Introduction to Lab-On-a-Chip

2018 1123 Liver on a chip (I) 肝臟與肝臟晶片技術簡介

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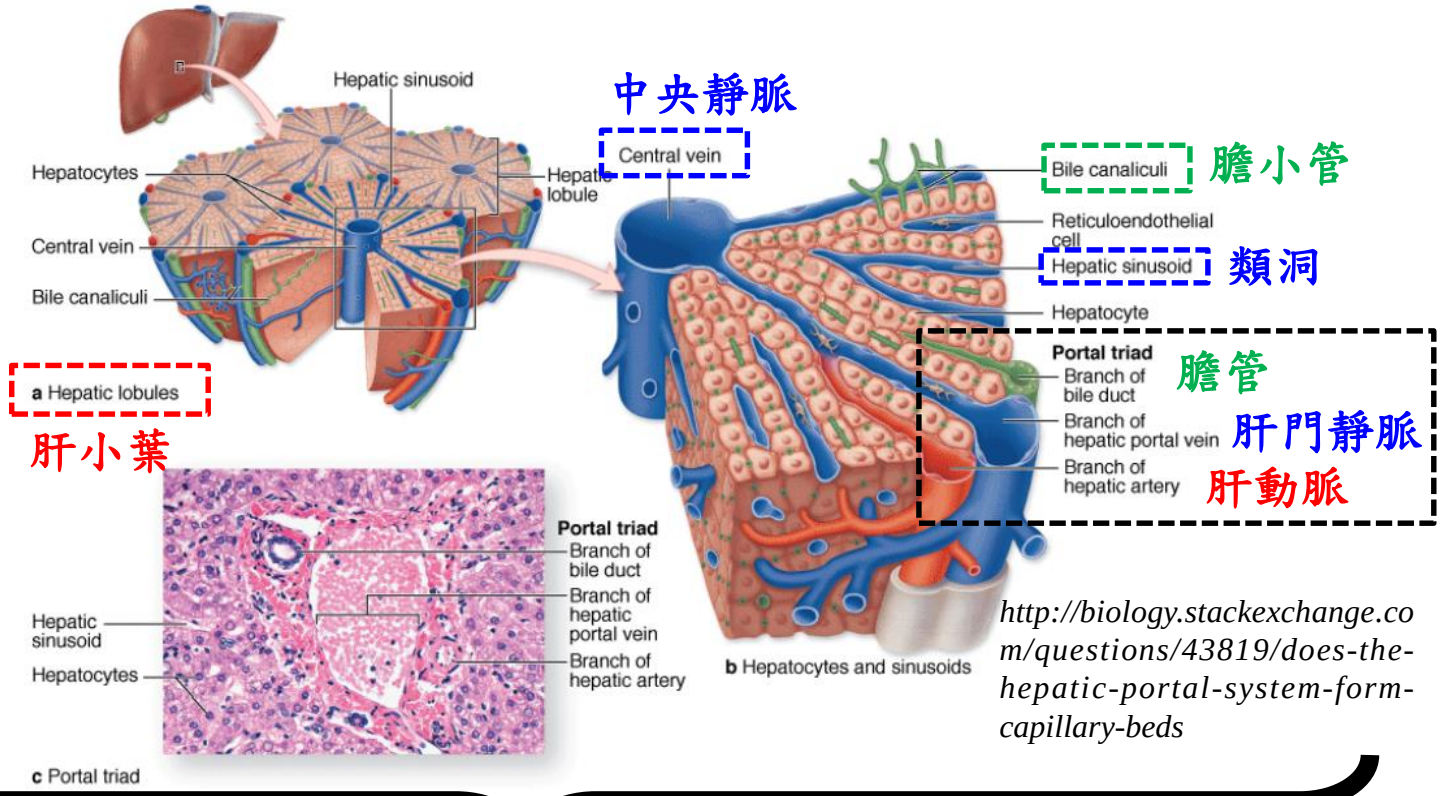
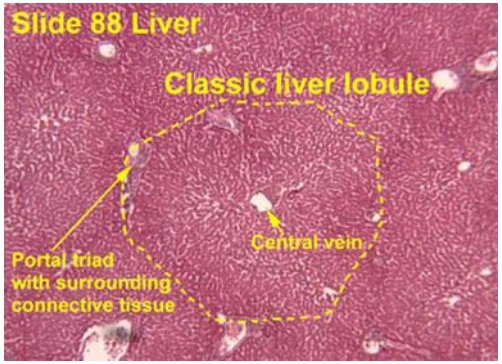
Liver structure

Hepatocyte optimal microenvironment

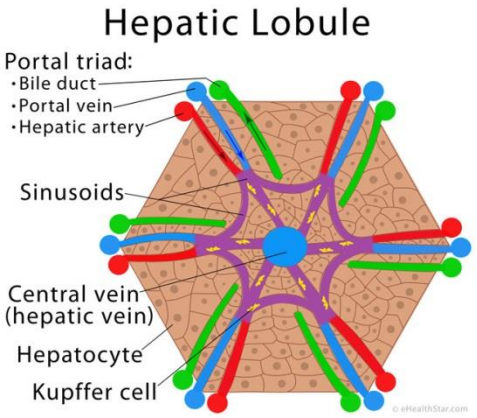
Hepatocytes interact with diverse extracellular matrix molecules, non-parenchymal cells, and soluble factors (for ex: hormones, oxygen)



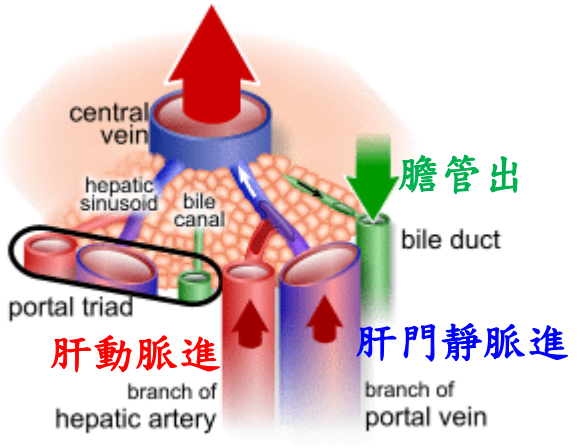
http://biology.stackexchange.com/questions/43819/does-the-hepatic-portal-system-form-capillary-beds



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

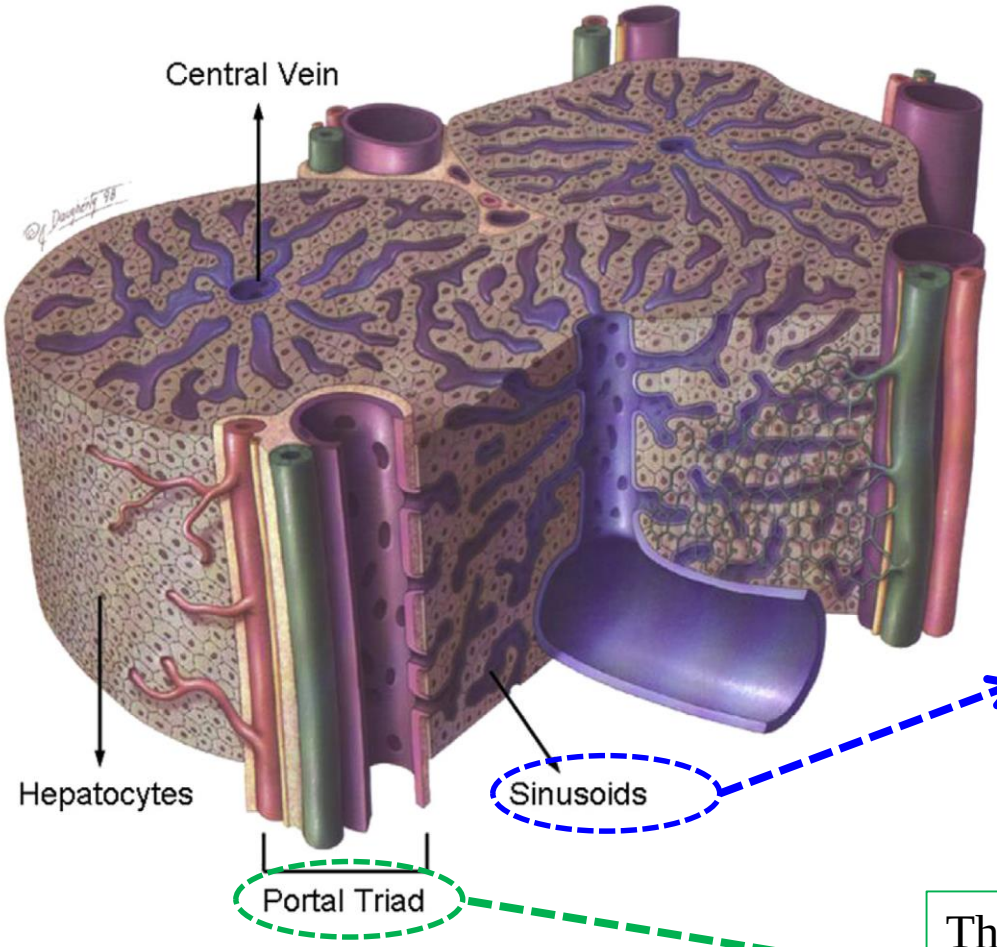


http://www.ehealthstar.com/anatomy/liver

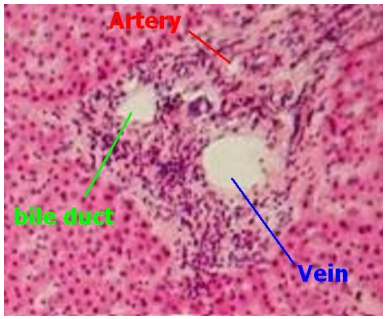


www.blobs.org

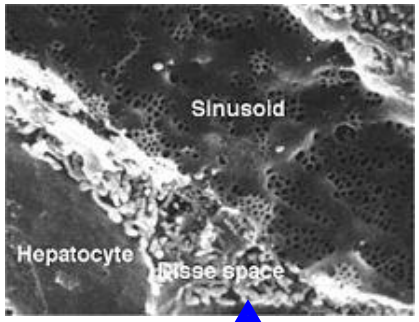
Liver structure (2)



Portal triad



Disse space



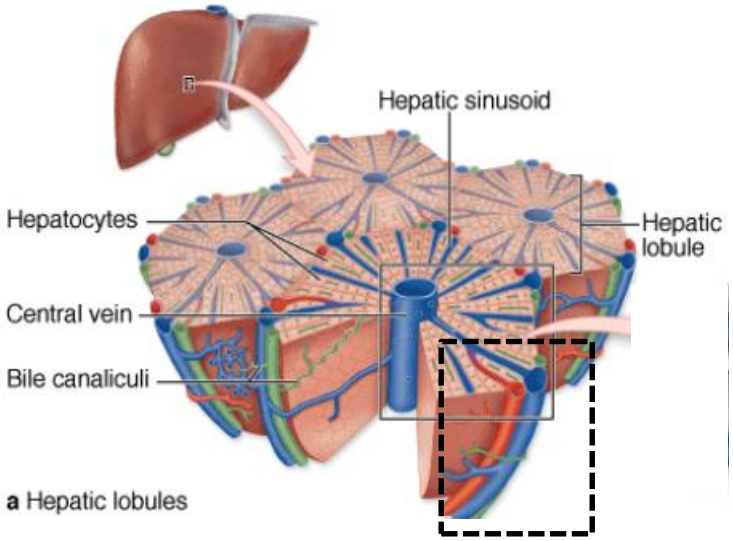
en.wikipedia.org

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

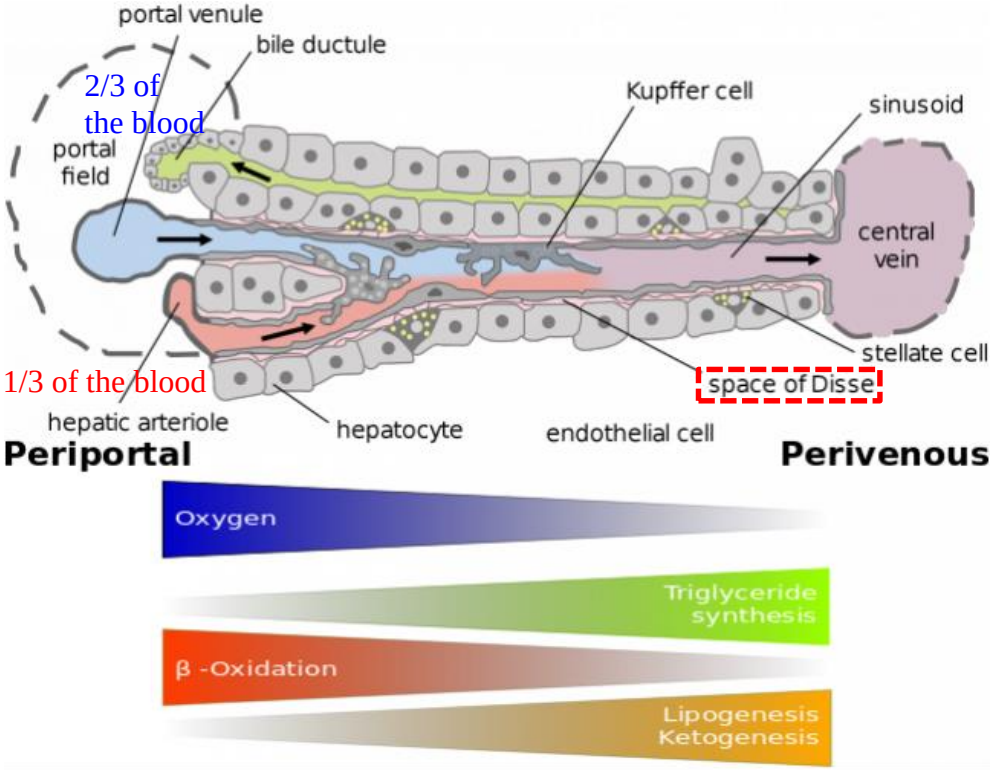
The two lobes of the liver contain repeating functional units called lobules (肝小葉), which are centered on a draining central vein (中央靜脈). Portal triads at each corner of a lobule contain portal venules (肝門小靜脈), arterioles (動脈) and bile ductules (膽管)

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

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)

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

Liver zonation

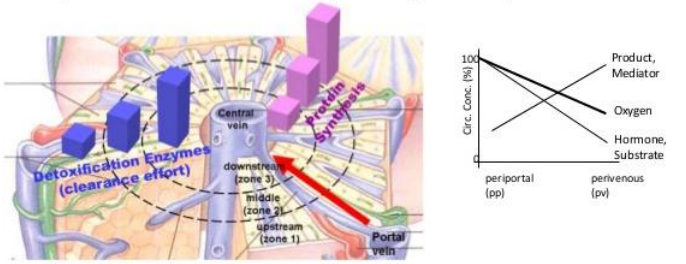
Within the liver lobule, hepatocytes are partitioned into three zones based on morphological and functional variations along the length of the sinusoid (zonation). Zonal differences have been observed in virtually all hepatocyte functions.

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.

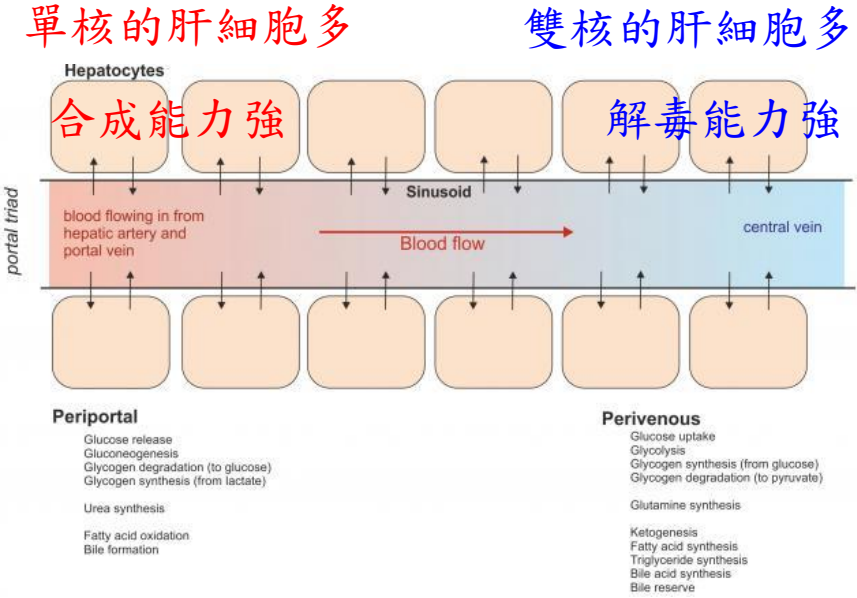
Liver Zonation

- 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

http://flipper.diff.org/app/items/3802

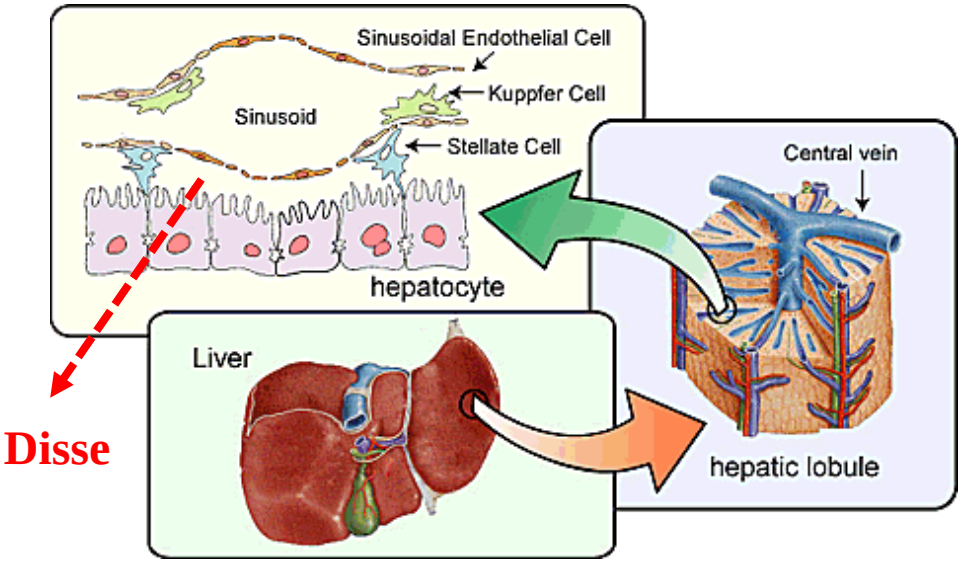


http://portal-vl.h-its.org/portal-view-v2/liver-function/liver-basics/health/lobule/en

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.

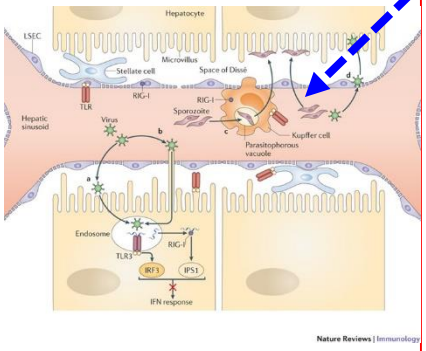
Later the so-called oval cells were added to the list [7, 8]. Liver hosts a population of stem/progenitor cells, which in rodents includes oval cells [9]. Cell fate experiments suggested that stellate cells can also be the precursors of liver epithelial cells [10, 11].

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 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

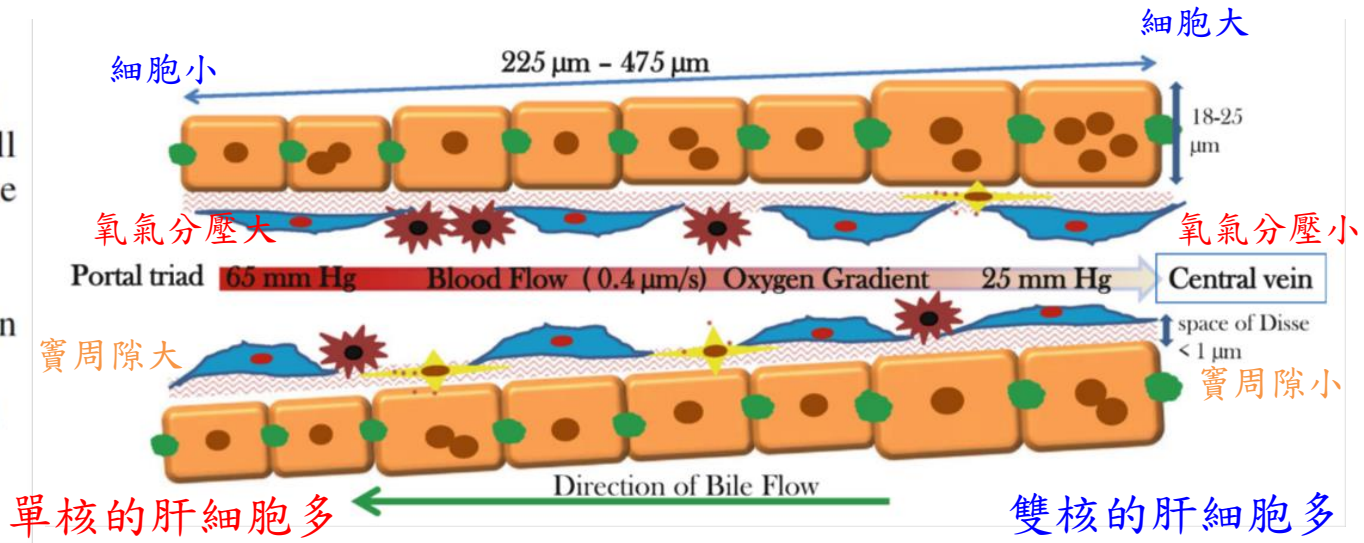
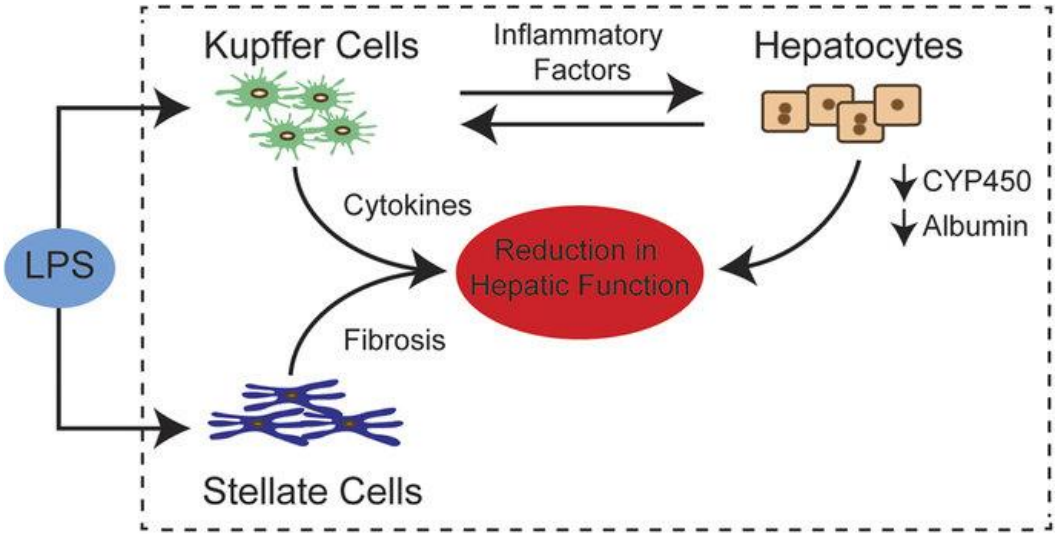


Fig. 1 Organization of a liver sinusoid depicting distribution and arrangement of various cell types. The blood flows from the portal triad toward the central vein, creating an oxygen gradient that leads to zonation in liver. The size of hepatocytes, sinusoidal lumen diameter and fenestrae changes from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

2014 Biomaterials for liver tissue engineering

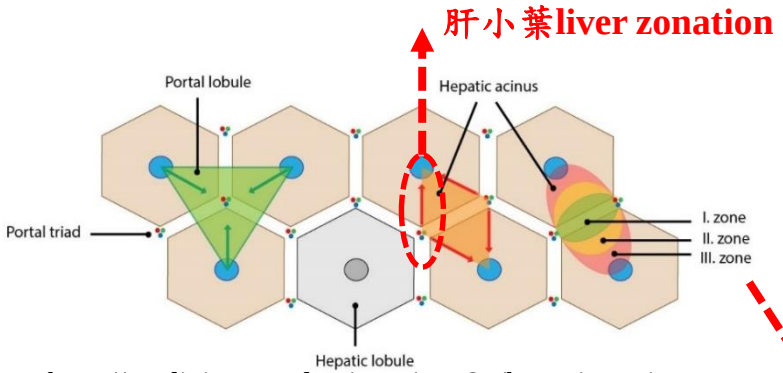


To evaluate the inflammatory response, we exposed the co-cultures to an inflammatory stimulus (LPS) and measured their response.

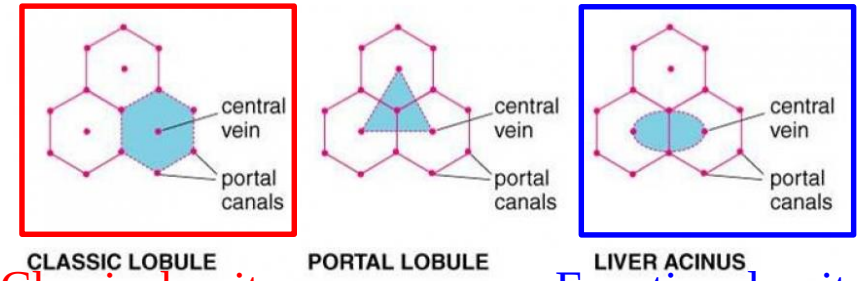
Figure 5. Interactions between hepatocytes and non-parenchymal cells in co-culture. External Stimuli and cellular secretions from non-parenchymal cells influence the behavior and overall outcomes. Capturing cellular interactions and evaluating the cumulative responses are essential to create *in vitro*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



http://medicine.academic.ru/99185/hepatic_acinus



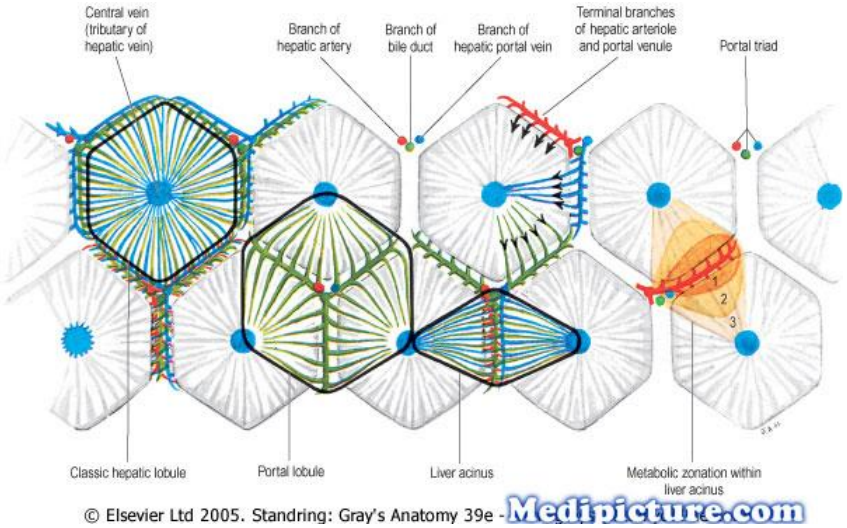
Classical unit

Functional unit

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

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



http://www.blogarama.com/medicine-remedies-blogs/303509-medicine-picture-fcps-mrcp-preparation-medici-blog/5112992-liver-microstructure-best-figure-from-grays-anatomy

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).

