

Optimizing High-Density Human iPSC Expansion

and Process Efficiency Using Next-Gen Bio-Processor

Introduction

The advent of Induced Pluripotent Stem Cells, or iPSCs, has substantially transformed cell-based disease modeling, regenerative medicine, and the stem cell-derived therapeutic industry. These advances also led to a sudden surge in demand for iPSCs and, hence, technologies that can help in iPSCs' expansion in an automated fashion (1).

Stämm Bio developed a novel bubble-free bioreactor that demonstrated significant advantages in space-time yields in high-density mammalian cell culture applications in adherent (HEK, MSC, iPSC) and suspension (CHO, JURKAT) cultures.

As cell therapies with iPSCs advance through clinical trials, the transition from traditional and labor-intensive manual culture systems (e.g., T-flasks, Cell Factories) to robust, scalable bioprocess platforms becomes critical (1, 2).

Conventional culture systems face severe scalability constraints, high footprint requirements, manual labor intensity, and increased contamination risks (3). Stämm eliminated most of them with the unique design and automation of the BFB (bubble-free bioreactor).

The Stämm BFB is a semi-automated, functionally closed bioprocessor that delivers optimized mass and gas transfer dynamics, achieving a 4X increase in peak viable cell density (VCD) and 4X specific productivity in mAb titer compared to conventional fed-batch bioreactors, while reducing batch-to-batch variability.

This application note demonstrates the operational advantages and performance capabilities of the BFB model Aries with a 6 mL volume run in a commercial incubator in human-induced pluripotent stem cells (hiPSCs) generated from cord blood-derived CD34+ progenitors.

Material and Methods

Gibco® Episomal hiPSC (A18944, Thermo Fisher Scientific) cells were cultured and maintained in Essential 8™ Flex (Thermo Fisher Scientific) on a Geltrex® coated surface throughout the cell culture process. Initial cultures after thawing from stocks were done on multiwell plates with the same media, followed by seeding in the BFB.

Control and BFBs were inoculated at 1×10^4 cells/cm² and operated at a media exchange rate of 1 volume per day (1 VVD). Cells were cultured in a controlled environment at 37°C with 5% CO² in an incubator. The details of the materials used are provided in *Table 1*.

Table 1. Materials used for adherent hiPSC cell culture

Componentes	Descriptions
Cell Line	hiPSC
Media	StemFlex™
Wash Solution	Proprietary
Dissociation Reagent	PBS
Quenching agent	TrypLE™ Express
Hardware	Incubator setup; Volume -6 mL; Available surface area 120 cm ²

Automated sampling was performed at 24 hrs interval to assess metabolite consumption.

After 5 days of expansion in the BFB, cells were harvested from the cartridge with TrypLE™ Express via automated fluid dispensing and collection. Automated cell extraction from the BFB does not need TFF.

One BFB was sacrificed on day 2, sectioned, and stained for alkaline phosphatase. Next, assessment of cell viability, identity, and pluripotency was performed with a trypan blue exclusion assay, cell cytometry (SSEA4 and TRA-1-60), and qPCR, respectively.

