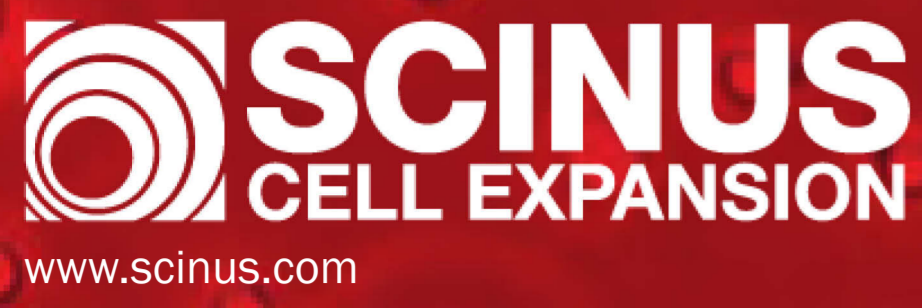


Closed, Suspension-based Expansion Of Human Induced Pluripotent Stem Cells In The Osilaris™ Bioreactor For Multiple Passages



Juda-EL Jno Baptiste-Sam¹, Sofia Carvalho², Caroline Borges-Pereira², Joana R. Loureiro², Ruud Das¹

1. Scinus Cell Expansion Netherlands B.V., Zeist, the Netherlands

2. Stematters, Biotecnologia e Medicina Regenerativa SA Parque de Ciência e Tecnologia Avepark, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION

Induced pluripotent stem cells (iPSCs) have great potential in cell therapy but manufacturing using traditional 2D culture methods are inefficient and expensive. We developed a closed-loop passing strategy for a cell-only aggregate-based hiPSC expansion workflow, lowering contamination risk and improving efficiency for GMP-compliant manufacturing using the Osilaris™ bioreactor (Figure 1).

