Embryoid body and microcarrier ratio optimization for hematopoietic cell production in bioreactors

Open per	*September 2024*
Duration	8 months
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1. General introduction

Blood transfusion is currently the most common cell therapy applied worldwide (WHO, 2022). Severe anemias, healthy red blood cell deficiency, can only be treated with blood transfusions and stem cell transplantations. However, a lack of immune-matched cells often affects the use of these therapies. Employing *in vitro*-produced red blood cells represents an attractive therapy that could overcome these risks.

The development of induced pluripotent stem cells (iPSCs) started a new era in regenerative medicine. iPSCs are artificial cells created by reprogramming human somatic cells and have the potential to produce patient-specific progenitor or functional cells (Martins Fernandes Paes et al., 2017). Hematopoietic cell lineages can be generated *in vitro* from iPSCs. The *in vitro* production of hematopoietic stem cells (HSC) and red blood cells (RBC) from iPSCs opens the possibility of curing anemias through transplantation and transfusion, respectively (Wilkinson et al., 2019).

This project is part of the TRACER consortium (**Tr**eating hereditary **a**nemias through stem **ce**ll **r**esearch) and aims to develop a bioreactor process to produce hematopoietic stem/progenitor cells (HSPC) from iPSCs. The protocol consists of several major experimental phases: i) iPSC maintenance, ii) embryoid body (EB) formation (3D cell aggregates), iii) hematopoietic organoid (HeO) formation and iv) expansion of HSPCs. The main goal is to standardize the expansion and differentiation of iPSCs to control the reproducible culture of (HSPCs) in bioreactors.

To induce the formation of HeO, microcarriers (MC) are used to support the growth and differentiation of EBs to HeOs. This process takes around three to four weeks. From week 4 to week 8, the HeOs start producing HSPC which are released into the supernatant of the culture. However, the HSPC attach to unused microcarriers. This leads to a lower yield of HSPCs . To maximize yield and process efficiency, the goal of this MSc thesis project is to optimize the microcarrier-EB ratio to minimize the HSPC-MC interactions and ensure an effective and efficient HSPC harvest.

2. Work packages

WP 1: Project scoping (M1-M2)

In this first work package you will get to know the research field and the topic of your thesis. You will perform a literature review and summarize your findings in a literature review report. You will also make a detailed short term planning up until end M2 (deadline: end M1) based on the templates provided by your supervisor(s). After delivery of your literature report and M2 planning, you will dive deeper into the experimental design phase. You will make a final experimental planning based on discussion with your

supervisor(s), lab introductions, and the initial project description. This is accompanied by making a long-term project plan until M8. The deadline for the experimental and full project plan is end of M2.

Key deliverable

- Summary of literature review: hematopoietic cell production, iPSC culture, microcarrier-cells interaction, etc.
- Short-term planning (until M2)
- Experimental plan
- Long-term planning (until M8)

WP 2: iPSC culturing training

iPSCs can be cultured adherently and in suspension. Culturing these cells requires a specific training to passage them between dishes and to produce single-iPSC for differentiation purposes. Furthermore, during this training, an overview of iPSC differentiation to HSPC will be given. Finally, an introduction about the use of flow cytometry for cell identification and making cytospins for cell physiology purposes will be provided.

Key deliverable

- Ability to independently culture and differentiate iPSC to hematopoietic cells.
- Standardized harvest protocol from microcarriers
- Proficiency in flow cytometry and cell physiology using cytospins

WP 3: HeO formation in different MC ratios

Based on the experimental planning, different microcarrier ratios will be tested to adhere EBs and differentiate them into HeO. Six-well plates will be used to test the MC ratios. The optimal EB-MC ratios will be scale-up into 125 mL shake flaks. The cultures will be analyzed weekly for cell density and cell identity. Furthermore, cytospins will be made to obtain a record on the cells physiology.

Key deliverable

- Small-scale reproducible protocol to obtain HeOs with different EB to microcarrier ratios
- Standardized analytical workflow

Experimental results per condition:

- Cell concentration
- Cell viability
- Flow cytometry to identify produced cells
- Cell physiology

WP 4: Results analysis

After performing the experimental plan, you will move to data analytics. In this work package, you will analyze flow cytometry results using the FlowJo™ software. In addition, you will create HSPC production graphs to compare the conditions tested and analyze the pictures of the cytospins produced.

Key deliverable

- Workflow and metrics for flow cytometry data analysis
- Workflow and metrics for cytospin data analysis
- Insight on the correlation between experimental conditions and product quality

3. Proposed time line

Months / Activity	M1	M2	M3	M4	M5	M6	M7	M8
WP 1: Project scoping								
Literature review								
Experimental planning								
WP 2: iPSC culturing training								
Learn to culture iPSC								
Learn to differentiate iPSC to HeO								
WP 3: HeO formation in different MC ratios								
6-well plate dishes experiments								
Shake flasks experiments								
WP 4: Results analysis								
Flow cytometry results								
Cell density graphs and cytospins								
Thesis writing + presentation								

4. Overview of deliverables

MEP deliverables

- Literature report (M1)
- Experimental plan + 9M planning (M2)
- 3M Progress report (M3)
- Intermediate presentation at TU Delft (M5)
- 6M Progress report (M6)
- Written MEP thesis and final presentation (M8)

Project deliverables

- Ability to independently culture and differentiate iPSC to hematopoietic cells
- Proficiency in flow cytometry and cell physiology using cytospins
- Small-scale reproducible protocol to obtain HeOs with different EB to microcarrier ratios
- Standardized analytical workflow
- Workflow and metrics for flow cytometry and cytospin data analysis
- Insight on the correlation between experimental conditions and product quality

5. References

Martins Fernandes Paes, B. C., Moço, P. D., Pereira, C. G., Porto, G. S., de Sousa Russo, E. M., Reis, L. C. J., Covas, D. T., & Picanço-Castro, V. (2017). Ten years of iPSC: clinical potential and advances in vitro hematopoietic differentiation. *Cell Biology and Toxicology*, *33*(3), 233–250. https://doi.org/10.1007/s10565-016-9377-2

WHO. (2022). *Blood transfusion*. https://www.who.int/news-room/facts-in-pictures/detail/blood-transfusion

Wilkinson, A. C., Ishida, R., Kikuchi, M., Sudo, K., Morita, M., Crisostomo, R. V., Yamamoto, R., Loh, K. M., Nakamura, Y., Watanabe, M., Nakauchi, H., & Yamazaki, S. (2019). Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature*, *571*(7763), 117–121. https://doi.org/10.1038/s41586-019-1244-x