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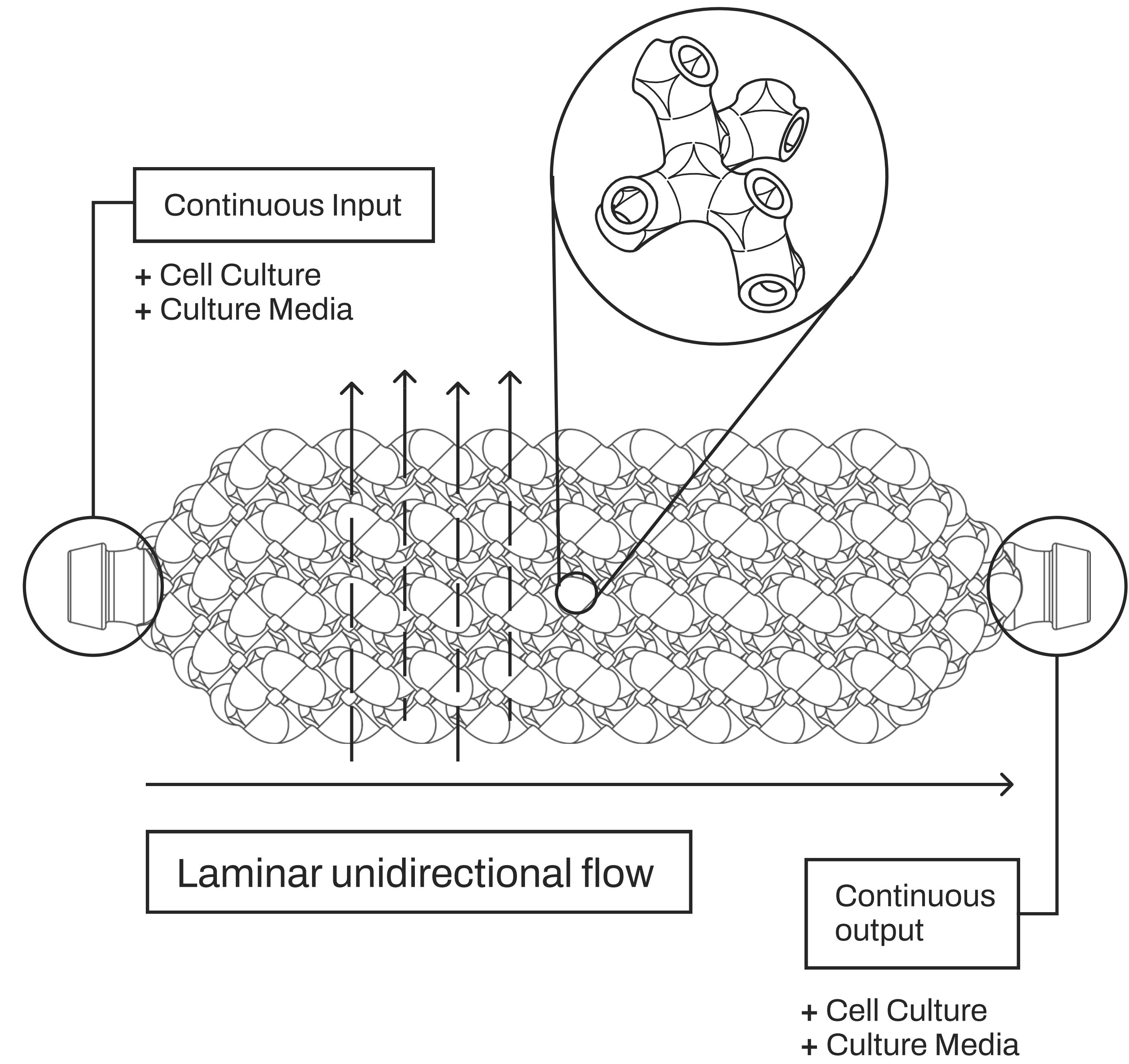


Image 1. Bubble-free Bioreactor model Aries 6 mL.

Results and Discussions

Human iPSCs seeded in the BFB for a 5-day expansion showed 6.4X growth, with peak cell density reaching 6.4×10^4 cells/cm² (Figure 1). The control set of cells reached 7.5×10^4 cells/cm² during the same growth period (Figure 1). While the cell growth in the BFB was slightly lower than the control, cell viability remained remarkably high at 95% and 97% for iPSCs expanded in the BFB and control, respectively (Figure 1, right axis).

To visualize cells cultured in the BFB, a 6 mL bioreactor cartridge with iPSCs seeded and grown for 48 hrs was cut into sections and stained with a fluorescent substrate for alkaline phosphatase (ALP) (Figure 2), whose activity is strongly upregulated in undifferentiated pluripotent cells (4). As seen in Figure 2, cell morphology and expression of the early pluripotency marker were maintained, with an even distribution of cells along the BFB inner walls.