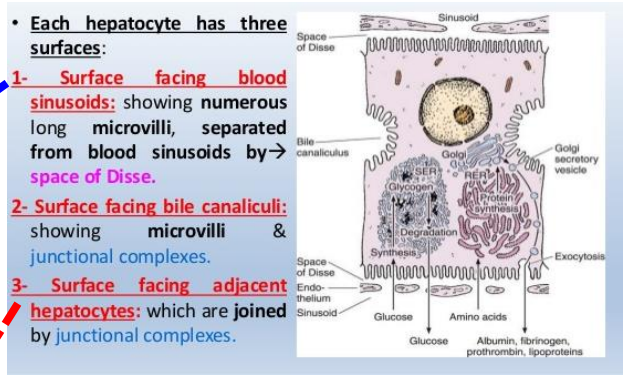
300×10^{10} cells

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 m^2

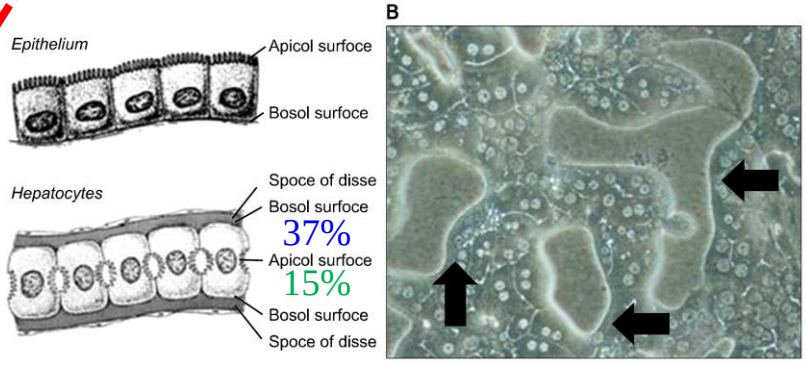
171×10^7 cells

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

Hepatic functions (1)

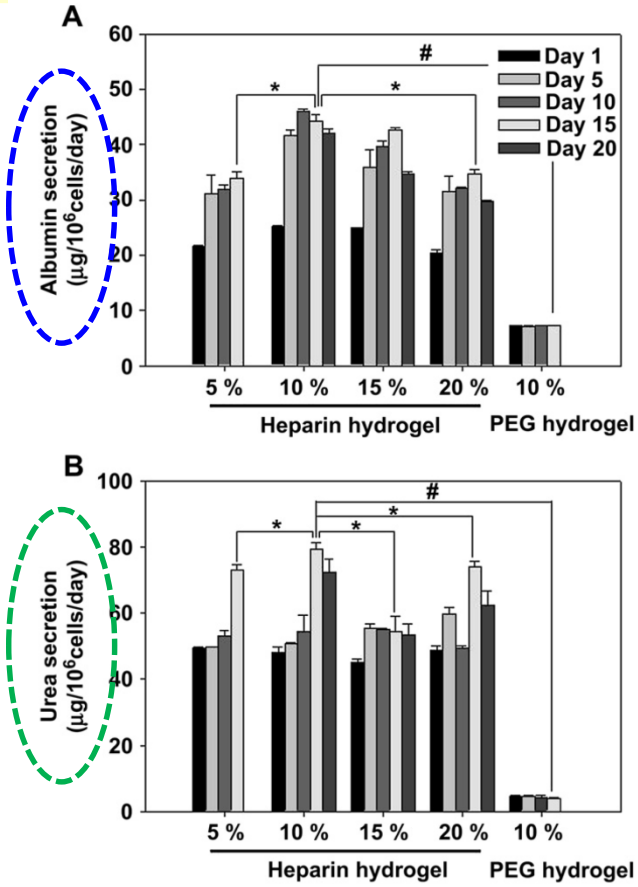
TABLE 46.2 Hepatic functions

Functional classification	Examples
Synthetic	Albumin Secretion Alpha-1-antitrypsin Secretion Coagulation Factor Production (II, IX, X) Lipoprotein and apoprotein synthesis Ceruloplasmin production Ferritin production Complement production
Metabolic	Ureagenesis and metabolism Bilirubin Metabolism Steroid Metabolism Gluconeogenesis/Glycogen Production 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	

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)

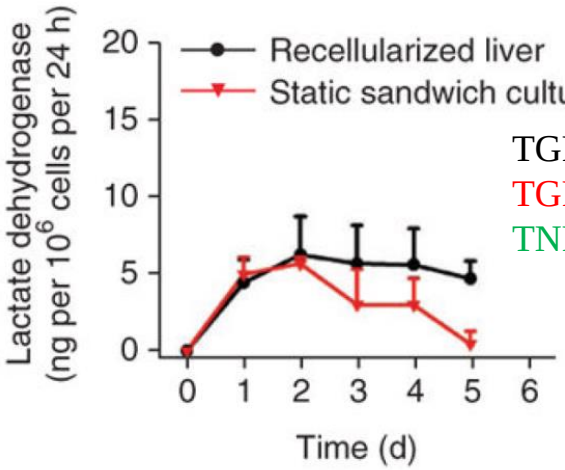
Other hepatic functions

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

TABLE 2. *Measured hepatocyte functions in cocultures*

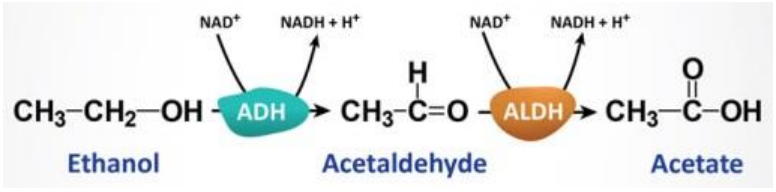
Albumin secretion
Cytochrome P-450 activity (isoenzymes 1A1, 2B1, 3A1) and inducibility
Glutathione S-transferase
Tight junctions (detection of ZO-1)
Gap junctions (detection of connexin 32, microinjection)
Other: pyruvate kinase, transferrin, DNA synthesis, UDP-glucoronyl transferase

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

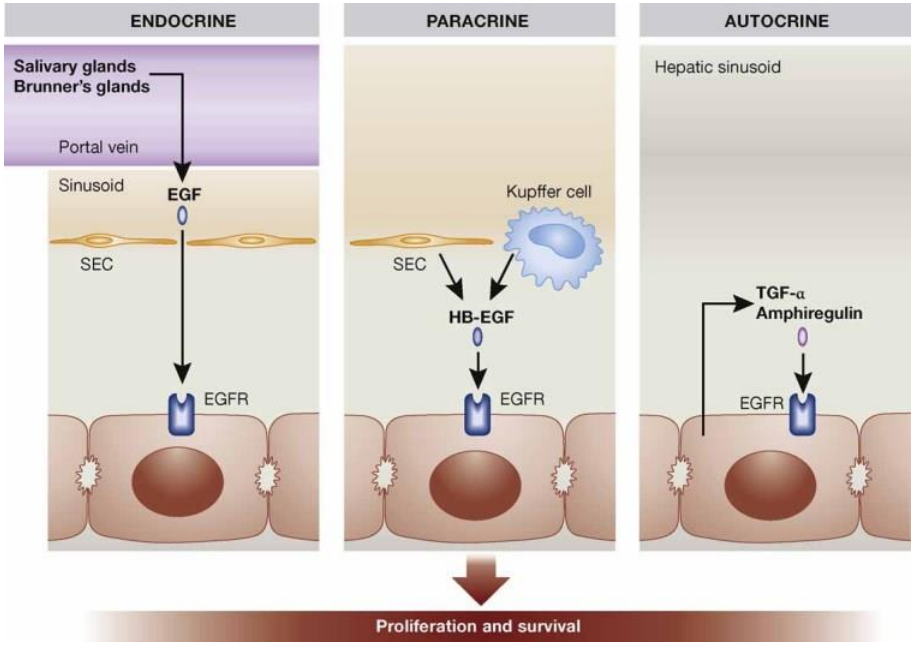


TGF-α→ 幫助DNA合成與細胞增殖
TGF-β-->抑制肝細胞生長
TNF-α→ 腫瘤壞死因子

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



http://themedicalbiochemistrypage.org/ethanol-metabolism.php



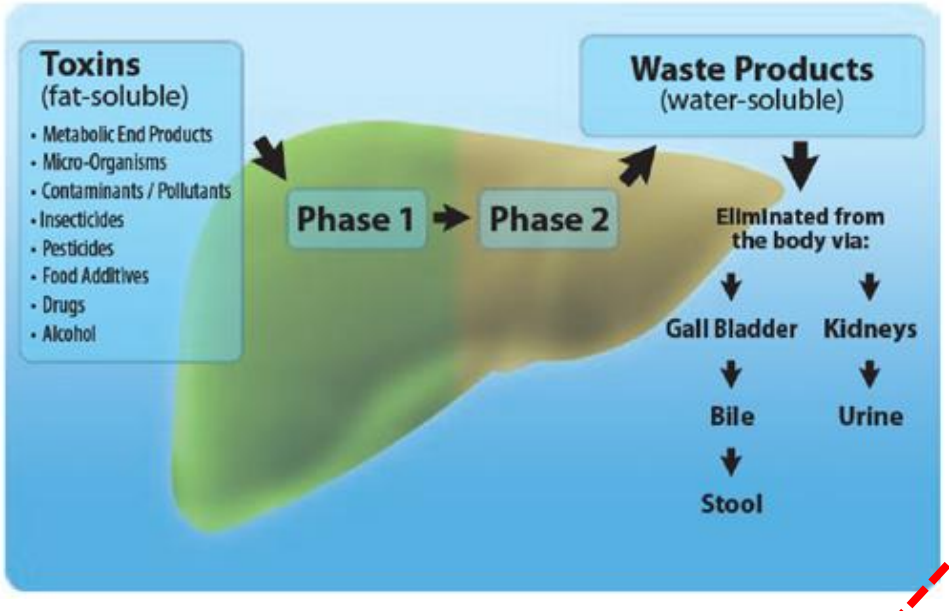
Hepatic functions (3)

Drug metabolism in liver

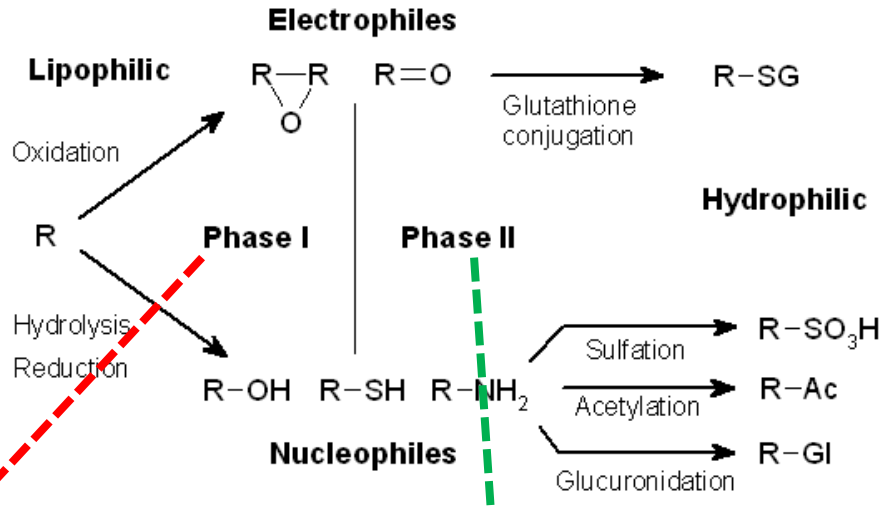
P450 reaction (負責有機受質的氧化作用):

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

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 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

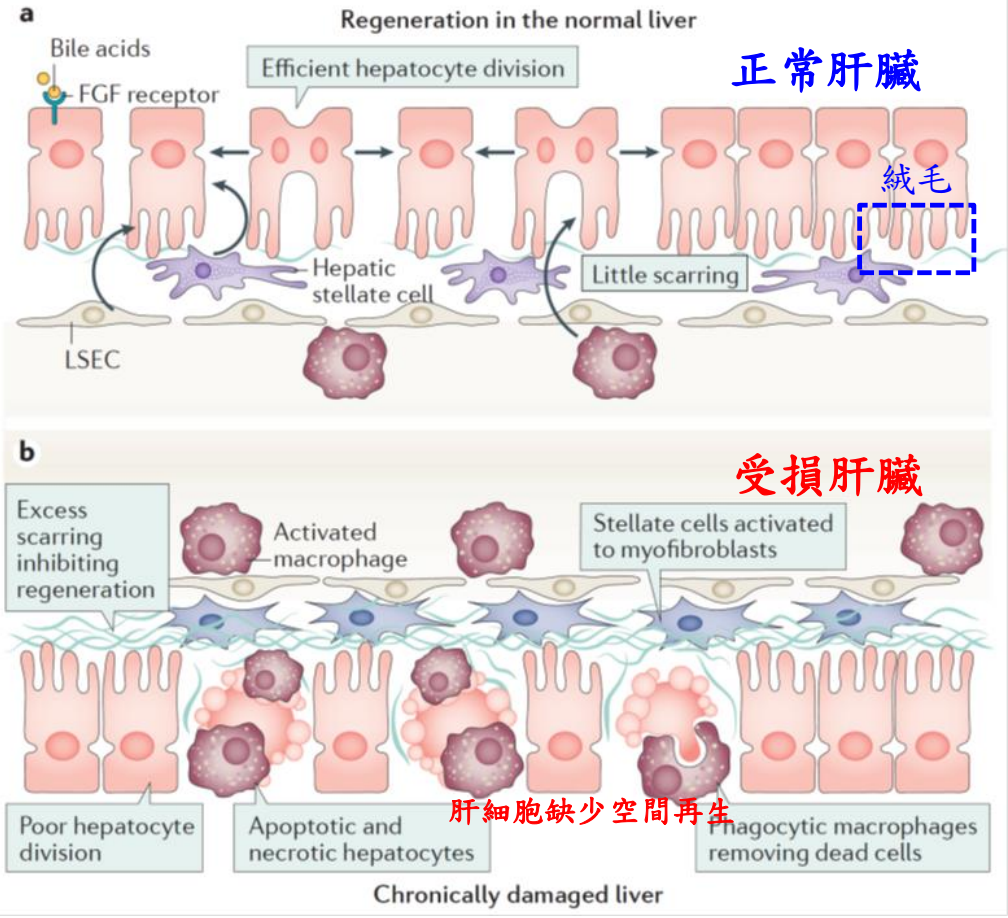


Figure 2 | **Schematic of normal and abnormal liver regeneration. a** | Regeneration in the normal liver follows partial hepatectomy or moderate liver injury. In this setting, bile acids are rapidly upregulated, and serum factors are able to rapidly induce regeneration in the liver. Non-parenchymal cells; 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.

- 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

2016 Liver regeneration — mechanisms and models to clinical application

肝硬化原理

The pathogenesis of liver fibrosis 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. The progression and reversal (反轉) of liver fibrosis and the formation of myofibroblast are given subsequently (Figure 2).

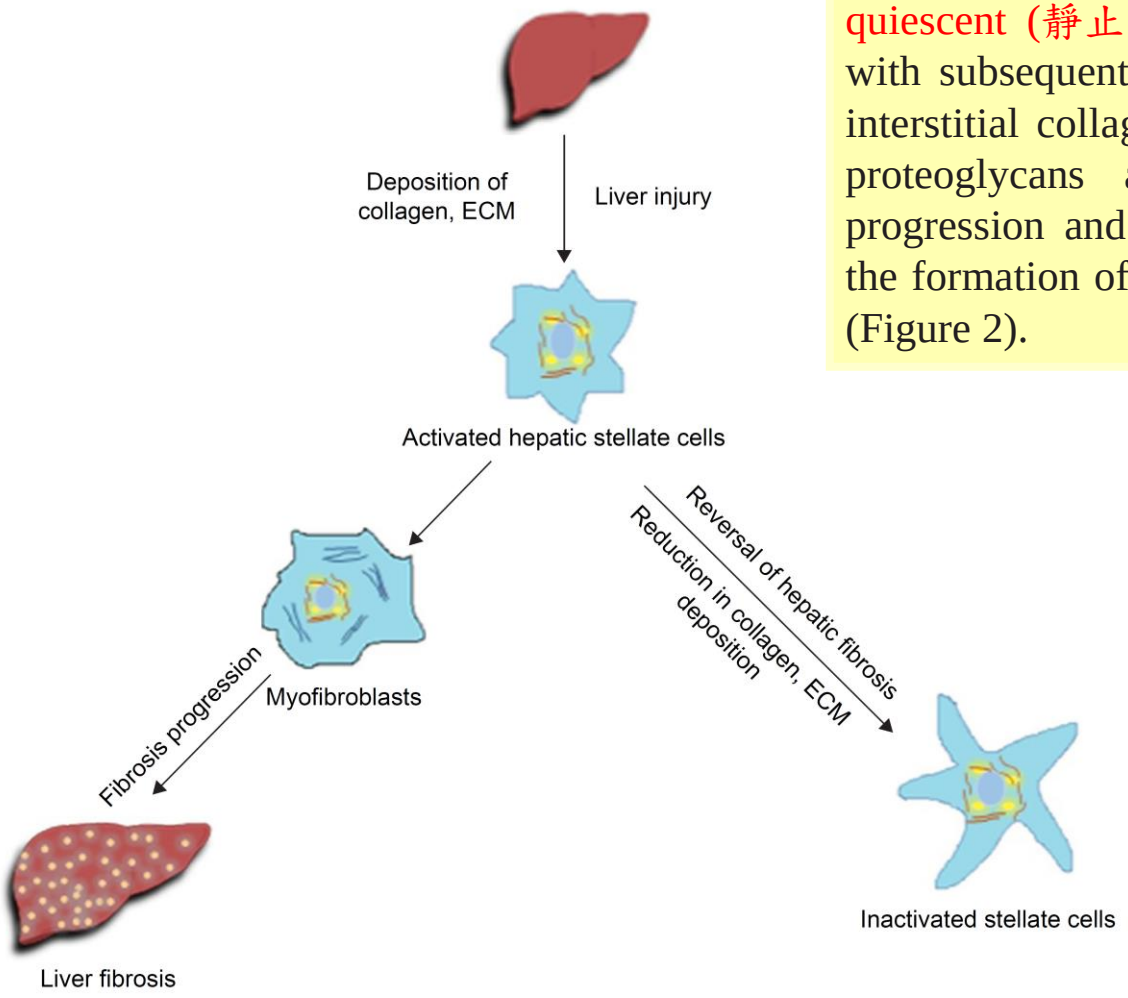
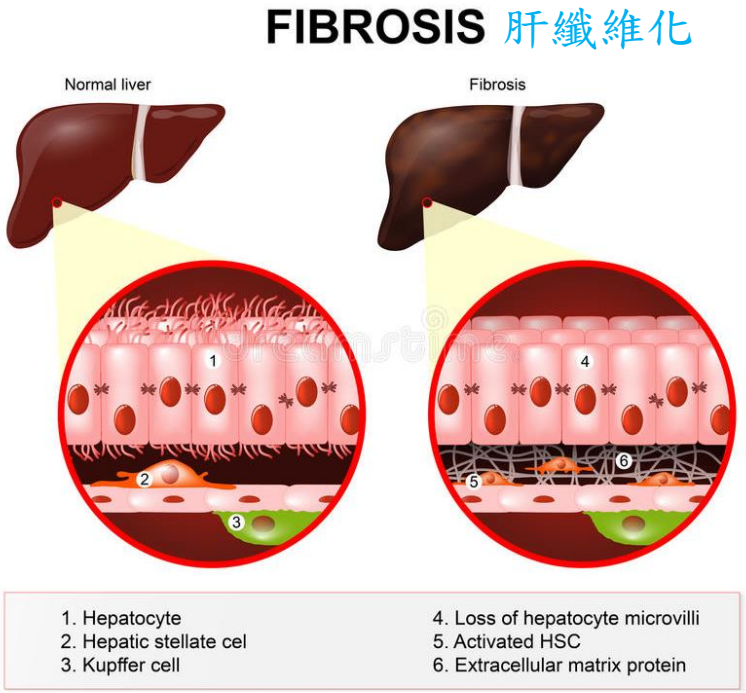


Figure 2 Formation of myofibroblasts and progression and reversal of hepatic fibrosis.
Abbreviation: ECM, extracellular cell matrix.

2017 Nanoparticles for the treatment of liver fibrosis

Liver fibrosis *V.S* Liver cirrhosis



https://www.dreamstime.com/stock-illustration-liver-fibrosis-cirrhosis-human-diseases-image83244245

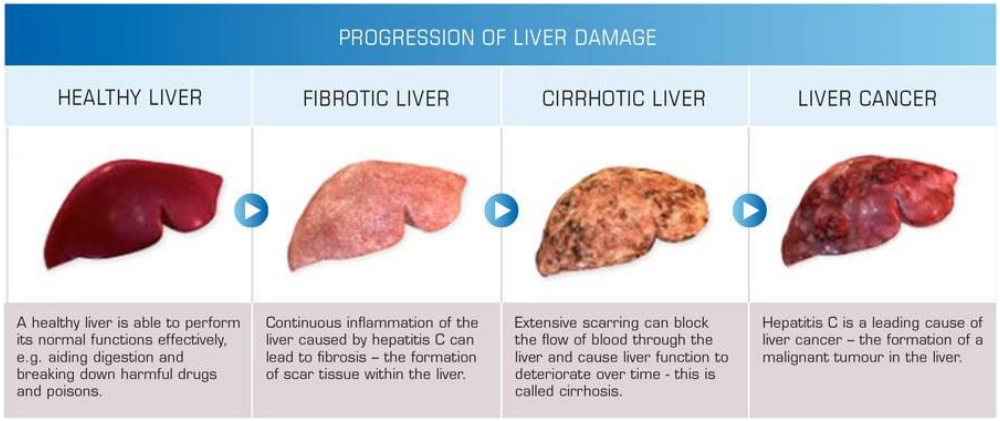
肝硬化

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://wasabi.org/liver-disease/

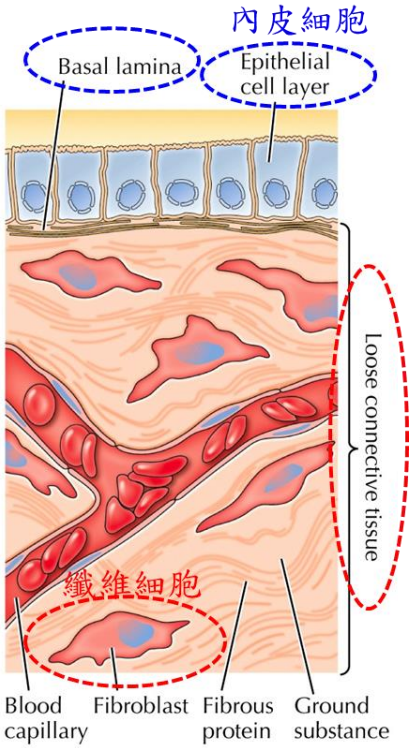
Proliferation of differentiated cells

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!!

