Hepatic Microtissues from Human Pluripotent Stem Cells

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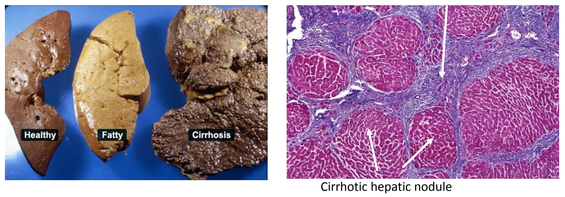
Introduction

A fundamental understanding of liver development is important for research areas in liver tissue regeneration, direct tissue replacement and alleviating chronic liver diseases. It is therefore critical to replicate human liver physiology with biological models that mimic its functions. We hypothesize that the structures and cues that initiate 3D liver formation can be mimicked with hepatic microtissues. Thus we aimed to engineer an in vitro organoid model of the liver diverticulum (LD) , a key structure that: 1) arises in mouse development (E9.5) and human development (d26) and 2) forms the 3D liver.

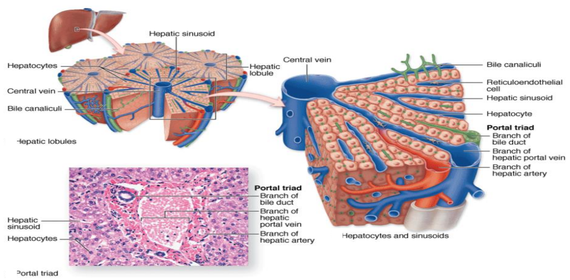
Liver cancer and cirrhosis:

a.



Liver Microarchitecture

b.



Early liver development

c.

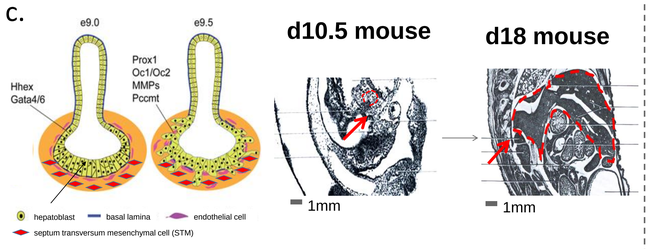
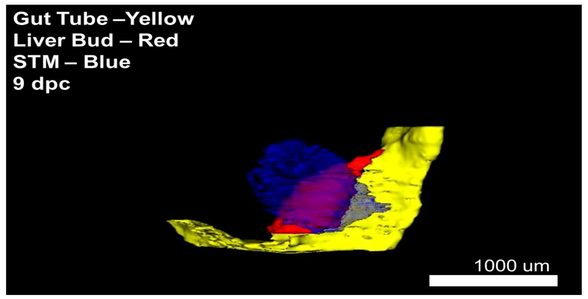


Figure 1: a) Liver cirrhosis is a growing epidemic worldwide characterized by the appearance of fibrotic tissue around hepatic nodules b) The liver is comprised of hepatic sinusoids that give rise to complex architecture c) The hepatic sinusoids are developed from hepatic cords

http://peir.path.uab.edu/library/picture.php?/11363

In silico analysis of liver diverticulum

a.



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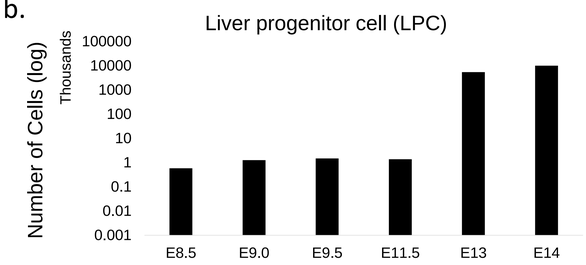

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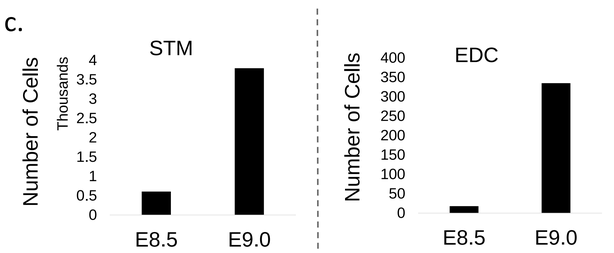
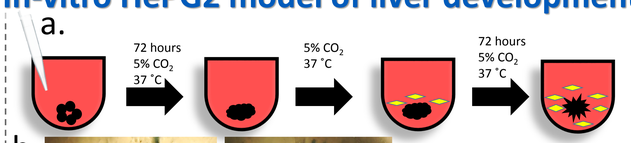


Figure 2: a) 3d image reconstruction of murine liver bud at 9 dpc b) Rapid increase in the number of hepatic progenitor cells (10^4 fold increase) c) Significant increase in the number of septum transversum mesenchyme cells (STM) and endothelial cells (EDC)

In-vitro HePG2 model of liver development

a.



