High-throughput Manufacturing of Embryoid Bodies for the Differentiation of Human iPSCs into Hematopoietic Stem Cells and Progenitor Cells

Xionghui Lin[1], Natacha Coppieters[2], Mariana Tsimacidis[2], Léa Leclercq[1], Julien Lemarque[1], Christina Nguyen[2], Cécile Augereau[1], & Stéphanie van Loo[2]
[1] Cellistic, Rue Edouard Belin 2, 1435 Mont-Saint-Guibert, Belgium, [2] LiveDrop, GIGA - Avenue de l'Hôpital 11, 4000 Liège, Belgium.

INTRODUCTION

In vitro differentiation of human induced pluripotent stem cells (iPSCs) into various lineages serves as a valuable tool not only for developmental biology but also for tissue engineering and regenerative medicine. One common approach involves guiding iPSCs through the embryoid bodies (EBs) stage, which comprises the three embryonic germ layers and mimics early embryonic development. To generate homogeneous EBs, conventional methods such as the hanging drop technique, 96-well plates, AggreWell™, or Elplasia™ are typically employed. However, these approaches are labour-intensive and challenging to scale up. Here, we present a novel application of the ModaFlow™ instrument to rapidly produce large quantities of iPSC aggregates, which can subsequently differentiate into hematopoietic stem cells and progenitor cells (HSPCs) via the EB stage. This method offers a scalable solution for future biomedical and therapeutic applications.

MATERIAL and METHODS

Human iPSCs were resuspended at 1.5x10^6 cells/mL and encapsulated in 5 nL droplets using a Modaflow™ instrument to obtain aggregates. Droplets containing cells were placed in a cell incubator for 3-4 hours to allow aggregation. The aggregates were subsequently recovered after breaking droplets and seeded into non-adherent 6-well plates with custom differentiation medium supplemented with various cytokines. The plates were put on a shaking platform inside a cell incubator for up to 18 days. During this period, EBs were formed and subsequently differentiated into HSPCs. Cell viability was assessed using an NC200 instrument, and HSPC identity was confirmed by flow cytometry (FACS).

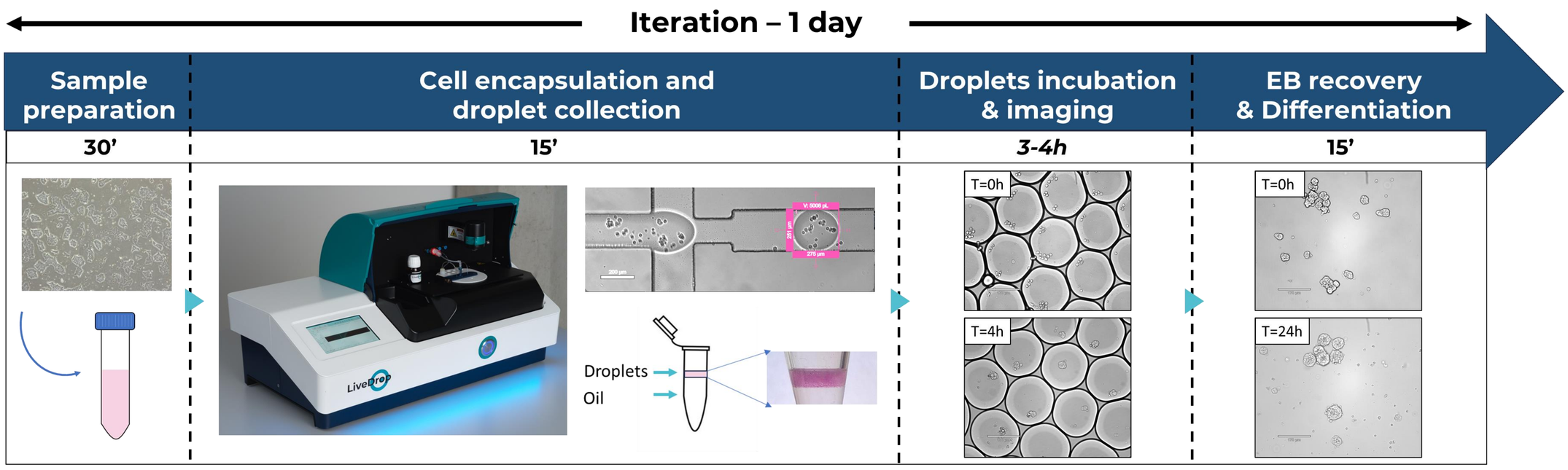

Figure 1. Complete Workflow for the High-throughput Manufacturing of Embryoid Bodies. A typical workflow takes 1 day and starts with the **preparation of cells** at a desired concentration leading to a given number of cells per droplet, and thus per cell aggregate. Cells get **encapsulated** in homogeneous droplets of predefined size (5 to 10 nL) using a ModaFlow™ instrument. Droplets containing cells are collected in a tube and **incubated** for 3 to 4 hours in a conventional incubator to allow time for aggregation. If desired, a small aliquot of droplets can be observed with a microscope to monitor aggregate formation. Once formed and compact, cell aggregates are released from droplets and placed in conditions specifically optimized for the differentiation into hematopoietic stem and progenitor cells.

