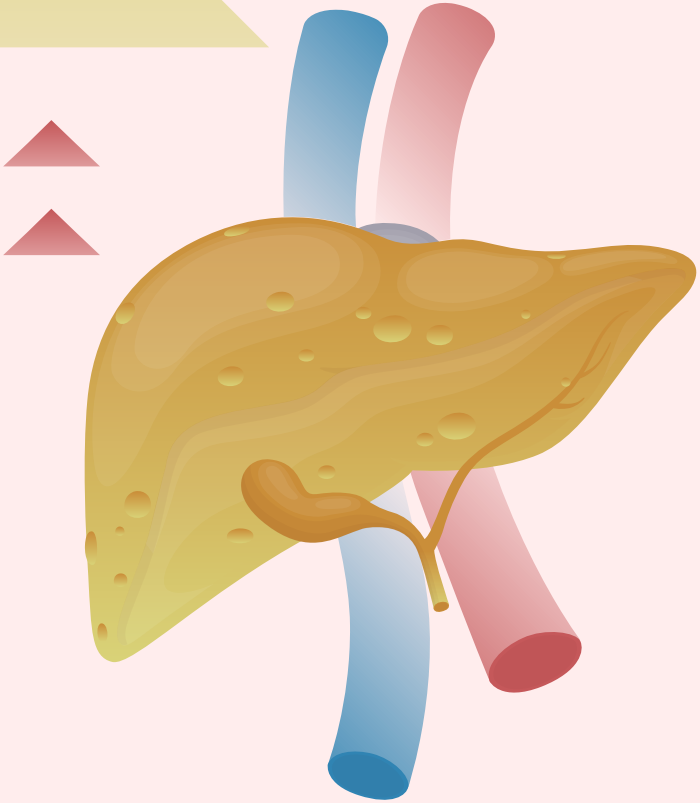


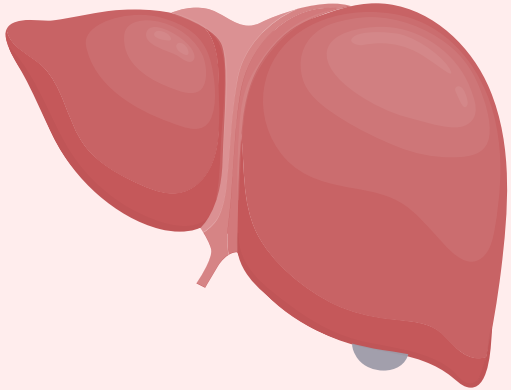
Liver On A Chip for Drug Toxicity Testing

Allison, Marguerite , Olasubomi, Samantha, and Semele

Table of contents

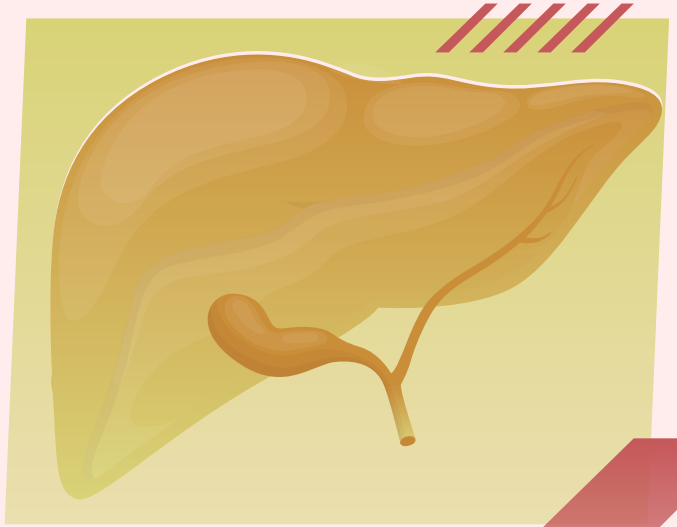
01

Drug Toxicity and Hepatotoxicity



Liver & Drugs

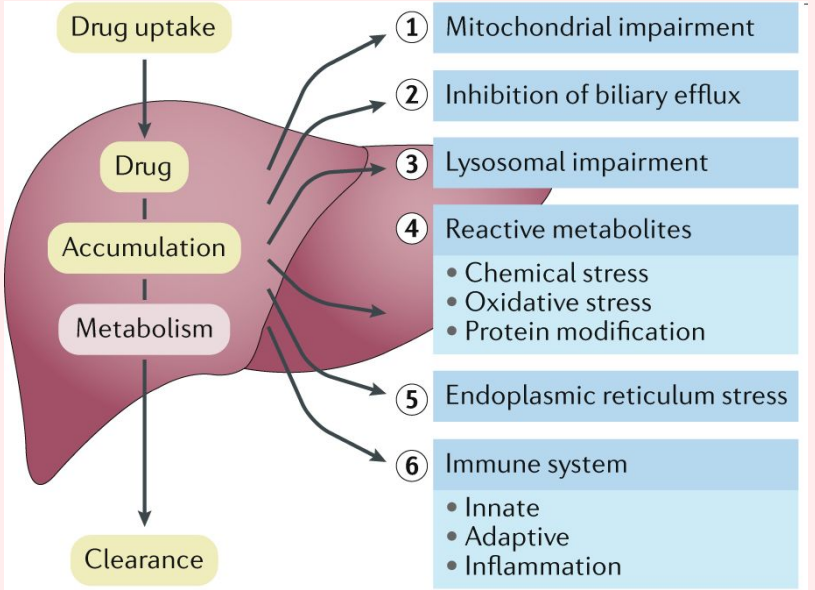
The liver is the second largest organ in the body behind your skin and it is the primary site for drug metabolization. It breaks downs drugs into forms that are easier for the body to use and are non toxic