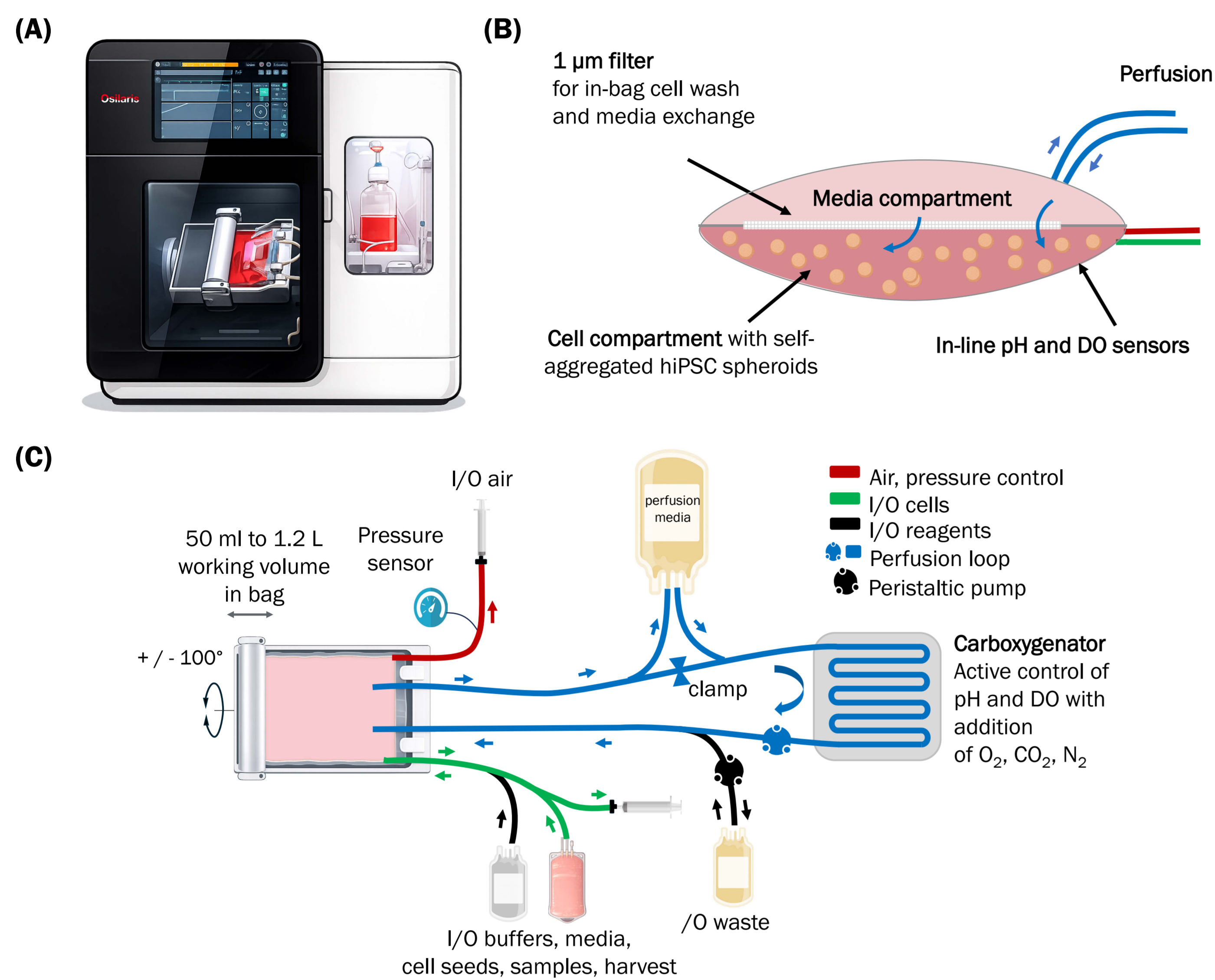


Figure 1: (A) The Osilaris™ bioreactor. (B) Cross-section view of the Osilaris™ suspension culture bag with spheroids. (C) Schematic of the fluidic pathway in the Osilaris™ cabinet.

MATERIALS AND METHODS

Cultivating WTC-11 iPSCs in the Osilaris bioreactor involved key steps outlined in Figure 2. Single cells were seeded into an Osilaris™ suspension bag at a density of 1×10^5 cells/ml in 300 mL Y-27632-supplemented StemScale medium. The development of self-aggregating spheroids were controlled in a dynamic environment with a rocking regimen of 350°/s at an angle of 90°.

Spheroid size was maintained by adjusting the rocker's vertical pause during growth to prevent clumping of spheroids the larger they become. To avoid necrosis, spheroid size was kept under 400 μm (± 50 μm) by enzymatic dissociation every 3-4 days using Accutase (Figure 2). In-bag dissociation steps were performed in a closed-loop setup by adjusting the Osilaris™'s flow paths. After sampling and counting, the bag was expanded to dilute the culture back to 1×10^5 cells/ml. Pluripotent capacity was confirmed through 2D characteristic retention, flow cytometry, and differentiation potential after 3 passages.