Liver regeneration

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

舉例來說：

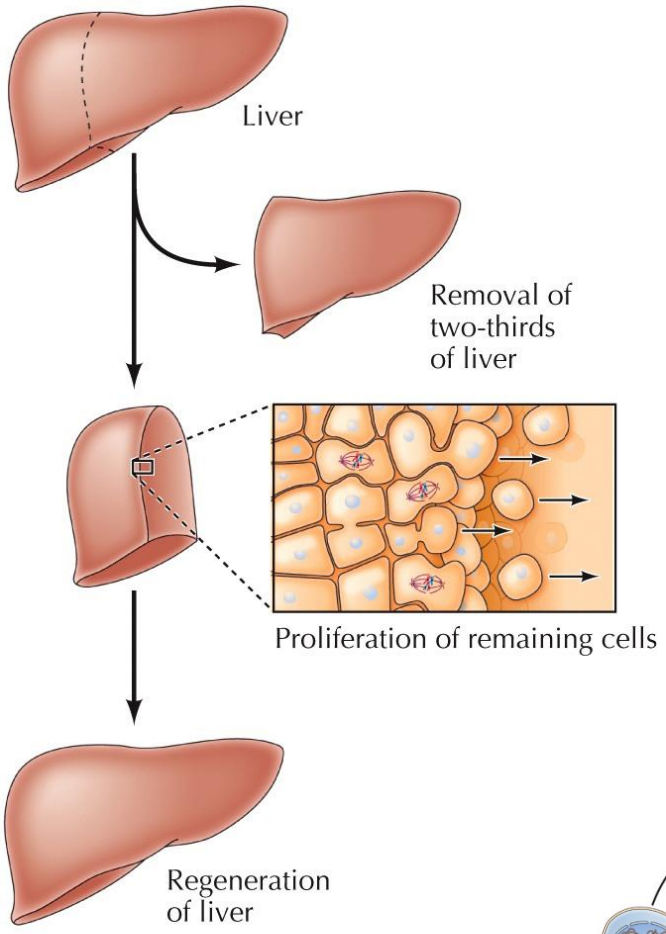
Liver

Liver cells

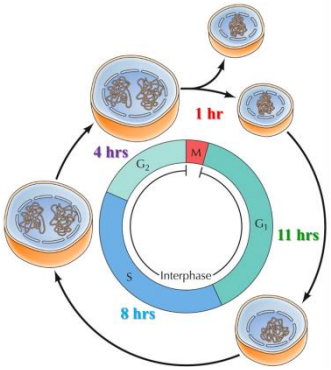
Liver cells are normally arrested in the G_0 phase of the cell cycle. However, if large numbers of liver cells are lost, the remaining cells are stimulated to proliferate to replace the missing tissue

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)

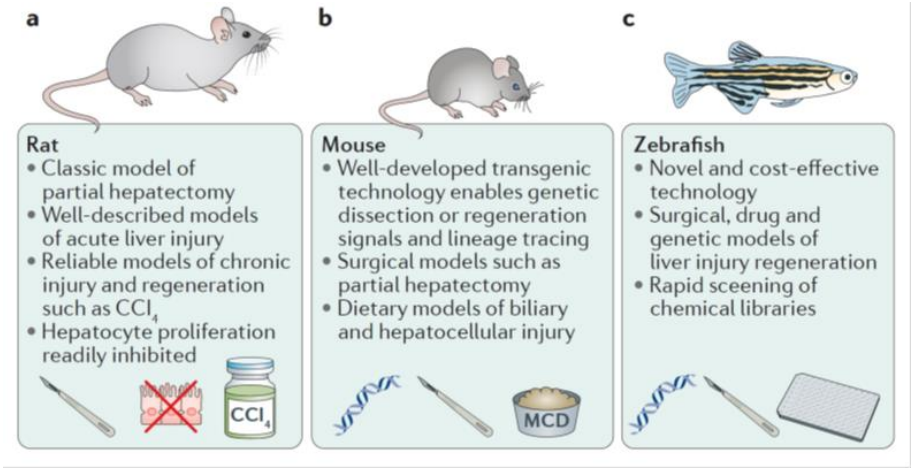


THE CELL: A MOLECULAR APPROACH 6e, Figure 17.15



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

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

肝再生原理

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

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

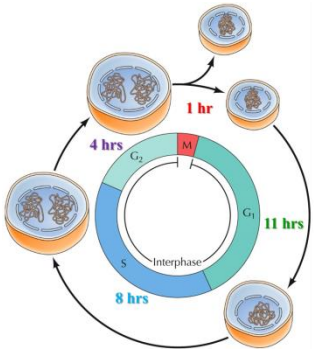
Liver targeting

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].

- 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

G_o
- Blue nuclear
- Blue cytoplasm

G0
No brown staining (nucleus or 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

Liver failure and current treatments

Liver failure can be generally separated into two major categories:

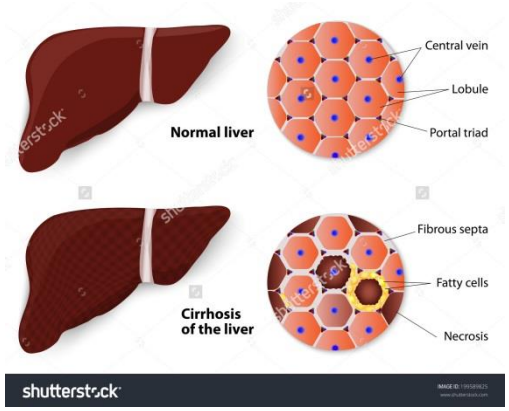
Fulminant hepatic failure

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://www.shutterstock.com/zh/image-illustration/cirrhosis-liver-normal-structure-diagram-199589825

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

Major causes of chronic liver diseases

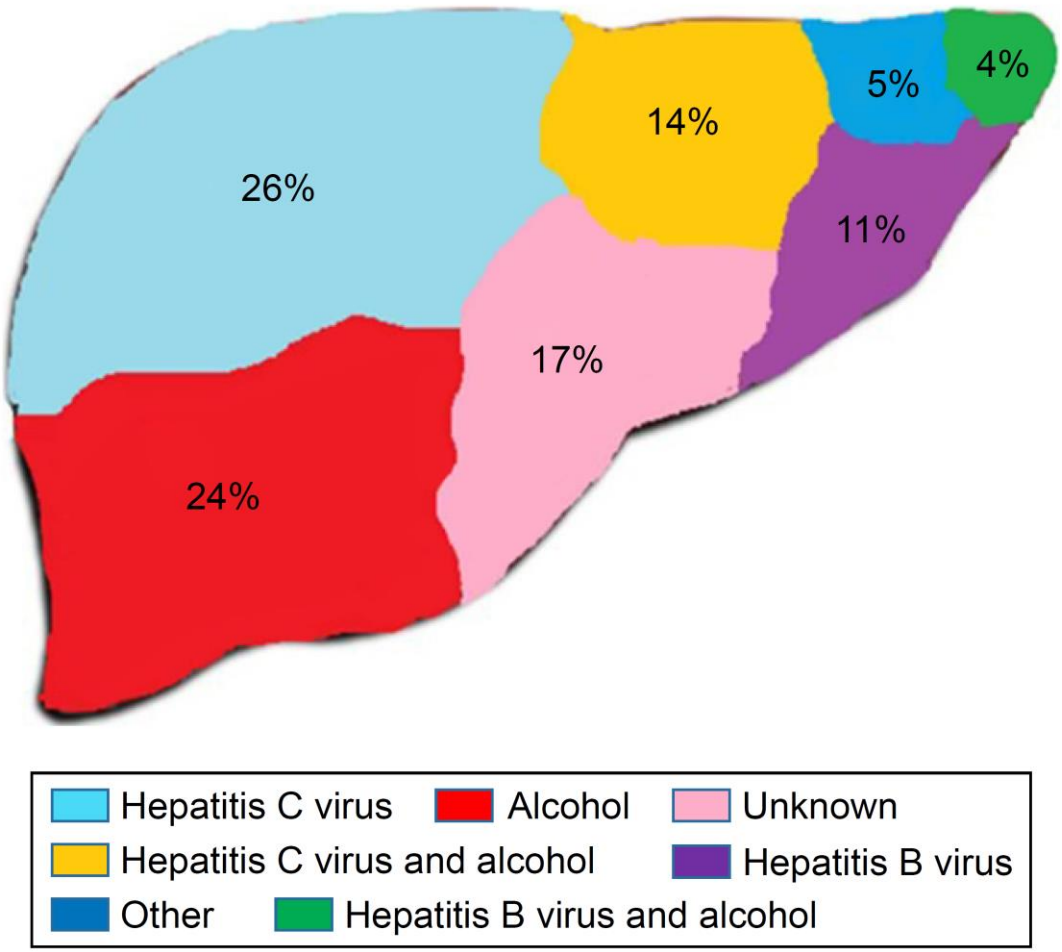
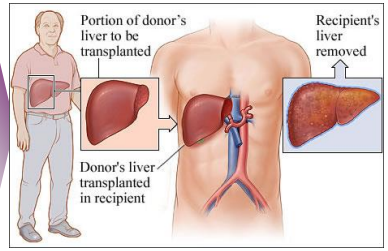


Figure 1 Major causes of chronic liver diseases

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

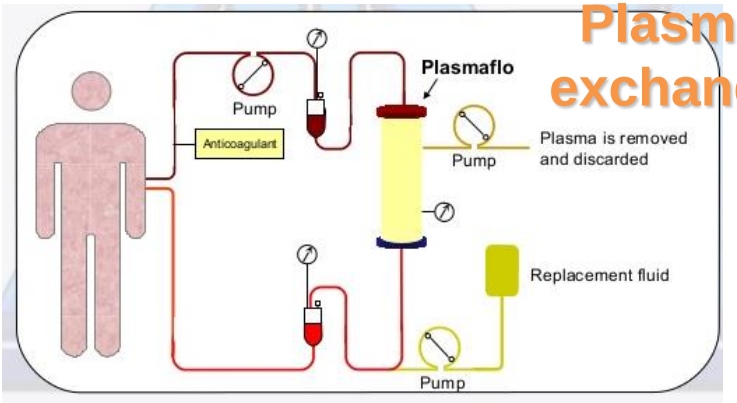
- Partial hepatectomy (PH) or chemical injury induces the proliferation of the existing mature cell populations.
- However, biliary and vascular complications are major concerns in these procedures

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



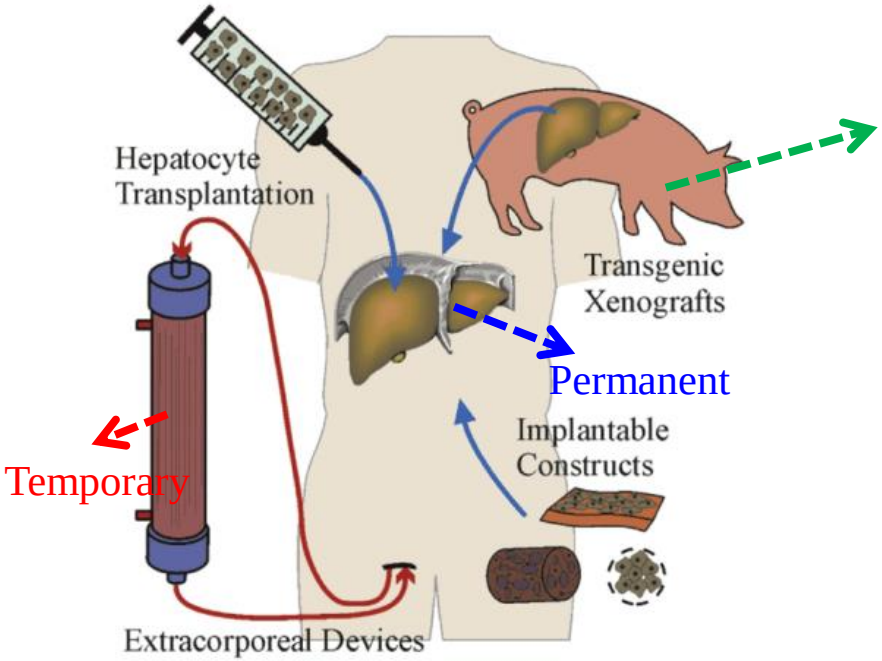
https://www.slideshare.net/WanMed1/plasma-therapy-training-presentation

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



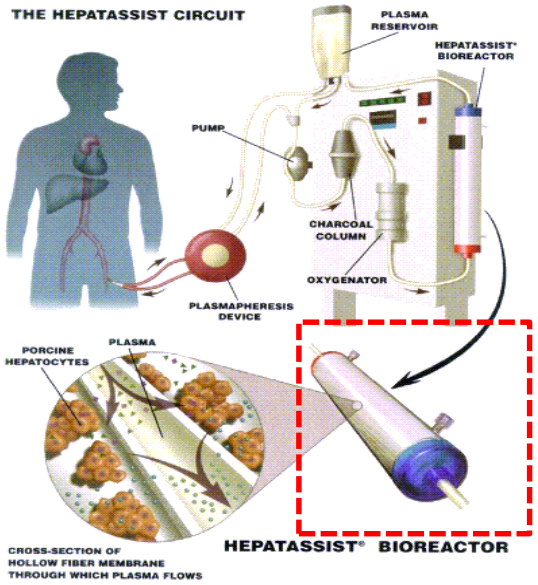
Primary porcine hepatocytes have been utilized in a range of BAL device configurations with some encouraging results. However, the utility of xenogeneic porcine cells for human liver therapies is restricted by immunogenicity and the potential for xenozoonotic transmission of infectious agents such as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



http://www.ufrgs.br/imunovet/molecular_immunology/exvivo.html

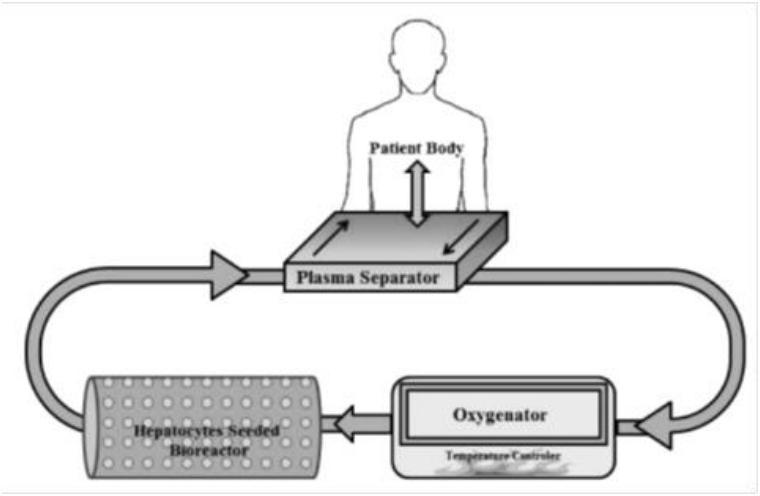


Figure 1. A proposed set up of bio-artificial liver (BAL) system. The system comprises the filtration of patient venous blood passes through plasma separator unit connected to oxygenator unit under a controlled temperature. Furthermore, the plasma is then passed through hepatocytes activated bioreactor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

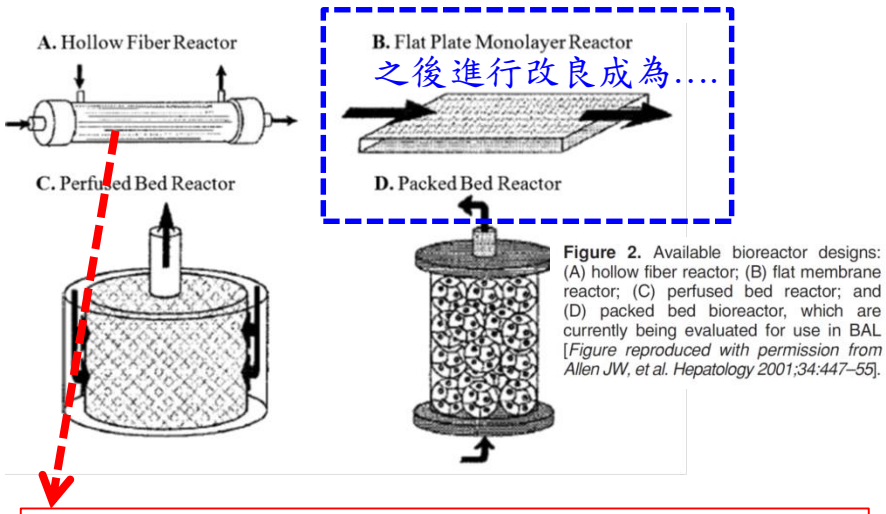


Figure 2. Available bioreactor designs: (A) hollow fiber reactor; (B) flat membrane reactor; (C) perfused bed reactor; and (D) packed bed bioreactor, which are currently being evaluated for use in BAL [*Figure reproduced with permission from Allen JW, et al. Hepatology 2001;34:447–55*].

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 Rat Humans	<1 month	*Advantages:* 1. Uniform cell distribution 2. Ease of scale up 3. In vivo like microenvironment *Disadvantages:* 1. Large dead volume 2. Low surface area/ volume ratio 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 Pig	<25 days	*Advantages:* 1. large surface area/ volume ratio 2. Efficient exchange of nutrients and waste material 3. Porous system for cell support 4. High product recovery 5. Immunoisolation 6. Ease of scale up 7. In vivo like microenvironment *Disadvantages:* 1. Expensive 2. Nonuniform cell distribution 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 Pig	<16 days	*Advantages:* 1. High surface area/volume 2. In vivo-like microenvironment 3. Ease of scale up 4. Immunoisolation 5. High capacity for cell mass *Disadvantages:* 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 Pig	<21 days	*Advantages:* 1. Cell-to-cell contact 2. Ease of scale up *Disadvantages:* 1. Limited oxygen and nutrient supply 2. Chances of viral infiltration	Preclinical trials on PUF-HALSS

使用時間都很短!

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.

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 Human adult and fetal, xenogeneic	Sourcing, expansion, phenotypic instability, immunogenicity, safety (xenozoonotic)
Immortalized hepatocyte lines Tumor-derived, SV40, telomerase, spontaneously immortalized	Range of functions, genomic instability, safety (tumorigenicity)
Stem Cells 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 Alpha-1-antitrypsin Secretion Coagulation Factor Production (II, IX, X) Lipoprotein and apoprotein synthesis Ceruloplasmin production Ferritin production Complement production
Metabolic	Ureagenesis and metabolism Bilirubin Metabolism Steroid Metabolism Gluconeogenesis/Glycogen Production 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. Implantable devices. Cell transplantation.	High host compatibility.	Reduced availability. Poor *in vitro* proliferation.
Porcine hepatocytes	Porcine liver	Bio-artificial liver. Implantable devices.	Enhanced availability compared with human hepatocytes.	Potential zoonotic disease transmission. Protein–protein incompatibility. Possible immune response.
Tumour derived human hepatocyte cell lines	Cell lines derived from human hepatic carcinoma.	Bio-artificial liver. Implantable devices.	Easy storage, maintenance and *in vitro* proliferation.	Tumorigenicity. Reduced functional performance.
Immortalized human hepatocyte cell lines	Human hepatocytes immortalized by gene transfection.	Bio-artificial liver. Implantable devices.	Easy *in vitro* proliferation, storage and maintenance. Reduced tumorigenicity compared with tumour derived cell lines.	Potential tumorigenicity. Long-term safety concerns. Reduced functional performance.
Adult stem cells (MSCs)	Adult tissues	Cell transplantation. Implantable devices.	Unlimited availability. Less safety concerns.	Transdifferentiation of MSCs to myofibroblasts. Lower efficiency of the available hepatic differentiation protocols. Longer duration of hepatic differentiation.
Oval cells	Human fetal liver	Cell transplantation.	Native liver stem cells. Can differentiate to both hepatocyte and biliary cells.	Cell isolation is difficult. Limited availability. Tumorigenic potential.
Human fetal hepatocytes	Human fetal liver	Bio-artificial liver. Implantable devices. Cell transplantation.	Easy cell isolation. Can undergo few cell divisions *in vitro*.	Lower functional efficiency. Reduced availability. Ethical concerns. Possible tumorigenicity.
Hepatoblast	Human fetal liver at early stage of gestation	Cell transplantation.	Extensive *in vitro* proliferation.	Ethical issues. 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. Implantable devices.	Unlimited availability and indefinite proliferation. Hepatic differentiation capability.	Ethical issues. 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

Advantage of HepaRG

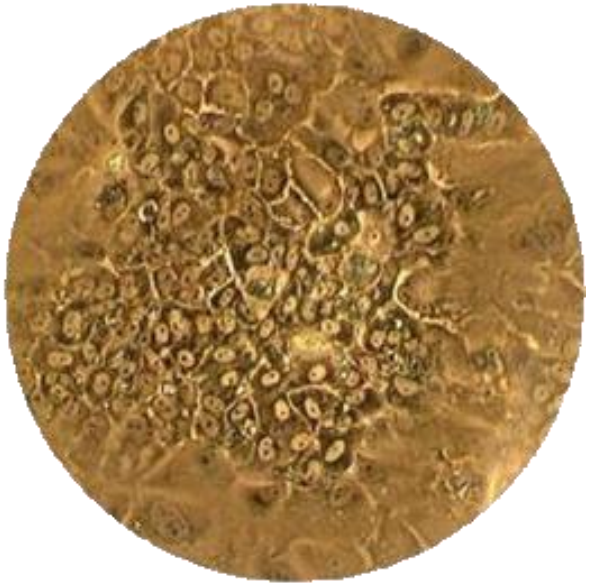
- **HepaRG** is the most innovative and useful hepatic cell line
- A unique and well established hepatic cell system able to produce early hepatic progenitor cells as well as completely mature human hepatocytes.

http://www.heparg.com/

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



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

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*

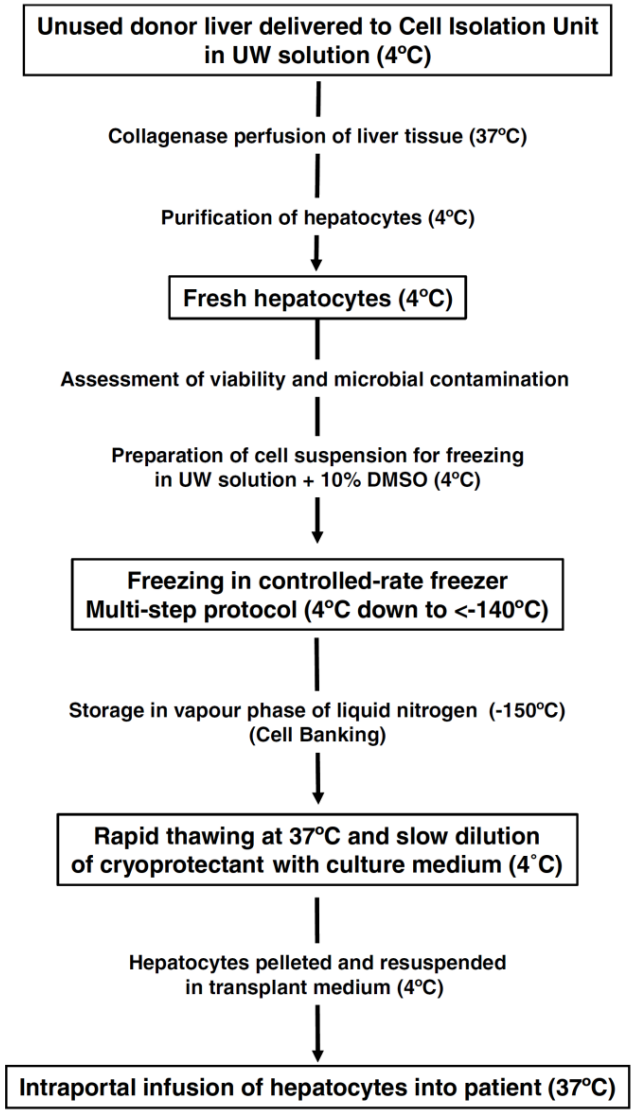
1. Loss of liver-specific functions
2. Significant proliferative capacity during regenerative responses is limited

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 multipotency or pluripotency and thereby serve as a possible source of hepatocytes, as well as other non-parenchymal liver cells.

Cryopreservation



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

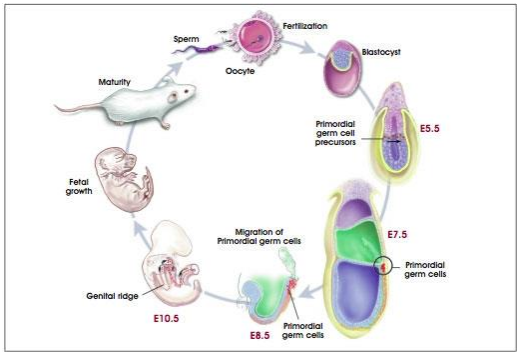
- A variety of techniques have been developed to enable the cryopreservation of human hepatocytes.
- This enables the large number of hepatocytes that are prepared from a single liver to be stored and thawed with reproducible cellular function. This option has opened the door to a variety of in vitro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

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

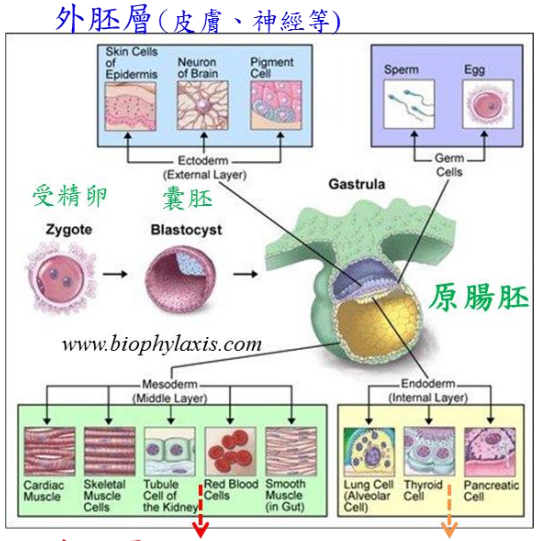
Fetal hepatoblasts (肝母細胞) are liver precursor cells present during development that exhibit a bipotential differentiation capacity, defined by the capability to generate both hepatocytes and bile duct epithelial cells. Sourcing problems associated with fetal cells have led researchers to search for resident 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

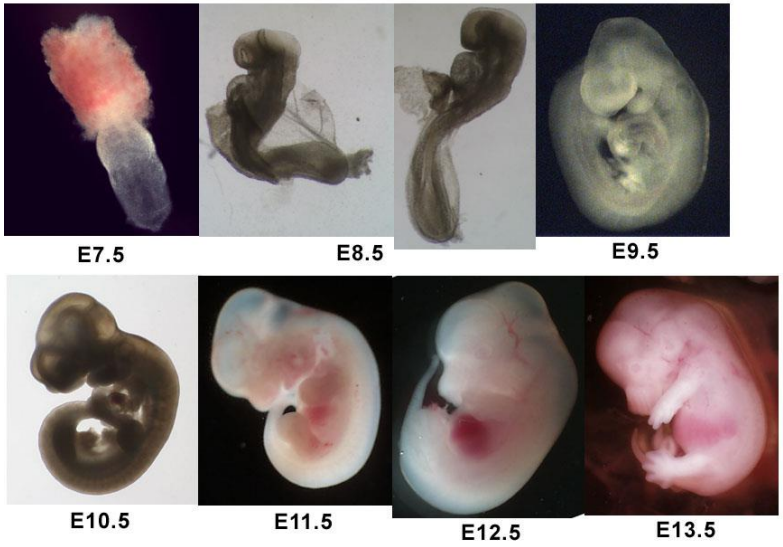
Mouse embyro



stemcells.nih.gov



www.biophylaxis.com



www.swarthmore.edu

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**

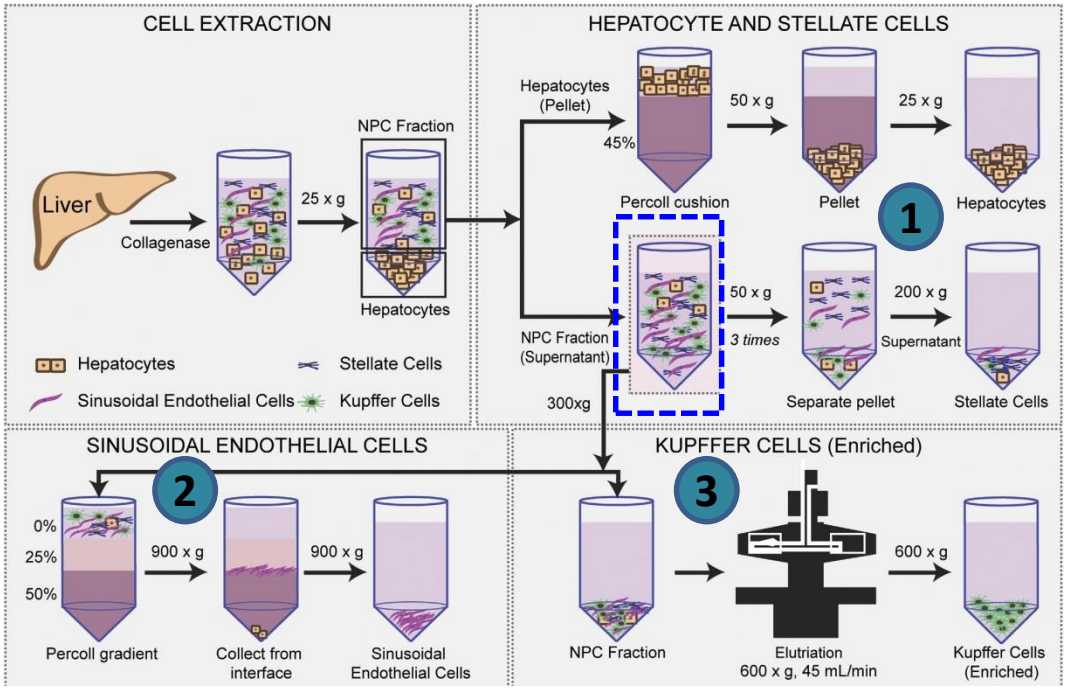
Difficult to isolate until about **day 11**

