Bilirubin induces the maturation of liver sinusoidal endothelial cells and human liver organoids

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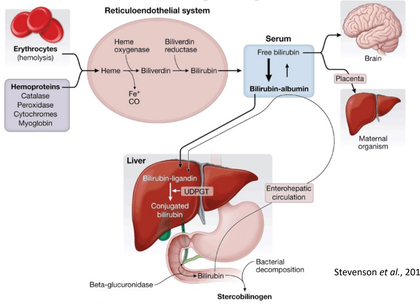
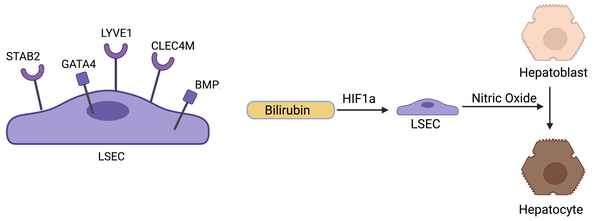
Background

The development of induced human liver organoids (HLOs) is a complex and important issue as it provides an avenue for liver disease treatment and research. HLO induction from induced pluripotent stem cells (iPSCs) has been modeled by fetal development. In vivo, liver development happens in three general phases: specification, budding, and growth and maturation.



Stevenson *et al.*, 2012

Using these stages, Shinozawa *et al.*, 2020 developed a model for HLO induction. However, the HLOs described lacked markers of maturation as well as prominent vasculature required for the enhanced function of a liver. Liver sinusoidal endothelial cells (LSECs) are a specialized endothelial cell that reside only in the liver. LSECs are highly fenestrated and have a unique set of receptors that function to help transport material in and out of the liver. Genetic specification for LSECs has remained ill defined by (De Haan *et al.*, 2020). This study focuses on LSEC marker genes: BMP2, GATA4, CLEC4M, STAB2, LYVE1.



Fetal development revealed that the production of fetal blood cells and thus bilirubin precedes the development of LSECs and hepatoblast differentiation. Suggesting that bilirubin may impact the hepatoblast differentiation to hepatocytes. Fetal bilirubin is a regulator of the HIF1a pathway. The HIF1a pathway stimulates the production of nitric oxide synthase by LSECs. Nitric oxide released by the LSECs will be used for the differentiation of hepatocytes from hepatoblasts (Poisson *et al.*, 2017).

Aims / Hypothesis

Hypothesis:

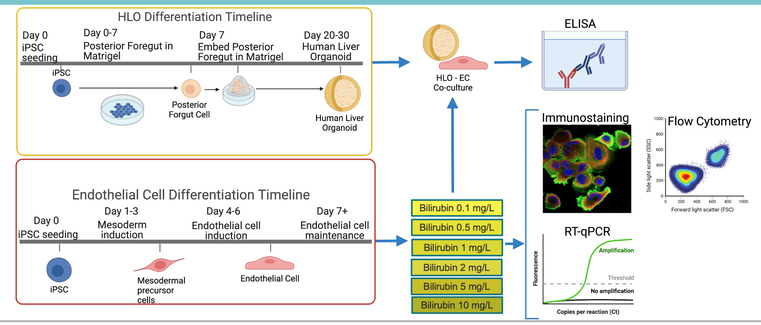
- Physiological levels of bilirubin promote the formation of LSECs from endothelial cell (EC) differentiation and promote differentiation of mature hepatocytes.

Aims:

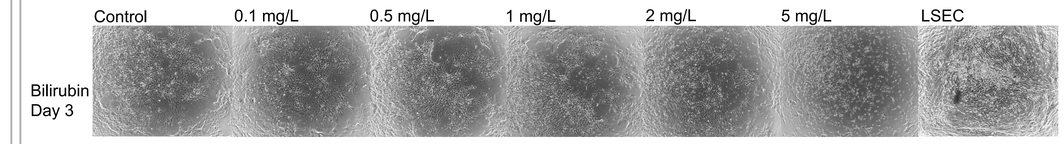
- Induce ECs, LSECs, and HLOs from stem cell precursors
- Analyze the effect of bilirubin as a metabolite for the maturation of LSECS and HLO maturation

Methods

iPSCs were differentiated into human liver organoids, endothelial cells, and liver sinusoidal endothelial cells. Bilirubin was added at varying concentrations to the endothelial cells for 3 days. After three days bilirubin treated endothelial cells were placed in a co-culture with the induced human liver organoids for an additional 7 days. iPSC derived LSECs were used as a control to compare morphology to bilirubin treated endothelial cells. Changes to bilirubin treated Ecs were observed using immunofluorescence, RT-qPCR, and flow cytometry. HLO maturation was analyzed via an albumin ELISA.



Results 1. Bilirubin treated endothelial cell morphology



Endothelial cells treated with bilirubin did not exhibit morphological changes. Endothelial cell morphology resembles that of LSEC morphology.

