Scalable Manufacturing of Functional Islets From hiPSCs Using GMP-Grade Reagents and Vertical-Wheel® Bioreactors

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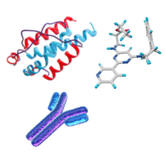
Background

Human induced pluripotent stem cells (hiPSCs) represent a promising and renewable source for generating insulin-secreting islets, offering a potential curative cell therapy for Type 1 diabetes. Despite this promise, significant challenges still limit the large-scale production of hiPSC-derived islets suitable for clinical use. Key obstacles include limited availability of GMP-grade reagents compatible with closed system, scalable bioprocessing platforms, and the lack of high-throughput, automated quality control tools necessary to ensure manufacturing consistency and critical quality attributes of the cell therapy products.

To address these hurdles, we present here a versatile and scalable workflow utilizing Vertical-Wheel bioreactors (VWB) in combination with GMP-grade reagents, enabling robust expansion and differentiation of hiPSCs into functional islets. Complemented by automated analytical technologies, this integrated approach supports the production of clinically relevant hiPSC-derived islets with consistent quality, thereby accelerating the translation of hiPSC-based cell therapies for diabetes treatment.

Materials and Methods

We first optimized culture conditions across multiple hiPSC lines to enable robust 3D expansion using GMP-grade reagents and instruments. Next, we developed a hybrid 2D–3D differentiation workflow in which hiPSCs were initially directed toward pancreatic progenitors in 2D culture, followed by further differentiation and maturation within Vertical-Wheel bioreactors to produce insulin-secreting islets. At key stages, cells were characterized for identity and function using flow cytometry, automated ELISA (Ella™), and immunofluorescence microscopy.



R&D Systems Reagent Portfolio

We utilized a panel of proteins, small molecules, and antibodies from the R&D Systems reagent portfolio to expand, differentiate, and characterize hiPSC-derived cell types.

PBS-0.1 Mini Vertical-Wheel Bioreactor

We used the PBS-0.1 Mini Vertical-Wheel bioreactor system to enable scalable 3D expansion and differentiation of hiPSCs.

Ella-Automated Immunoassay Platform

We measured insulin secreted by beta cells in culture supernatants using the Ella automated ELISA platform with the Simple Plex™ Human Insulin cartridge, enabling sensitive, precise, and high-throughput quantification.

R&D Systems Reagents Used in the Study

hiPSC Maintenance and Expansion	Catalog #
ExCellerate™ iPSC Expansion Medium, Animal-Free, GMP	CCM036-GMP
Cultrex™ UltiMatrix Reduced Growth Factor Basement Membrane Extract	BME001-005
Y-27632 dihydrochloride, GMP	TB1254-GMP

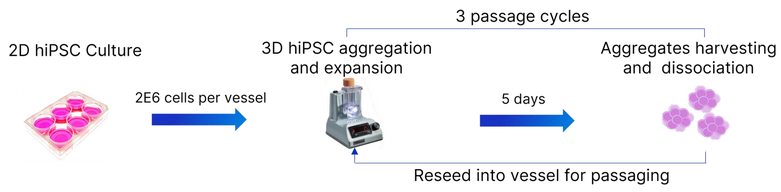
Beta Cell Differentiation	Catalog #
Recombinant Human/Mouse/Rat Activin A GMP Protein	338-GMP
Recombinant Human KGF/FGF7 GMP Protein	BT-KGF-GMP
CHIR 99021, GMP	TB4423-GMP
Retinoic acid	TB0695-RMU
LDN 193189, GMP	TB6053-GMP
TPPB	5343
SANT-1	1974
RepSox	TB3742-RMU
T3	TB6666-RMU
DAPT	TB2634-RMU
L-Ascorbic acid	TB4055-RMU
Heparin sodium salt	2812