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**

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



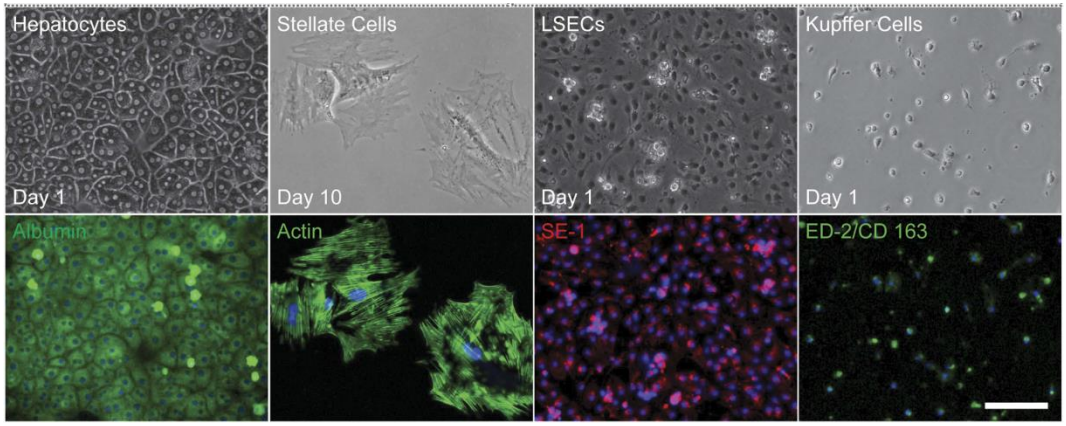


Figure 1. Isolation and characterization of primary hepatocytes and non-parenchymal cell fractions from rat livers. Rat livers were extracted from rats and digested using collagenase and cells obtained as a suspension. Hepatocytes were pelleted and enriched using a percoll cushion (45%), and pelleted.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

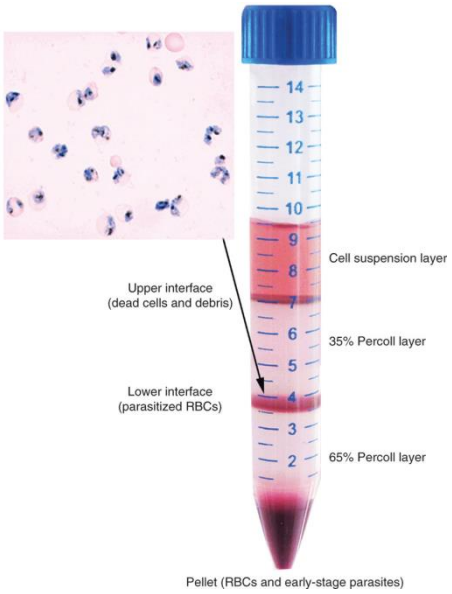
- Stellate cells were isolated using centrifugation (pelleted at 200 × g) and cultured for 7–10 days with 1 passage before use.
- Sinusoidal Endothelial cells were isolated using a percoll gradient (interface between 50% and 25%).
- Kupffer cells were isolated using centrifugal elutriation (at 600 ×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

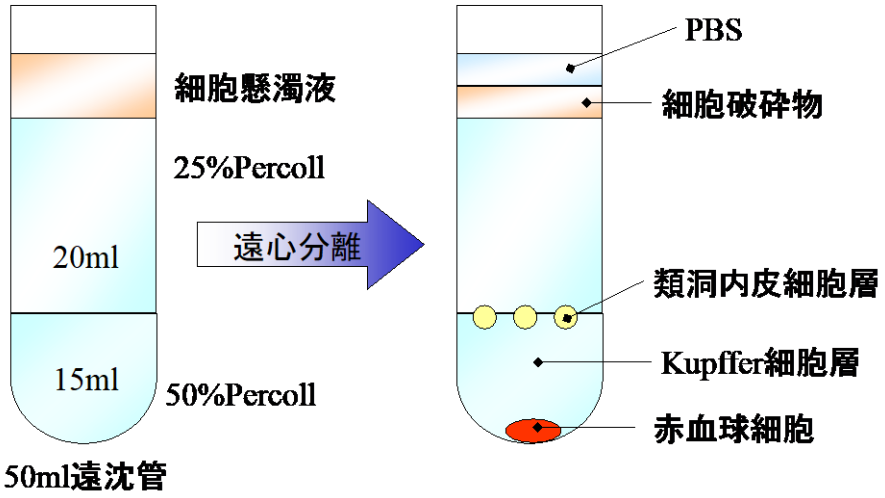
Cell separation by density

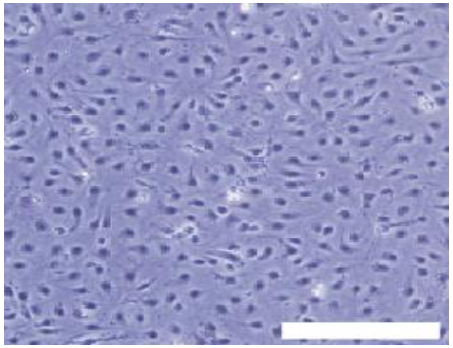
Spinning the cells at such a high g force may damage them. In addition, Percoll may be taken up by cells, so it is preferable to layer cells on top of a preformed gradient



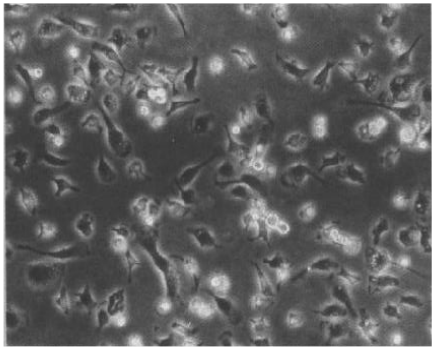
https://www.researchgate.net/figure/49809842_fig1_Figure-1-Purification-of-parasitized-RBCs-on-a-Percoll-step-gradient-consisting-of-an

https://www.fishersci.com/shop/products/ge-healthcare-percoll-plus-centrifugation-media-2/p-3753328





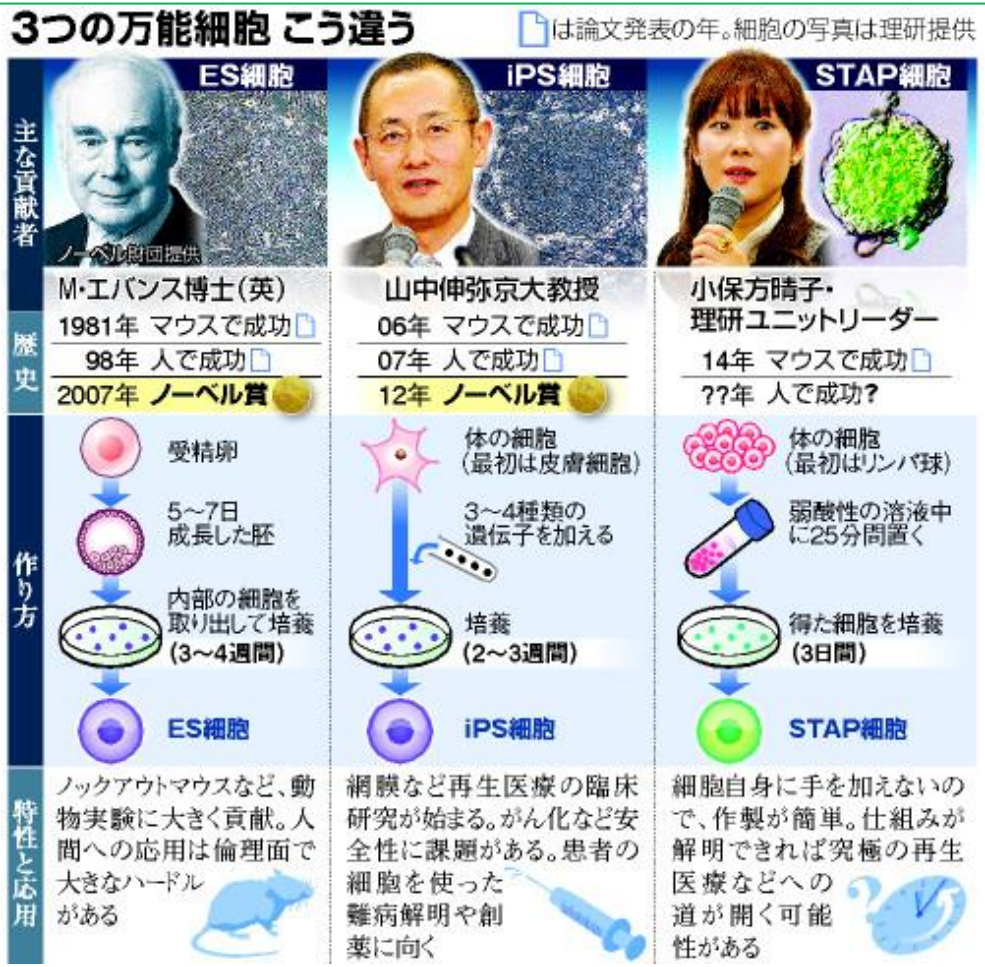
Sinusoidal endothelial cells



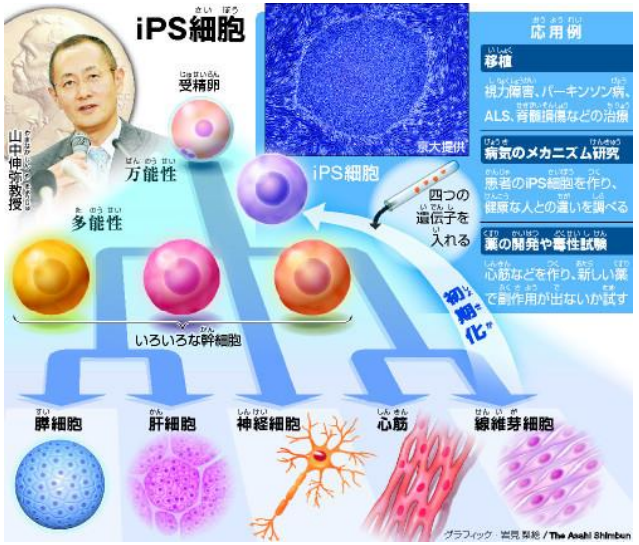
Kupffer cells

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



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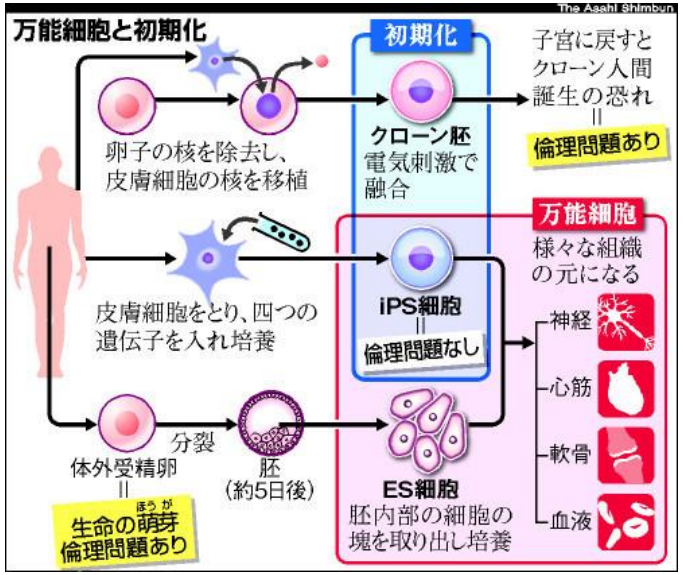


www.asahi.com

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

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）

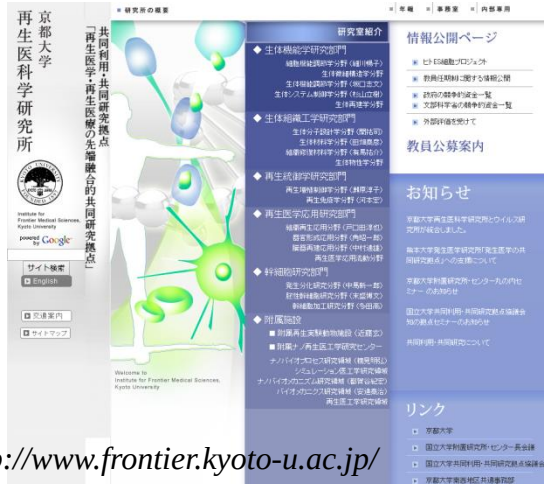
http://www.youtube.com/watch?v=gcxnpc7qNQ4

4. iPS細胞からヒト肝臓作製　横浜市立大が発（2013/07/04）

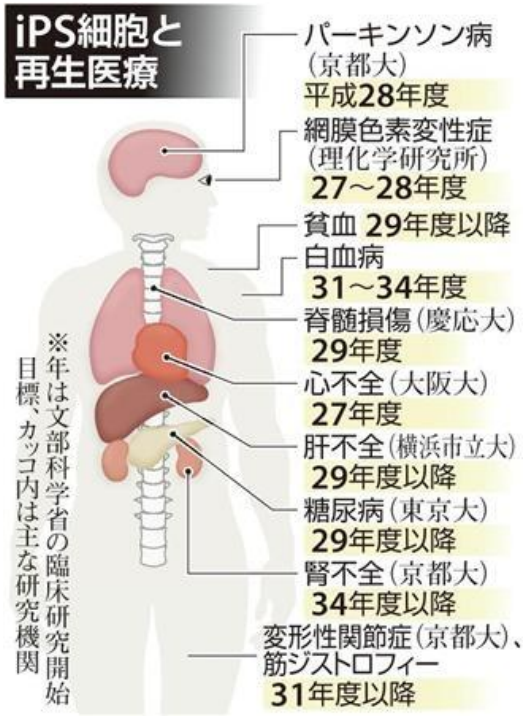
http://www.youtube.com/watch?v=ze_oQDpakrs

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。

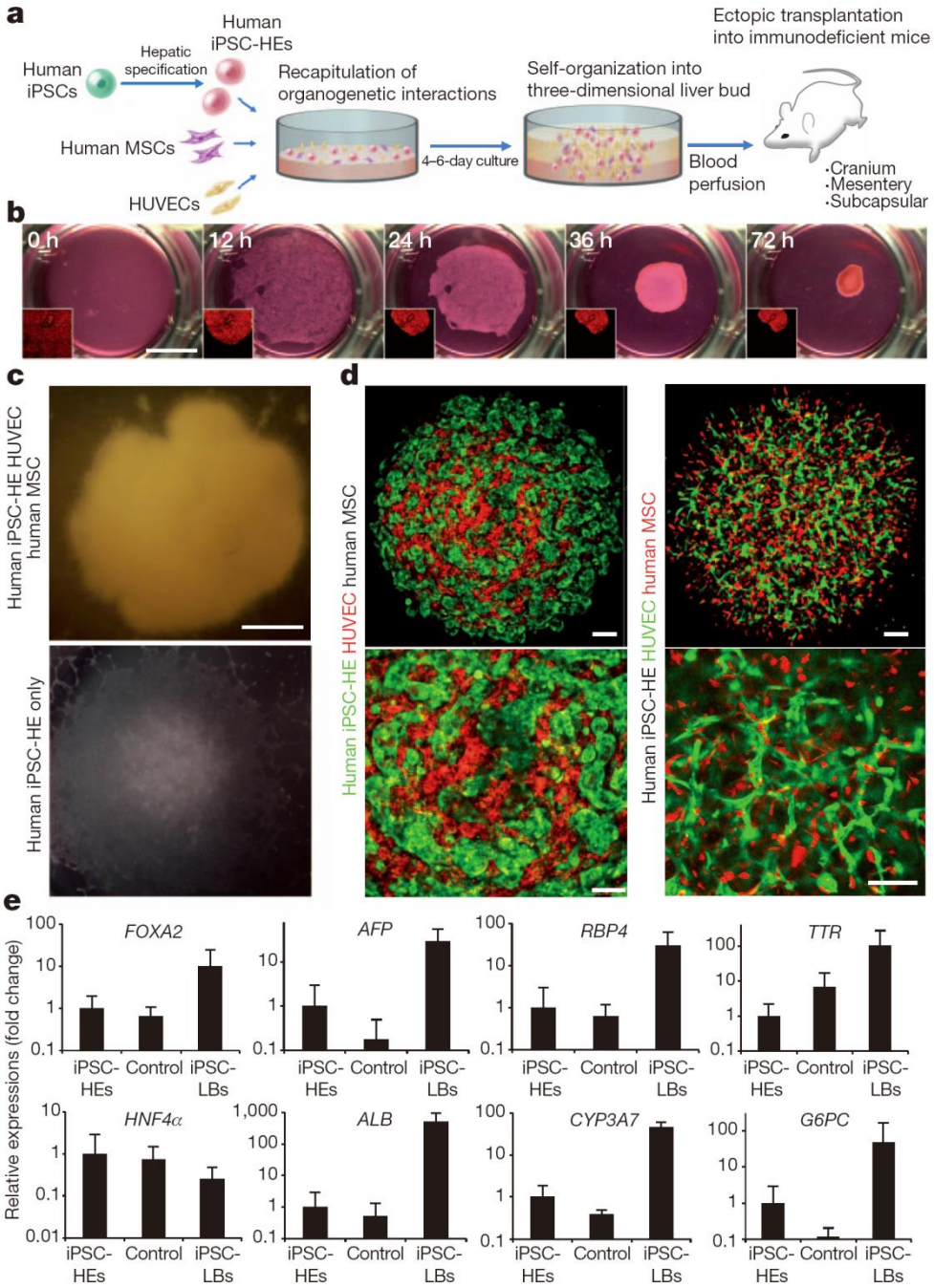
http://www.nikkei.com/article/DGXLZO81854920S5A110C1TJM000/

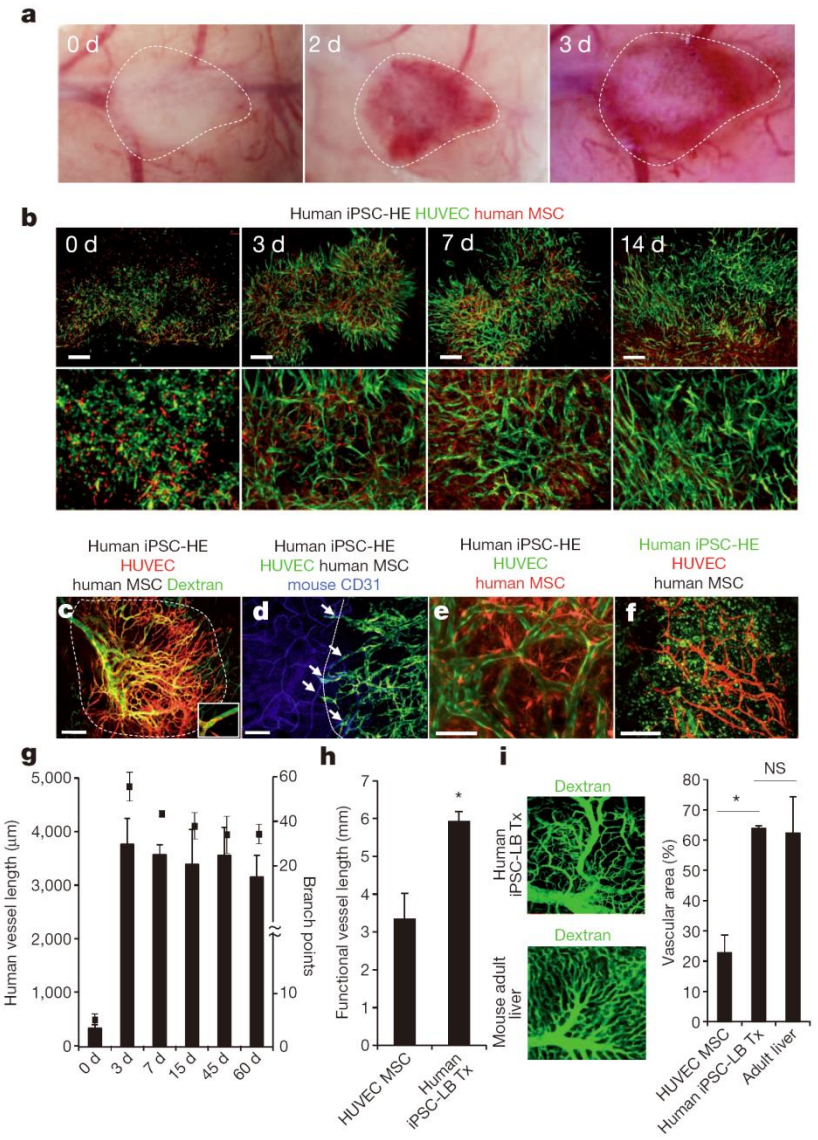
To date clinic application

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

In vitro hepatic culture models (2D culture)

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

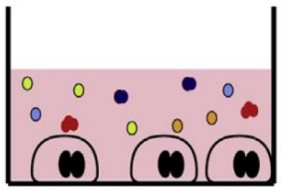
1. Soluble factors (medium composition)
2. Cell-matrix interactions
3. Heterotypic cell-cell interactions with non-parenchymal cells.

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

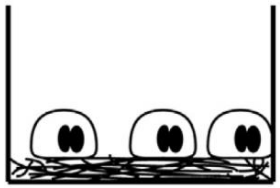
Certain mitogenic factors



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)

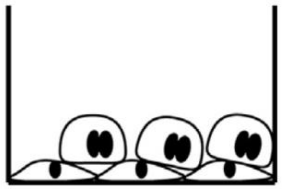
Heterotypic cell-cell interaction



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

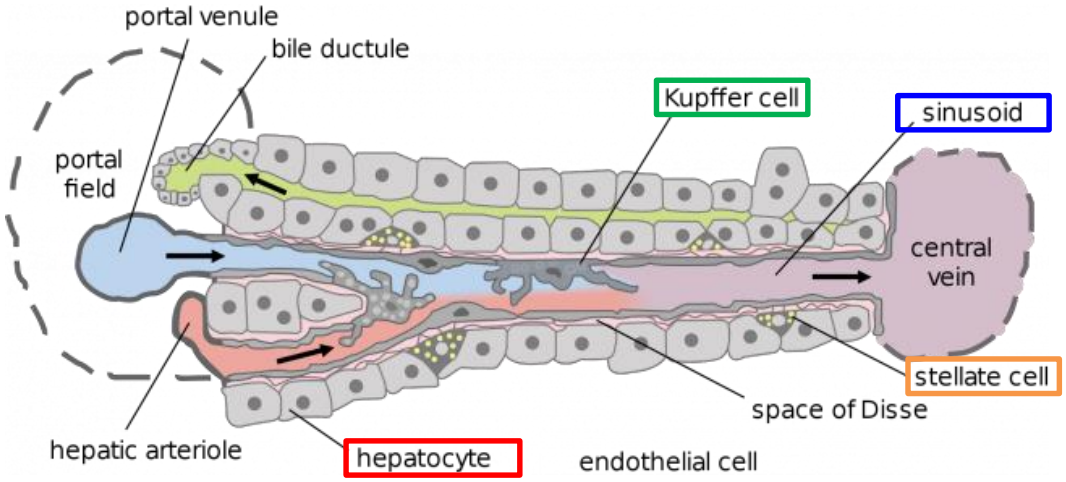
A variety of ECM coatings



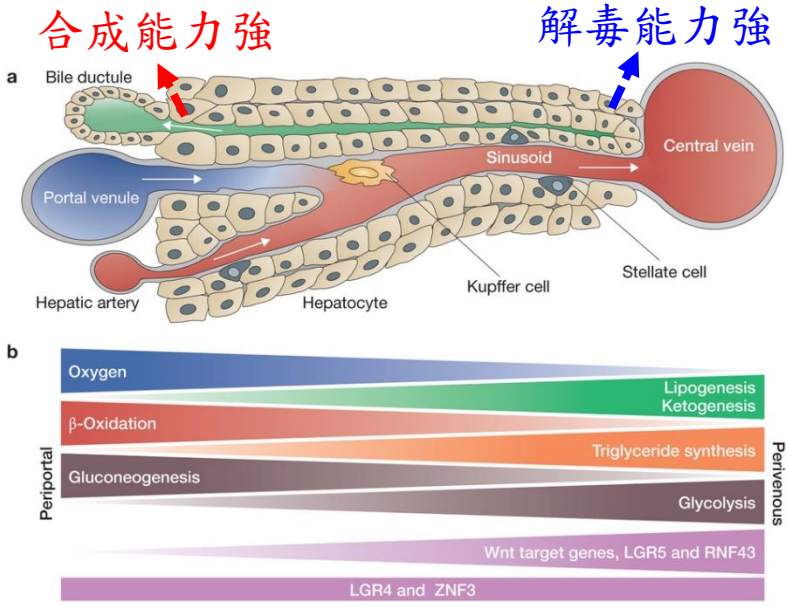
Co-Cultivation

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

In vitro hepatic culture models (2D culture)



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



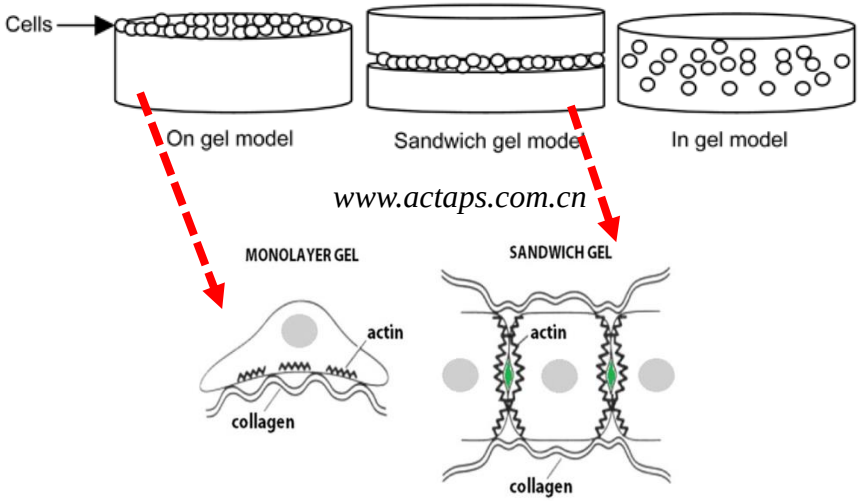
2016 Orchestrating Wnt signalling for metabolic liver zonation

- 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 .