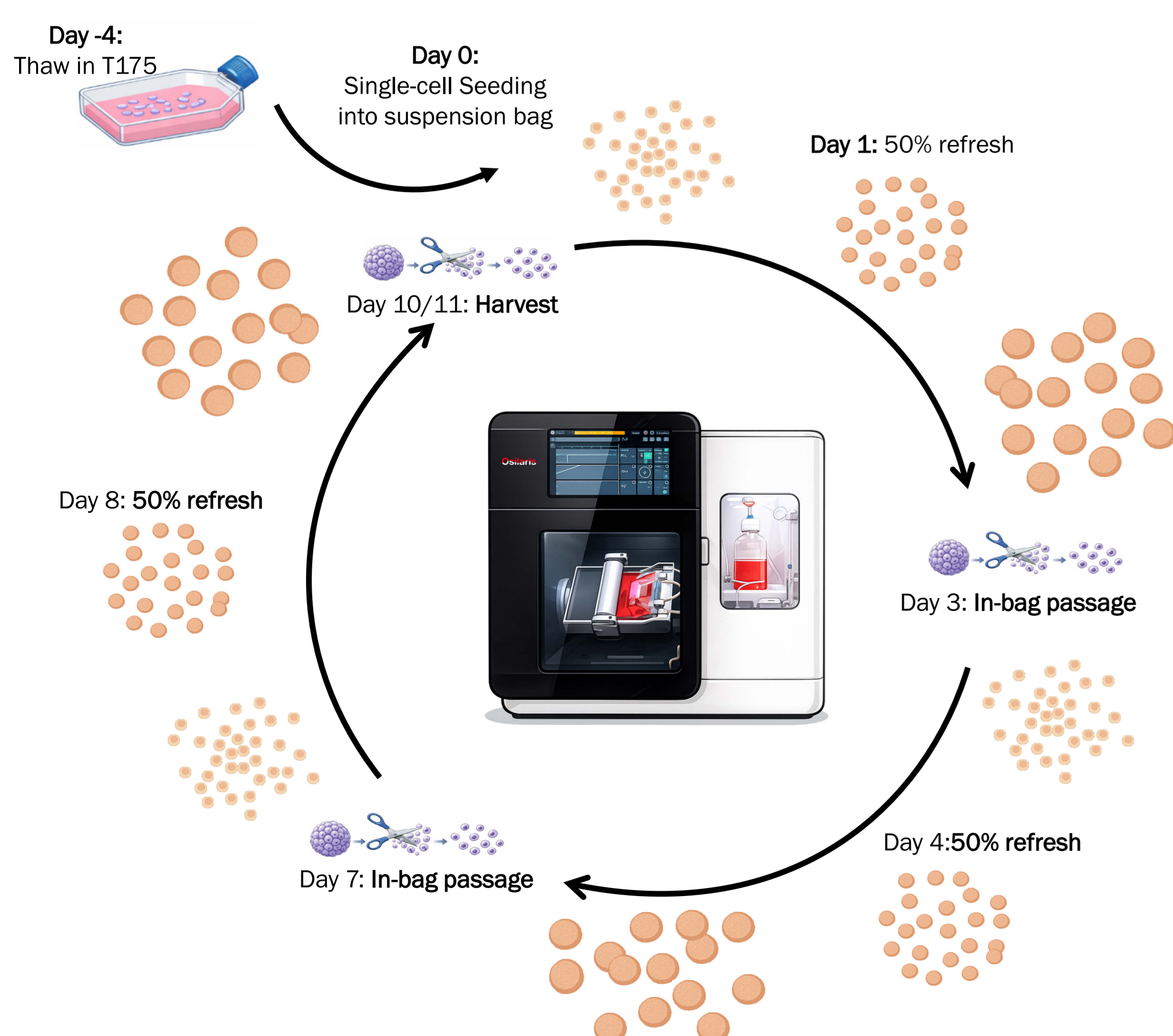


Figure 2: Process overview of hiPSC expansion in the Osilaris™ bioreactor. A single cell inoculum is seeded directly into the suspension bag and over 3-4 days, spheroids form (100-200 μm) and grow (300-400 μm). In-bag dissociation then is executed by using the bag's built-in filter to drain media, replace with Accutase, digest spheroids back to single cells, and finally dilute the culture back to the initial cell density, leveraging the unique expansion roller. This process is repeated 2 times before harvest at day 10/11 when the expanded hiPSCs are collected for downstream analysis.

RESULTS

hiPSC Expansion

An average of 4.1×10^8 single cells ($\pm 1.3 \times 10^8$ cells, n=3) were yielded from Osilaris™ run (Figure 3B), representing a fold change of up to 18.33 in 10/11 days. Figure 3 represents results of one donor, but similar results were obtained for multiple donors.

