Hepatology Snapshot:

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2D and 3D liver models

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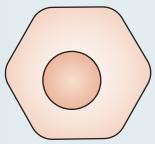
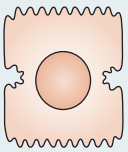
Hepatic cell types

Hepatic cell lines



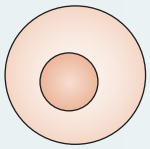
- Expandable, affordable
- Imperfect replicate of hepatocyte biology

Primary human hepatocytes



- Limited by cell sourcing
- Short-lived hepatic phenotype and functions

iPSC-derived hepatocytes



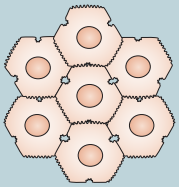
- Potentially unlimited cell source
- Only fetal-like hepatocyte state attained by current protocols

2D culture methods

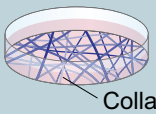
Strategies to improve and maintain longevity, function and/or phenotype

Mimicking hepatic microenvironment

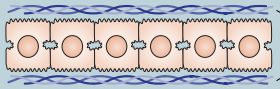
High density culture



ECM-coated surface

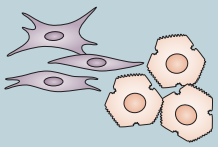


Sandwich culture



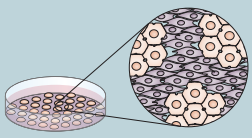
Addition of other cell populations

Non-parenchymal cell co-culture



Prolongs hepatic function of most hepatic cell types

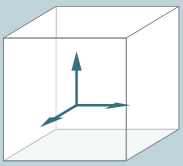
Micro-patterned co-culture



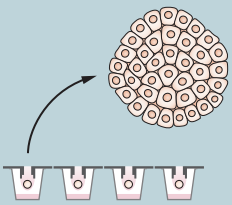
- Spatial patterning of different cell populations
- Requires expertise and equipment

3D culture methods

Techniques to create 3D microtissues

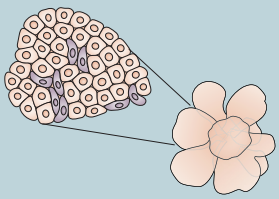


Spheroids



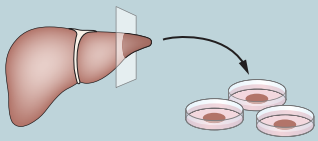
- Prolongs hepatic phenotype and function

Organoids



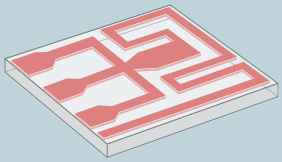
- Potential for long-term models
- Currently functionally immature or biliary in phenotype

Liver slices



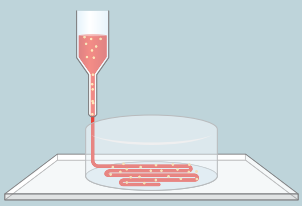
- Cultured for up to 2 weeks
- Retain cellular diversity
- Patient-to-patient variability

Microfluidic devices



Recapitulate important mechanical stimuli missing from *in vitro* models

3D printed tissues



Bioprinted models incorporate parenchymal and support cells in spatially patterned arrangements

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The liver performs hundreds of essential functions for the human body, including protein synthesis, nitrogen metabolism, detoxification, and drug metabolism. Yet hepatocytes, the primary functional cell of the liver, have been historically difficult to culture *ex vivo*. To overcome this hurdle, a variety of complementary *in vitro* liver models have been introduced. These models, which can be broadly categorized into 2D *vs*. 3D models, now offer a sizable toolkit with which to study human hepatic biology and disease. Culture format restrictions and hepatic functionality (*e.g.,* albumin secretion, cytochrome induction, and urea secretion) stratify each model, though none of them are both simple to use and effective for predicting all hepatic functions. Future work must focus on model affordability/accessibility, throughput, and long-term maintenance of a broad range of hepatic functions.

2D adherent cultures

Cells from various hepatic cell sources are plated onto a cell culture dish as a monolayer. The simplicity of 2D approaches makes them particularly amenable for high-throughput studies, such as pharmaceutical screening.