Hepatotoxicity

Drug induced liver injury (DILI)

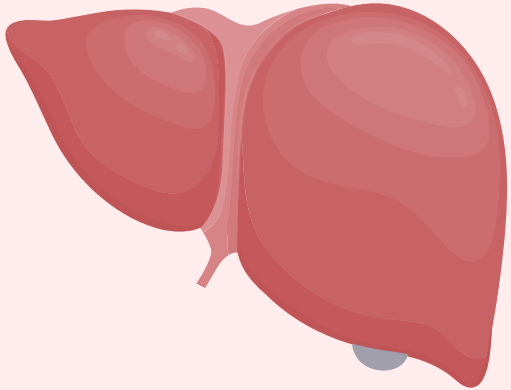
- Failure of the liver to clear drugs leading to accumulation and hepatocyte death
- Impairs mitochondrial function
- Accounts for 13% of all acute liver failures in the US
- Currently on the rise in developed nations



https://www.nature.com/articles/s41573-019-0048-x

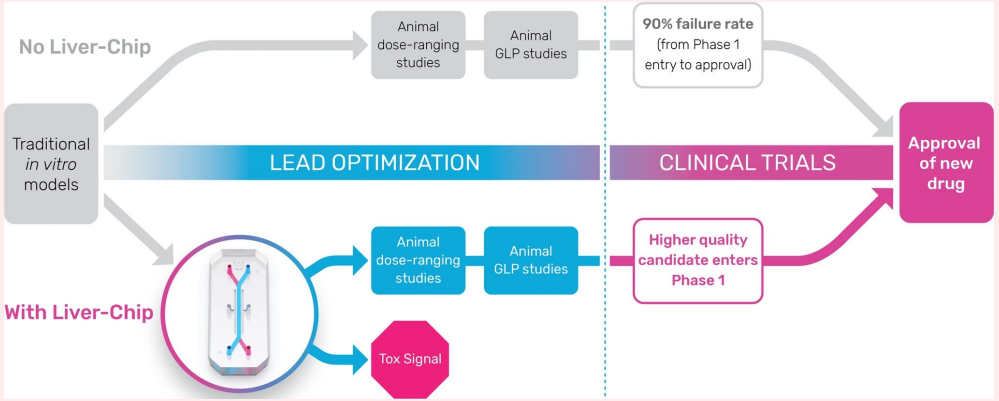
02

Relevance of Liver on a Chip in Toxicology



Drug Safety & Efficacy Testing

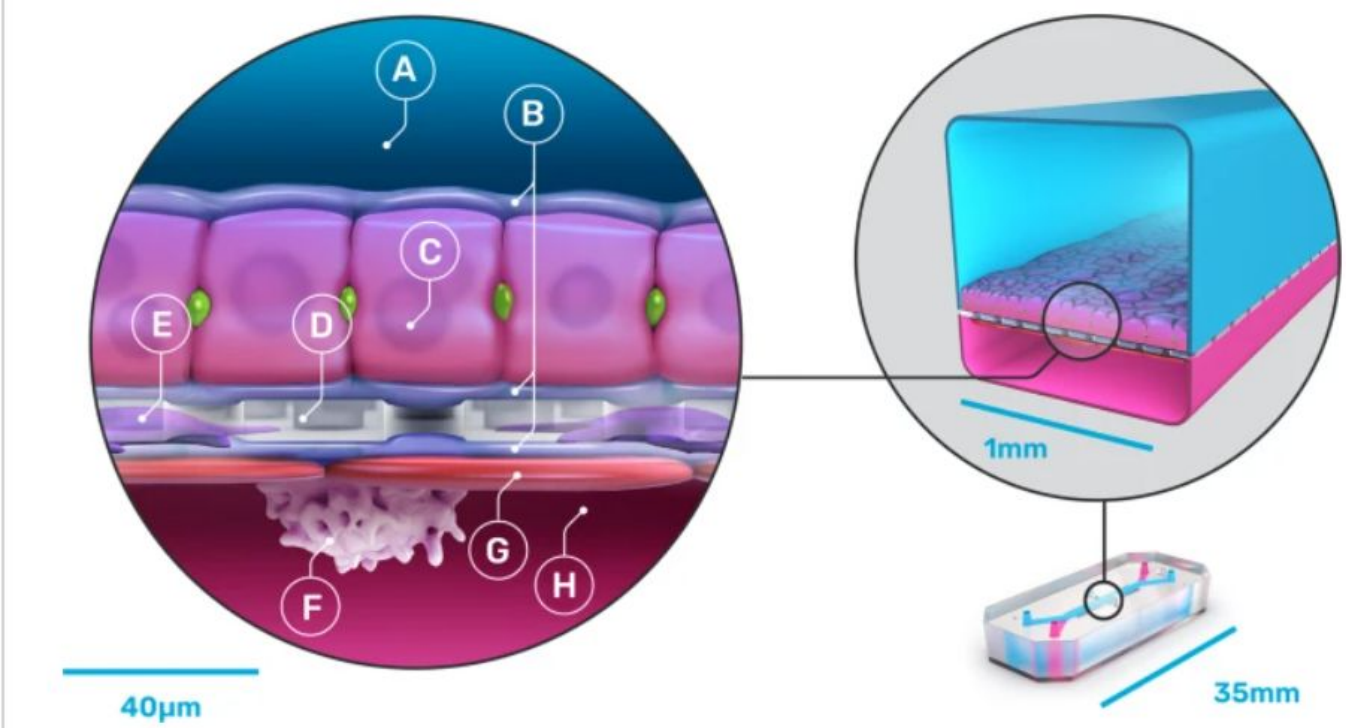
- Human liver toxicity is the most common cause of failure for a drug in preclinical trials
- Organ-on-a-chip models have been able to faithfully recreate complex functions and pathophysiology of human organs
- Species-specific Liver-on-a-Chip models have been shown to recapitulate human models better than cross-species live models
- Provide an ethical alternative to live animal models



https://www.nature.com/articles/s43856-022-00209-1

Comparison to current models

- Live animals models only correctly predict toxicity in humans around 70% of the time