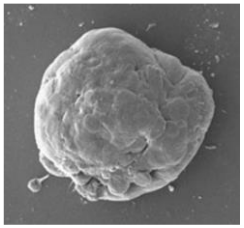
In vitro hepatic culture models (3D culture)

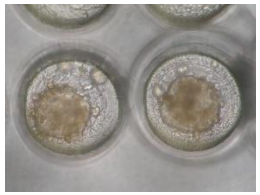
3D gel culture

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

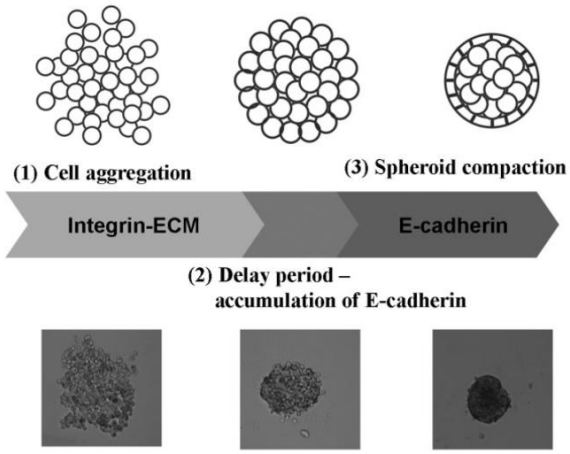
- Under spheroidal culture conditions, hepatocyte survival and functions are improved over standard monolayers on collagen.
- Potential mechanisms underlying these effects include an increased extent of homotypic cell-cell contacts between hepatocytes, the retention of a three-dimensional cyto-architecture, 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.



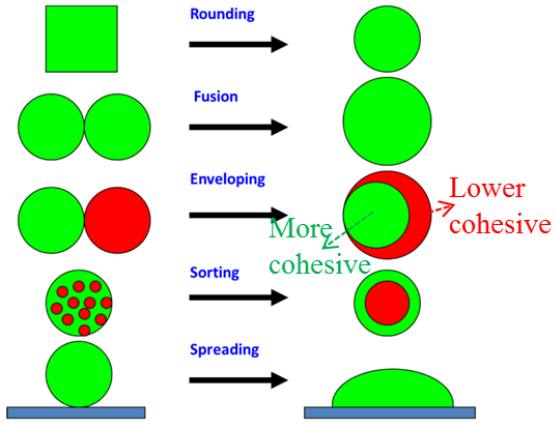


Fig. 5.3. Evidence of fluidity of tissue spheroids.

Video: 3D spheroid formation in InSphero GravityPLUS system

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

Histotypic culture: Spheroid (2)

Spheroid Preparation method

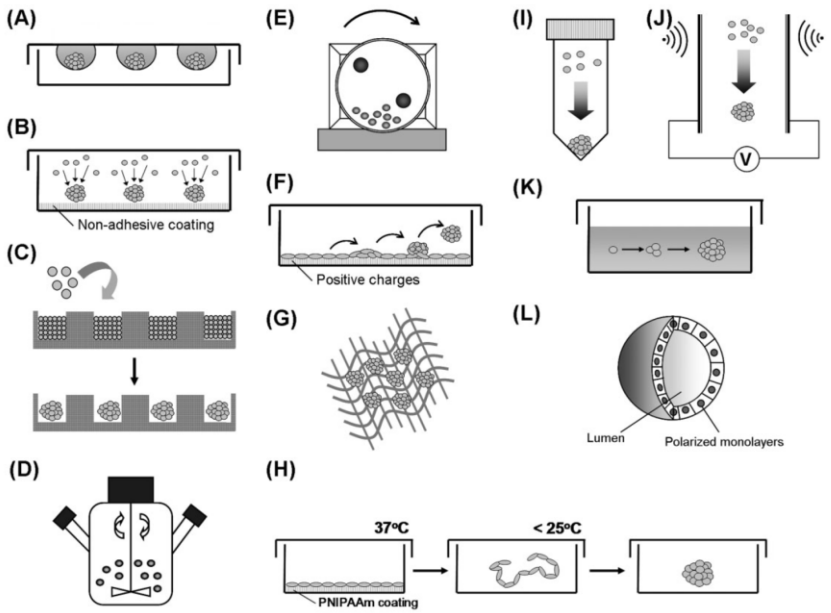
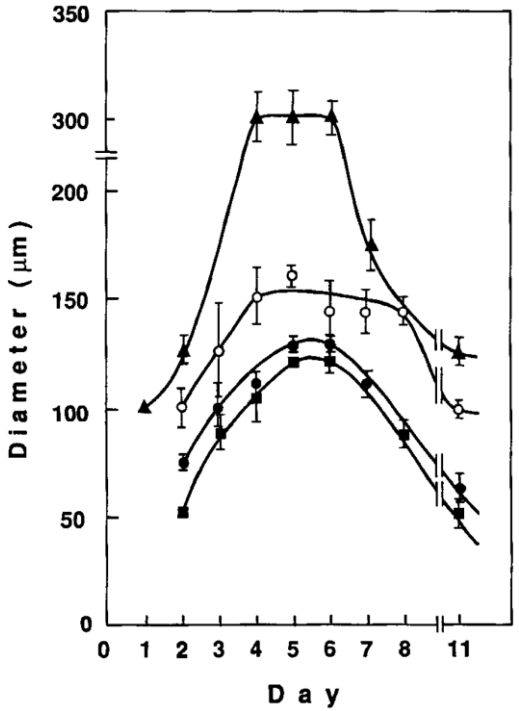
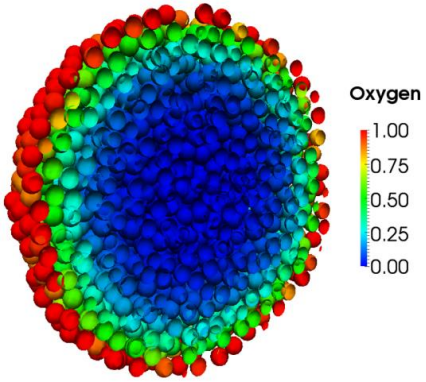


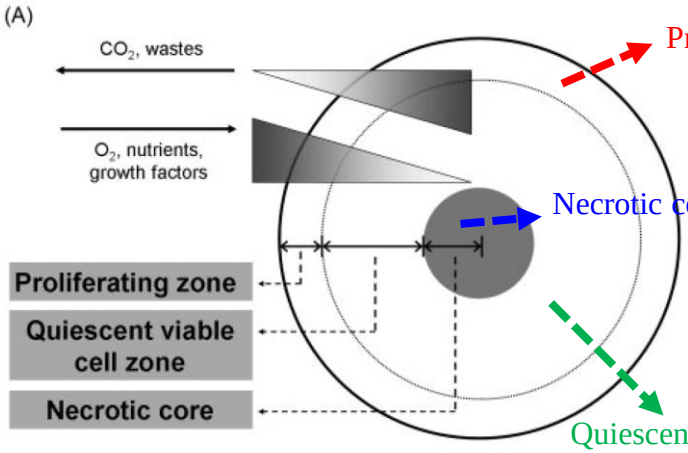
Figure 3. Methods for MCS generation. (A) Hanging-drop culture. (B) Single cell culture on nonadhesive surface. (C) Micromolding techniques. (D) Spinner flask culture. (E) Rotary cell culture systems. (F) Hepatocyte self assembly on Primaria dishes. (G) Porous 3-D scaffolds. (H) The use of PNIPAAm-based 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





www.ploscollections.org



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

Histotypic culture: Spheroid (3)

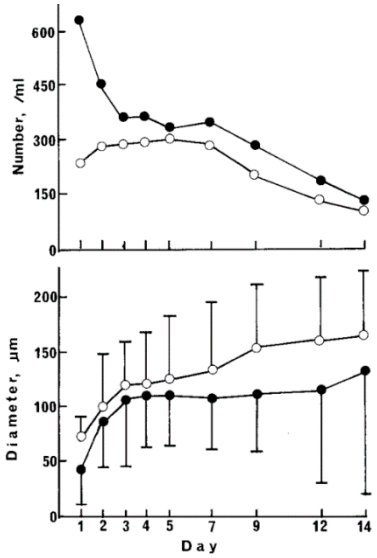


FIG. 2. Changes in number (top) and diameter (bottom) of multicellular spheroids during 2 weeks of culture. (○) An uncoated Primaria dish; (●) Falcon 3001 dish coated with proteoglycan fraction. Each point represents the mean of triplicate cultures with standard deviation indicated as a vertical line.

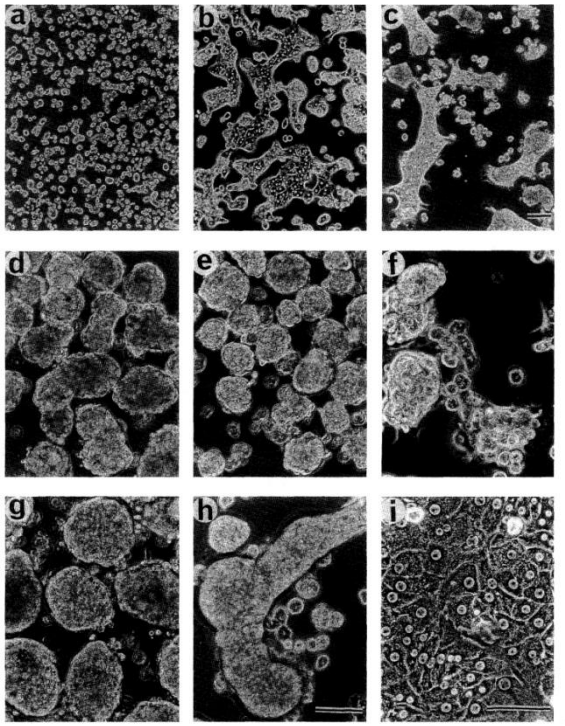


FIG. 1. Phase-contrast microscopic features of multicellular spheroids of adult rat hepatocytes. (a–d) Uncoated Primaria dish at 0 h (a); 24 h (b); 52 h (c) 4 days (d) after seeding; (e) Falcon 3001 dish coated with proteoglycan fraction at Day 4; (f) Falcon 1008 dish at Day 4; (g) Falcon 3001 dish coated 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

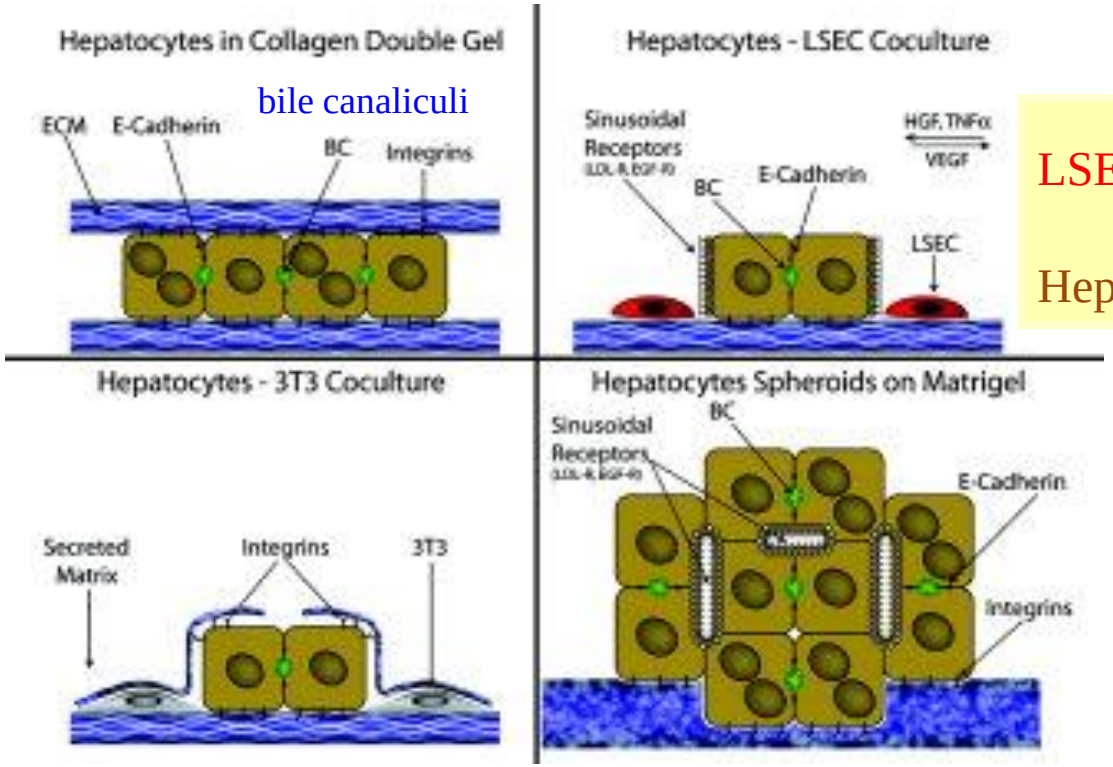
Solid-State Regulation of Cell Assembling of Adult Rat Hepatocytes in Primary Culture

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)



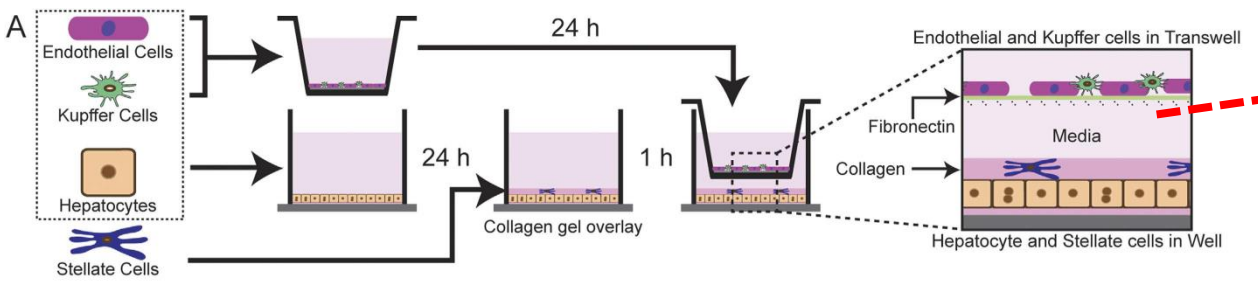
HGF, TNF-α
LSEC→Hepatocyte

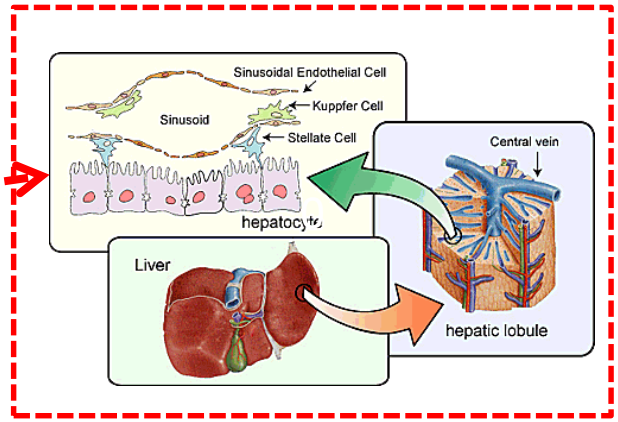
VEGF
Hepatocyte→LSEC

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.

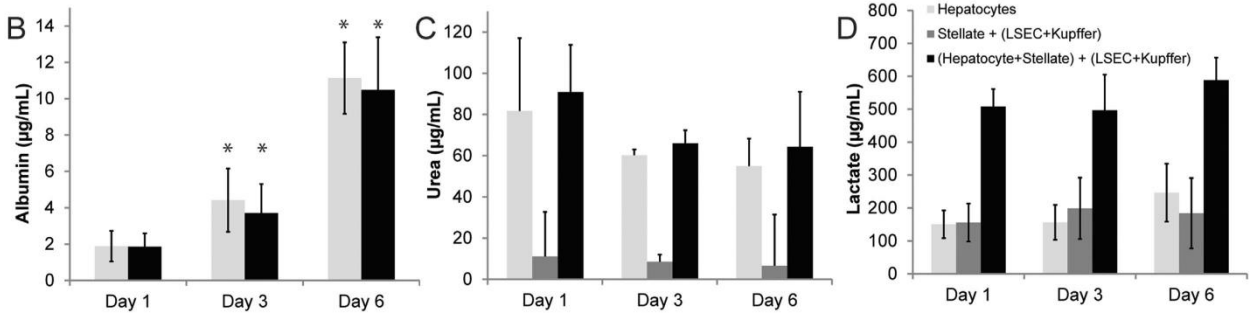
https://www.researchgate.net/figure/7334024_fig2_Figure-8-Schematic-of-a-hypothetical-model-showing-the-polarization-of-hepatocytes-in

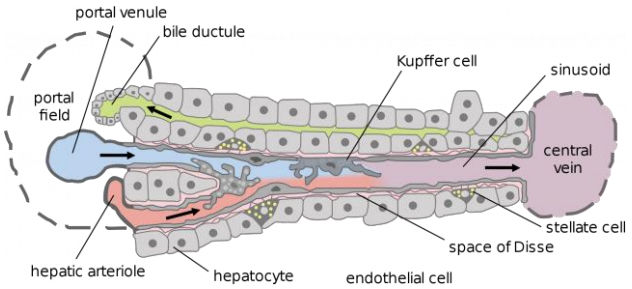
In vitro hepatic culture models (Co-culture)





www.pinterest.com





https://goo.gl/4ycF6J

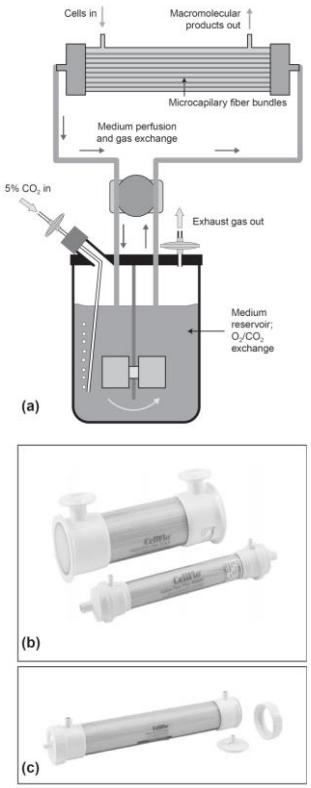
Figure 2. Co-culture setup, and functional activity of hepatocyte monocultures, NPCs and hepatocyte-NPC formats. (**A**) Hepatocytes, LSECs and KCs were isolated from a rat liver and seeded on collagen coated well and fibronectin coated transwell respectively and cultured overnight. SCs are overlaid 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).

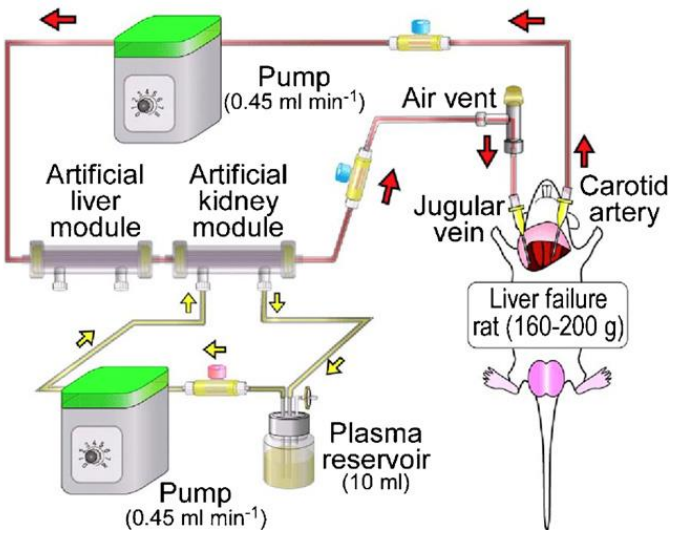
2016 Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response

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

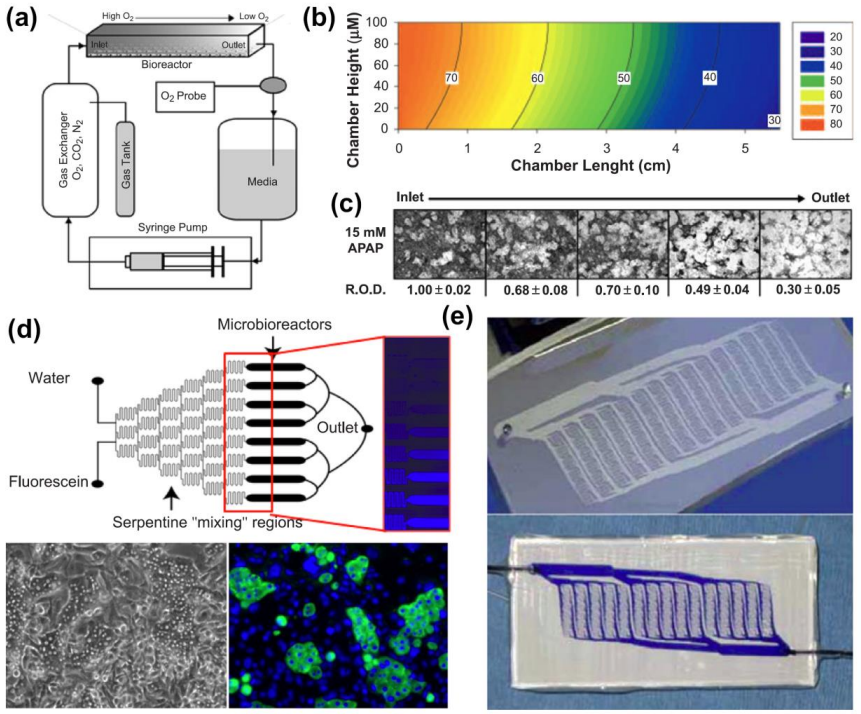


FIGURE 46.4

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

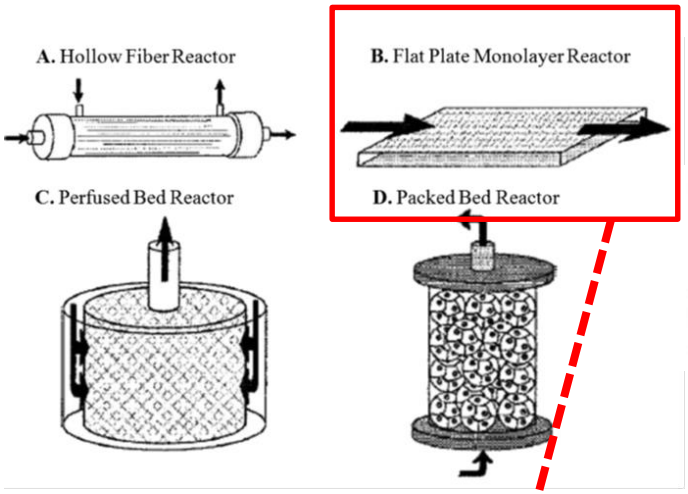
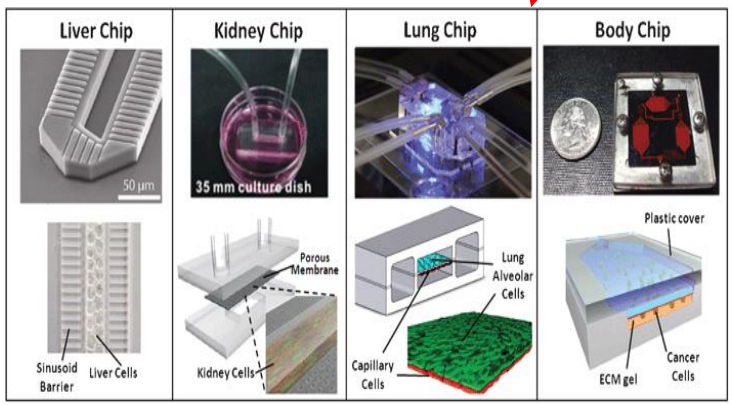
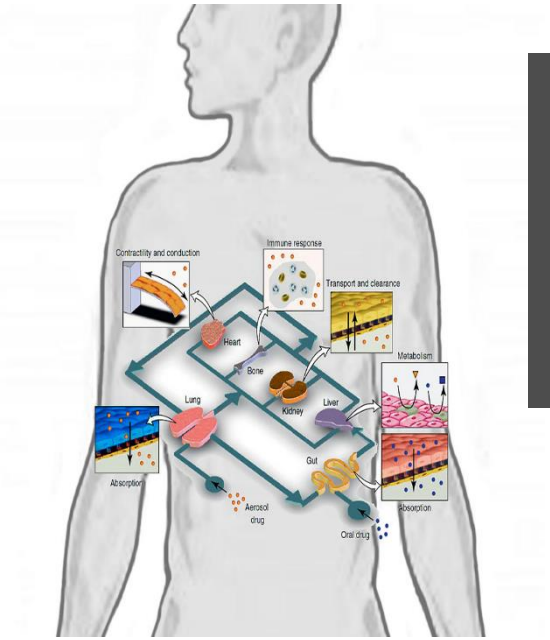


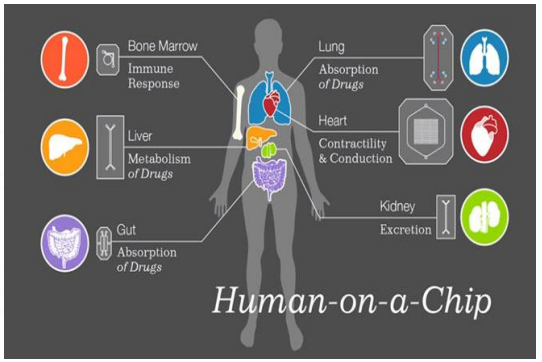
Figure 2. Available bioreactor designs: (A) hollow fiber reactor; (B) flat membrane reactor; (C) perfused bed reactor; and (D) packed bed bioreactor, which are currently being evaluated for use in BAL [*Figure reproduced with permission from Allen JW, et al. Hepatology 2001;34:447–55*].

