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Differentiation of StemScale hPSC suspension cultures to definitive endoderm lineages

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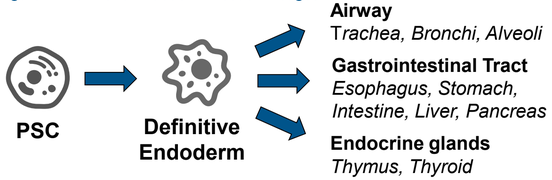
ABSTRACT

StemScale PSC Suspension Medium supports the suspension culture of pluripotent stem cells (PSCs) self-assembled into spheroids. Suspension culture simplifies handling and scale-up of PSC growth and differentiation workflows. PSC spheroid cultures can be differentiated to definitive endoderm (DE) cells in suspension using the PSC Definitive Endoderm Induction Kit. Effective DE induction is demonstrated in both human induced-PSC and embryonic stem cells. The utility of differentiated DE cells is demonstrated by further differentiation to lung epithelial cell organoids.

INTRODUCTION

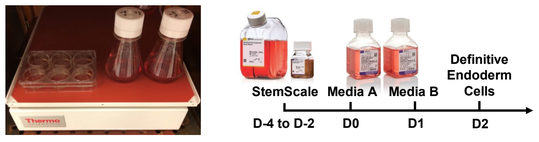
The definitive endoderm is one of the three primary embryonic germ layers and generates the epithelial cells lining the airway and digestive tract, and also contributes to vital organs including the lungs, liver, pancreas, thymus, and thyroid[1]. Thus, reprogramming pluripotent stem cells into definitive endoderm is the first step in generating PSC-derived models of many critical tissues. Production of definitive endoderm cells and their downstream lineages at scale may contribute to advances in disease modeling, cell and gene therapy, as well as drug discovery and safety screening[2]. Suspension culture enables reproducible and scalable growth of PSCs, which self-assemble into spheroids. Emerging evidence demonstrates that part or even all of some PSC differentiation protocols can be performed in suspension to leverage these same advantages.

Figure 1. Definitive Endoderm Tissue Lineages



MATERIALS AND METHODS

Figure 2. Suspension Workflow for Definitive Endoderm Induction

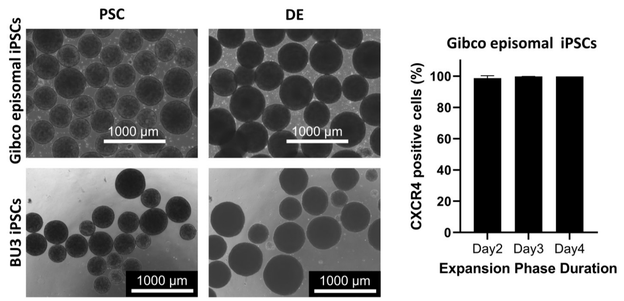


Cell culture.
The Gibco™ Human Episomal iPSC, BU3 iPSC, and WA09 hESC lines were cultured in StemScale™ media in non-TC treated vessels, agitated at 70 rpm on a Thermo Scientific™ CO_2 resistant orbital shaker placed within an incubator at 37°C and 5% CO_2. Differentiation to definitive endoderm was performed using the Gibco™ PSC Definitive Endoderm Induction Kit according to the manufacturer's protocol.

Cell analysis.
Flow cytometry was performed on the Invitrogen™ Attune NxT Flow Cytometer. Fluorescence imaging was performed on the CellInsight™ CX7 High Content Analysis Platform. RNA was isolated using the Invitrogen™ PureLink™ RNA Mini Kit and reverse transcribed using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit. Gene expression levels were measured using predesigned TaqMan™ Gene Expression Assays and the QuantStudio™ 12K Flex Real-Time PCR System. TEER measurements were made using a WPI EVOM2.

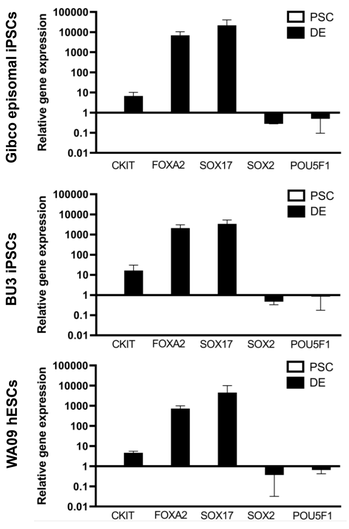
RESULTS

Figure 3. Efficient Induction of Definitive Endoderm in Suspension



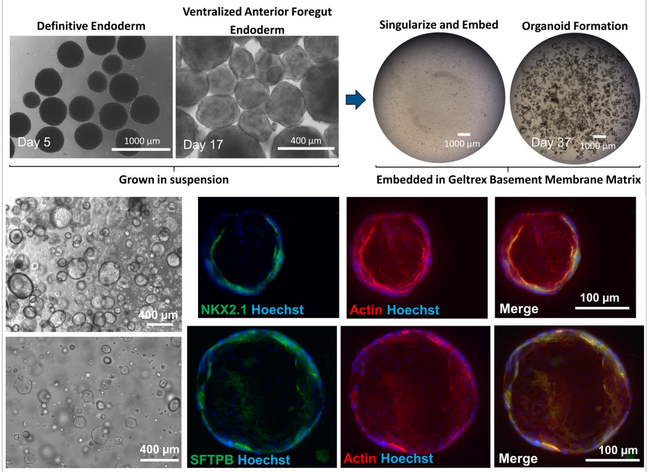
Suspension growth and differentiation of PSCs to definitive endoderm.
PSCs grown in suspension in StemScale (left column) were differentiated to definitive endoderm (right column) using the Gibco Definitive Endoderm Induction Kit. To determine if there is an optimal timing to begin this differentiation, PSC spheroids were passaged and then expanded in StemScale for 2, 3, or 4 days prior to DE induction. The resulting definitive endoderm spheroids were dissociated to single cells and performance was assessed quantitatively by flow cytometry for CXCR4, a definitive endoderm marker. >95% of cells from each condition were CXCR4 positive. Efficient definitive endoderm induction at each of these time points provides flexibility for incorporating this method into a differentiation workflow.