- The ability to predict DILI is much lower, as there is lower concordance between animal-human models
- While testing bosentan-mediated DILI that same plasma concentration of bosentan was found in the liver-on-a-chip model that induced toxicity in human patients
- In a comparison to 3D hepatocyte spheroids, a commonly used FDA clinical trial for drugs, liver-chips were about to predict DILI in 12/15 (80%) drugs while the spheroids only predicted in 8/19 (42%)
- Liver-chip models did not mark any false positives were as 3D hepatic sprehorids did (with 67% specificity)

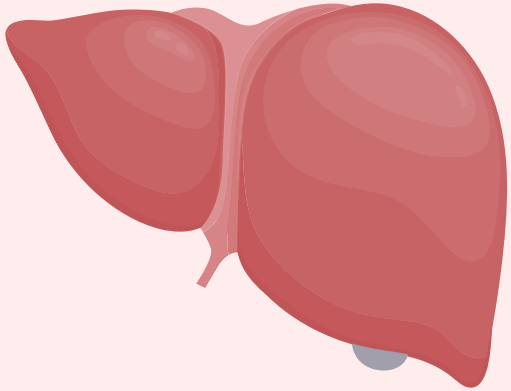


This diagram shows primary human hepatocytes (C) that are sandwiched within an extracellular matrix (B) on a porous membrane (D) within the upper parenchymal channel (A), while human liver sinusoidal endothelial cells (G), Kupffer cells (F), and stellate cells (E) are cultured on the opposite side of the membrane in the lower vascular channel (H).

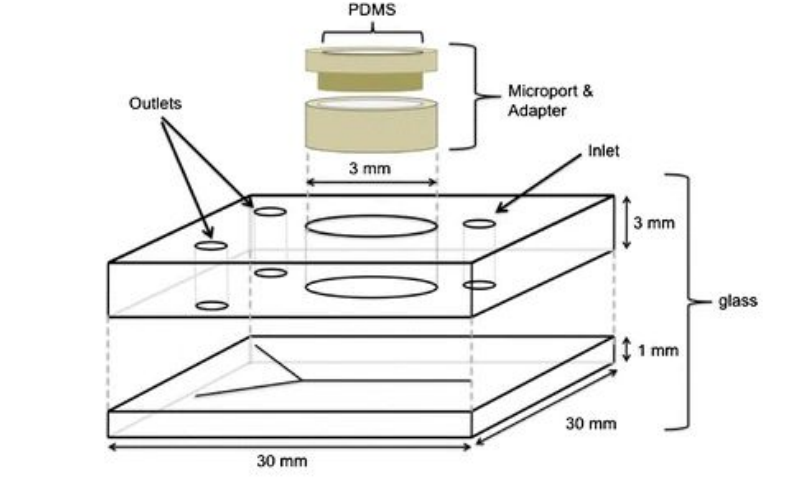
https://www.nature.com/articles/s43856-022-00209-1