Cell Lineage Characterization	Catalog #
FlowX FoxP3/Transcription Factor Fixation & Perm Buffer Kit	FC012
Human/Mouse Oct-3/4 APC-conjugated Antibody	IC1759A
Human Nanog PE-conjugated Antibody	IC1997P
Human/Mouse SOX2 APC-conjugated Antibody	IC2018A
Human/Mouse SSEA-4 APC-conjugated Antibody	FAB1435A
Human SOX17 APC-conjugated Antibody	IC1924A
Human HNF-3 beta/FoxA2 Alexa Fluor® 488-conjugated Antibody	IC2400G
Human/Mouse PDX-1/IPF1 PE-conjugated Antibody	IC2419P
Human C-Peptide Antibody	MAB14171
DA ZP1	7444

Functional Characterization of Islets	Catalog #
Simple Plex Human Insulin Cartridge	SPCKB-PS-000507
Ella Automated Immunoassay System	600-100

Results

3D Expansion of hiPSCs in Vertical-Wheel Bioreactors



We developed a robust 3D hiPSC expansion protocol in the PBS-0.1 Mini bioreactor by optimizing key culture parameters such as agitation speed and cell seeding density[1,2]. This enabled scalable and reproducible expansion of hiPSC aggregates across multiple cell lines while maintaining expression of pluripotency markers. The method supported consistent growth kinetics, demonstrating its suitability for scalable hiPSC production.

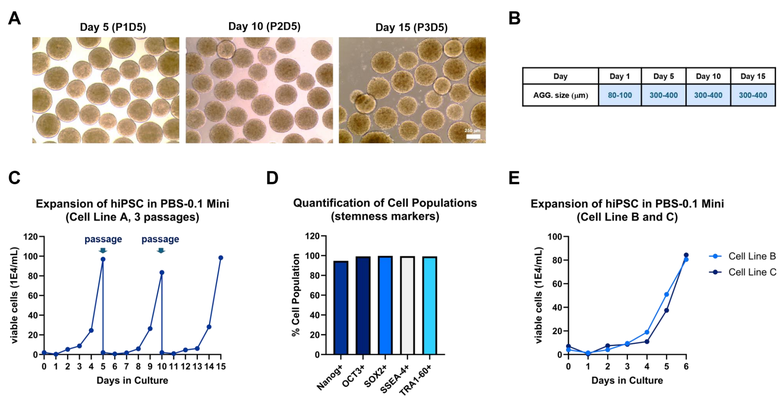
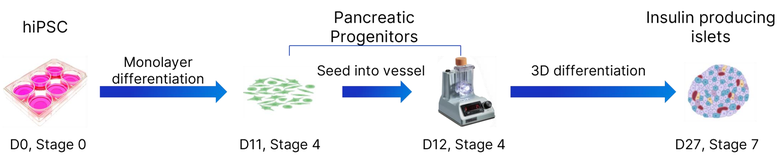


FIGURE 1. 3D expansion of hiPSCs in Vertical-Wheel bioreactors. (A) hiPSCs (Cell Line A) were expanded in PBS-0.1 Mini bioreactors over three consecutive passages. Aggregate morphology was assessed at the end of each passage using an EVOS Cell Imaging System. **(B)** Expanded hiPSC aggregates exhibited a homogeneous size distribution, ranging from 300–400 µm. **(C)** Quantification of viable hiPSCs (Cell Line A) demonstrated robust and consistent expansion across three passages. **(D)** Flow cytometry analysis of expanded hiPSCs (Cell Line A) at day 15 confirmed high expression of pluripotency markers. **(E)** Two additional hiPSC lines (Lines B and C) were evaluated and showed comparable expansion performance in PBS-0.1 Mini bioreactors.

2D-3D Differentiation of hiPSCs into Islets in Vertical-Wheel Bioreactors



We next established a hybrid 2D-3D differentiation protocol to generate hiPSC-derived beta cells[3-5]. Beginning with monolayer differentiation, hiPSCs were guided into pancreatic progenitor cells by day 12. These progenitors were then aggregated and further differentiated and matured into pancreatic beta cells within 3D culture using the PBS-0.1 Mini bioreactor until day 28. The resulting pancreatic islet-like clusters exhibited positive DTZ staining, indicative of abundant insulin granules.

