Introduction to the Liver

Lecture 1

Presenter

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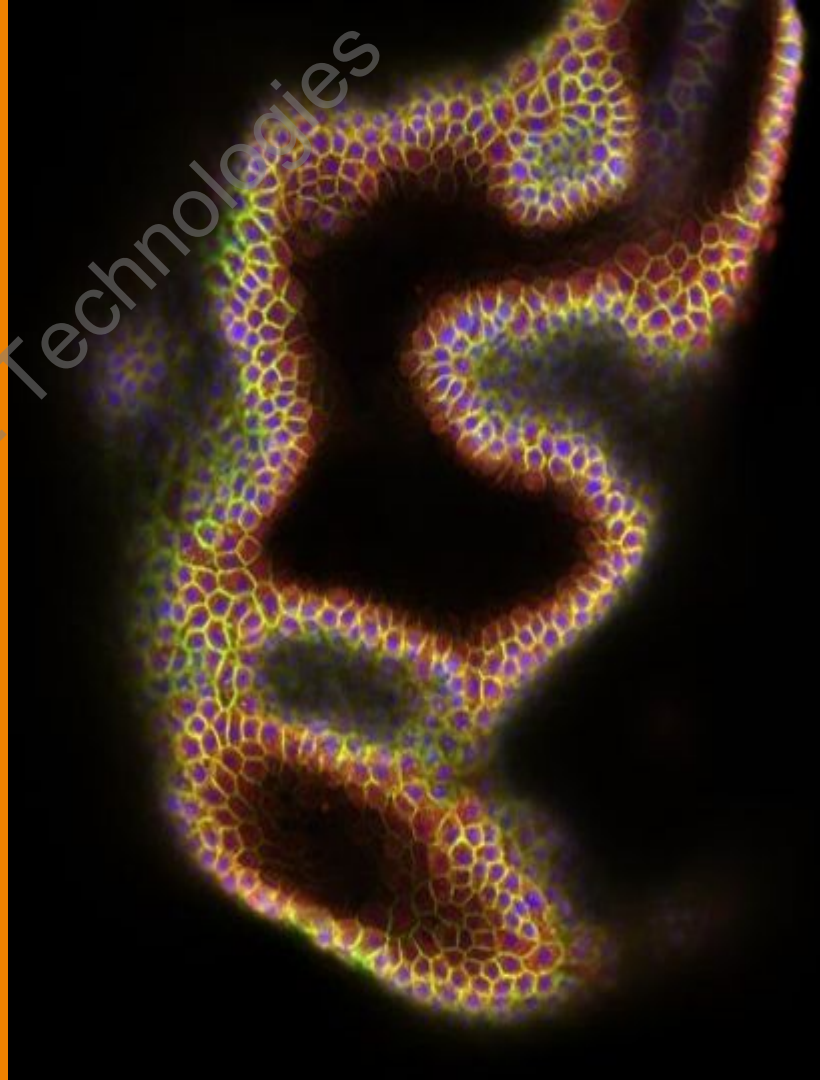
Scientist, Scientific Support, Epithelial Cell Biology



Learning Objectives

After this session, you should be able to:

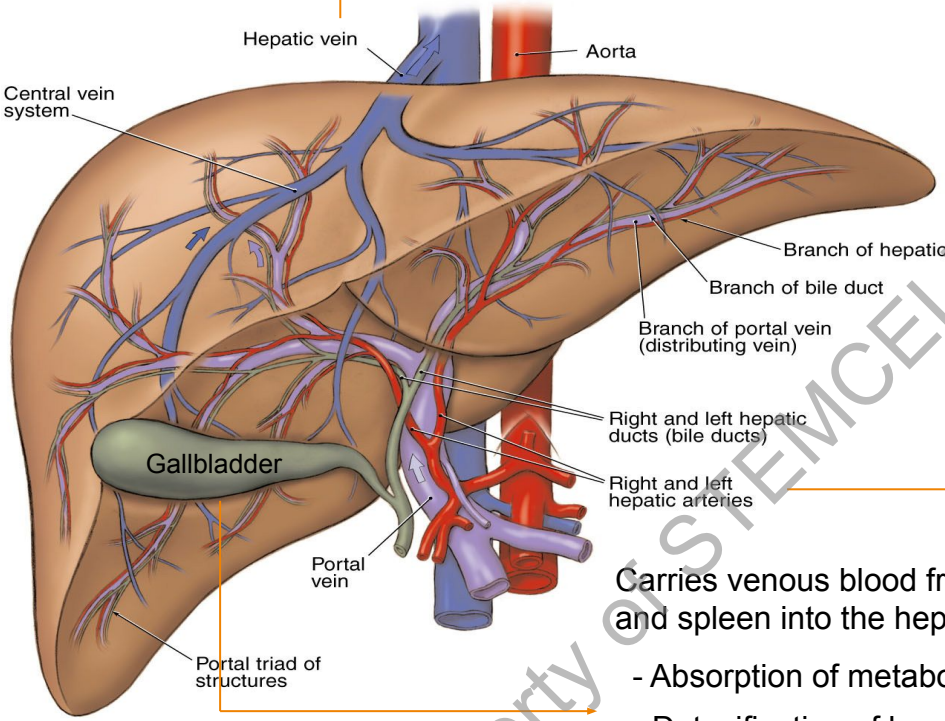
- Understand the basic biology and functions of the liver
- Describe the liver research landscape and the history of organoid systems
- Describe different liver culture methods

Outline

1. Introduction to Liver Biology
2. Liver Culture and Model Systems

Section 1 | Introduction to Liver Biology

Introduction to the Liver: Hepatic Anatomy and Vasculature



Carries deoxygenated blood and blood which has been filtered by the liver to the inferior vena cava and into the heart.