03

BioMEM Techniques used for Organ Specificity and Building



Study of ethanol induced toxicity in liver explants using microfluidic devices



Hattersley, S.M., Greenman, J. & Haswell, S.J. Study of ethanol induced toxicity in liver explants using microfluidic devices. *Biomed Microdevices* **13**, 1005–1014 (2011). https://doi.org/10.1007/s10544-011-9570-2

Microfluidic chip fabrication

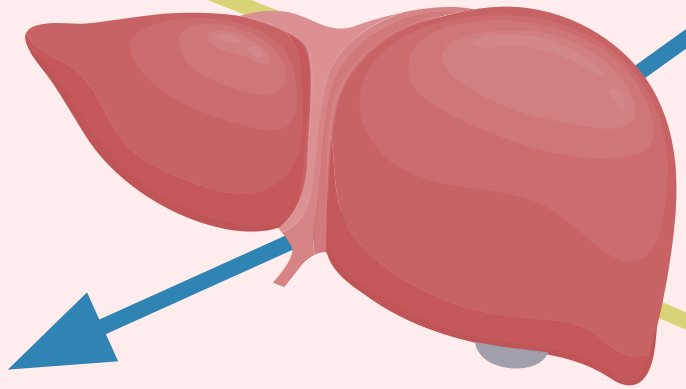
- Choosing the glass
- Cutting the glass
- Preparation of mask
  - AutoCAD LT software
  - Photo-reduction of laser print drawing
- Exposure to photoresist
- Development of pattern
  - 50% microposit and 50% DI water
- Wet etching
  - Hard bake → 4 hr → 120 °C
  - 1% hydrofluoric acid with 5% ammonium fluoride solution at 65 °C
  - Etch rate 100 µm/hr
- Post-etch cleaning
- Preparation of top plate
  - Thermal bonding → 600 °C

McCreedy, T. (2001). Rapid prototyping of glass and PDMS microstructures for micro total analytical systems and micro chemical reactors by microfabrication in the general laboratory. *Analytica Chimica Acta*, *427*(1), 39-43. https://doi.org/10.1016/S0003-2670(00)01174-0

Primary Cell Types to Replicate

Hepatocytes

Liver Sinusoidal Cells (LSECs)

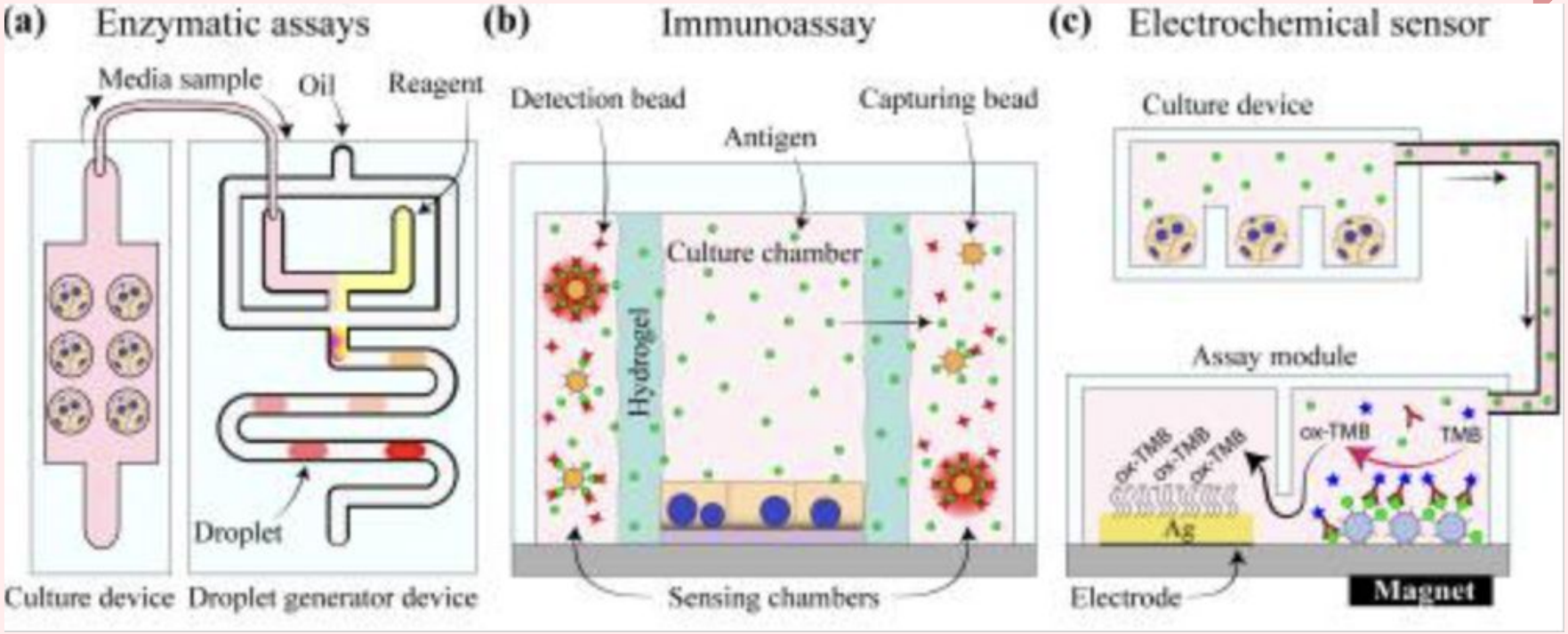


Hepatic Stellate Cells (HSCs)

