Optimizing the Differentiation of hiPSC Derived Hepatocyte-Like Cells via Mechanical Stimulation

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Mastering the differentiation process from human induced pluripotent stem cells (hiPSCs) to mature hepatocytes is a crucial obstacle to overcome to advance the development of the organ-on-a-chip platform. This device will be essential for the future of pre-clinical tests for drug development as it integrates multiple cell lines onto a single device [1]. Hepatocytes are essential for drug metabolism and thus would be a great asset to study in the organ on a chip. The chemical and mechanical environments during the differentiation process have been studied for several cell lines differentiated from hiPSCs, but as for hepatocytes most of the focus has been diverted to chemical stimulants, resulting in insufficient hepatocyte maturation [2]. The aim of this project is to mimic the *in vivo* conditions of the differentiation of hepatocytes during the early embryonic stage of human development where these cells are situated adjacent to the heart [3]. The recreation of the beating of the heart as a physical stimulus should lead to differentiated hepatocyte-like cells that more closely resemble real primary hepatocytes. The hiPSCs will be cultured in a poly-dimethyl siloxane (PDMS) microfluidic device that applies strain and shear stress to the cells via pulsations of the adhering surface. After 12 consecutive days of stimulus application, the mature hepatocyte-like cells will be analyzed using qRT-PCR against real primary human hepatocytes. The data collected from this experiment will improve our understanding of the differentiation of hepatocytes from hiPSCs and it will further assist in the manipulation of this process.

[1] Kamei, K. *et. al.* (2017). Integrated heart/cancer on a chip to reproduce the side effects of anti-cancer drugs in vitro. *RSC Advances,7*(58), 36777-36786. doi:10.1039/c7ra07716e

[2] Pan, D. *et. al.* (2015). The role of mechanical stimuli in the vascular differentiation of mesenchymal stem cells. *Journal of Cell Science, 128*(14), 2415-2422.

[3] Zorn, A.M., Liver development (October 31, 2008), StemBook, ed. The Stem Cell Research Community, StemBook, doi/10.3824/stembook.1.25.1.

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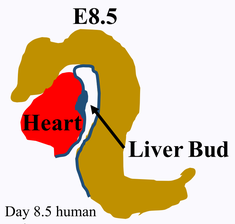
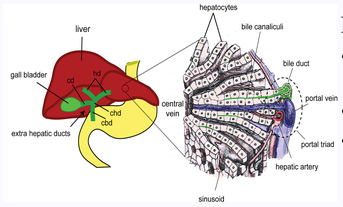
Hepatocyte and Liver

Hepatocytes

- Essential cells for drug metabolism
- Major focal point for pharmaceutical companies
- Compose over 80% of the liver mass[1]
- **Adjacent to heart during early human embryonic development[1]**



Day 8.5 human embryo.



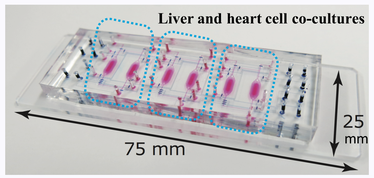
Enhanced view of hepatocyte organization in liver[1].

Pharmaceutical Application

- Successful pre-clinical testing requires fully functioning cells
- Difficult to obtain hepatocytes
- **Problems:**
  - Donor limitations
  - Varied sample quality of primary hepatocyte cells
- **Solution:**
  - Differentiate hepatocyte cells from human induced pluripotent stem cells (hiPSCs)

Organ-On-A-Chip

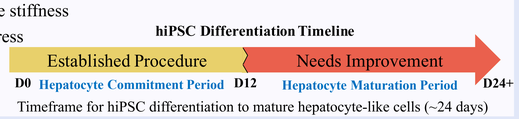
- Integrates multiple cell/tissue lines into one device
  - Observe interactions
- Drug metabolic effects of hepatocytes could greatly benefit any co-culture study
- Future of pre-clinical tests
  - ↑ Reliability
  - ↑ Cell/tissue consistency
  - ↓ Need for animal testing



Organ-on-a-chip model developed by Dr. Kamei[2].