Hepatic/portal triad

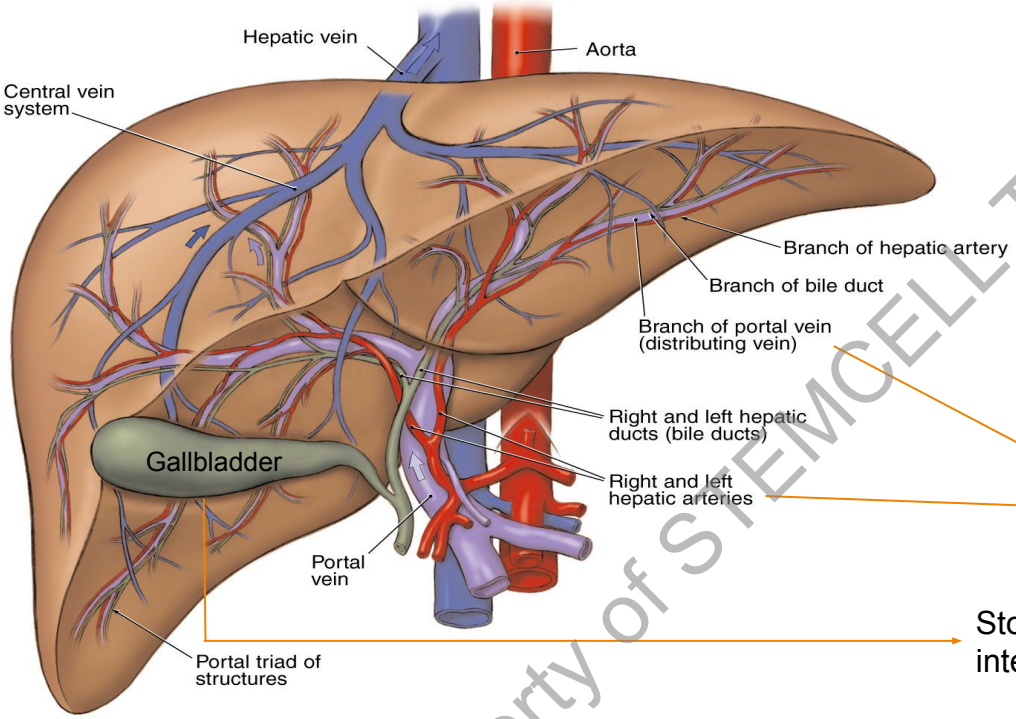
Supplies oxygenated blood to the liver

Carries venous blood from the gastrointestinal tract, gallbladder, pancreas, and spleen into the hepatic sinusoids and drained by the hepatic vein

- Absorption of metabolic substrates and nutrients
- Detoxification of harmful ingested substrates before reaching the systemic circulation

Source: @2007 MediVisuals, Inc.

Introduction to the Liver: Hepatic Anatomy and the Biliary System



Drain and collect bile produced by hepatocytes and carry it to the gallbladder

Stores bile and releases it into the small intestine when food is being digested

Source: @2007 MediVisuals, Inc.

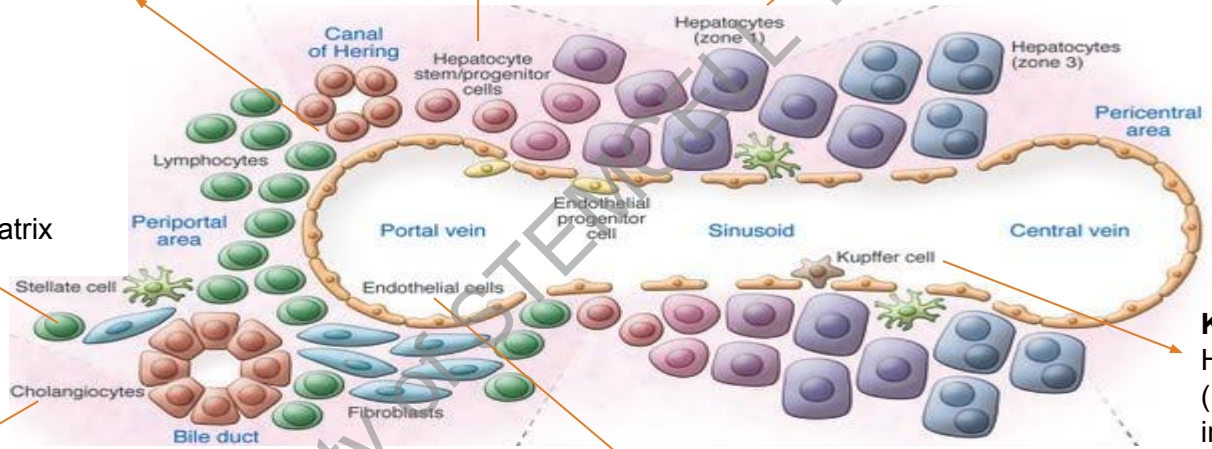
Introduction to the Liver: Hepatic Cell Types