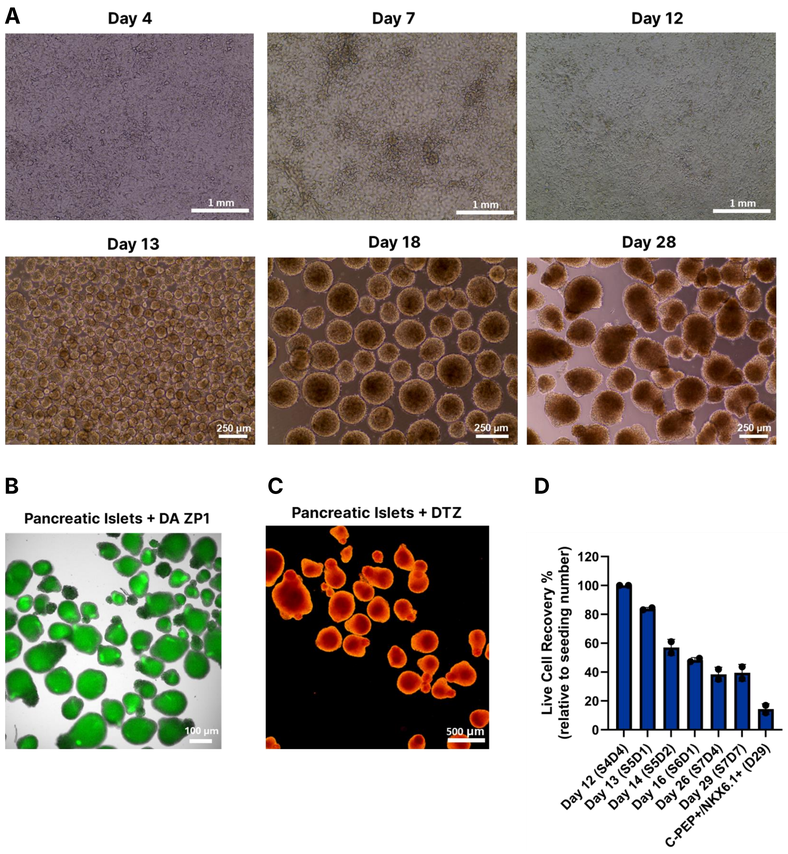
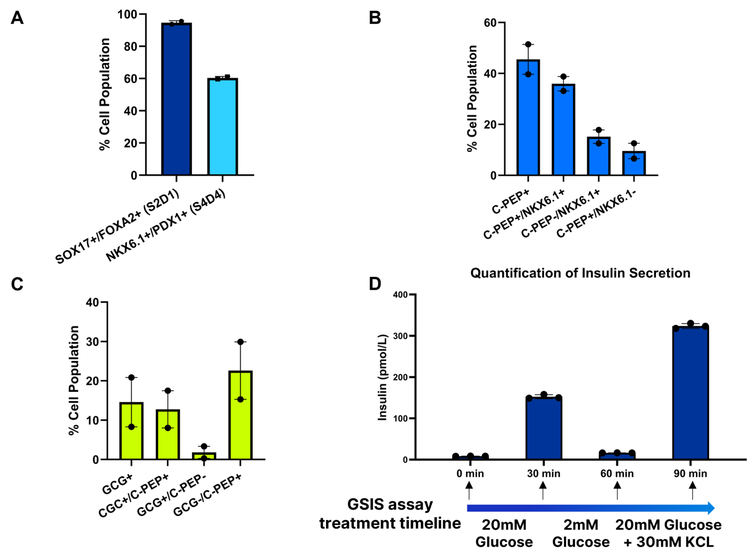


FIGURE 2. 2D–3D differentiation of hiPSCs into pancreatic islet–like clusters in Vertical-Wheel bioreactors. (A) hiPSCs were differentiated using a hybrid 2D–3D workflow. Representative brightfield images show cell morphology across key stages of differentiation, captured using an EVOS Cell Imaging System. **(B)** hiPSC-derived pancreatic islet–like clusters were stained with DA ZP1 and imaged using a Leica THUNDER Imaging System. **(C)** hiPSC-derived pancreatic islet–like clusters were stained with dithizone (DTZ) and imaged using an EVOS Cell Imaging System. **(D)** Cell recovery from Stage 4 through Stage 7 was quantified relative to the initial seeding density (Exp1: 0.5E6/mL; Exp2: 1.2E6/mL) in the PBS-0.1 Mini bioreactor. Data are presented as the mean of two biological replicates.