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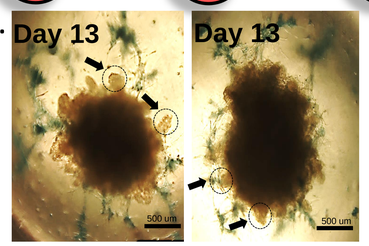

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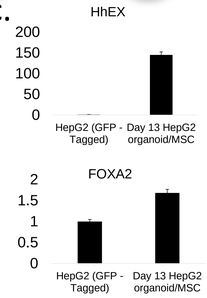
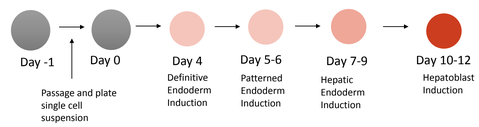


Figure 3: a) Schematic of tissue formation b) Phase contrast image tissue showing bud formation c) Upregulation of hepatic transcriptional markers

Hepatic Progenitor Cell Induction from hPSCs

a.



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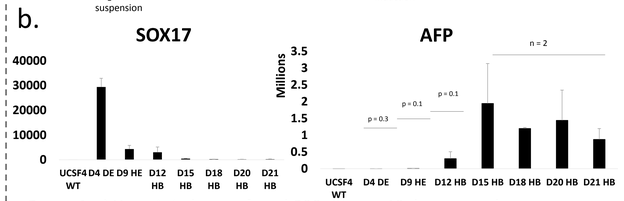
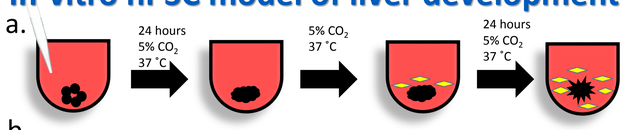


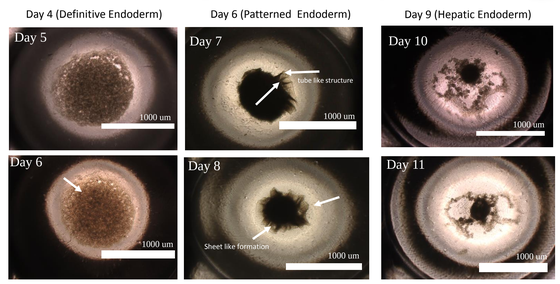
Figure 4: a) Hepatic induction from hPSCs using a 12 day protocol
b) Downregulation of Endoderm markers and upregulation of hepatic markers

In-vitro hPSC model of liver development

a.



b.



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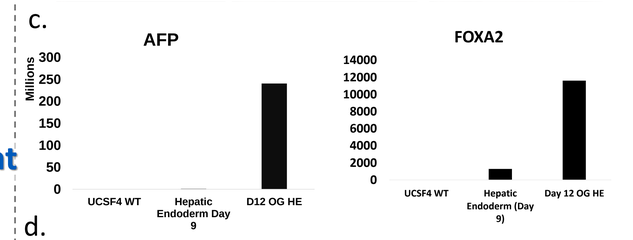
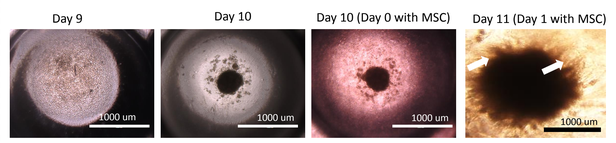


d.



e.

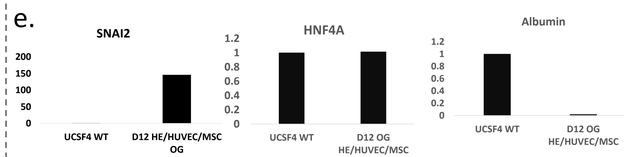
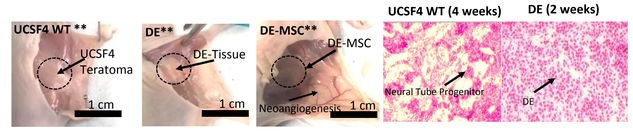


Figure 5: a) Schematic of tissue formation b) Phase contrast of tissue formation c) Upregulation of hepatic markers d) Phase contrast images of multicellular tissue formation e) Upregulation of liver migratory markers

In-vivo transplantation of hPSC cells

a.

b.



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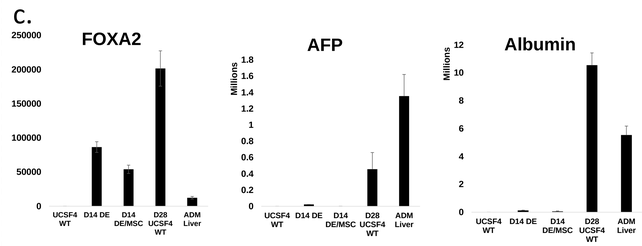

Figure 6: a) Injected cells subcutaneously over left hind-limb which form visible tissues b) H&E stain of hPSC derived tissue c) Transcriptional upregulation of hepatic markers post murine injection.

Conclusion

- Stem cell derived hepatic progenitor cells as organoids express significant levels of AFP, however liver specific markers such as albumin are absent.
- Modifications to existing protocol are required to solve these remaining challenges.
- Organoid models of early liver development show promising cord-like projections similar to in-vivo morphogenesis.
- 3D tissue models need improved tissue growth and morphogenesis

Future Work

- In-vivo transplantation of in-vitro derived hepatic organoids.
- Development of cholangiocytes as an additive cell for improved organoid model.
- Development of epithelial sheets using existing differentiation protocols to mimic epithelial architecture of developing liver bud.

Acknowledgements

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