2011 Extracorporeal Bioartificial Liver for Treating Acute Liver Diseases



http://pubs.rsc.org/en/content/articlehtml/2012/lc/c2lc4008

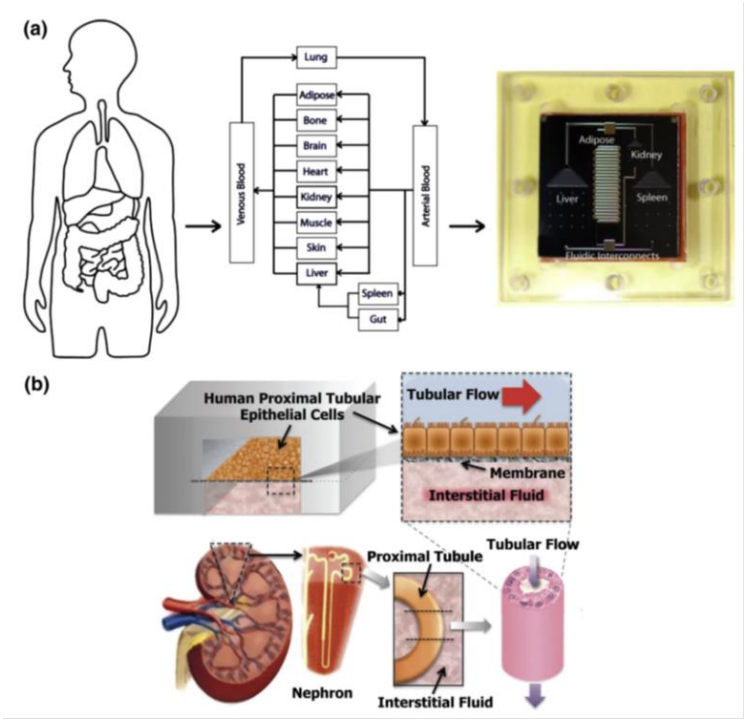




2011 From 3D cell culture to organs-on-chips, Trends in Cell Biology

Organ on a chip

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.



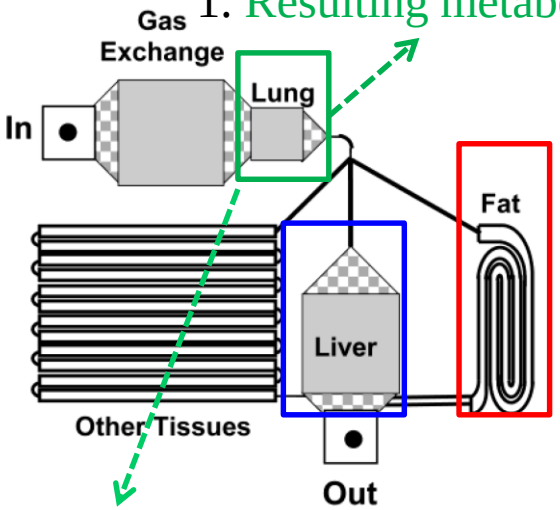
Organ on a chip主要用途

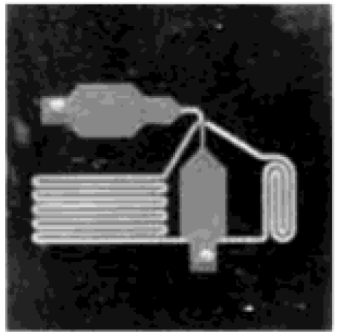
- First advantage is greatly favorable when applied as a drug-testing platform. Conventional drug-testing techniques, such as *in vitro* culture and animal tests, often do not provide environments that are physiologically relevant to humans, whereas organ-on-a-chip does.
- The second advantage is greatly 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).)*

Shuler and colleagues' animal-on-a-chip

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





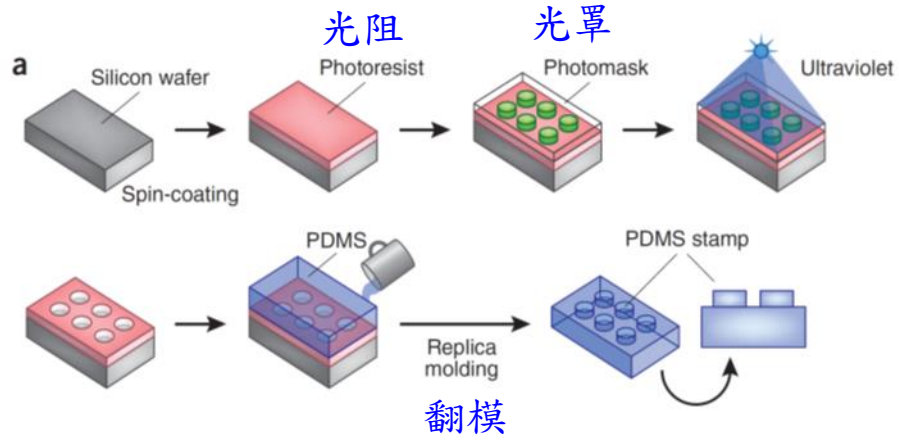
Naphthalene is fat soluble

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

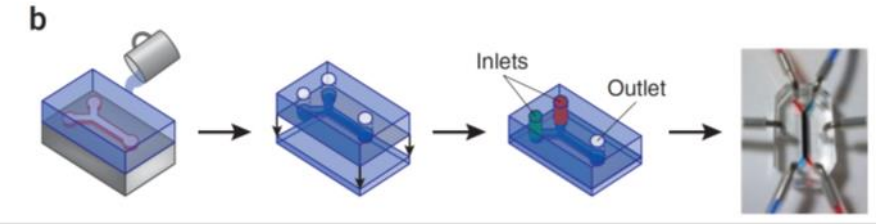
2. 1,2-環氧萘會與glutathione 結合排出

- 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**).

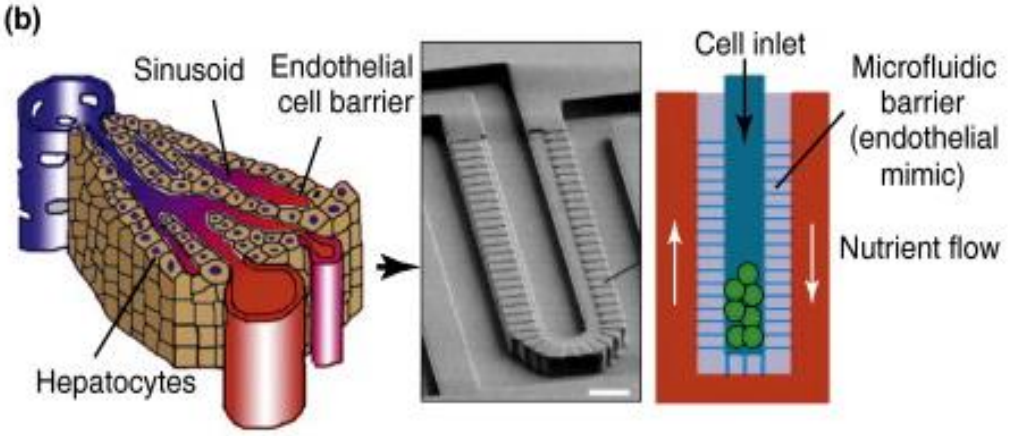


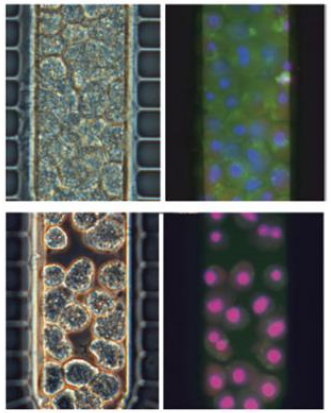
This approach was modified by inverting the PDMS mold and conformally sealing it to a flat smooth substrate, such as glass, to create open cavities in the form of small (cross section < 1 mm × 1 mm), linear, hollow chambers, or ‘microfluidic channels,’ with openings at both ends of the polymer block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

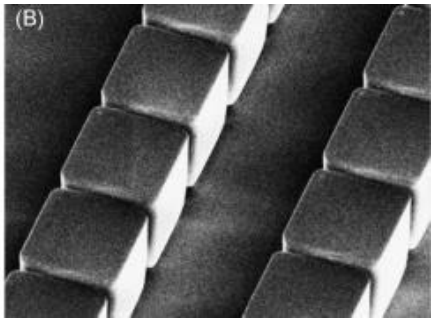


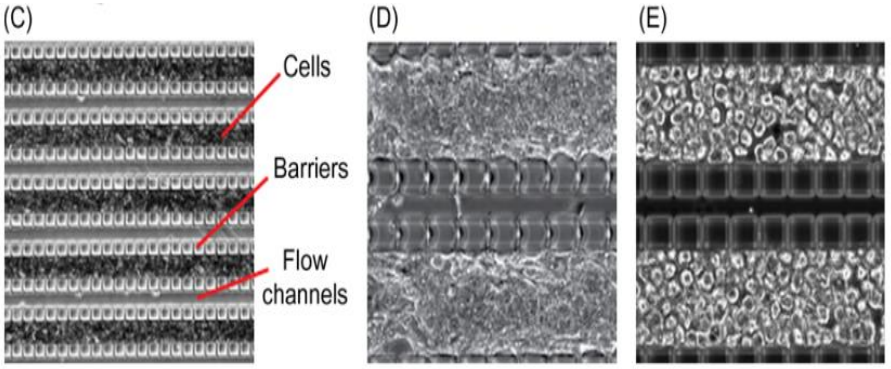


- Scale bar : 50μm
- **Chamber大約50μm**
- Barrier 的間隙2μm
- 特點：
- 間隙特別小，
- 模擬肝臟in vivo環境

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



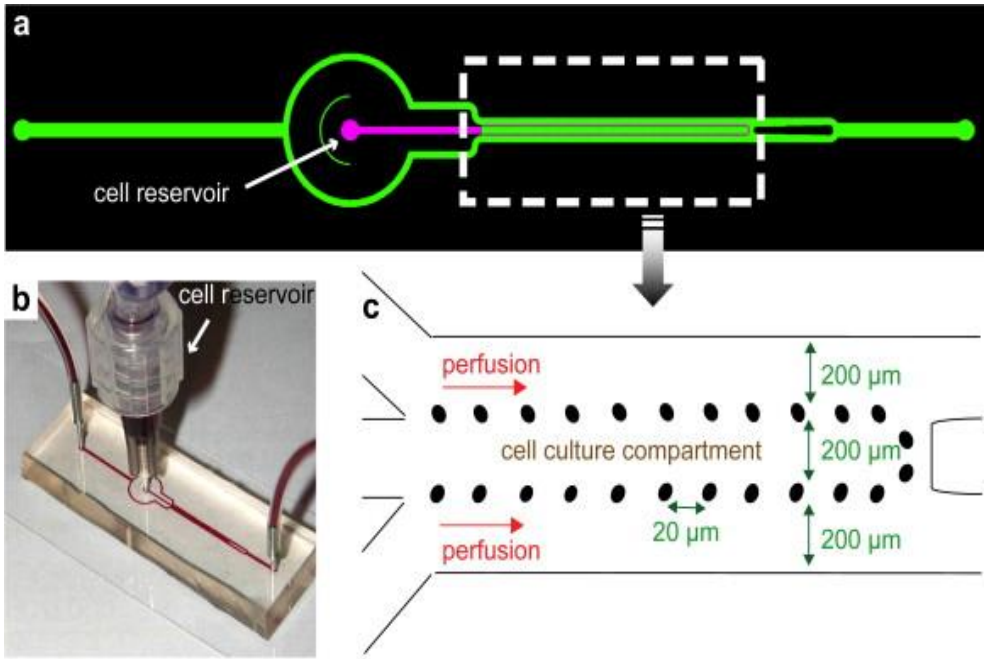


2013 Microfluidic Cell Culture Systems Chapter 14 – Microfluidic Hepatotoxicity Platform,2013

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

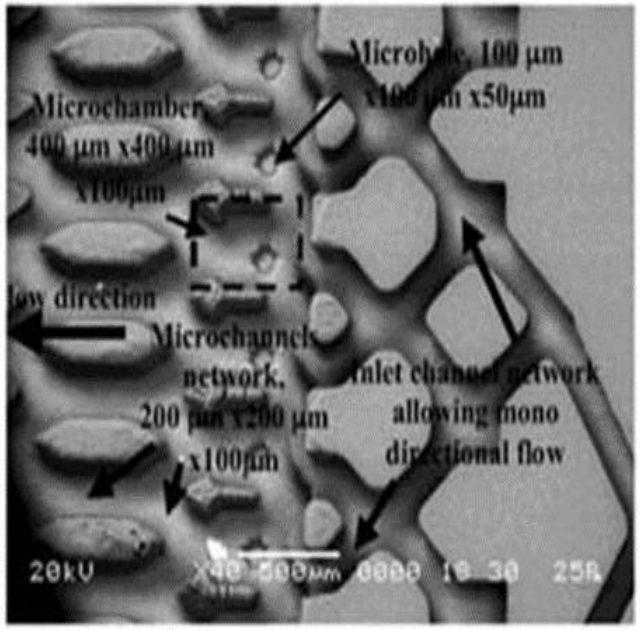
Some examples of Liver on a chip

- 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



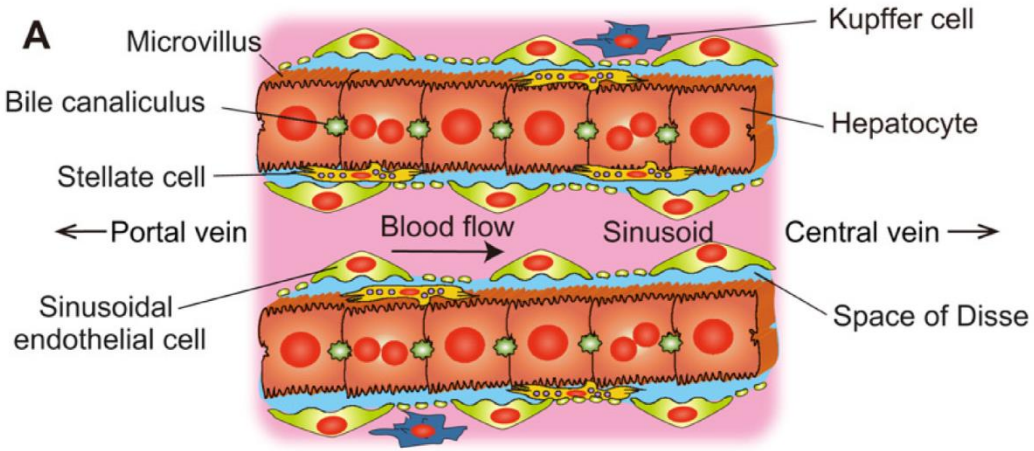
2008 A gel-free 3D microfluidic cell culture system

- 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

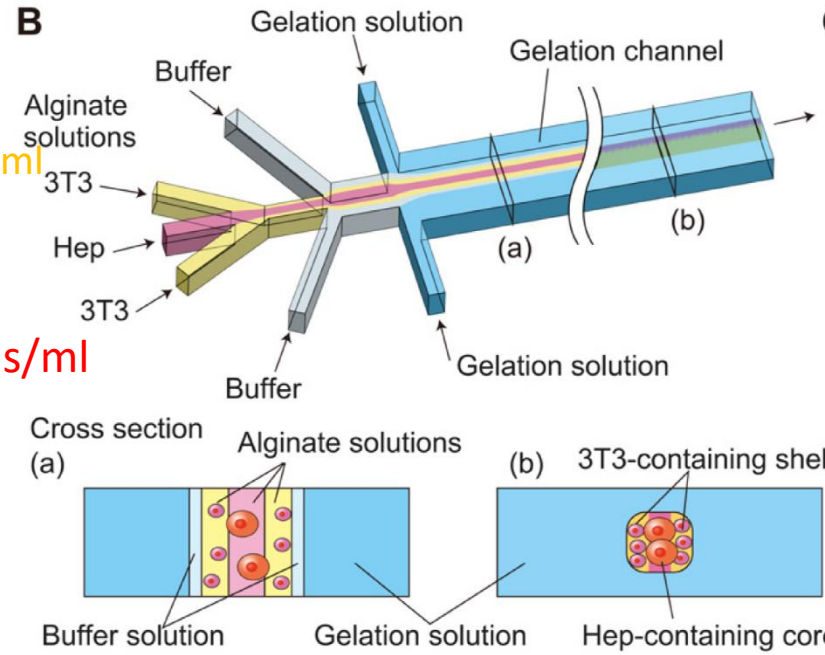


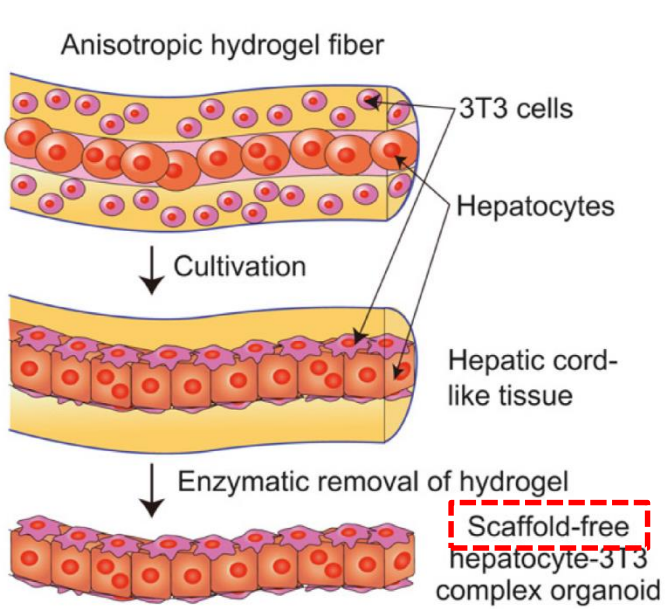
2008 Microfluidic PDMS (Polydimethylsiloxane) Bioreactor for Large-Scale Culture of Hepatocytes

Some examples of Liver on a chip



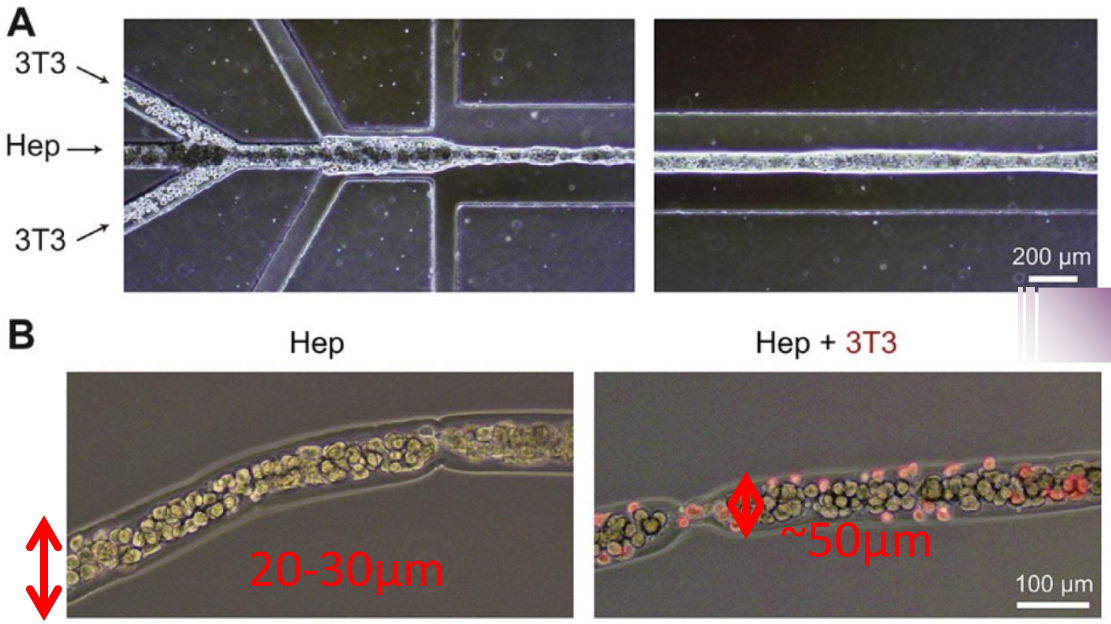
- Width: 400µm
- Length: 50mm
- Depth: 160µm

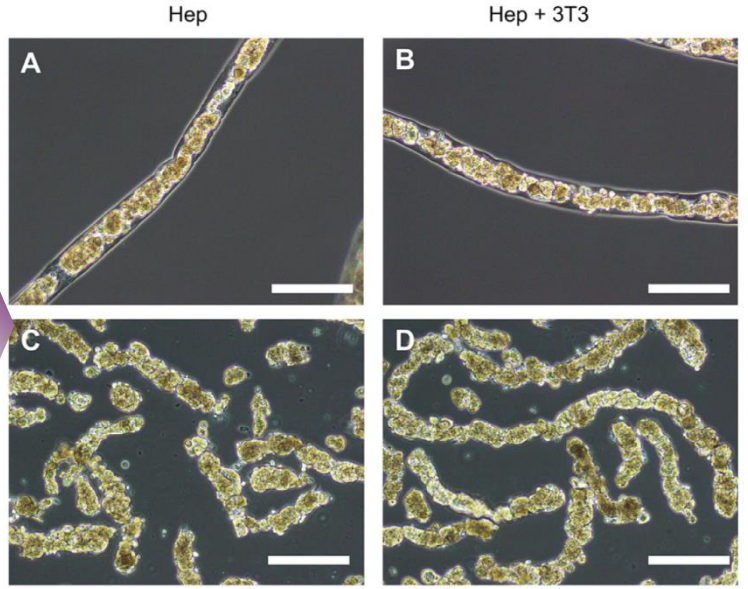




2012 Controlled Formation Of Heterotypic Hepatic Micro-organoids In Anisotropic Hydrogel Microfibers For Long-term Preservation Of Liver-specific Functions

