3-D Cultured Organoids as an Insight to Stem Cell Therapy

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INTRODUCTION

- Early stages of human embryonic blood development arises from hemogenic embryonic cells (HECs) within the yolk-sac (YS) to produce hematopoietic cells.
- The fetal liver (FL) then provides niche of hematopoietic stem/progenitor cell (HSPC) development after five weeks of gestation.
- Various culture systems, including embryoid body (EB) differentiation and 2-D cultures of HECs have been common models to recapitulate embryonic blood development. However, the introduction of a novel 3-D stress free culture system provides a new angle of approach.
- Using human embryonic stem cells (ESC), our aim with this experiment was to improve upon these previous models to simulate *in vivo* growth in an *in vitro* organoid culture.

METHODS and MATERIALS

- Human ESCs, H9 cell lines were used. This cell line includes the transgenic construct *Runx1* enhancer reporter (Fig. 1). H9 was aggregated in microwells (Aggrewell 400, Stem Cell Technologies, Fig. 2a) O/N followed by transferring to Aggrewell 800 with supportive MS5 stromal cells O/N.
- The yolk-sac organoids (YSO) were confirmed to form the spheroid structure and transferred to the ClinoReactor®.
- This 3-D culture was maintained with media previously used to culture human organoids and a cytokine pattern previously reported to induce YSO.
- The organoids and the supernatant were collected and analyzed for HSPC and endothelial expression.
- The ClinoStar® incubator (Fig. 2b) uses a combination of gravity and rotation to suspend the organoids within the media. It has been reported to simulate and replicate *in vivo* environments.

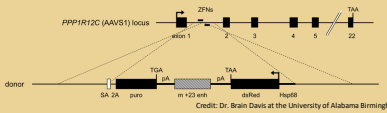


Figure 1: The *Runx1* + 23enh reporter construct

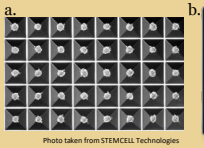


Figure 2: a. The Aggrewell microwells to start the 3-D organoid culture. **b.** The ClinoStar® incubator, housing up to 6 ClinoReactor® dishes.

METHODS and MATERIALS

	Day 0	Day 2	Day 4	Day 6~
CHIR99021	2 μM			
BMP4	80 ng/mL			
VEGF	80 ng/mL	80 ng/mL	15 ng/mL	10 ng/mL
SB431542		1μM		
SCF		50 ng/mL	100 ng/mL	10 ng/mL
bFGF		25 ng/mL		
TPO				10 ng/mL
IGF				25 ng/mL
G-CSF				30 ng/mL
EPO				2 U/mL

Figure 3: Cytokine pattern and timing of media changes. The EBs were supplemented with fresh Advanced DMEM/F12 media in the ClinoReactor®. The timing and dosage of the growth factors were reported to support YS growth and hematopoietic cell production.

RESULTS

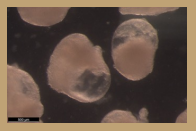


Fig 4: EB formation verified by brightfield microscope at D8 (left) and *Runx1* enhancer reporter DsRed (right)

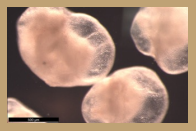
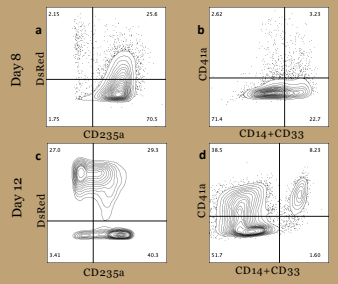


Fig 5: EB formation verified by brightfield microscope at D12 (left) and *Runx1* enhancer reporter DsRed (right)

Figure 6: (a., c.) Erythrocytes expressed by CD235a were detected in the supernatant at both d8 and d12, suggesting the presence of primitive hematopoiesis. **(b., d.)** There also was a population marked by CD14+CD33+ of myeloid cells with a noticeable change of distribution of CD14+CD33+CD41a- to CD14+CD33+CD41a+ between days 8 and 12.



RESULTS

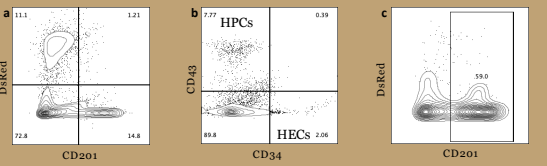


Figure 7: a. There are a distinct population of DsRed+ cells from the dissociated EBs, suggesting *Runx1* presence and activation. DsRed+CD201+population presents evidence of endothelial HSCs within the EBs from day 12. **b.** A population of CD34+CD43+ hematopoietic progenitor cells (HPC) were also detected in the dissociated EBs on day 12. **c.** Hemogenic endothelial cells are marked as CD34+CD43-CD201+.

CONCLUSION

- The results are promising to simulate the growth of organoids *in vitro* as EBs in a 3-D environment as shown by the differentiation of HPCs and endothelial cells within the EBs. We detected the emergence of CD235a primitive erythropoiesis, followed by CD34+CD43+ HPC production. In addition, the culture also produced CD14+CD33+ myeloid cells and CD41a+ megakaryocytes. The use of the CelVivo 3-D stress free culture recapitulated YS hematopoiesis.

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REFERENCES

Tamaoki, N et al. (2023). Self-organized yolk sac-like organoids allow for scalable generation of multipotent hematopoietic progenitor cells from induced pluripotent stem cells. *Cell Reports Methods*, *3*(4), 100460. https://doi.org/10.1016/j.crmeth.2023.100460