Quantitative and Functional Characterization of hiPSC-Derived Beta Cells

Comprehensive analyses were conducted to characterize cell identities at intermediate stages and to assess the composition of the final cell product. The differentiation protocol successfully generated over 90% definitive endoderm (DE) cells and 60% pancreatic progenitor (PP) cells. By the end of the process, around 40% of the population consisted of C-PEP⁺/NKX6.1⁺ beta cells. Immunostaining of the islet-like clusters revealed a heterogeneous cellular composition, comprising beta cells (C-peptide⁺), alpha cells (glucagon⁺), and delta cells (somatostatin⁺), reflecting the cellular diversity and functional architecture within the islets. Importantly, these islets demonstrated glucose-responsive insulin secretion, confirming their functional maturity.



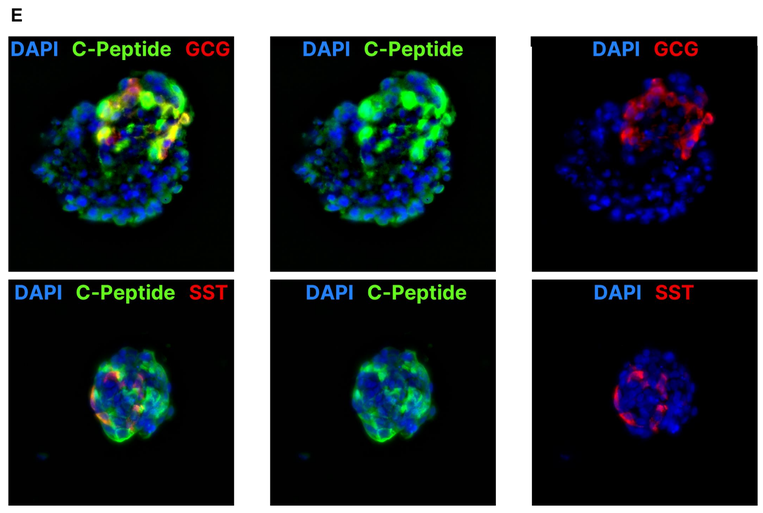


FIGURE 3. Characterization of the hiPSC-derived beta cells. (A) Definitive endoderm cells were stained with anti-SOX17 and anti-FOXA2 antibodies and quantified by flow cytometry. Pancreatic progenitor cells were stained with anti-PDX1 and anti-NKX6.1 antibodies and similarly quantified. **(B)** Beta cells were stained with anti–C-peptide and anti-NKX6.1 antibodies and analyzed by flow cytometry. **(C)** Alpha cells were stained with anti–C-peptide and anti-glucagon (GCG) antibodies and analyzed by flow cytometry. **(D)** Glucose competence was assessed by a glucose-stimulated insulin secretion (GSIS) assay. Culture supernatants were analyzed using the Ella automated ELISA platform with Simple Plex Human Insulin Cartridge. **(E)** hiPSC-derived pancreatic islet–like clusters were fixed, cryo-sectioned, and stained for C-peptide, glucagon (GCG), and somatostatin (SST). Images via Leica THUNDER Imaging System. Data are presented as the mean of two (A-C) and three (D) biological replicates.

Conclusions

We established a scalable and reproducible 3D hiPSC expansion and hiPSC-derived beta cell differentiation workflow in Vertical-Wheel bioreactors that yields functionally mature islets.

GMP-grade, animal component-free R&D Systems proteins and small molecules supported robust cell growth, lineage specification, and maturation throughout the workflow, reinforcing translational readiness.

Quantitative, high-throughput characterization using R&D Systems antibodies and the Ella automated immunoassay platform enabled confident assessment of cell identity and glucose competence.

Together, this integrated manufacturing and analysis workflow offers a robust platform for large-scale production and evaluation of hiPSC-derived islets, supporting continued advances in diabetes research and cell therapy applications.

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