The liver is composed of several cell types, of which hepatocytes make up the most cell mass:

Hepatocytes (~60-80%):
- Hormone production
- Synthesis of bile, serum proteins, lipids, carbohydrates
- Detoxification
- Absorption and storage of nutrients

Progenitor cells:
Regeneration

Lymphocytes:
B cells, T cells and NK cells



Stellate cells:
Maintain extracellular matrix

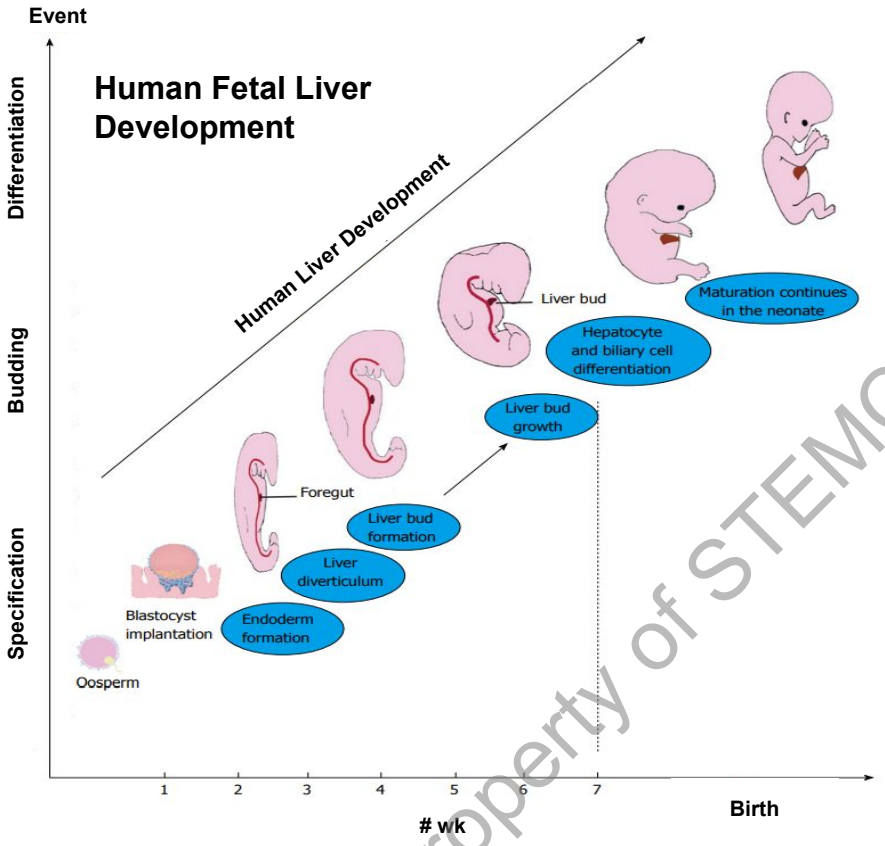
Kupffer cells:
Hepatic macrophages (phagocytosis and immune response)

Cholangiocytes:
- Line the bile ducts
- Control bile flow rate and bile pH

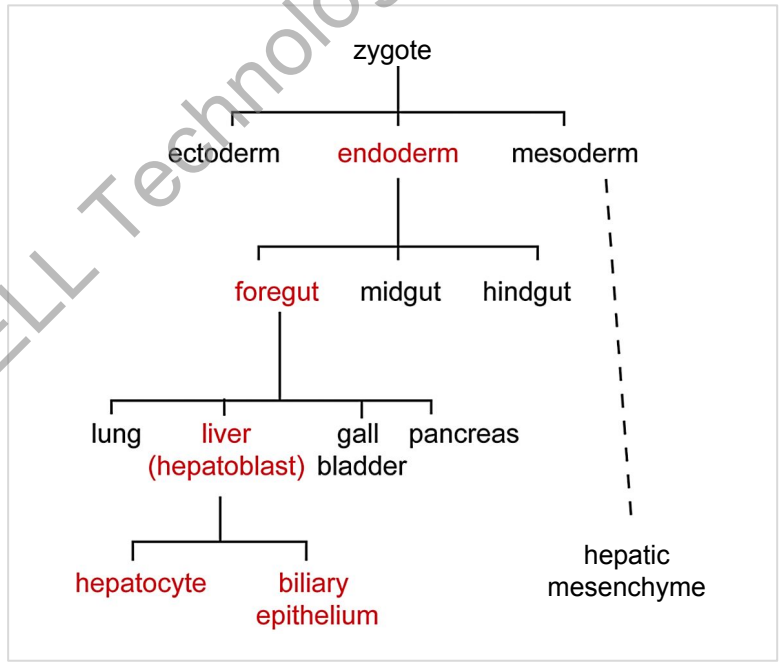
Endothelial cells:
Vasculature formation

Yamashita T et al (2013) J Clin Invest. 123(5): 1911-8.