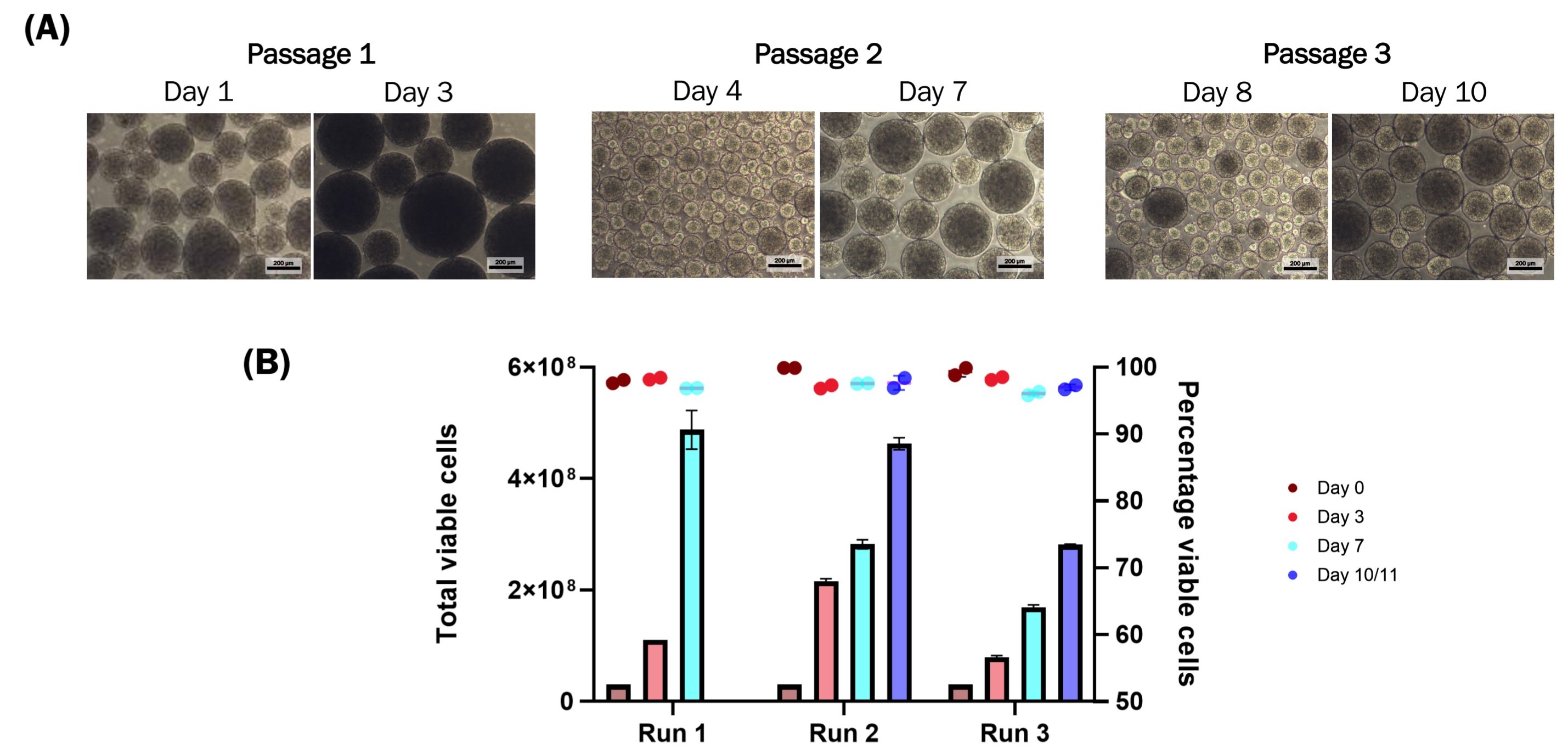


Figure 3: (A) Representative visual inspections of hiPSC spheroids over a typical 10-day expansion (scale bar 200 μm). (B) Total viable cell counts (left y-axis, bars) and cell viability (right y-axis, dots) of dissociated spheroids after each in-bag dissociation step for 3 separate expansion runs.

Cell processing and characterization

Following in-bag passages, a sample of monocellular suspension was reseeded in LN511-coated plates (2D) and demonstrated iPSC-like morphology with dense colonies and high nucleo-cytoplasmic ratio (Figure 4C). The population doubling time remained under 24 hours, consistent with typical iPSC behavior.

Flow cytometry revealed >95% expression of pluripotency-associated transcription factors Oct3/4, SOX2, and Nanog, as well as surface markers SSEA-4 and TRA-1-60, while differentiation marker CD13 was expressed at <1% (Figure 4A). Although lower efficiency towards ectoderm was observed, trilineage differentiation was successful and confirmed the culture's capacity to differentiate into three germ layers (Figure 4B).