Objective: Optimize hiPSC Differentiation

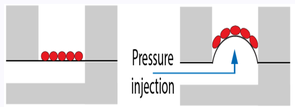
- Obtain differentiated hepatocytes that more closely resemble real primary human hepatocytes
- Optimize the differentiation process of hiPSCs to mature hepatocyte-like cells via chemical and **mechanical** control
  - Chemical cues have been heavily researched with promising findings
  - Recently physical cues have begun to be investigated:
    - Substrate stiffness
    - Shear stress
    - Strain



Timeframe for hiPSC differentiation to mature hepatocyte-like cells (~24 days)

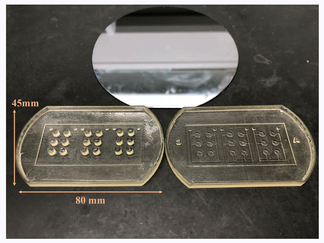
Approach: Physical Stimulus Application

- Simulate beating heart *in vitro*
- Stretching of hepatocyte cells in culture continuously from day 14
- Cyclic pressure in 5 second intervals
- Increase resemblance of differentiated hepatocytes to real primary human hepatocytes

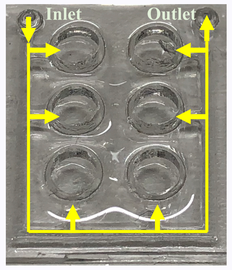


Premature hepatocytes (red) on pulsating membrane[3].

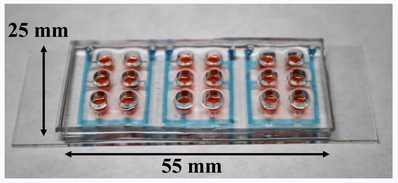
Microfluidic Device and Mechanical Stimulus



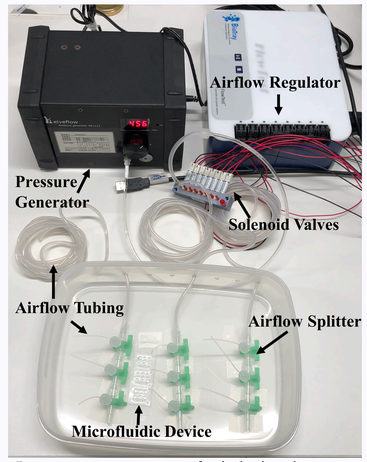
3D printed moulds and silicon wafer used to assemble the device.



Airflow diagram in assembled microfluidic device, air flow tubing inserted in the "inlet".

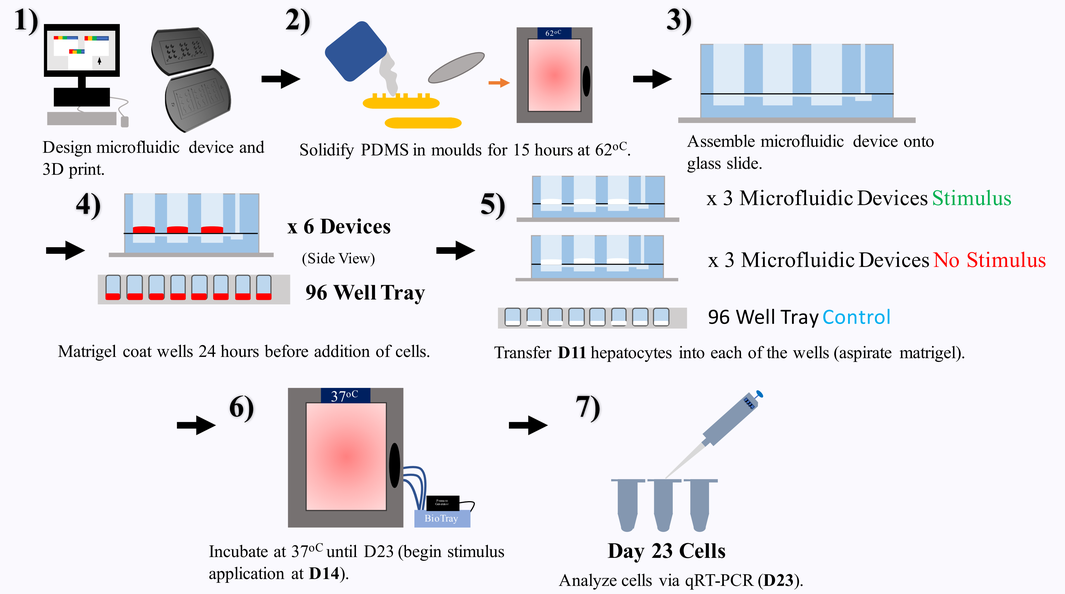


Completed microfluidic device with dyes to indicate the culture wells and air flow chambers.