Introduction to the Liver: In Vivo Organogenesis



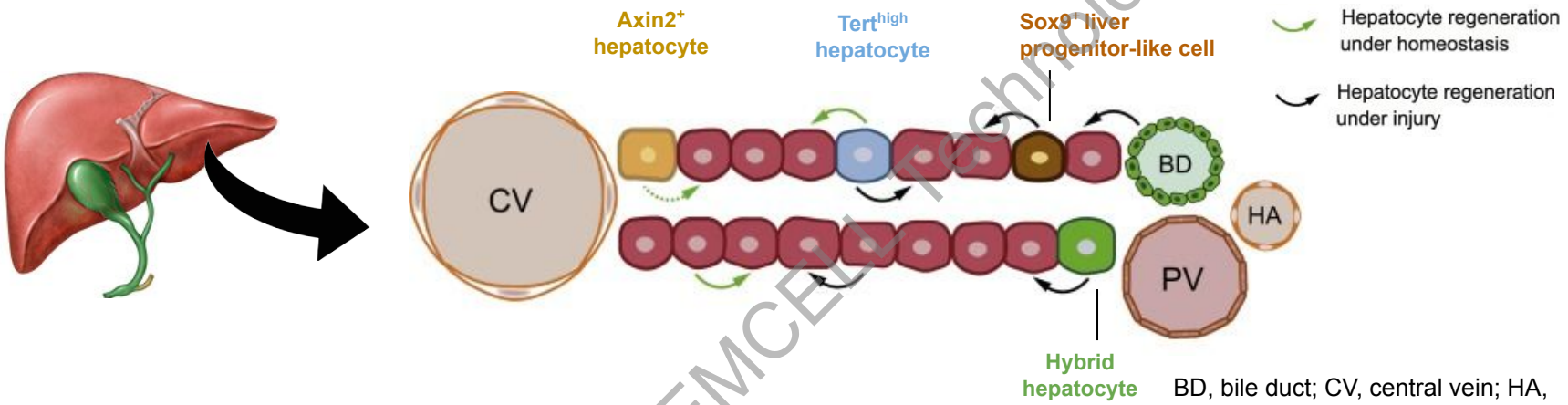
Zhou WL et al. (2012). World J Gastroenterol. 18(17): 2018-25.



Liver Development *in vivo* is directed through:
Endoderm → Foregut → Hepatoblasts

Figure adapted from StemBook

Introduction to the Liver: Homeostasis and Regeneration



BD, bile duct; CV, central vein; HA, hepatic artery; PV, portal vein.

- Hepatocyte regeneration during **homeostasis**
  - slow self-duplication of existing hepatocytes
- Hepatocyte regeneration after **injury**
  - increased self-duplication of existing hepatocytes
  - transdifferentiation of biliary epithelial cells

Type and location of injury direct the cell source involved in hepatic regeneration

Li W et al. (2020) Trends Cell Biol. 30(4):329-338.