After expansion for 4 days, iPSCs were harvested with TrypLE™ Express, and total RNA was extracted to analyze the expression of pluripotency markers SOX2, OCT4, and NANOG by qRT-PCR (5). Crucially, all markers showed a similar expression profile when compared to the control (Figure 3), confirming that the cells successfully maintained their pluripotency within the BFB. Further fluorescence-activated cell sorting (FACS) was performed to identify cell surface markers SSEA4 and TRA-1-60, which are highly expressed on undifferentiated pluripotent cells (6). Both control and BFB-expanded iPSCs showed high expression (>80%) of these cell surface antigens (Figure 4).

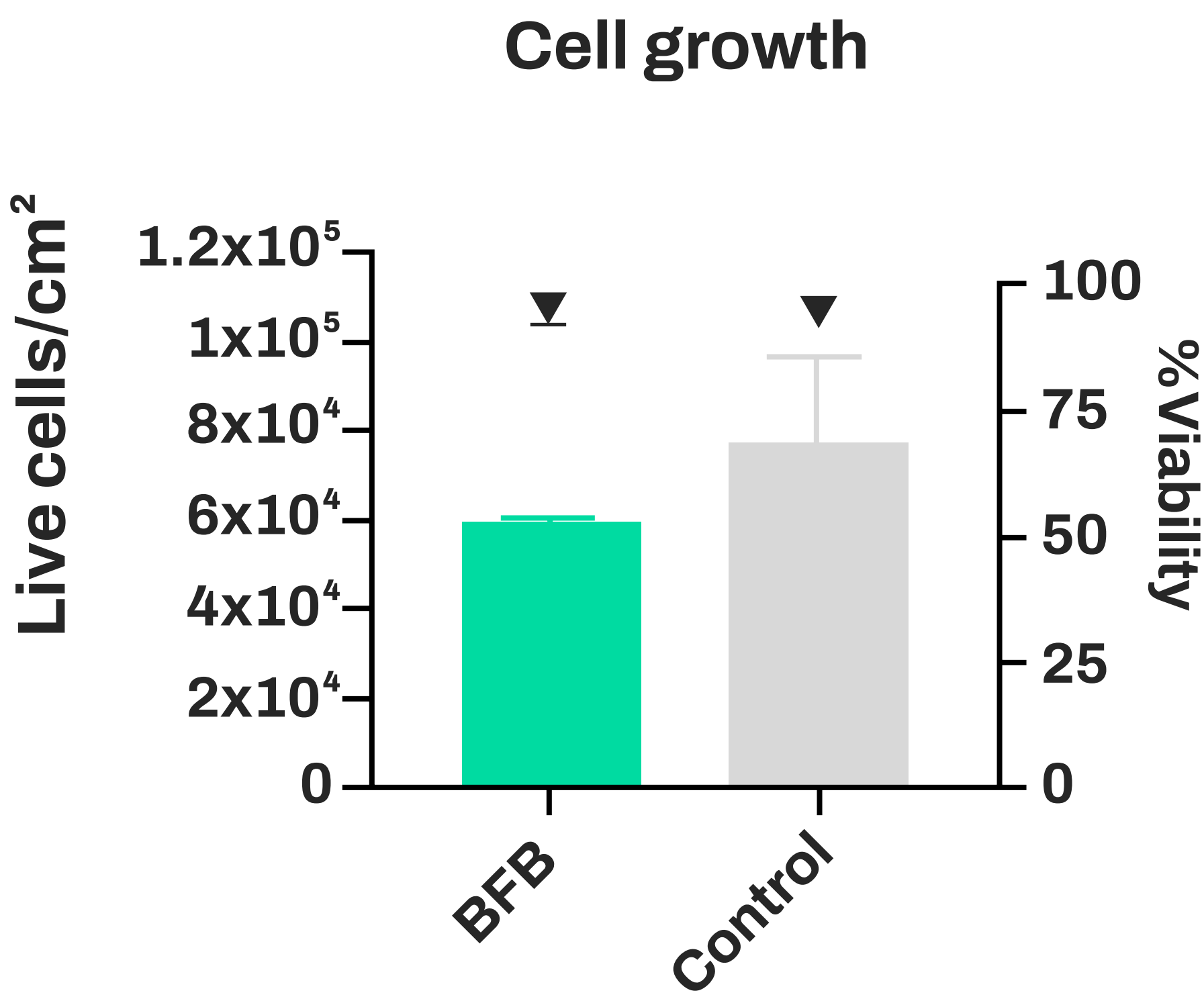


Figure 1. iPSCs culture expansion rate after 5 days. Viability over 95%. Control: Geltrex-coated 12-well plate. Bar graphs depict mean cell density with error bars representing SD (n=6).