Some examples of Liver on a chip



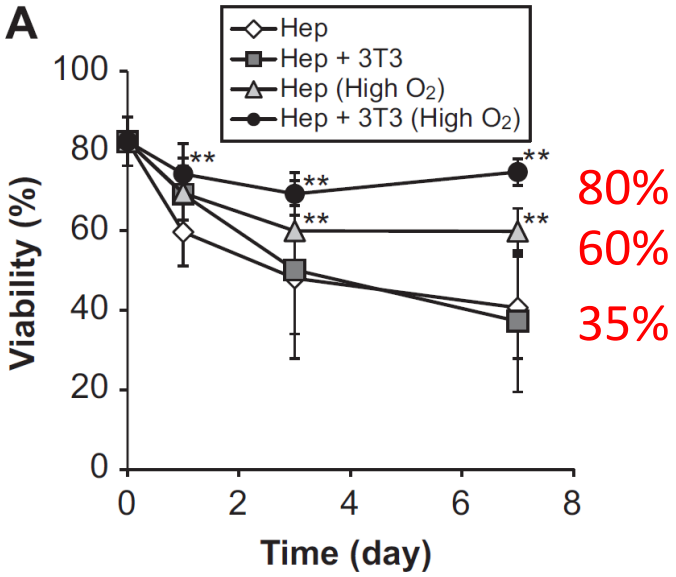


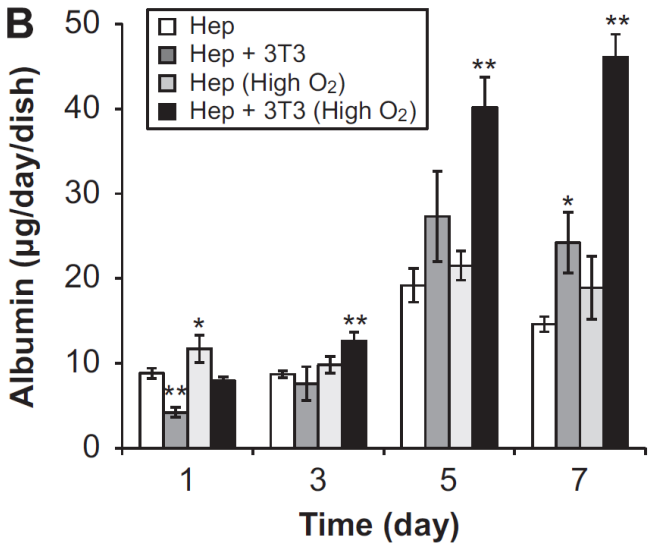
201±102 μm

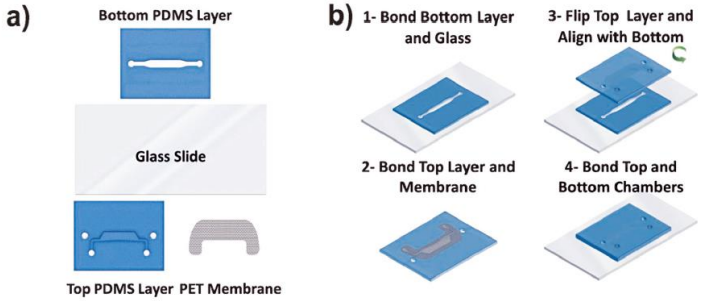
viability : 50%

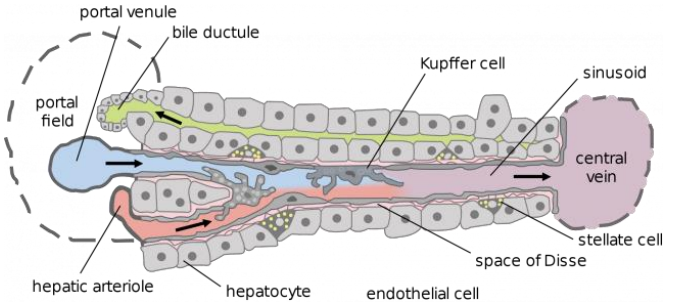
179±102 μm

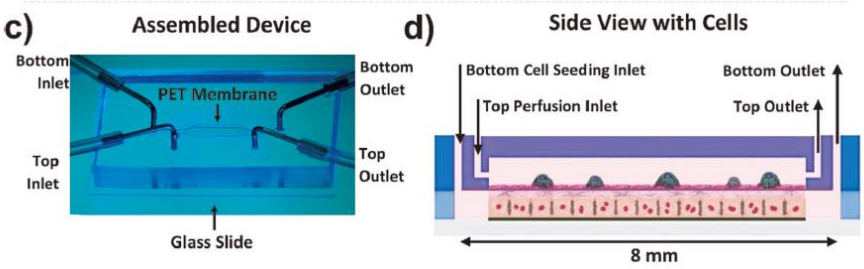
viability : 80%

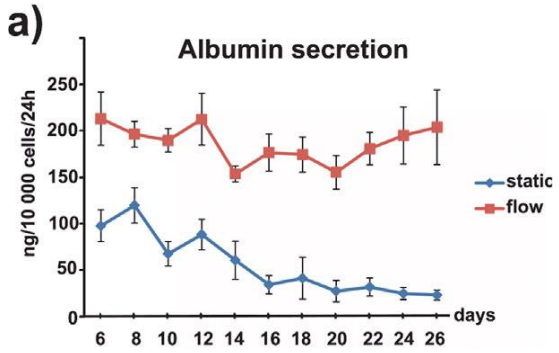


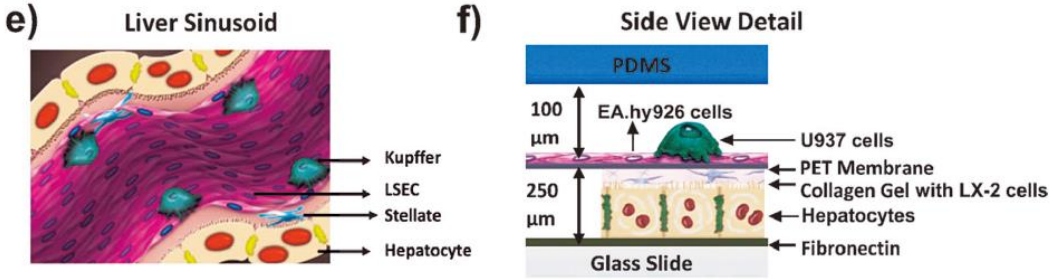


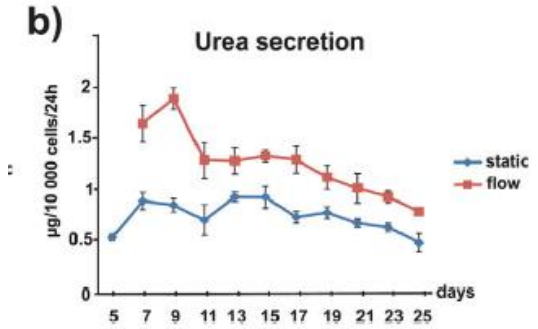


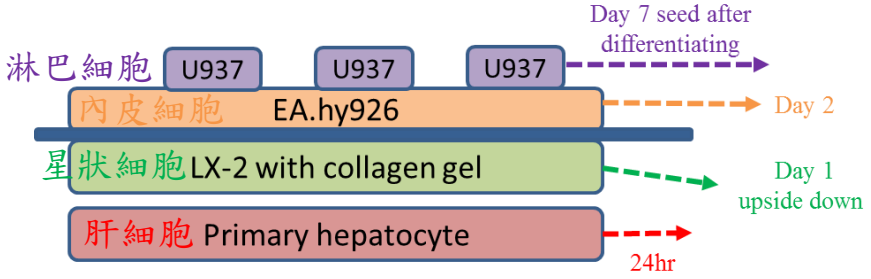








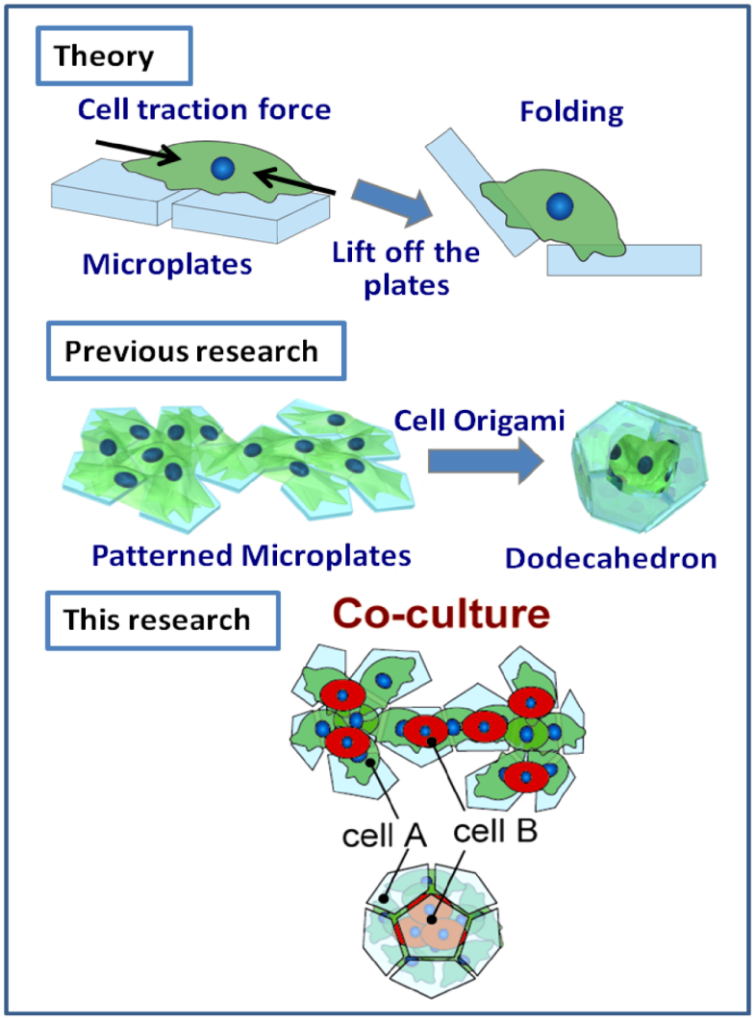


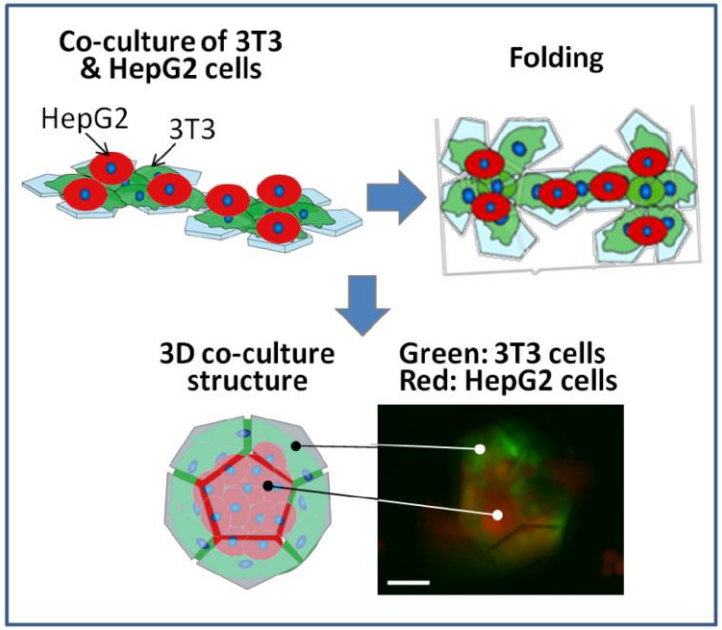


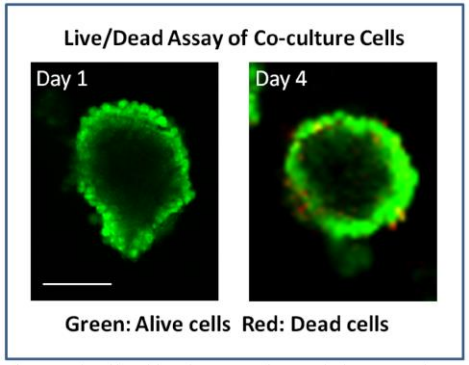
U937: lymphocyte in lung
EA.hy926 : umbilical vein endothelial cells
LX-2 : Human Hepatic Stellate Cell

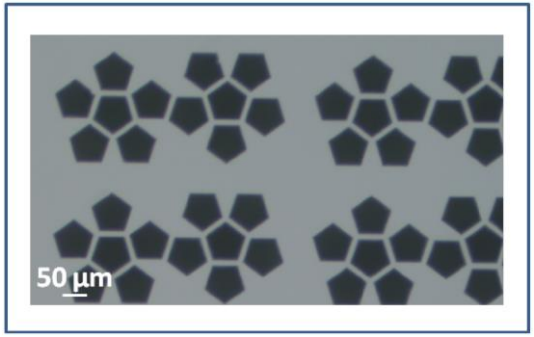
2016 Long-Term Maintenance of a Microfluidic 3D Human Liver Sinusoid

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

Some examples of Liver on a chip

Diameter= 169 ± 6 μm

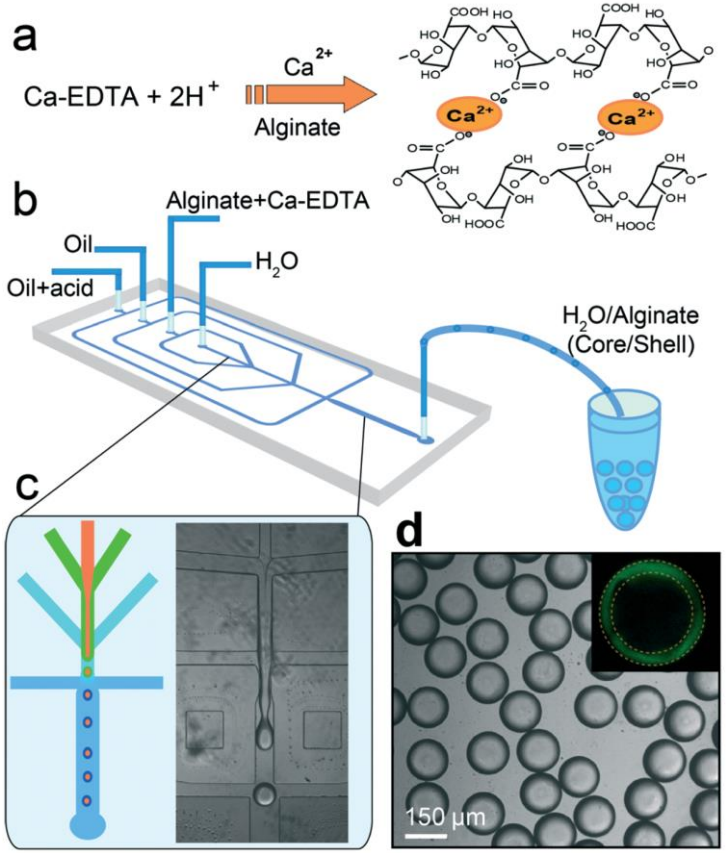


Fig. 1 Construction of the 3D scaffold in a drop consisting of an aqueous core and a hydrogel shell. a) Crosslink of the alginate network by triggered release of Ca^{2+} from the Ca-EDTA complex. b) Schematic diagram of the PDMS device. c) Fabrication of core–shell droplets using w/w/o double emulsions 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.

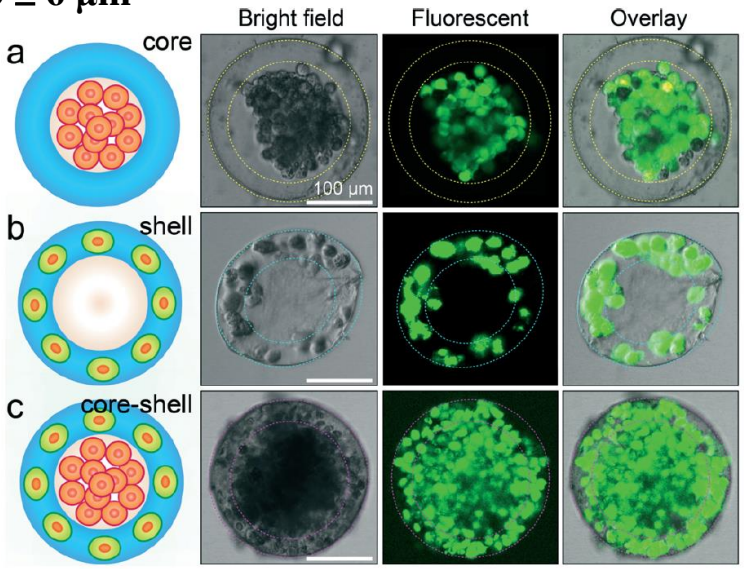
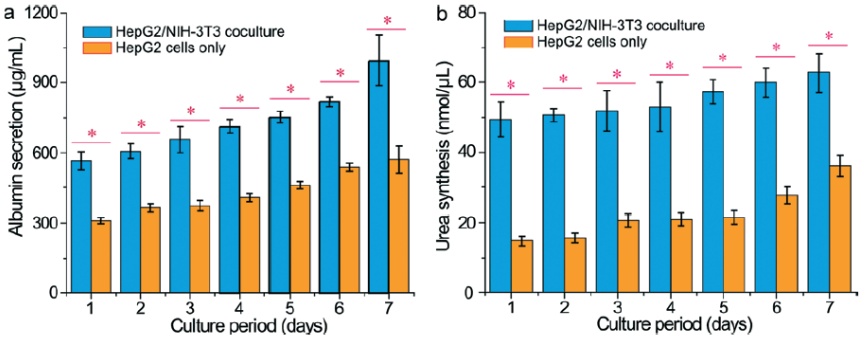


Fig. 2 Spatial assembly of different cells in the 3D core–shell scaffold. a) HepG2 cells confined in the core by the hydrogel shell. b) NIH-3T3 fibroblasts immobilized by the crosslinked alginate network in the shell. c) Simultaneous assembly of hepatocytes in the core and fibroblasts in the shell, forming 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

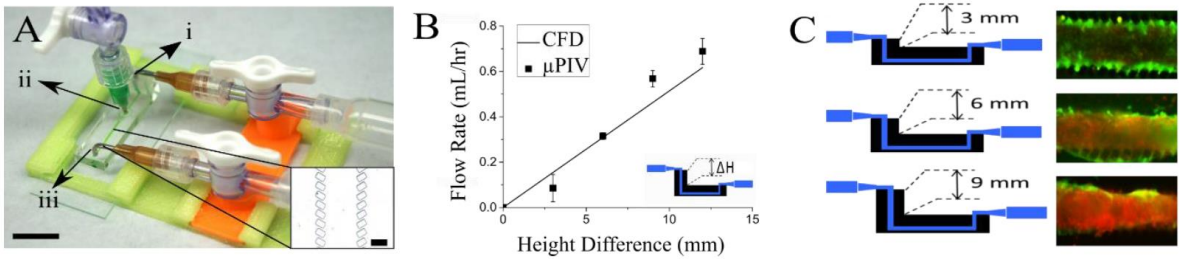


Figure 1: (A) Pump free microfluidic culture device setup. (i) Media inlet. (ii) Seeding inlet. (iii) Media outlet. (B) Live-dead staining of microfluidic culture primary rat hepatocyte with different flow rate. (C) Simulated flow rate by CFD and micro-particle imaging velocimetry of the pump free microfluidic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

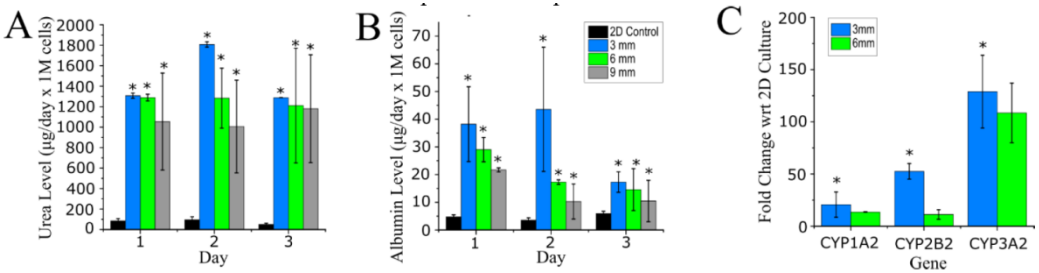


Figure 2: (A) Urea synthesis of primary rat hepatocytes in microfluidic cultures and in 2D static cultures. **(B)** Albumin synthesis of primary rat hepatocytes in microfluidic cultures and 2D static cultures. **(C)** Cytochrome P450 metabolic functions of primary rat hepatocytes under perfusion cultures.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

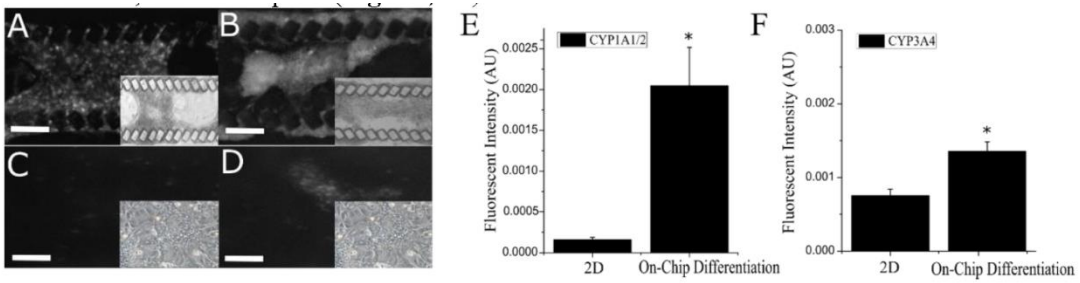


Figure 3: CYP1A2 activities of HepaRG-hepatocytes after 14 days differentiation in **(A)** 3D microfluidic device and **(B)** 2D static culture as indicated by ethoxyresorufin-0-deethylase (EROD) fluorescent enzymatic assay. **(C-D)** CYP3A4 activities of HepaRG-hepatocytes after 14 days differentiation 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.

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/

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

Some examples of Liver on a chip

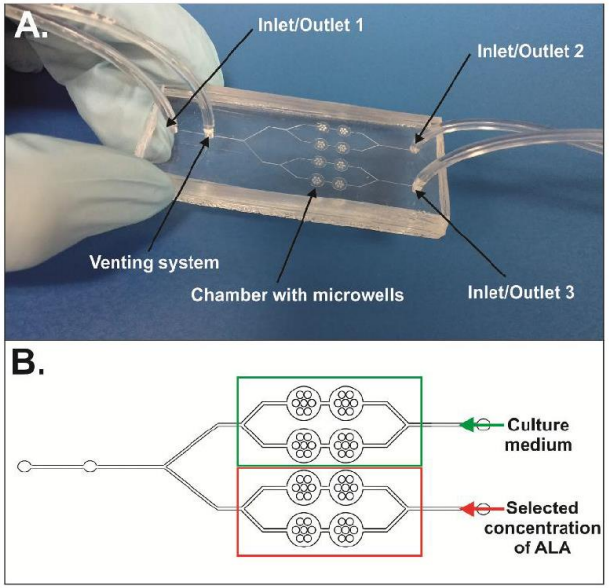
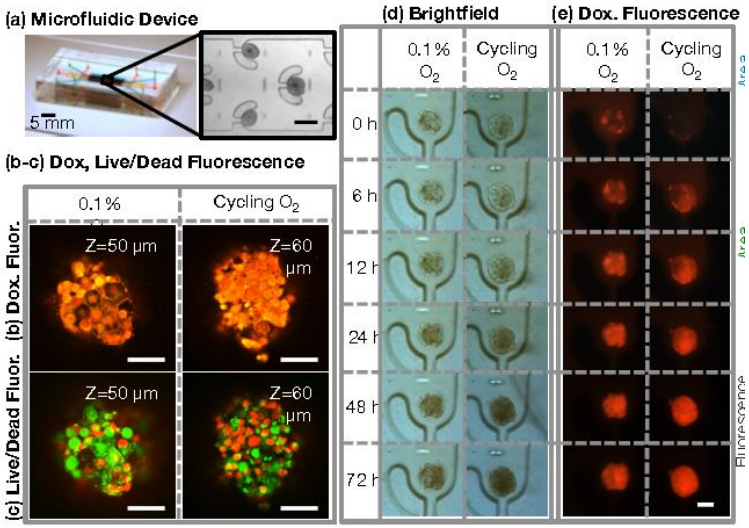
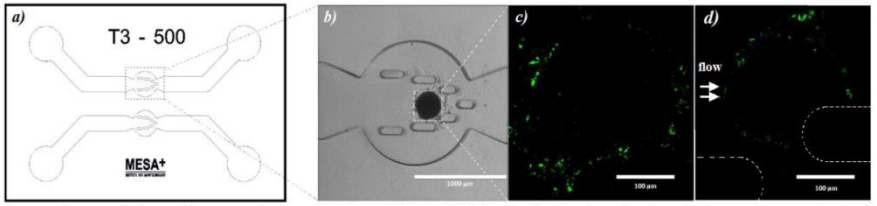


Figure 1: (A) The microfluidic system (B) Scheme of microchannels and microchambers network. Distribution of control and selected ALA concentrations in the microsystem.

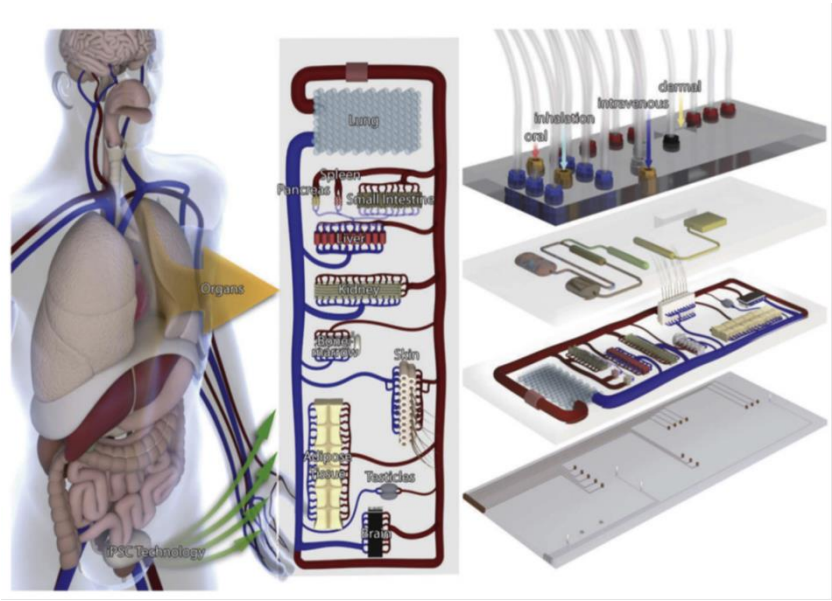


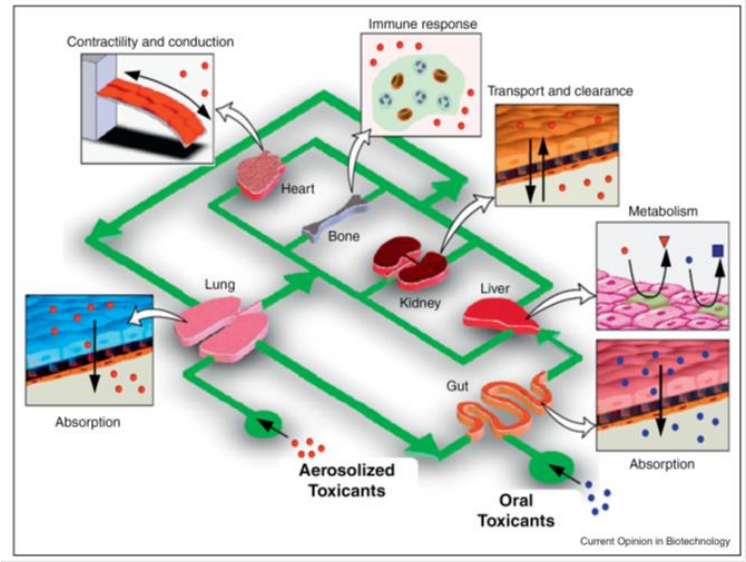


dynamic conditions by creating a laminar flow (24µl.min^{-1}) in the device led to an accelerated penetration of NPs deeper in the tissue, and longer exposure led to even deeper penetration.

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).

Future work of Organ on a chip





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

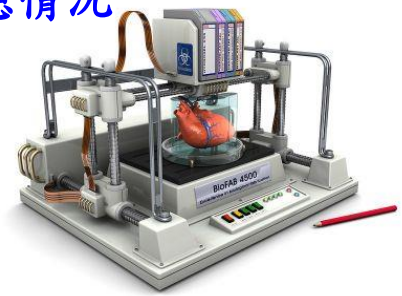
- This proposed human-on-a-chip system consists of 11 organ equivalents connected by one close loop circulation system. This device has multiple routes of administration and sensing as well as mechanical actuators to mimic organ motions such as heartbeat, intestine movements, and lung breathing motions.
- 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

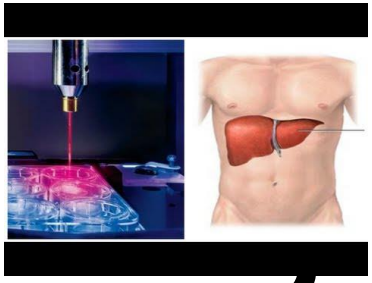
Prospective design of a "human-on-a-chip". *Figure reproduced from Materne et al. (2013).*

3D printed liver

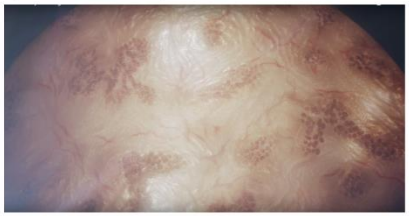
理想情況

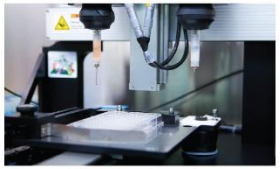


真實情況



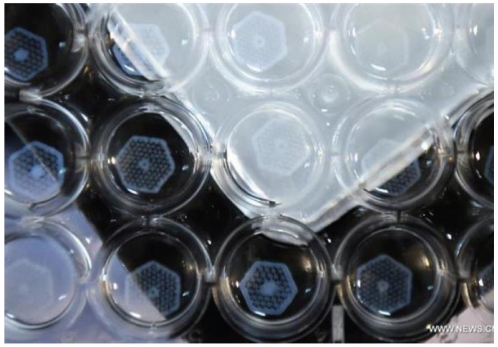
organovo



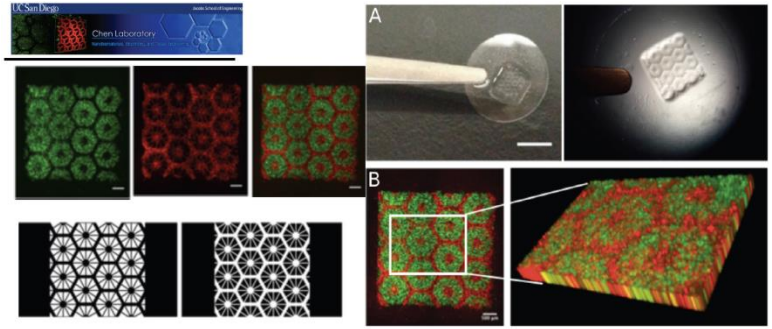


・該公司能列印出星狀細胞、內皮細胞等
・協助客戶進行藥物測試

Regenovo 杭州捷诺飞生物科技有限公司 Regenovo Biotechnology Co., Ltd.