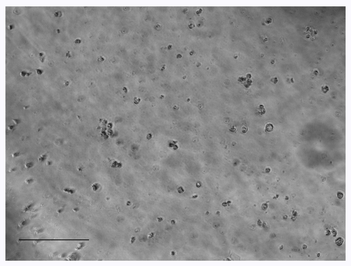


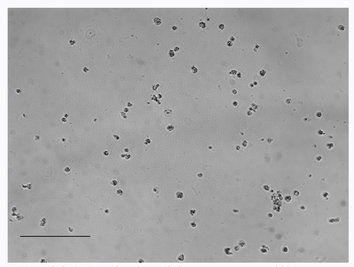
Pressure generator setup for inducing the pulsating well physical stimulus upon the treatment group.

Maturation Protocol on Microfluidics



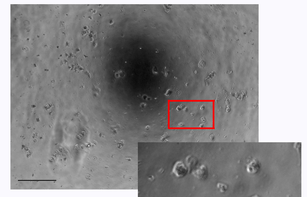
Hepatocyte Adhesion Complications

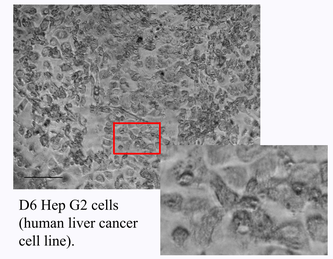

D14 hiPSC derived hepatocyte-like cells on microfluidics.


D14 hiPSC derived hepatocyte-like cells in 96 well tray.

Most cells died after lack of adhesion within 3 days.

hiPSC Derived Hepatocytes vs. HepG2


D17 hiPSC derived hepatocyte-like cells.


D6 Hep G2 cells (human liver cancer cell line).

Determine if the microfluidics and/or human error is causing the cells to die by culturing hiPSCs and HepG2 ("strong cells") side by side.

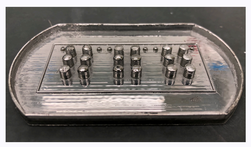
Conclusion

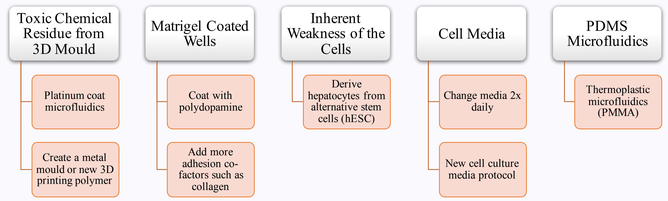
- Mechanical stimulus could not be investigated due to the hepatocyte-like cells not adhering to PDMS surface
- HepG2 cells were able to adhere and survive on the surface for over one week
  - Suggests an inherent characteristic of hiPSCs is hindering adhesion
- New cell culture protocol changes may be influencing the ability of the cells to adhere to the surface

Future Directions

Cell Adhesion Predicament

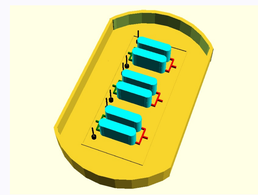
- Investigate the cause of a lack of cell adhesion to PDMS surface
- 5 possible problems to consider (with proposed solutions)


Platinum coated microfluidic mould.



Alternative Mechanical Stimuli

- Liquid shear stress has been shown to improve hiPSC differentiation
- Incorporate pulsating wells with liquid shear stress
  - Ideally mimic entire development process *in vitro*


Pressure chambers, culture chambers, liquid input, liquid output

References

[1] Zorn, A.M., Liver development (October 31, 2008), StemBook, ed. The Stem Cell Research Community, StemBook, doi/10.3824/stembook.1.25.1.
[2] Kamei, K. et. al. (2017). Integrated heart/cancer on a chip to reproduce the side effects of anti-cancer drugs in vitro. RSC Advances,7(58), 36777-36786. doi:10.1039/c7ra07716e
[3] Minier, N. (2017). Mechanical stimulation for optimization of hepatocyte differentiation in body-on-a-chip. Unpublished.

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