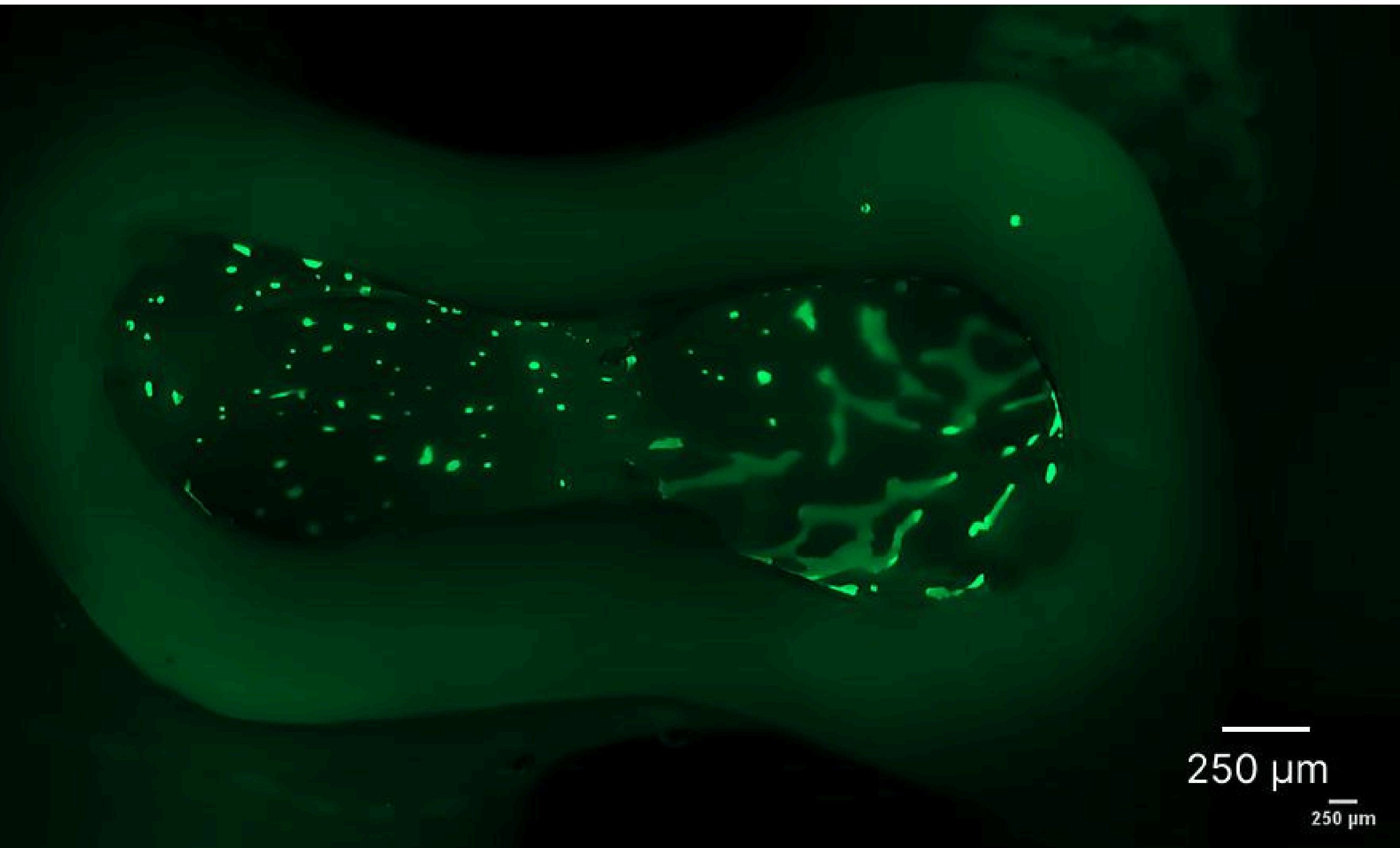


Figure 2. Fluorescent micrograph of a BFB channel section. iPSCs cultured for 48 hours and stained (green) for the pluripotency marker alkaline phosphatase. Scale bar: 250 microns