Results 2. Endothelial cells express LSEC markers at physiological levels of bilirubin

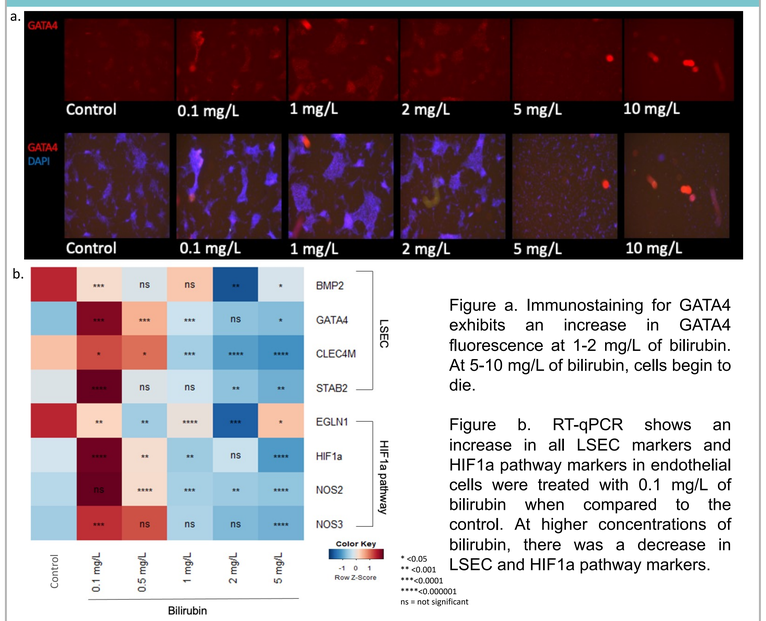


Figure a. Immunostaining for GATA4 exhibits an increase in GATA4 fluorescence at 1-2 mg/L of bilirubin. At 5-10 mg/L of bilirubin, cells begin to die.

Figure b. RT-qPCR shows an increase in all LSEC markers and HIF1a pathway markers in endothelial cells were treated with 0.1 mg/L of bilirubin when compared to the control. At higher concentrations of bilirubin, there was a decrease in LSEC and HIF1a pathway markers.

Results 3. Flow cytometry analysis reveals an increase in LYVE1 population in bilirubin treated endothelial cells

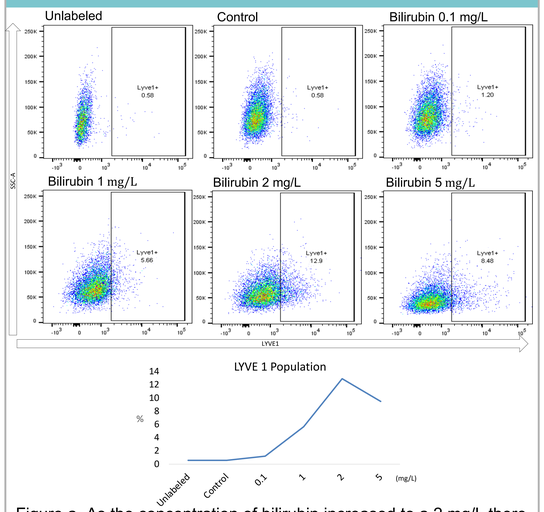
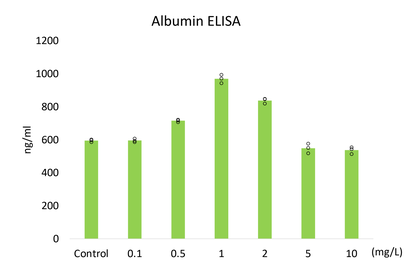


Figure a. As the concentration of bilirubin increased to a 2 mg/L there was a higher percentage of cells expressing LYVE1 with the highest percentage of LYVE1 cells at 12.9%. At 5 mg/L the percentage of cells expressing LYVE1 began to decrease to 8.48%.

Results 4. HLOs-Endothelial Cell co-cultures show an increase in maturation



Albumin production within the HLOs was at it's highest in HLOs co-cultured with 1 mg/L bilirubin treated endothelial cells demonstrating an increase in HLO maturation.

Conclusions

At physiological levels of bilirubin, there is an increased in LSEC characteristics as exhibited by PCR and Flow Cytometry. Additionally, PCR indicates that at physiologic levels of bilirubin could activate the nitric oxide pathway allowing for the induction of LSEC and hepatocyte differentiation. The maturation of HLOs was also indicated by an increase in production of albumin with physiologic levels of bilirubin treated endothelial cells that were cocultured with HLOs. This study shows that physiologic levels of bilirubin induce LSEC characterization of endothelial cells as well as increase the maturation of HLOs.

References

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Acknowledgements

This study was supported in part by NIH grant T35DK060444 and DP2 DK128799-01.