Kupffer Cells (KCs)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6843249/

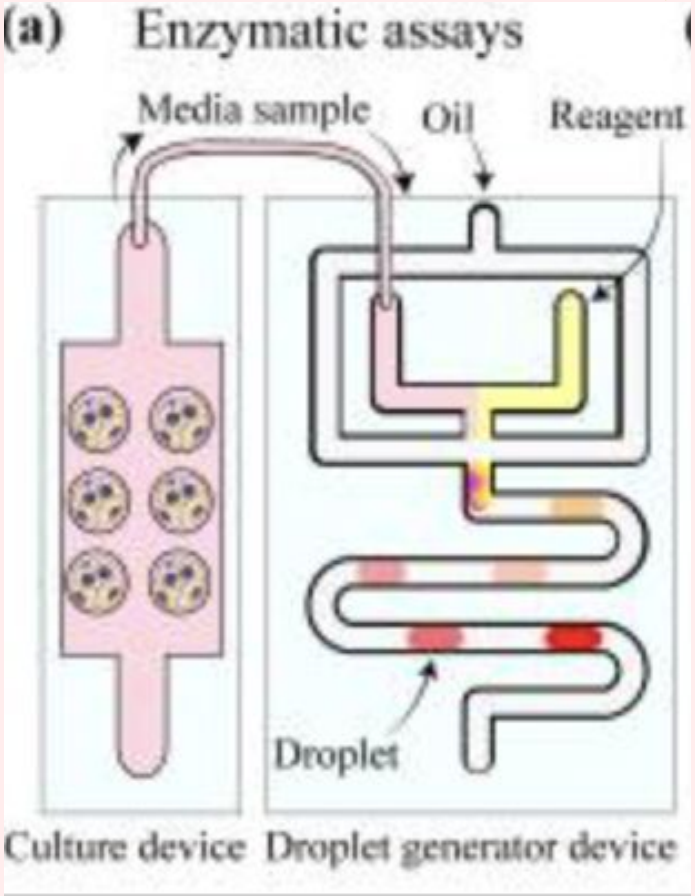
Functional Analysis – Biosensing



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8519630/

Analysis Method 1

A microfluidic droplet generator device used for analysis of media conditioned by hepatocytes.

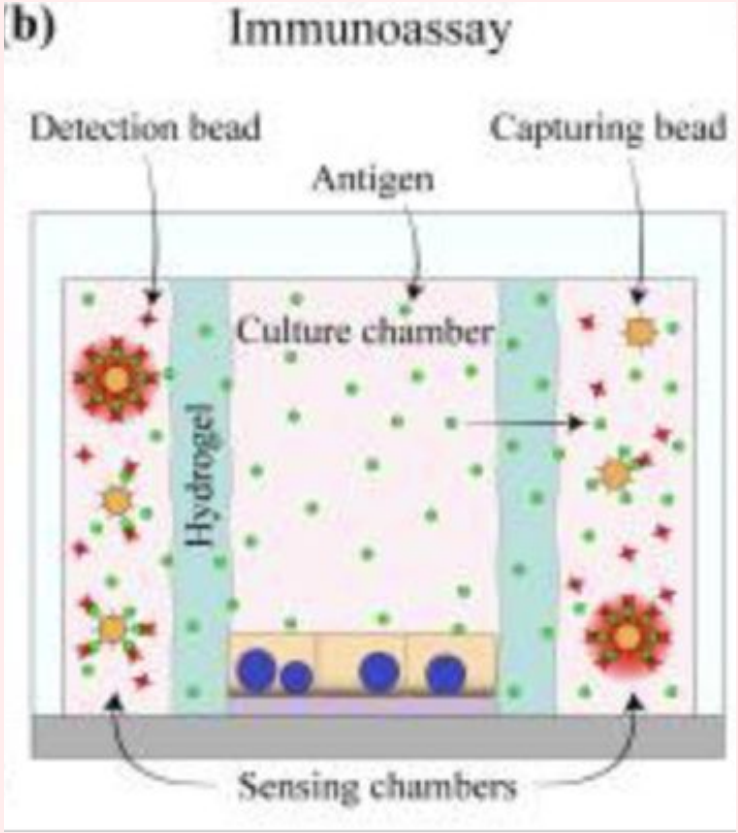


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8519630/

Analysis Method 2

A microfluidic hepatocytes culture device with a **hydrogel permeable layer** allowing for diffusion of proteins.