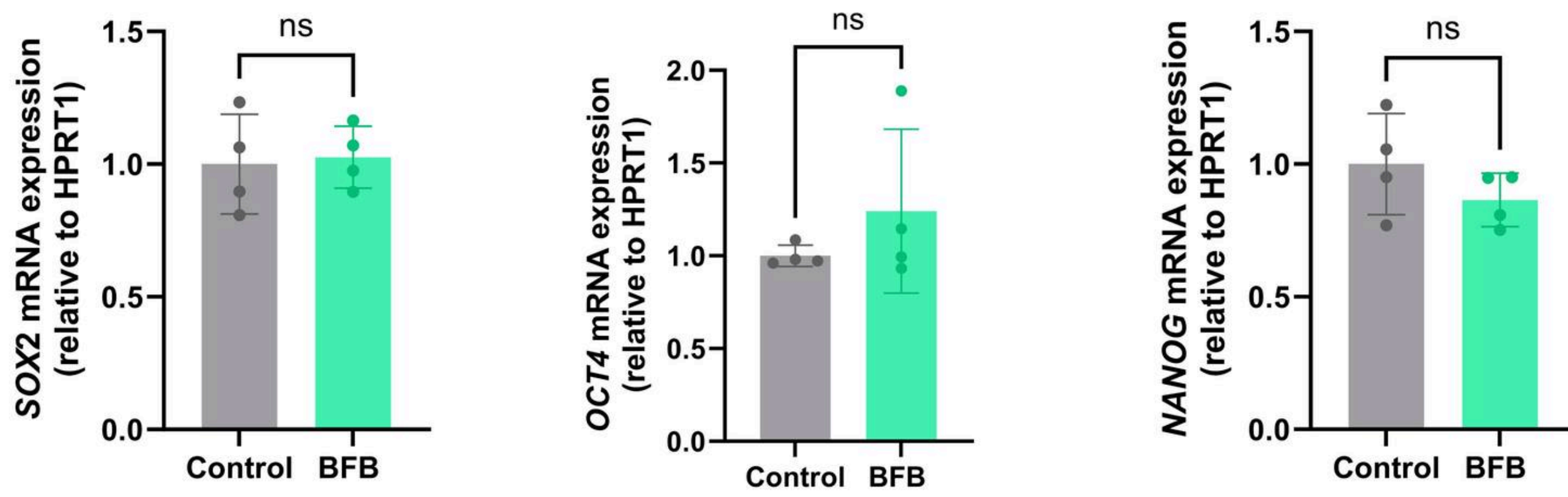


Figure 3. Gene expression was quantified by qPCR. SOX2, OCT4, and NANOG were evaluated. Cells harvested from the BFB and control plates were cultured for 5 days in multiwell plates before analysis. The bar graph represents mean expression values (n=4) normalized to the HPRT1 gene. Error bars represent SD. Test of significance: ns (non-significant), p-value=0.05.