Hepatic cell lines have been developed from liver tumors and immortalized hepatocytes, including HepaRG, HepG2, HuH-7, and others. These cell lines are expandable and affordable, making them especially useful for studies that require large numbers of cells. However, most cell lines exhibit abnormal liver-specific functions (*e.g.,* drug metabolism), are genetically abnormal, and imperfectly replicate all aspects of hepatocyte biology.[1]

Primary human hepatocytes can be isolated from human or other mammalian livers. These cells retain their hepatic phenotype and functions for a short period of time (days) in a 2D monolayer but then progressively dedifferentiate.[2] Primary hepatocyte morphology and functional stability can be prolonged in 2D by incorporating elements that mimic the hepatic microenvironment, such as culturing hepatocytes at high-density to increase cellular interactions, and/or growing cells on a collagen-coated surface or between 2 layers of extracellular matrix, known as *sandwich culture*.[1] However, even in these formats hepatic function declines within a few days in culture, limiting use to short-term studies of function and acute toxicity. Furthermore, this model continues to be limited by cell sourcing, as long-term expansion of functional adult primary human hepatocytes *in vitro* has not yet been achieved.

iPSC-derived hepatocytes: Induced pluripotent stem cells (iPSC) can be differentiated into hepatocyte-like cells by recapitulating developmental signaling networks *in vitro*. While iPSC-derived hepatocytes offer a potentially unlimited cell source, current differentiation protocols attain only a fetal-like hepatocyte state, which precludes studies of adult hepatic biology.[1,3]

Addition of other cell populations in co-culture: Addition of a variety of stromal or non-parenchymal liver cell populations to hepatic cultures prolongs hepatic function of most of the hepatic cell types above. Additionally, further spatially patterning these different cell populations in 2D culture (*e.g.*, *micropatterned co-culture*[2,4]) stabilizes hepatic morphology and function for weeks, enabling chronic toxicity and metabolism studies not possible with other 2D models. However, this method requires expertise and equipment not easily accessible to all laboratories.[1]

3D culture

3D culture offers functional and morphological advantages by recapitulating cell-cell and cell-extracellular matrix interactions that are absent in 2D culture, though these methods are often more difficult to translate into high-throughput approaches.

Spheroids: Primary hepatocytes can form spheroids through culture in hanging drops, microwells, non-adherent plates, or other aggregation strategies. Spheroid culture prolongs maintenance of hepatic phenotype and function, which is further improved by co-aggregation with stromal or non-parenchymal cells.[4–6]

Liver organoids: Hepatic cells can transiently proliferate and self-organize to form liver organoids that mimic the structure and function of human liver when cultured under specific media and matrix conditions. Various organoid models have been developed from numerous hepatic cell types that vary in their similarity to human hepatocytes, including iPSC-derived hepatoblasts,[7] hepatic stem/biliary cells,[8] and primary fetal and pediatric hepatocytes.[9] Though hepatic organoid culture shows potential to generate long-term models that conserve liver structure and function, current organoids remain functionally immature or biliary in phenotype.

Liver slice culture: Resected liver biopsies can be sliced into thin tissue sections and cultured *ex vivo* for up to 2 weeks. Liver slices retain the cellular diversity of the liver and maintain *in vivo* cellular architecture but are subject to major patient-to-patient variability and cellular death after resection and culture.[10]

Organ on a chip and microfluidic models: Using fabrication methods such as soft lithography, hepatocytes with or without non-parenchymal cells can be spatially patterned in 3D. Fluid flow can be introduced to recapitulate important mechanical stimuli that are missing from most other *in vitro* models. While such models offer physiologic improvements, they can be labor-intensive to make and maintain, limiting their throughput.[1,4]

Bioprinted liver: By marrying 3D printing technologies with cytocompatible biological “inks”, engineers can bioprint tissue models that incorporate parenchymal and support cells in spatially patterned arrangements. For example, advances in stereolithography[11] and sacrificial scaffolds[12] have enabled early models of the liver, though future improvements to printing materials and resolution are still needed to accurately replicate liver architecture.

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Authors’ contributions

SHS and KS: conceptualization, writing, and figure design.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.06.022.

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Author names in bold designate shared co-first authorship

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