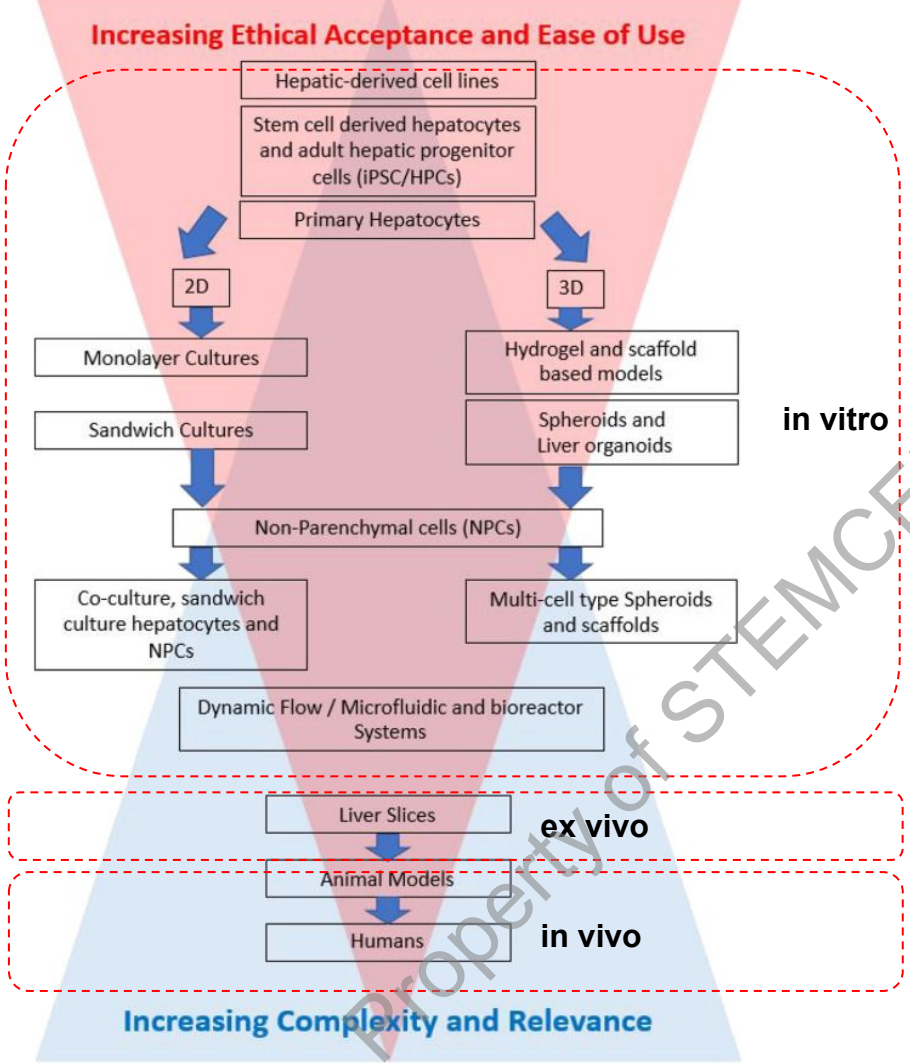
Metabolic Functions for the Liver

Albumin Secretion	• Most abundant blood protein in mammals (12 g/day in humans) • Buffers pH; regulates oncotic pressure in blood vessels • Water-soluble, monomeric, plasma carrier (e.g. hormones, fatty acids, bilirubin, drugs, etc)
Detoxification via CYP3A4	• Metabolizes ~60% of prescribed drugs • Absent in fetal liver, CYP3A4 increases to ~72% of adult levels by 12 months • Substrate activation varies between species, gender, etc
Bile Acid Synthesis	• Produced via P450-mediated oxidation of cholesterol • Total bile acids comprised of cholic acid and chenodeoxycholic acid • Absent until final stages of fetal development, incompletely developed function at birth
Urea Synthesis	• Product of amino acid metabolism, for excretion of nitrogenous waste • Glutamine → ammonia → urea, via the urea cycle

Summary

- The liver is composed of two parenchymal cell populations: hepatocytes and cholangiocytes
- Non-parenchymal cells interact with hepatocytes/parenchymal cells for function
- The liver has diverse functions: synthesis and secretion, storage and biotransformation
- The liver is a relatively stable organ, with slow turnover under homeostasis. Hepatic injury can trigger the regeneration of up to 3/4 of the organ mass

Section 2 | Liver Culture and Model Systems



Introduction to Liver Culture Methods

- Range of models used for liver studies, including in vitro, ex vivo and in vivo models
- Models range in complexity, physiological relevance, and ease of use
- More complex and relevant models are often technically challenging to work with

Kyffin JA et al. (2018) Toxicol In Vitro 48: 262-275.

Introduction to Liver Culture Methods

Cell Source	**Immortalized Cell Lines (i.e. HepG2)**
Culture Formats	• Adherent monolayers • Spheroids • Organ-on-a-chip
Features	• Cheap, convenient, proliferative • Ease of scale-up • Well-established assay protocols • Historical characterization • Compatible with cryopreservation
Challenges	• Limited maturity, physiological relevance • Karyotypically abnormal • Represents a single donor profile • Serum-free workflows lacking

Introduction to Liver Culture Methods

Cell Source	Pluripotent Stem Cells (PSCs)
Culture Formats	• Adherent monolayers • Spheroids • Organoids • Organ-on-a-chip
Features	• Renewable source of liver cells • Varied donor profiles, no need for primary tissue access • Relevance for developmental studies • Protocols simple to follow • Suitable for the generation of complex isogenic models • PSCs and organoids compatible with cryopreservation and banking
Challenges	• High variability between protocols • Fetal phenotype • Limited protocols for cryopreservation, sorting, or scaling in 2D • Organoids require Matrigel® (or equivalent ECMs)

Introduction to Liver Culture Methods

Cell Source	Primary Hepatocytes (PHHs)
Culture Formats	• Adherent monolayers • Spheroids • Organoids • Organ-on-a-chip
Features	• Current gold standard • Representative of *in vivo* functionality and maturity • Well-established assay protocols • Historical characterization • Characterized in complex disease models • Can represent varied patient profiles
Challenges	• Limited/costly access to donor samples • Complex isolation and culture protocols • Subject to rapid dedifferentiation in culture • Single-use, cannot be cryopreserved once thawed • Lack of protocols supporting robust cell proliferation • Organoids require Matrigel® (or equivalent ECMs)

Introduction to Liver Culture Methods

Cell Source	**Hepatic Tissue (e.g. biopsies, resections)**
Culture Formats	• Intrahepatic Cholangiocyte Organoids (ICOs) • Suspension using dilute ECM • Organ-on-a-chip • Ex-vivo tissue slice cultures
Features	• Retention of liver structure, cells, and function • ICOs highly proliferative, genetically stable, compatible with cryopreservation, banking • Representative of in vivo metabolic maturity • Represent varied patient profiles • Suitable for patient-representative disease models and precision medicine studies
Challenges	• Challenging to access tissue • Culture success affected by ischemia • ICOs must be differentiated for mature functionality • Limited assay protocols for ICOs • Organoids require Matrigel® (or equivalent ECMs) • (Ex-vivo slices) short duration of studies (< 5 days) due to necrosis

Introduction to Liver Culture Methods

Cell Source	Animal Models
Culture Formats	• Not applicable
Features	• Systemic model for research questions, includes immune cells • Supports lineage-focused studies • Suitable for long-term studies investigating safety and efficacy
Challenges	• Costly and time consuming to set up • Limited throughput • Often does not translate to humans • Ongoing efforts to implement 3R principles (replacement, reduction, and refinement) for animal research

FDA Modernization Act

- The FDA Modernization Act 2.0 provided more clarity on the acceptability of in vitro tests, making it explicit that they are accepted in lieu of animal studies as long as the data submitted proves they are equally capable of assessing risk

- The act gives companies the ability to submit results obtained with different testing methods, such as computer models and in vitro assays, instead of animal testing when they are making new drug submissions

