**Abstract**

Recent advancements in developmental biology and tissue engineering have greatly contributed to pharmaceuticals and drug testing by allowing for the development of more efficient and accurate in vitro models of human tissues. In particular, the Wells lab, at the Cincinnati Children’s Hospital Medical Center, has developed a protocol for the differentiation of human induced pluripotent stem cells (hiPSCs) into intestinal organoids. However, this protocol, which mimics the in vivo microenvironment of developing definitive endoderm and mid-hindgut, often results in the random generation of gut spheroids, which later mature into organoids. The complex development of hindgut does not allow for finer analysis of the local environment pertinent to the development of an individual spheroid. To address this problem, we employed hydrogel-based microwells. By attempting to differentiate hiPSCs into mid-hindgut tissue in microwells of varying 2D dimensions, depth, and spacing, we were able to deduce more optimal conditions for the in vitro development of individual spheroids. This method will allow for improved analysis of the local factors important for spheroid generation because it reduces the previously complex culture over a relatively large surface area to the smallest-budding unit. Using this approach to gain a better understanding of the local environment may lead to an improved differentiation protocol that will result in more organized intestinal tissue useful for drug testing and disease modelling.

**Background: Intestinal Differentiation**

Previously, the Wells lab has developed a protocol to differentiate human induced pluripotent stem cells into intestinal organoids in vitro.\(^1\)\(^2\)

**Background: Microwells**

PEG-based microwells have been used to culture stem cells in an effort to produce homogeneous embryoid bodies.\(^3\) Here, we make microwells of varying dimensions using an ink template to pattern UV-induced polymerization of PEGDA.

**References**


**Using Microwells for Controlled Intestinal Differentiation of hiPSC Cells**

**Objective and Motivation**

The current protocol for intestinal differentiation of hiPSCs is complex and produces hundreds of spheroids over 2 cm\(^2\), making it difficult to identify key components for spheroid budding in the microenvironment. Our goal is to use microwells to culture the smallest-budding unit of intestinal spheroids to better analyze the microenvironment pertinent to spheroid formation and to model the budding process for more reproducible and controllable differentiation.

**Three Experimental Challenges**

1. **Differentiating hiPSC cells on a glass surface.**
   - Has not been done before
   - Standard procedure requires plastic surface

2. **Maintaining hiPSCs’ pluripotency in microwells.**
   - Pluripotency is a stem cell’s ability to differentiate into any cell type in the body

3. **Differentiating hiPSCs into hindgut in microwells.**
   - That is, generating spheroids (eventually finding the smallest-budding unit)

**Results**

1. **Can hiPSCs adhere to and be differentiated on a silanized glass surface?**
   - Cultured hiPSCs on a silanized glass surface for 4 days.
   - Fixed and stained for pluripotency markers Sox2 and Oct4 (Fig. 3A).
   - Compared the differentiation of hiPSCs on plastic and glass surfaces (Fig. 3B) and stained for definitive endoderm markers after 4 days.

2. **Can hiPSCs remain pluripotent in microwells?**
   - hiPSCs were seeded onto matrigel-coated microwells of various dimensions
   - After 4 days, the cells were fixed and stained for Sox2 and Oct4 (Fig. 4A).

3. **Evaluating success of differentiation protocol in microwells.**
   - Compared differentiating cells in microwells with differentiating cells on a standard plastic surface (Fig. 5A).
   - Stained for definitive endoderm (FoxA2 and Sox17) and hindgut markers (Cdx2 and Sox2) 4 and 8 days into differentiation, respectively (Fig. 5B)\(^3\)\(^2\), note the improved expression in the large break.

**Conclusion**

- hiPSCs can remain pluripotent in microwells and are likely able to be differentiated into definitive endoderm and (subsequently) into hindgut in microwells.
- Larger well and thinner walls have been more effective for maintenance of pluripotency and for differentiation. Could the PEGDA be absorbing larger amounts of growth factors and intercellular signals in microwells with thicker walls?

**Future**

1. Examine effects of wall thickness/depth and combine different dimensions and wall sizes on one grid.
2. Optimize microwells to culture smallest-budding unit for intestinal spheroids.
3. Analyze the microenvironment pertinent to spheroid generation.
4. Model budding process for more reproducible and controllable differentiation.