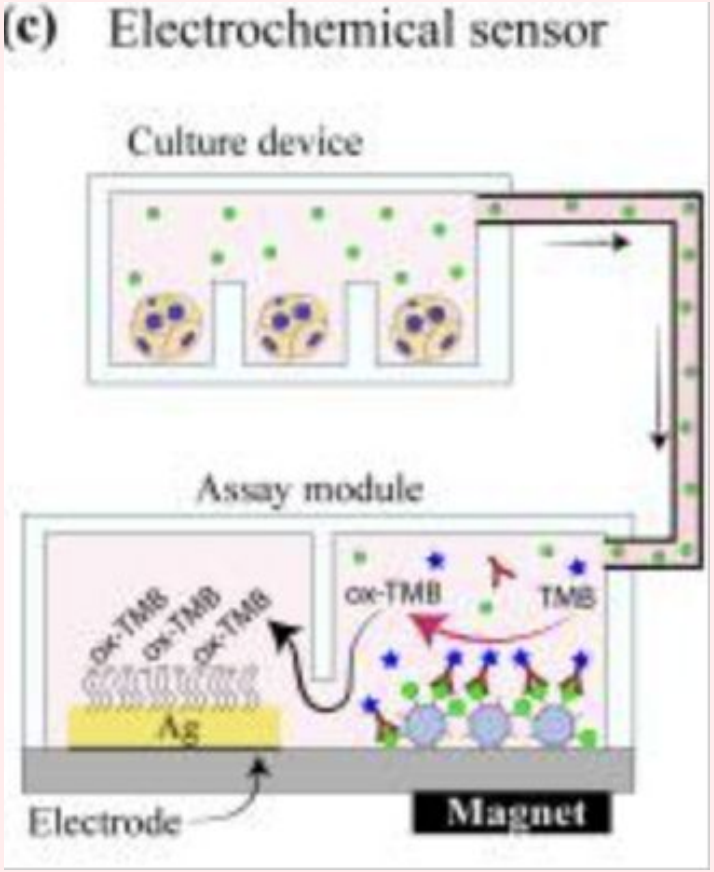
Bead based immunoassay to detect secreted growth factors.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8519630/

Analysis Method 3

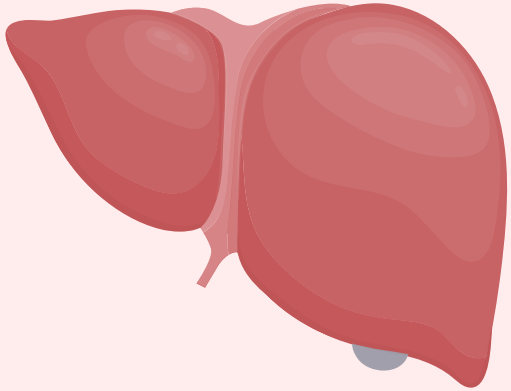
Magnetic beads capture proteins, label with HRP, and color with TMB



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8519630/

04

Methods for Testing Biocompatibility

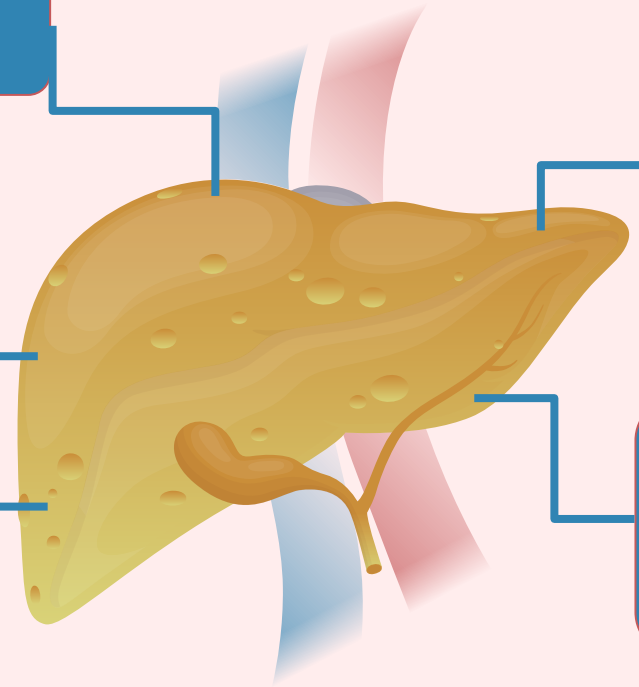


What is Biocompatibility?

- Is the material safe?
  - Replaces animal testing
  - More accurate account of human interaction
- Does it have the necessary components for its intended function?
  - Includes human hepatocytes, liver sinusoidal endothelial cells, kupffer cells, and stellate cells
  - Extracellular matrix that separates the model
  - A porous membrane to allow for drug delivery

https://wyss.harvard.edu/news/liver-chip-identifies-distinct-drug-toxicities-in-human-rat-and-dog-models/

Better than Animal Testing?