RESULTS

A high number of cell aggregates, homogeneous in size were successfully generated in less than 4 hours (Fig. 2A and 2B). After collection, cell aggregates were utilized for HSPC differentiation through EB stage. After 11 days of specific treatments, floating single cells emerged from EBs, with continuous release observed through day 17 (Fig. 2C). Flow cytometry was used to evaluate the viability of these floating cells and the expression of HSPC markers after 14, 17 and 18 days of differentiation. Results revealed that the viability of these cells was very high (>95% viability) and that they were triple positive for the markers, CD45, CD34, and CD43. These cells also remained CD38 negative (Fig. 2D), confirming they were HSCs/HSPCs. The viability of the floating cells and the purity of triple-positive HSPCs increased over time (Fig. 2E and 2F).

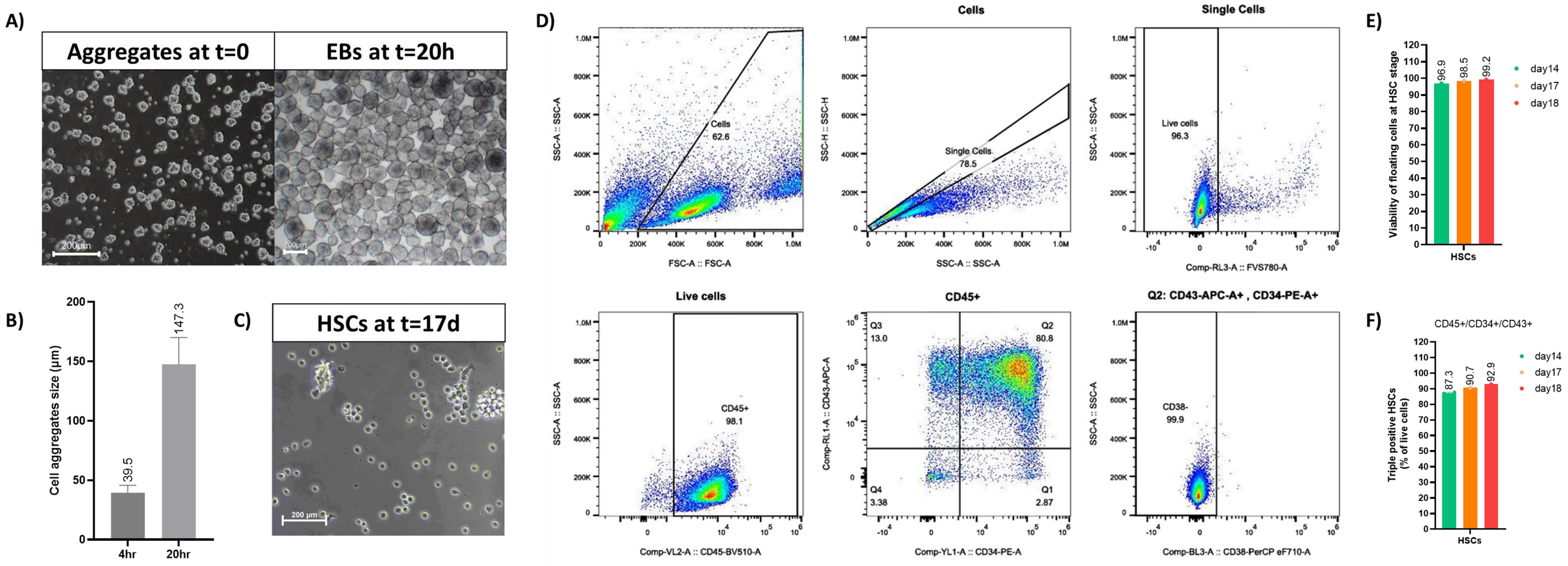

Figure 2. Differentiation of EB into high-purity triple-positive (CD45+/CD34+/CD43+) HSPCs. Embryoid bodies (EBs) generated with the ModaFlow™ were utilized for hematopoietic stem and progenitor cells (HSPC) differentiation. A) Representative images of EBs just after release from droplets and after an overnight incubation. B) Graph showing the average size of EBs (+/- SD). C) A representative image of HSC at day 17. D) Flow cytometry analysis confirming that floating single cells had high viability and were triple positive for CD45, CD34, and CD43 while remaining CD38 negative. E & F) Graphs showing the viability and the purity of HSCs increased from day 14 to day 18 of the differentiation. Results are given with the SD. Scale bars represent 200 µm.

DISCUSSION and CONCLUSION

Generating high numbers of homogeneous EBs is crucial for the *in vitro* differentiation of human iPSCs into various cell lineages. Using the ModaFlow™ instrument, we successfully manufactured over 1 million of EBs homogeneous in size in less than 4 hours with two independent iPSC lines, leading to ultra-pure, viable HSPCs ready to be further differentiated into different immune cell types including NK or T cells. This method offers the possibility to produce EB at large scale for future biomedical and therapeutic applications.

Turning Months into Days—Efficiency Redefined