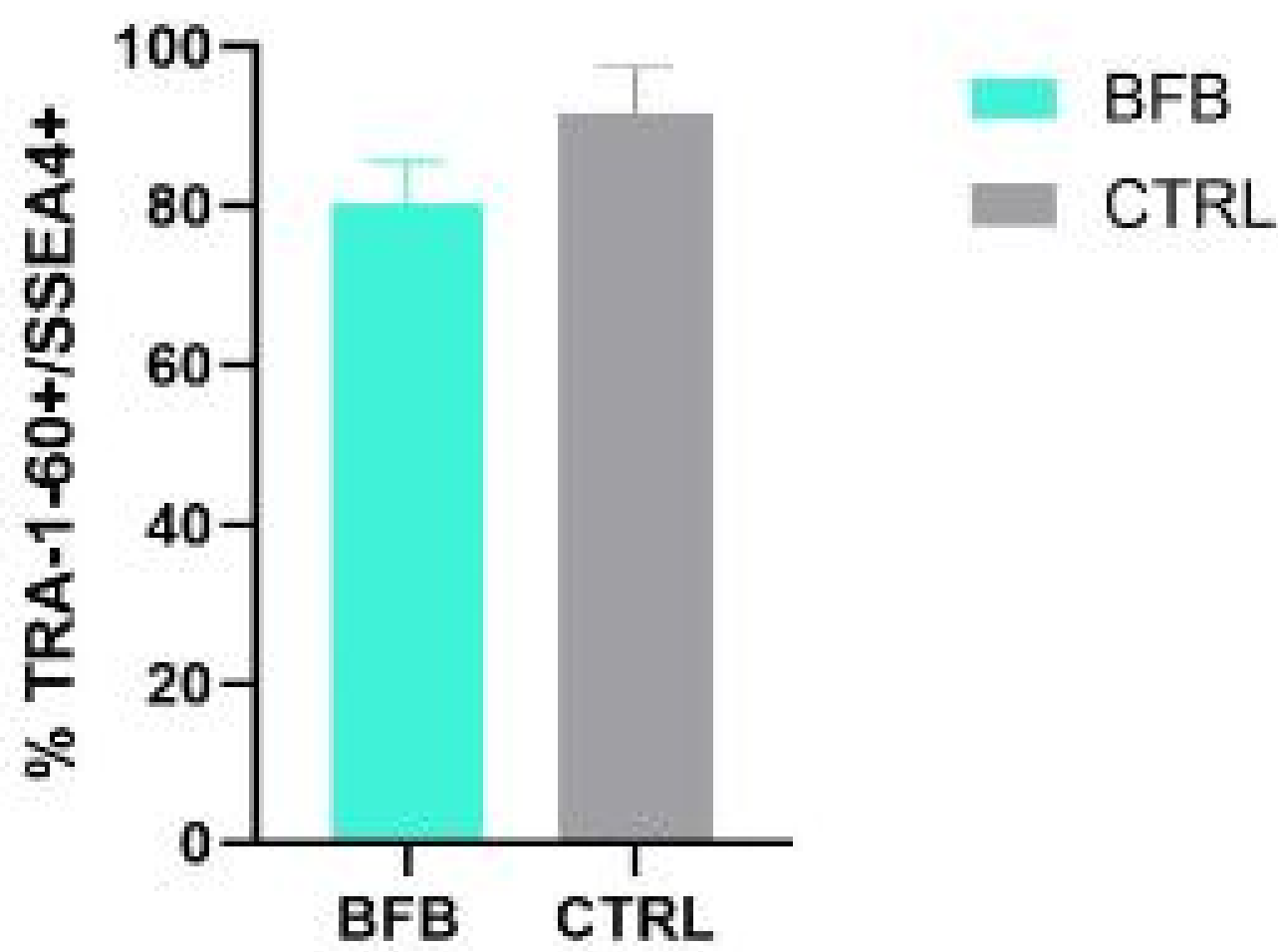


Figure 4. Identity markers of iPSCs cultured in BFB and control plates. Cells harvested from the BFB and control plates were cultured for 5 days in multiwell plates before. TRA-1-60/SSEA ratio of positive cells

Conclusions

Stämm's BFB provides an effective solution to the bottlenecks of scalable, automated, and functionally closed manufacturing systems for iPSCs. The expansion could be obtained within 5 days with high pluripotency, excellent cell viability, and functional cellular health and quality.

Within 4 days, similar cell expansion to the reference can be achieved. Besides, the high surface-to-volume ratio (20 cm²/mL) of the cell culture cartridges supports key identity and pluripotency markers.

References

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