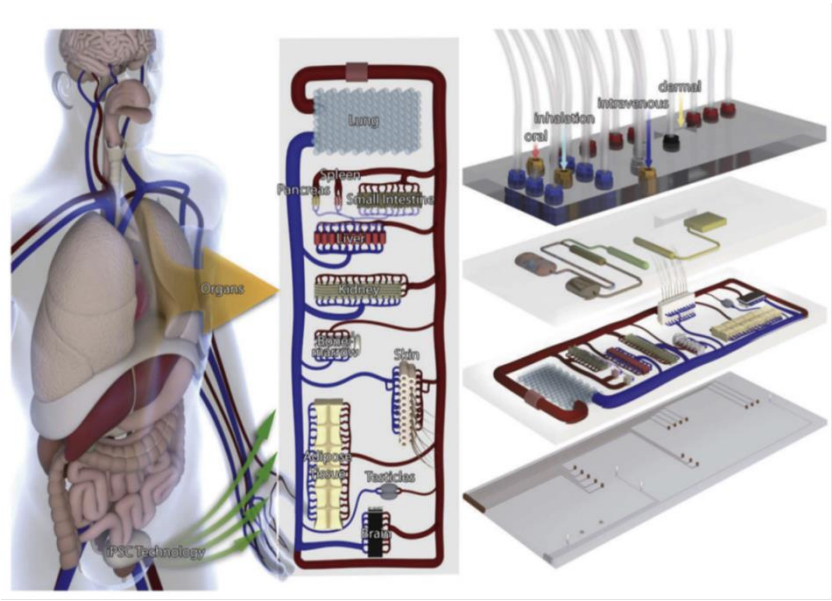
View Full Story

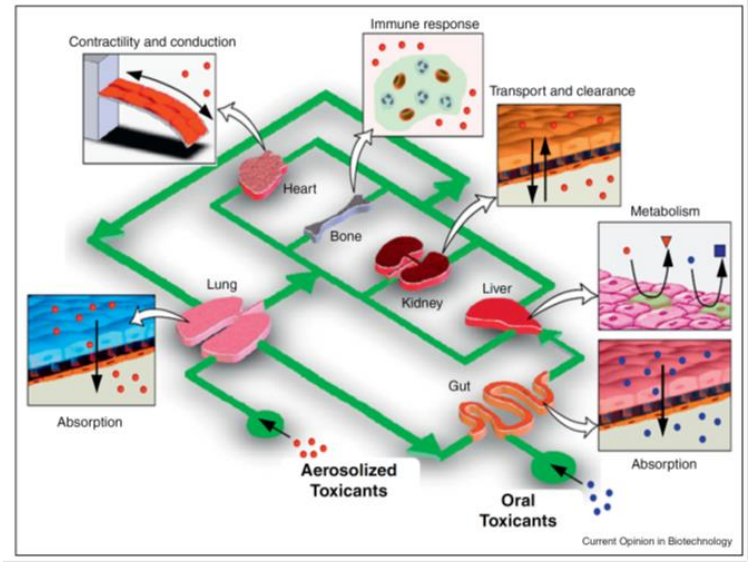
The integration of a bioprinted vascularized construct with a bioreactor into the original organ-on-a-chip design help to create a more realistic liver model

*2017 0815 https://www.animalsinscience.org/non-animal-alternatives/harvard-mit-liver-chip-narrows-gap-vitro-vivo-drug-tests/*

# Future work of Organ on a chip






*2017 Organ-on-a-chip for assessing environmental toxicants*

- This proposed human-on-a-chip system consists of 11 organ equivalents connected by one close loop circulation system. This device has multiple routes of administration and sensing as well as mechanical actuators to mimic organ motions such as heartbeat, intestine movements, and lung breathing motions.
- Nevertheless, these future goals remain dependent on the further development of individual organ-on-a-chip systems. Recreating fundamental organ functions, such as transportation in blood vessels and gas exchange in the lung, should be a major point of emphasis going forward. Progress in this field would not only serve as a basis for more effective integrative studies but also advance the cardinal goal of organ-on-a-chip studies—the high-fidelity recapitulation *in vivo* phenomena within a controllable, miniaturized *in vitro* platform.

**FIGURE 20.10**

Prospective design of a "human-on-a-chip". *Figure reproduced from Materne et al. (2013).*