Figure 4. Definitive Endoderm Gene Expression



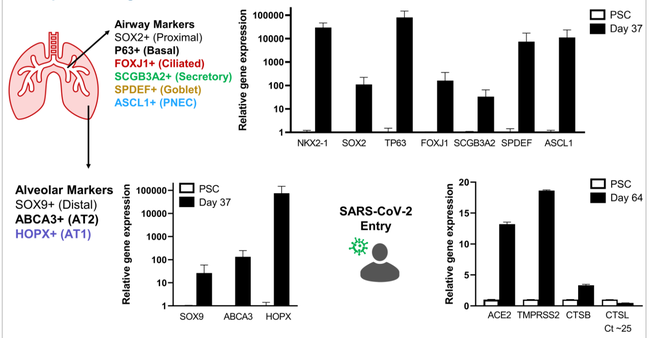
Consistent gene expression changes across PSC lines.
Two iPSC lines and one hESC line were grown in StemScale and then differentiated to definitive endoderm in suspension. The resulting definitive endoderm spheroids were dissociated and RNA was isolated from the cells so that gene expression changes could be compared to undifferentiated PSCs using qPCR (2ΔΔCt method). In all three lines, the definitive endoderm markers CKIT, FOXA2, and SOX17 were significantly upregulated in definitive endoderm cells. In contrast, expression of the pluripotency markers, SOX2 and POU5F1 (OCT4), was decreased in definitive endoderm cells.

Figure 5. Differentiation of Definitive Endoderm Cells to Lung Epithelial Organoids



Leveraging suspension culture during differentiation.
Definitive endoderm cells were differentiated to ventralized anterior foregut endoderm while in suspension. The resulting spheroids were dissociated to single cells and embedded in Geltrex basement membrane matrix domes, where they formed organoids containing lung epithelial-like cells that expressed the lung and thyroid transcription factor, NKX2.1, as well as surfactant protein B (SFTPB)[3].

Figure 6. Lung Epithelial Cell Markers and SARS-CoV-2 entry receptors in Lung Epithelial Organoids

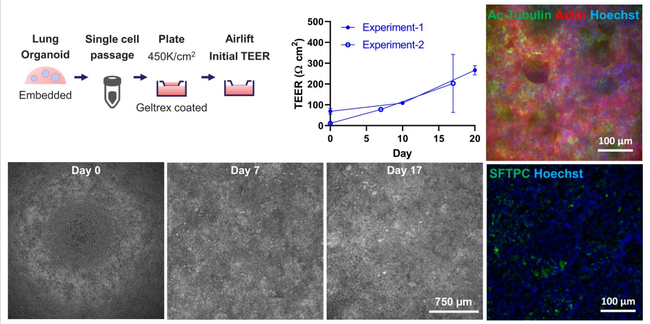


Ct ~25

Definitive endoderm-derived lung epithelial organoids express airway and alveolar markers.
Lung epithelial organoids derived from definitive endoderm cells were dissociated to single cells and lysed for RNA extraction. qPCR revealed the enrichment (compared to undifferentiated PSCs) of key lung-related transcription factors: NKX2.1, as well as SOX2 and SOX9, which mark proximal and distal airway cells, respectively[3]. Markers for the major airway epithelial constituents were expressed, demonstrating differentiation to lung epithelial-like cells.

Lung epithelial organoid cells also expressed the major proteins responsible for SARS-CoV-2 infection in human lungs, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), as well as cathepsin proteases that play a role in SARS-CoV entry[4].

Figure 7. Air-Liquid Interface Culture of PSC-Derived Epithelial-Like Cells



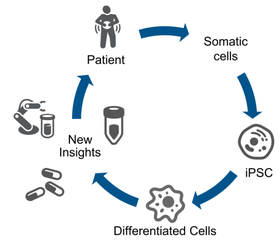
PSC-derived lung epithelial-like cells grow at air-liquid interface.
Cells dissociated from PSC-derived lung epithelial organoids were plated on cell culture inserts and then grown at air liquid interface. Cells form a monolayer initially that becomes multi-layered by day 7, corresponding to steadily increasing trans-epithelial electrical resistance (TEER). Immunofluorescence reveals expression of acetylated alpha-tubulin and surfactant protein C (SFTPC) at the apical surface.

CONCLUSIONS

StemScale PSC suspension cultures are compatible with the Definitive Endoderm Induction Kit and provide workflow and scalability advantages for stem cell differentiation.

PSC-derived definitive endoderm-derived cells may be an important tool for generating models of a wide range of tissues and diseases. StemScale PSC suspension culture provides a consistent and cost-effective method for growth, scale-up, and banking of PSC lines. Differentiation of PSC suspension cultures may be used to scale up important models for banking, performing large high-throughput screening or for regenerative medicine applications.

Figure 8. Potential Impact of iPSC-derived Models



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TRADEMARKS/LICENSING

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