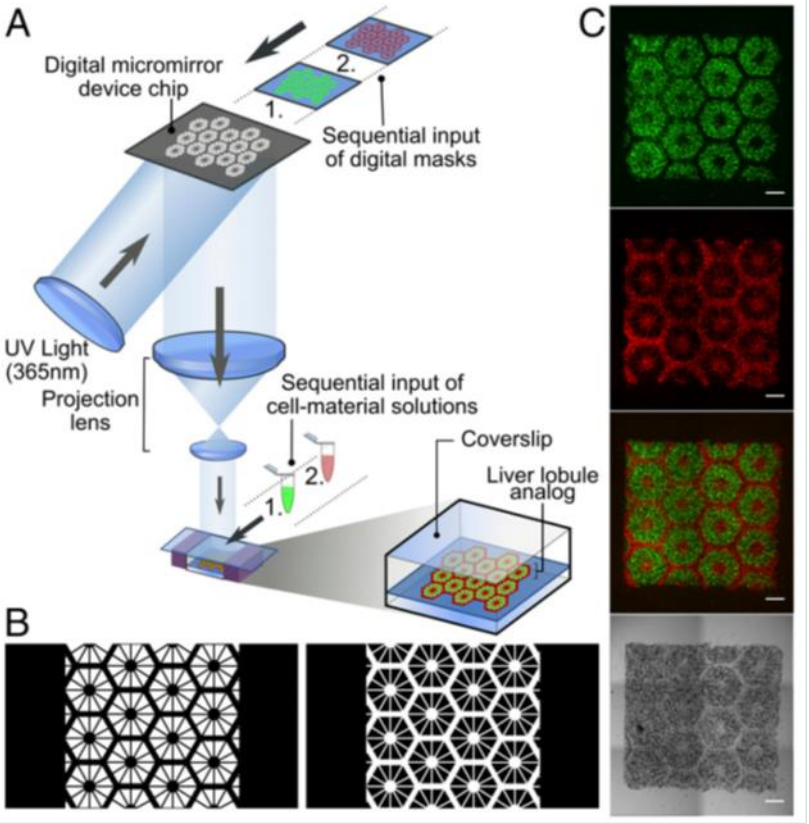
・徐銘恩教授列印出「肝單元」
・一個完整的肝臟大約是由50萬至100萬個肝單元組成



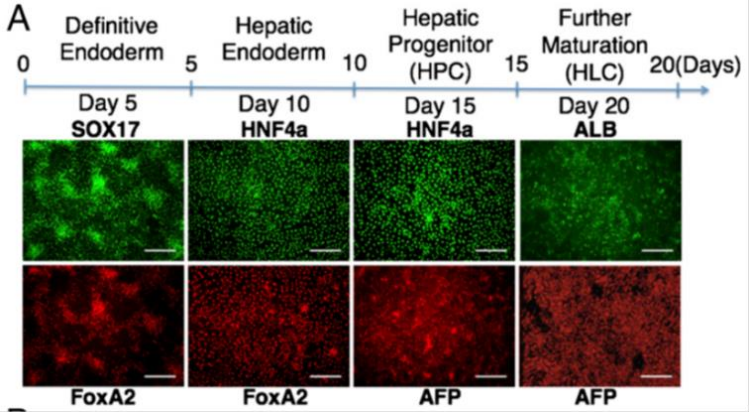
・利用幹細胞加上星狀/內皮細胞發展成肝組織
・進行更有效率的藥物測試
・GelMA (Methacrylic anhydride)甲基丙烯酸酐
・GMHA(glycidyl methacrylate hyaluronic acid) 甲基丙烯酸縮水甘油酯 玻尿酸

2015 Deterministically patterned biomimetic human iPSC derived hepatic model via rapid 3D bioprinting

3D printed liver

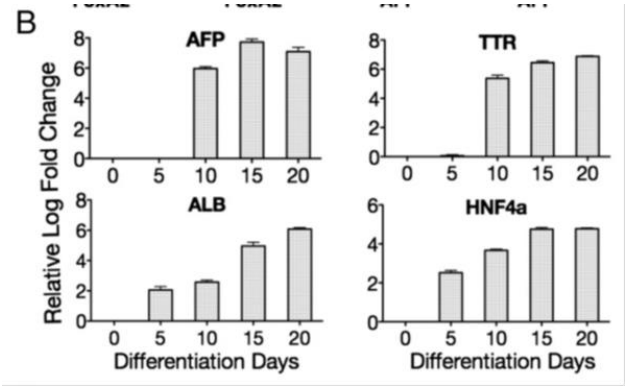


hiPSC-HPCs (green) in 5% (wt/vol) GelMA



supporting cells (red) in 2.5% (wt/vol) GelMA with 1% GMHA

To spatially pattern multiple types of cells and hydrogels, the digital masks were applied in a two-step sequential manner to create a first layer of hiPSC-derived hepatic cells supported by 5% (wt/vol) GelMA followed by a second complementary layer of supporting cells supported by 2.5% (wt/vol) GelMA and 1% GMHA (Fig. 1A).



Polymers and matrix used for liver tissue engineering

Table 1 Polymers and matrix geometry used for fabrication of scaffolds for liver tissue engineering

Material composition	Matrix geometry	Advantage	Disadvantage
PVA	Film coating [100]	Inert hydrophilic matrix, preserved functions of cryopreserved hepatocytes	Lacks any cell binding motif
PLGA	3D printed flow channels [101] foams [49, 50], collagen-coated nanoporous scaffold [102]	Biocompatible and biodegradability can be modulated by change in ratio of PLLA:PLGA; conducive environment for stem cell differentiation	Acidic degradation product, initiate peptide degradation and inflammation
PLLA	0.3 % collagen-coated nanoporous [52]	Maintained hepatic function for a period of 2–4 weeks, promote MSC differentiation to hepatocyte	
PLLA or PLGA coated with PVA	Porous scaffold [51]	Improved seeding due to hydrophilic coating	
Polydimethyl-sulfoxide	Membrane in microfabricated devices [98, 103, 104]	Oxygen permissible membrane, allow high cell seeding density	Highly hydrophobic thus can absorb biomolecules and reticulated PDMS might come into circulation. Permeable to water vapors thus can cause complete water evaporation and drying of devices
Polyurethane	Foam [105]	Easy chemical and mechanical modification, can be made biodegradable, facilitate mouse ES culture at high density and differentiation into hepatic lineage in BAL device	Degradation products are toxic
Polycaprolactone	Porous scaffold [57], nanofibers [106]	Inert, biocompatible and biodegradable	Highly hydrophobic thus uniform seeding of cells is difficult. Slow degradation rate
Polyethylene glycol	Hydrogels [107] microfabricated brushes [55], hydrogel microspheres for modular assembly [108] microencapsulation [28]	Hydrophilic, resistant to protein adsorption, amenable to chemical modification especially diacrylate facilitated crosslinking, addition of bioactive ligand, and modulation of polymer molecular weightt, can be polymerized in presence of cells	
Poly(*N*-isopropyl-acrylamide)	Grafted polymer chains [48]	Cell sheets can be obtained by modulation of surface properties via temperature	Becomes inelastic at physiological temperature
Polyethylene-terphalate	Films coated with ECM components [67] conjugation of galactose, RGD ligands [66]	Inert surface easily modified with bioactive ligand, oxygen permeable, used in drug screening platforms and BAL devices	Absence of cell adhesion ligand, non-biodegradable
Polyacrylamide	Inverted colloid crystal hydrogel [109]	Generates spheroid of controlled size and high yield	Non-biodegradable
Elastin-like polypeptides	Polyelectrolyte multilayer [58]	Precise control over scaffold composition, biodegradable, biologically relevant functionalization, stimuli responsive	Not cell adhesive needs functionalization
Natural polymers			
Chitosan	Hydrogel [34], porous scaffold, membrane [36], microfibers [37], nanofibers [38], microcarrier [35]	Resemble glycosoaminoglycans, promote spheroid formation Rat hepatocytes form immobile, 3D, flat aggregates on nanofibrous matrix and exhibit superior cell bioactivity with higher levels of liver specificity	Low mechanical strength, may be immunogenic
Fibrin gels	Hydrogel [110]	Hydrolytically degradable, coculture of human fetal liver cells and endothelial cells promote vascularization	Rapid degradation, low mechanical strength, immunogenic
Heparin	Hydrogel [61]	Natural, biocompatible, bioactive binds to growth factors suitable for implantable constructs	Low mechanical strength which can be modulated by incorporation of PEG
Matrigel	Coatings, films, gels [27, 111, 112]	Promotes spheroidal geometry and high expression of liver specific functions	Composition varies greatly
Self-assembling peptide	Nanofiber [113]	Promotes spheroid formation and	
Collagen	Dried films, gels [43, 114], sponges, foams [81]	Ample cell binding motif, native to liver, low antigenicity	Low mechanical strength and expensive
Alginate	Microencapsulation [115] porous hydrogel, microfluidic channels [88]	Hydrophilic, promotes spheroid formation, good for microencapsulation, cell seeding, is easy and less time consuming	Hydrophilic thus low adherence, may be immunogenic
Hyaluronic acid	Sponges [116], hydrogels [117], non-woven fabrics [45, 46]	Natural component of liver matrix, good substrate for coculture of hepatocytes, biocompatible	Low mechanical strength, highly viscous, difficult to work, fast tissue clearance
Native ECM	Decellularized biomatrix [62, 63]	Intact functional and structural components of native liver biomatrix promotes efficient cell function in vitro	Transplanted graft survival time is 2–8 days, initiates clotting in vivo due to exposed collagen

2014 Biomaterials for liver tissue engineering

Natural scaffold chemistry and modifications

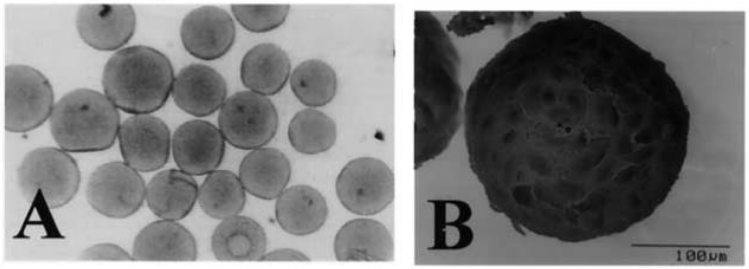
A wide variety of naturally-derived material scaffolds have been explored for liver tissue-engineering applications, including materials like collagen, peptides, fibrin, alginate, chitosan, hyaluronic acid, cellulose, decellularized liver matrix and composites of these

Early efforts in developing implantable hepatic constructs utilized collagen-coated dextran microcarriers that enabled hepatocyte attachment since hepatocytes are known to be anchorage-dependent cells

TABLE 46.5 Scaffolds utilized for hepatocellular constructs

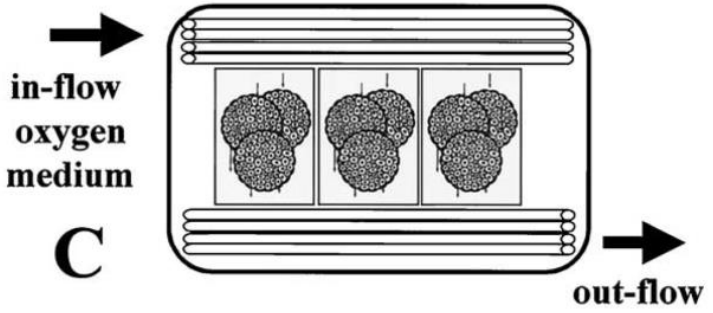
Category	Composition
Natural	Collagen, chitosan, collagen/chitosan composites, alginate, alginate composites, liver-derived biomatrix, peptides, hyaluronic acid, fibrin, gelatin, agarose
Synthetic	PLLA, PLGA, PLLA/PLGA composite, PGA, PEG, PCL, PET, PVA

Peptide(GRGSD)-modified cellulose

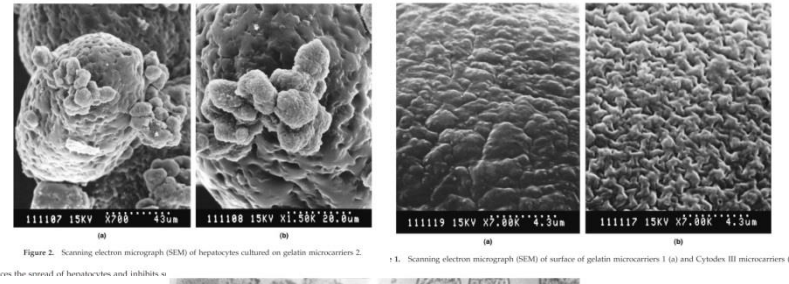


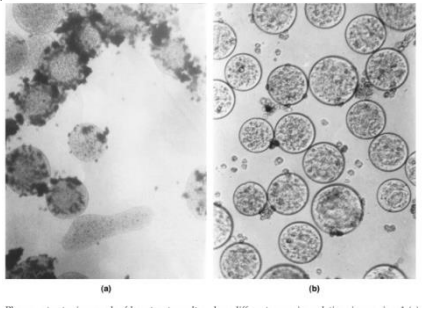
Cell attachment

Collagen-coated or **peptide**-modified cellulose, gelatin, and **gelatin**-chitosan composite microcarrier chemistries have also been explored for their capacity to promote hepatocyte attachment



Gelatin



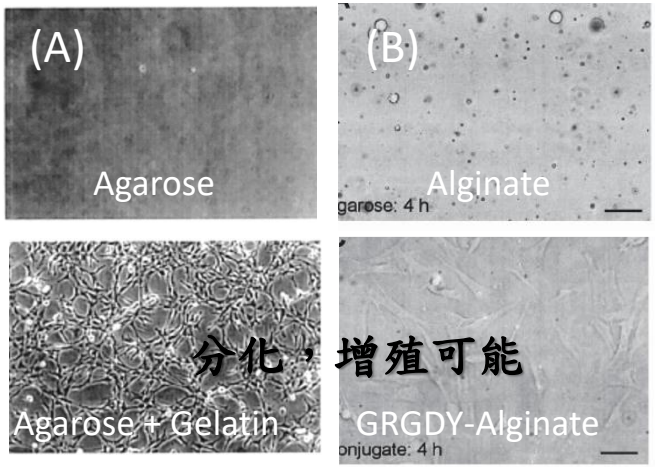


2003 Development of a cellulose-based microcarrier containing cellular adhesive peptides for a bioartificial liver

2003 Preparation and culture of hepatocyte on gelatin microcarriers

Poorly cell adhesive

Materials that are poorly cell adhesive like alginate have been exploited for their utility in promoting hepatocyte-hepatocyte aggregation



2000 Hepatocyte behavior within three-dimensional porous alginate scaffolds

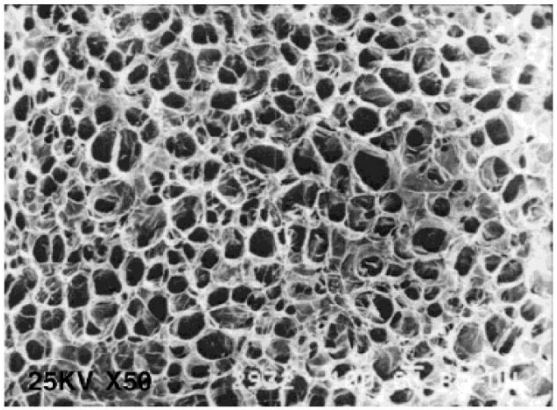
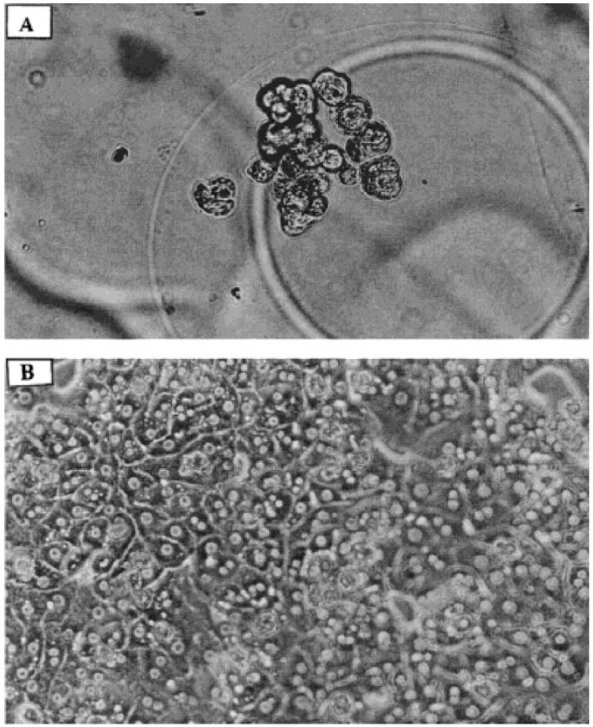


Figure 1. Scanning electron micrograph of a cross-section of the alginate sponge used in the study (—100 μm).



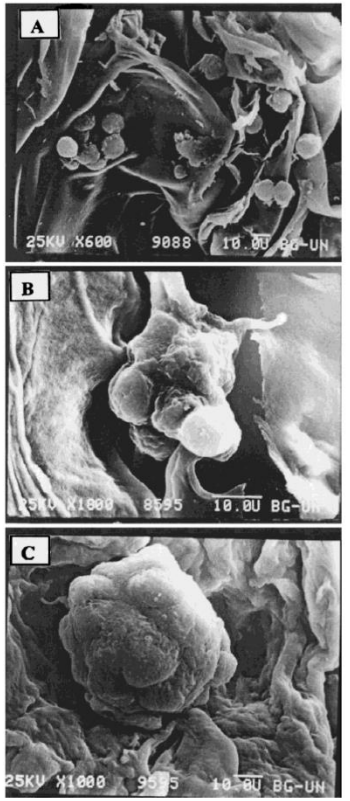


Figure 4. Scanning electron micrographs of hepatocytes within the alginate sponges at different times post seeding: (A) 1 day; (B) 4 days; (C) 7 days.

Synthetic scaffold chemistry

In contrast to biologically-derived material systems, synthetic materials enable precisely customized architecture (porosity and topography), mechanical and chemical properties, and degradation modality and kinetics

Synthetic materials that have been explored for liver tissue engineering include:

1. poly(L-lactic acid) (PLLA)
2. Poly(D, L-lactide-co-glycolide) (PLGA)
3. poly(Ɛ-caprolactone) (PCL)
4. poly(ethylene glycol) (PEG)

Advantage

The potential to finely tune its degradation time due to differences in susceptibility to hydrolysis of the ester groups of tis monomeric component (lactic acid and glycolic acid)

Disadvantage

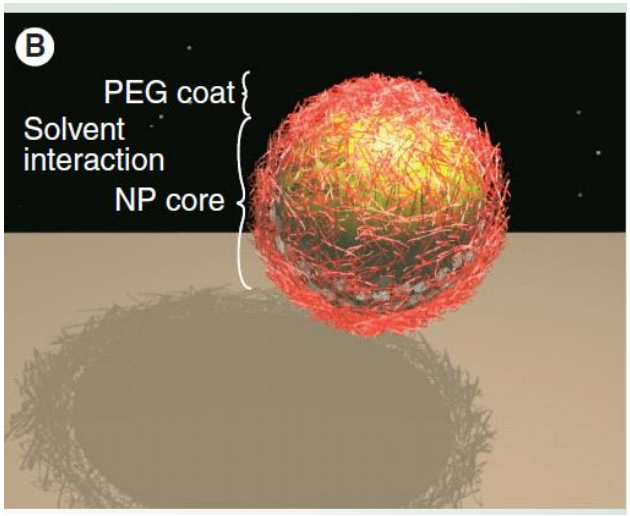
The accumulation of hydrolytic degradation products (lactic acid and glycolic acid) has been shown to produce an acidic environment within the scaffold which initiates peptide degradation and stimulates inflammation, which may affect hepatocyte function

PEG-based hydrogels

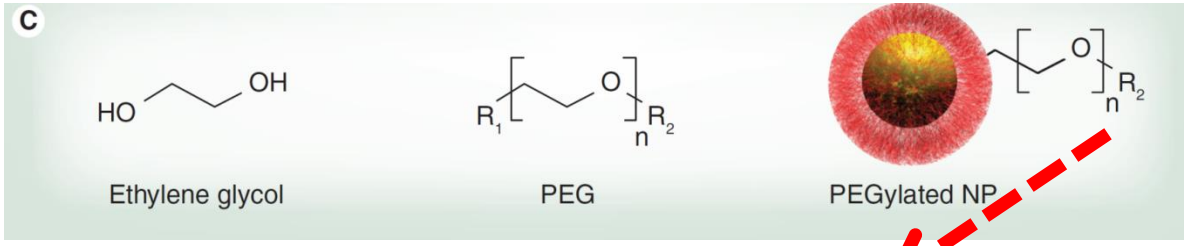
PEG-based hydrogels have been increasingly utilized in liver tissue-engineering applications due to their high water content, hydrophilicity, **resistance to protein adsorption**, biocompatibility, ease of chemical modification, and the ability to be polymerized in the presence of cells, thereby enabling the fabrication of 3D networks with uniform cellular distribution

- PEG-based hydrogels have been used for the encapsulation of diverse cell types, including immortalized and primary hepatocytes and hepatoblastoma cell lines.
- The encapsulation of primary hepatocytes requires distinct material modifications (e.g., 10% w/v polyethylene glycol (PEG) hydrogel, inclusion of RGD adhesive motifs) as detailed below, as well as, analogous to 2D co-culture systems, the inclusion of nonparenchymal supporting cell types such as fibroblasts and endothelial cells

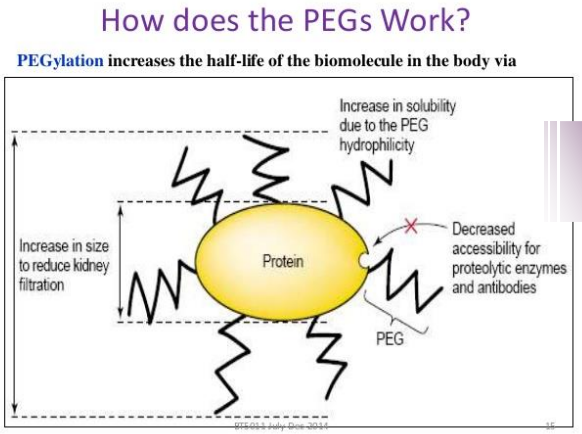
PEGylation



Reticuloendothelial system (RES) 網狀內皮系統



PEG contains the linkage group (R_1) and a terminus that interacts with solvent (R_2)



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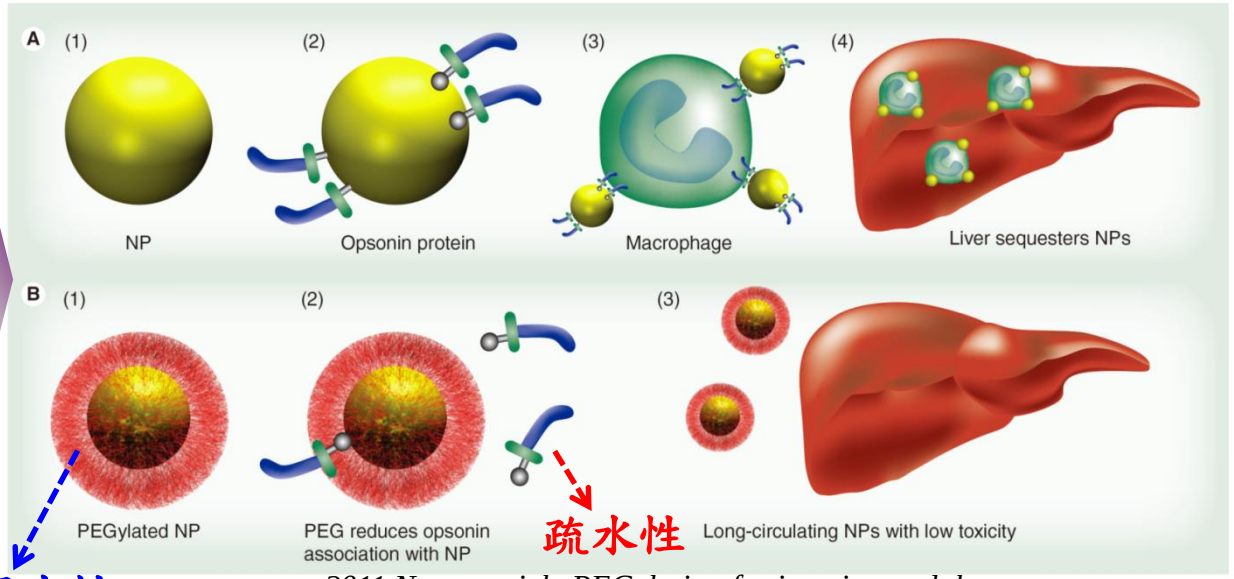

2011 Nanoparticle PEGylation for imaging and therapy