- It is likely that this act will push pharmaceutical companies to explore, invest in, validate, and use a variety of in vitro systems including organoids, organs-on-chip, and PSC-derived models

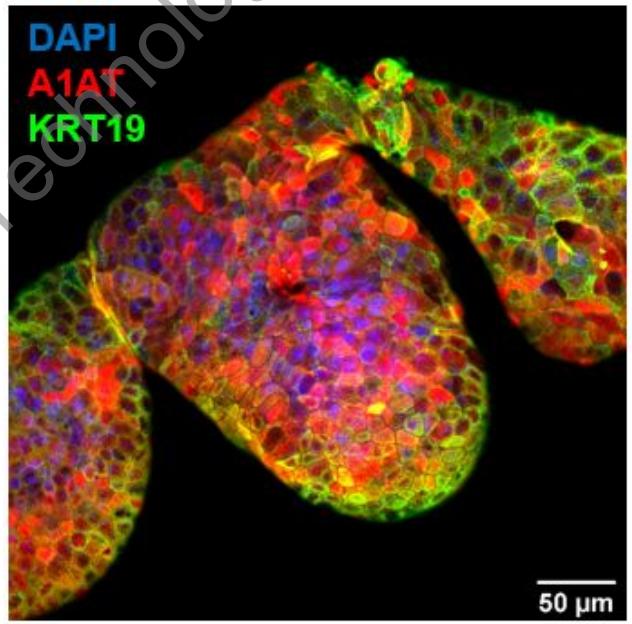
Due to the FDA modernization act, in vitro culture methods like organoids could become more attractive.

Organoid Definition

Organoid or·gan·oid (ôr'gə-noid')
Resembling an organ. n.
Complex, organized, and functional

1. Contains multiple organ-specific cell types.
2. Capable of recapitulating certain functions of the organ.
3. Cells are grouped together and spatially organized similar to an organ.

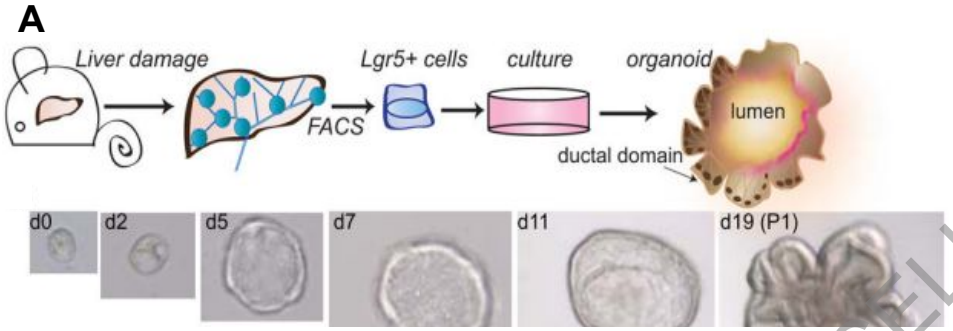
Can be derived from iPSCs, ESCs, adult-derived stem cells, or differentiated cell types



Hepatic organoids cultured in HepatiCult™ Organoid Differentiation Media (Human) (STEMCELL Technologies).

Lancaster MA et al (2014) Science 345(6194): 1247125.
Marsee A et al (2021) Cell Stem Cell 28(5): 816-832.

First publication of Mouse Liver Organoids

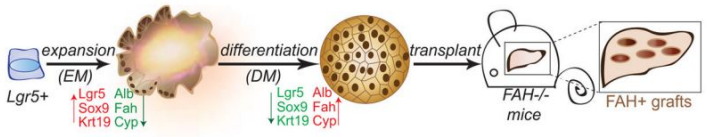


In vitro expansion of Lgr5 cells from adult liver tissue

Published in final edited form as:
Nature. 2013 February 14; 494(7436): 247–250. doi:10.1038/nature11826.

In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration

Meritxell Huch[1,*], **Craig Dorrell**[3,*], **Sylvia F. Boj**[1], **Johan H. van Es**[1], **Marc van de Wetering**[1], **Vivian S.W. Li**[1], **Karien Hamer**[1], **Nobuo Sasaki**[1], **Milton J. Finegold**[4], **Annelise Haft**[3], **Markus Grompe**[3], and **Hans Clevers**[1,5]

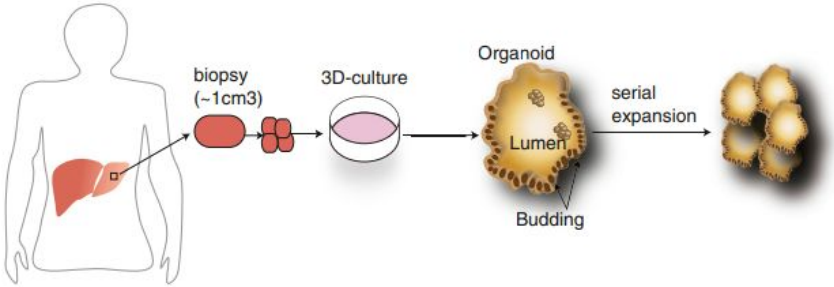


Schematic showing transplantation

Huch M et al. (2013) Nature 494 (7436): 247-50.

