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\textsuperscript{[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\textsuperscript{[2]}. The aim of this project is to mimic the \textit{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\textsuperscript{[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.


Hepatocytes

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

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 lines into one device
- Observe interactions
- Drug metabolic effects of hepatocytes could greatly benefit any co-culture study
- Future of preclinical tests

- Reliability
- Cell-tissue consistency
- Need for animal testing

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
  - Stretch

hiPSC Differentiation Timeline

- Established Procedure
- Needs Improvement
- Hepatocyte Maturation Period
- Timeframe for hiPSC differentiation to mature hepatocyte-like cells (~24 days)

Approach: Physical Stimulus Application

- Stimulate 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

Microfluidic Device and Mechanical Stimulation

- 3D printed mould and silicon wafer used to assemble the device.
- Airflow diagram in assembled microfluidic device, airflow tubing inserted in the "ultra".
- Pressure generator setup for inducing the pulsating well physical stimulus upon the treatment group.

Maturation Protocol on Microfluidics

1) Design microfluidic device and 3D print.
2) Solidify PDMS in moulds for 15 hours at 62°C.
3) Assemble microfluidic device onto glass slide.
4) x 6 Devices (Side View)
5) x 3 Microfluidic Devices Stimulus
6) Incubate at 37°C until D23 (begin stimulus application at D14).
7) Day 23 Cells

Hepatocyte Adhesion Complications

- Most cells died after lack of adhesion within 3 days.

hiPSC Derived Hepatocytes vs. HepG2

- Determine if the microfluidic 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)

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

References

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