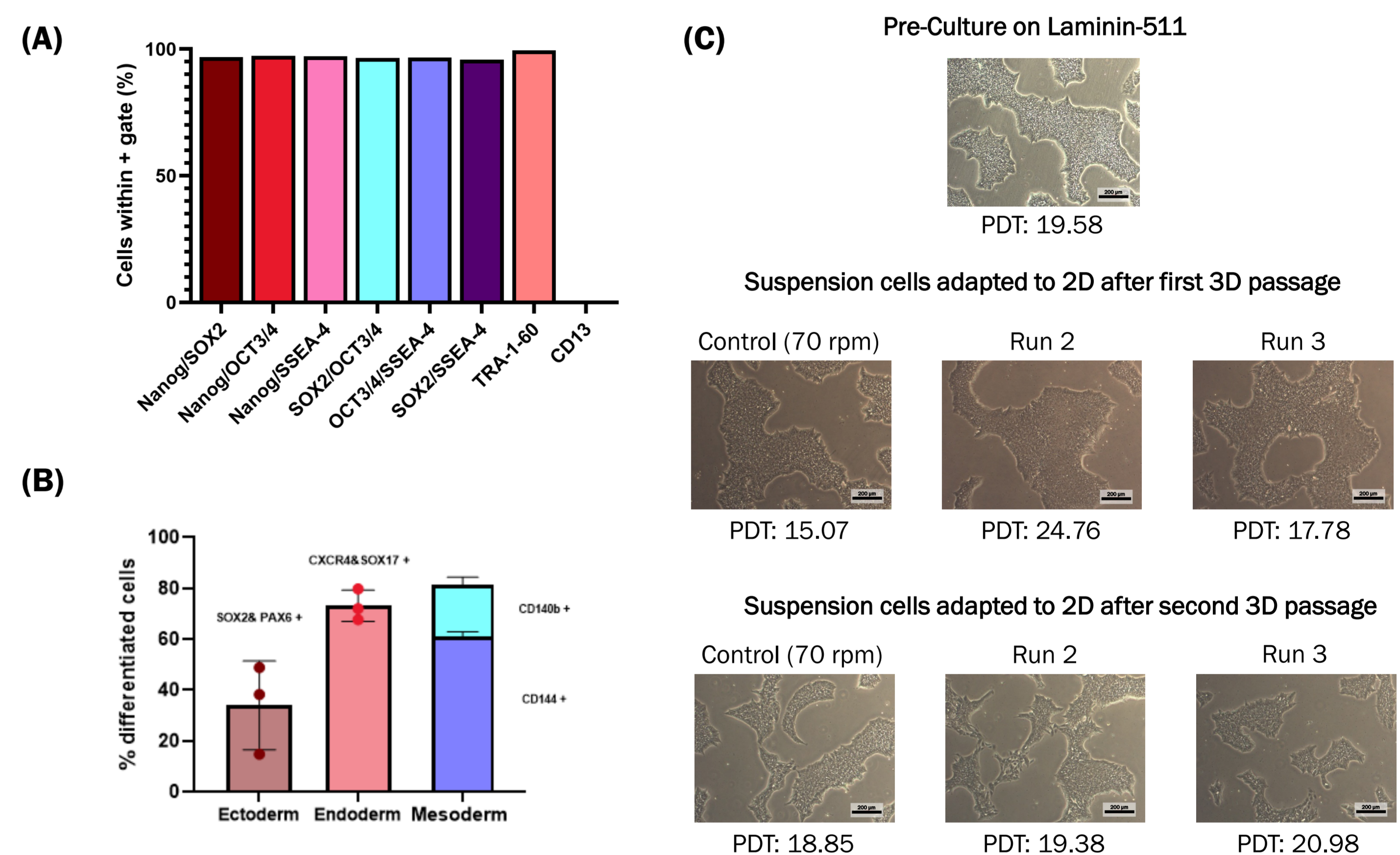


Figure 4: Characterization of hiPSCs cultured in the Osilaris™. (A) Population percentage of hiPSCs positive for pluripotency-associated transcription factors (OCT3/4, SOX2, Nanog), pluripotency-associated surface markers (SSEA-4, TRA-1-60), and differentiation (CD13). (B) Population percentage of cells differentiating into cells of each germ layer. (C) Adaptation of dissociated spheroids on LN511-coated surfaces, exhibiting typical hiPSC morphology and population doubling time <24 hrs.

CONCLUDING REMARKS

The workflow described in this study, positions the Osilaris™ bioreactor as a unique platform enabling a GMP-aligned, closed workflow. A fully closed, single-use bag system enables streamlined, end-to-end hiPSC manufacturing compatible with GMP-compliant productions demanding flexible and controllable culture parameters.

This workflow enables robust and reproducible expansion of hiPSCs, while preserving key pluripotent characteristics, including growth profile, morphology, marker expression, and tri-lineage differentiation. Ongoing studies are aimed at validating this process with multiple donors to evaluate its suitability for scalable generalized hiPSC-manufacturing using the Osilaris™ bioreactor.