More species specific

Accelerates drug development process

More cost effective as research goes on

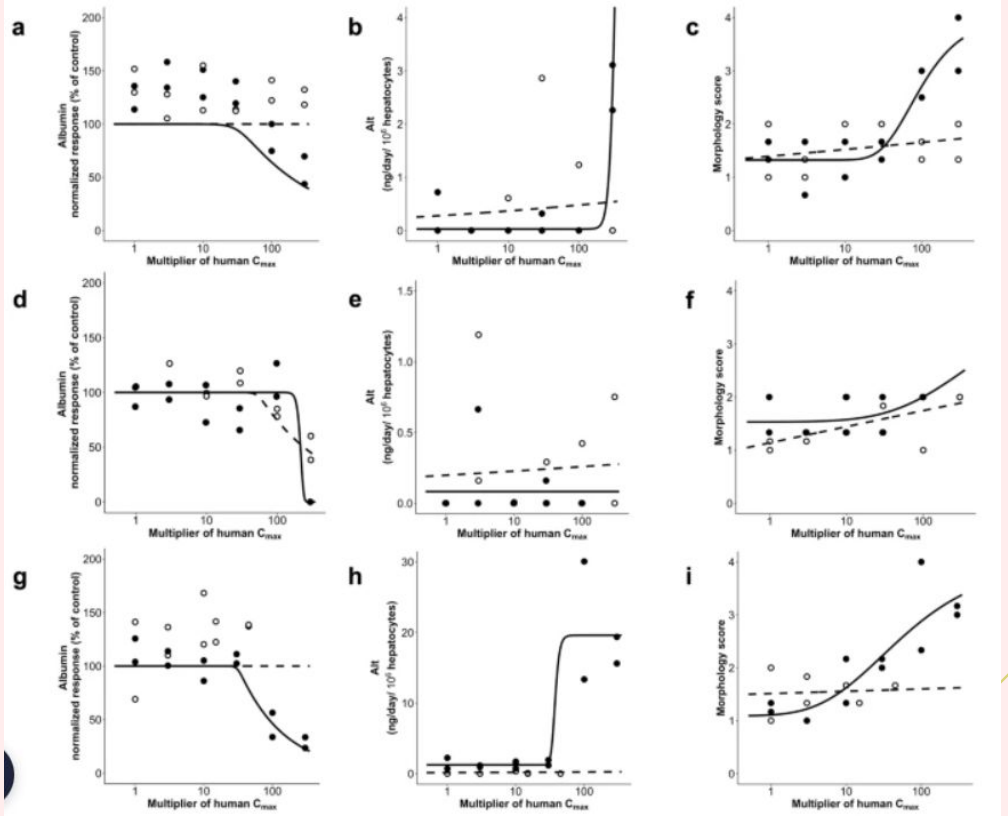
Precision medicine (per patient basis)

More ethically sound

Ability to Predict Liver Injury

- 870 chips analyzed
- Ability to detect benchmark molecules was recorded
- Liver-on-chip met guidelines across a set of 27 hepatotoxic and non-toxic drugs with 87% sensitivity and 100% specificity (Ewart et al., 2022).
- Study concludes that integrating liver-on-chip into drug development could improve the process and introduce safer and more effective medicine onto the market (Ewart et al., 2022).

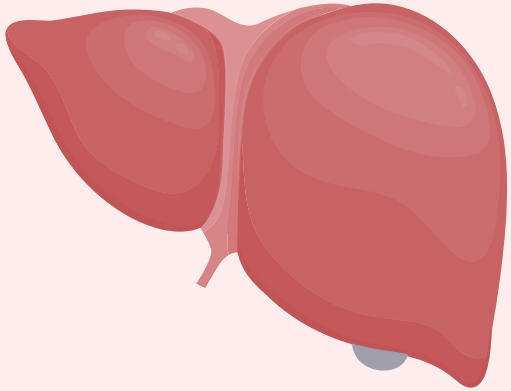
Fig. 4: Detection of drug concentration-dependent toxicity and liver injury.



https://www.nature.com/articles/s43856-022-00209-1

05

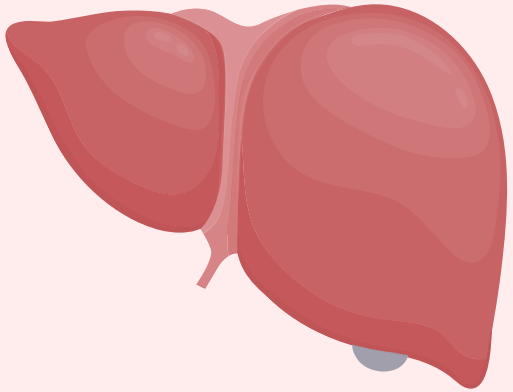
Limitations with Current Technology and Processes