First publication of Human Liver Organoids

Protocol Overview

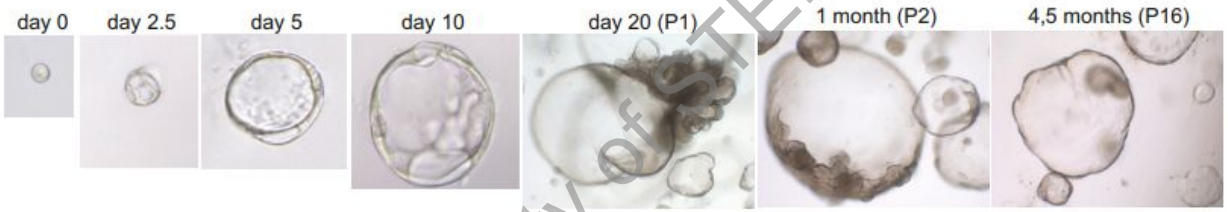


Article

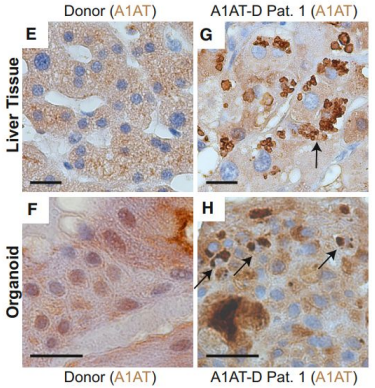
Long-Term Culture of Genome-Stable Bipotent Stem Cells from Adult Human Liver

Meritxell Huch,[1,9,10,*] Helmuth Gehart,[1,9] Ruben van Boxtel,[1,9] Karien Hamer,[1] Francis Blokzijl,[1] Monique M.A. Verstegen, Ewa Ellis,[7] Martien van Wenum,[3] Sabine A. Fuchs,[4] Joep de Ligt,[1] Marc van de Wetering,[1,8] Nobuo Sasaki,[1] Susanne J. Boers,[3] Hans Kemperman,[5] Jeroen de Jonge,[2] Jan N.M. Ijzermans,[2] Edward E.S. Nieuwenhuis,[4] Ruurdtje Hoekstra,[3] Stephen Strom,[6] Robert R.G. Vries,[1,8] Luc J.W. van der Laan,[2] Edwin Cuppen,[1] and Hans Clevers[1]
[1]Hubrecht Institute-KNAW, University Medical Centre Utrecht, CancerGenomics.nl, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands

Organoid Establishment and Expansion



Disease Modeling



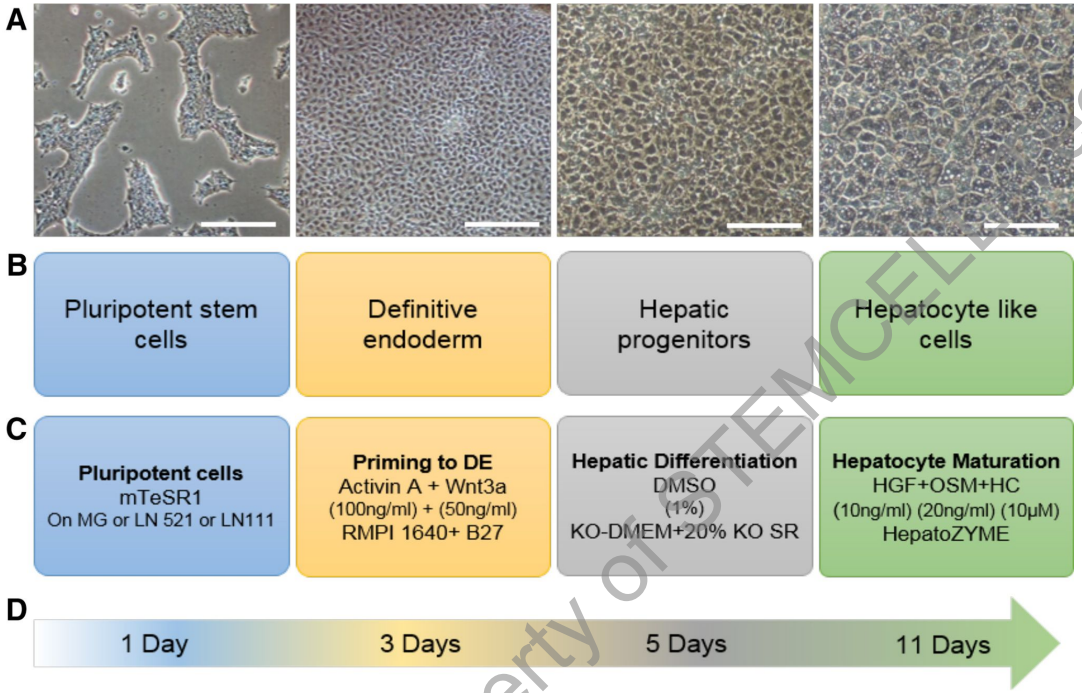
Huch et al (2015) Cell 160(1-2): 299-312.

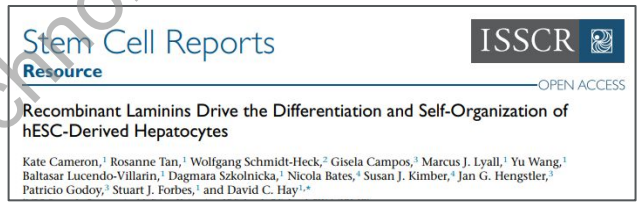
Publications Generating “Hepatocyte-Like Cells” from PSCs/iPSCs

Due to challenges accessing primary tissue and hepatocytes, and due to the renewable and donor-representative nature of hPSCs, researchers have explored the possibility of generating human hepatocytes from hPSCs through directed differentiation.

Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells	**Production of hepatocyte like cells from human pluripotent stem cells**	**Recombinant laminins drive the differentiation and self-organization of hESC-derived hepatocytes**	**Hepatic differentiation of human pluripotent stem cells in miniaturized format suitable for high-throughput screen**
Si Tayeb et al (2010)	Hannan et al (2013)	Cameron et al (2015)	Carpentier et al (2016)

Differentiation of PSCs Towards Hepatocytes




Stem Cell Reports
Resource
ISSCR
OPEN ACCESS

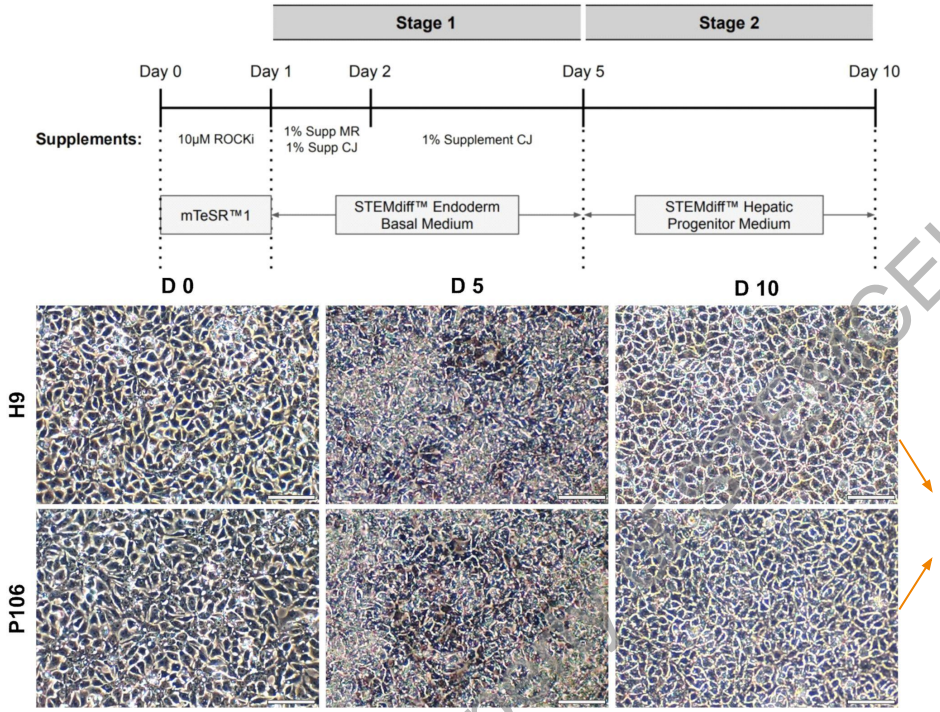
Recombinant Laminins Drive the Differentiation and Self-Organization of hESC-Derived Hepatocytes

Kate Cameron,[1] Rosanne Tan,[1] Wolfgang Schmidt-Heck,[2] Gisela Campos,[3] Marcus J. Lyall,[1] Yu Wang,[1] Baltasar Lucendo-Villarin,[1] Dagmara Szkolnicka,[1] Nicola Bates,[4] Susan J. Kimber,[4] Jan G. Hengstler,[3] Patricio Godoy,[3] Stuart J. Forbes,[1] and David C. Hay[1,*]

- Directed differentiation of hPSCs towards hepatocyte-like cells under defined conditions
- Improvement in hepatocyte phenotype and functionality using two laminin isoforms, laminin 521/laminin 111

Cameron K et al. (2015) Stem Cell Reports 5(6): 1250-1262.

Hepatic Progenitor Specification from PSCs Using a Defined Differentiation System



Key Stages

- Seed PSCs (H9)/iPSCs (P106) as single cells into LN-521 (laminin)-coated plates
- **Stage 1** = Differentiation into Definitive Endoderm (DE) using STEMdiff™ Definitive Endoderm Kit
  - Day 5 = Assess for DE induction
- **Stage 2** = Hepatic Specification using STEMdiff™ Hepatic Progenitor Medium
  - Day 10 = Detect expression of hepatic progenitor-specific markers

Hepatic progenitors display a cobblestone-like cell morphology

This JOVE paper is a result of a collaboration between STEMCELL and David Hay (University of Edinburgh) using the recently launched STEMdiff™ Hepatocyte Kit

Meseguer-Ripolles J et al. (2020) J Vis Exp: 159

Summary

- There are a variety of culture systems commonly used to study the liver, including:
  - Immortalized cell lines
  - Primary hepatocytes
  - Hepatic cells derived through the directed differentiation of PSCs
  - Animal models

- Published protocols describe the generation of primary tissue-derived mouse and human liver organoids capable of reproducing mature hepatic functionality

- These organoids have demonstrated applications in disease modeling, compound screening, and transplantation in mouse models

- PSC-based directed differentiation protocols can:
  - Recapitulate liver organogenesis in vitro
  - Provide an alternate source of liver cells
  - Serve as a model system for the study of liver development

Thank You

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