- Current,LOCs can only simulate partial structure/limited functions of liver
  - Lack of Bile structure: Bile acids crucial for liver function; absence affects long-term viability and drug metabolism
- Limited liver cell sources
  - Most LOCs use hepatocytes
- Lack of efficient large-scale production methods

06

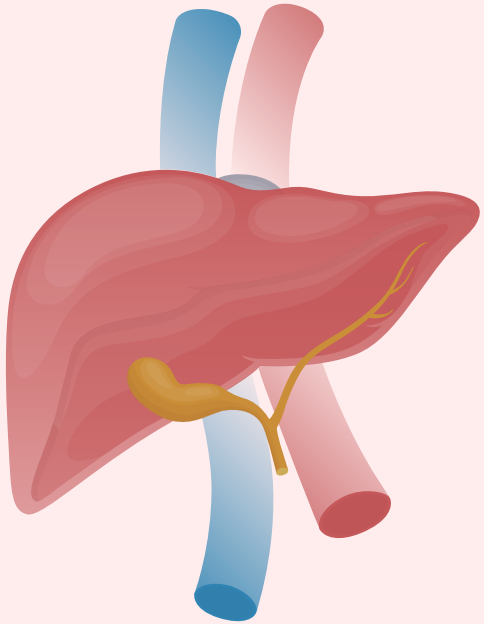
Future Directions to Address Limitations



- Integration of other liver cells
  - endothelial cells, Kupffer cells, & stellate cells
  - Mutant human embryonic stem cells and induced pluripotent stem cells (iPSC) -Heps cell
    - Add cell stacking layers
- Integration of multiple biosensors

Thanks

Do you have any questions?



References

- Caplin, J.D., Granados, N.G., James, M.R., Montazami R., Hashemi N. (2015) Microfluidic Organ-on-a-Chip Technology for Advancement of Drug Development and Toxicology. *Adv Healthc Mater*, 4(10): 1426-1450. https://pubmed.ncbi.nlm.nih.gov/25820344/
- Cong, Y., Hang, X., Chen, Z., Lu, Y., Liu, T., Wu, Z., Jin, Y., Luo, Y., Zhang, X. (2020) Drug Toxicity Evaluation Based on Organ-on-a-Chip Technology: A Review. *Micromachines*,11(4): 381. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7230535/
- Deng, J., Wei, W., Chen, Z., Lin, B., Zhao, W., Luo, Y., & Zhang, X. (2019, October 7). *Engineered liver-on-a-chip platform to mimic liver functions and its biomedical applications: A Review*. Micromachines. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6843249/
- De Hoyos-Vega, J.M., Hong, H.J., Stybayeva, G., Revsin, A. (2021) Hepatocyte cultures: From collagen gel sandwiches to microfluidic devices with integrated biosensors. *APL Bioeng*, 5(4): 41504. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8519630/
- Ewart, L., Apostolou, A., Briggs, S. A., Carman, C. V., Chaff, … Levner, D. (2022). Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. *Communications Medicine*, *2*(1), 1–16. https://doi.org/10.1038/s43856-022-00209-1
- Hattersley, S.M., Greenman, J. & Haswell, S.J. Study of ethanol induced toxicity in liver explants using microfluidic devices. *Biomed Microdevices* **13**, 1005–1014 (2011). https://doi.org/10.1007/s10544-011-9570-2
- Jang, K., Oteino, M.A., Ronxhi, J., Lim, H., … Hamilton, G.A., (2019). Reproducing human and cross-species drug toxicities using a Liver-Chip. *Sci Transl Med*, 11(517), https://www.science.org/doi/10.1126/scitranslmed.aax5516
- Knowlton, S., Tasoglu, S. (2016). A Bioprinted Liver-on-a-Chip for Drug Screening Application. *Trends Bioltechnol*, 34(9): 681-682. https://pubmed.ncbi.nlm.nih.gov/27291461/
- LifeSciencesIntelligence. (2023, September 26). *Comparing Chip vs. Animal Models in Medical Research*. LifeSciencesIntelligence. https://lifesciencesintelligence.com/features/comparing-chip-vs-animal-models-in-medical-research
- *Liver-Chip Identifies Distinct Drug Toxicities in Human, Rat, and Dog Models*. (2019, November 6). Wyss Institute. https://wyss.harvard.edu/news/liver-chip-identifies-distinct-drug-toxicities-in-human-rat-and-dog-models/
- McCreedy, T. (2001). Rapid prototyping of glass and PDMS microstructures for micro total analytical systems and micro chemical reactors by microfabrication in the general laboratory. *Analytica Chimica Acta*, *427*(1), 39-43. https://doi.org/10.1016/S0003-2670(00)01174-0
- Suk, K.T., Kim, D.J. (2012). Drug-induced liver injury: present and future. *Clin Mol Hepatol,* 18(3): 249-257. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3467427/