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**Practical choice for robust and efficient differentiation of human pluripotent stem cells**

Fang M *et al*. Efficient differentiation of hPSCs

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**Abstract**

Human pluripotent stem cells (hPSCs) have the distinct advantage of being able to differentiate into cells of all three germ layers. Target cells or tissues derived from hPSCs have many uses such as drug screening, disease modeling, and transplantation therapy. There are currently a wide variety of differentiation methods available. However, most of the existing differentiation methods are unreliable, with uneven differentiation efficiency and poor reproducibility. At the same time, it is difficult to choose the optimal method when faced with so many differentiation schemes, and it is time-consuming and costly to explore a new differentiation approach. Thus, it is critical to design a robust and efficient method of differentiation. In this review article, we summarize a comprehensive approach in which hPSCs are differentiated into target cells or organoids including brain, liver, blood, melanocytes, and mesenchymal cells. This was accomplished by employing an embryoid body-based three-dimensional (3D) suspension culture system with multiple cells co-cultured. The method has high stable differentiation efficiency compared to the conventional 2D culture and can meet the requirements of clinical application. Additionally, *ex vivo* co-culture models might be able to constitute organoids that are highly similar or mimic human organs for potential organ transplantation in the future.

**Key words:** Human pluripotent stem cells; Three dimensional; Embryoid body; Differentiation; Efficient; Three germ layers

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**Core tip:** Identifying a practical way to efficiently differentiate pluripotent stem cells is essential in regenerative medicine. After considering the advantages and limitations of current approaches, we summarize the ideal conditions and systems. We also provide potential choices for efficiently and robustly differentiating human pluripotent stem cells into target cells and tissues in different germ layers.

**INTRODUCTION**

The first five lines of human embryonic stem cells (hESCs) were obtained in 1998 from the inner cell mass of a 3- to 5-day-old fertilized embryo[1]. Subsequently, induced pluripotent stem cells (iPSCs) were created by reprogramming fibroblasts[2]. Human pluripotent stem cells (hPSCs), including hESCs and human (h)iPSCs, have the ability to self-renew and differentiate into any cell type from all germ layers[3], driving the development of regenerative medicine. The cells and organoids derived from hPSCs have various potential applications including complex diseases studies, cell-based drug screening, and limitless transplantation treatments[4,5]. With the rapid development of regenerative medicine technology, many differentiation approaches based on hPSCs have been explored. However, some challenges remain. To meet the needs of clinical application and basic research, high efficiency and stability are the key objectives during hPSC differentiation into high-quality target cells. Thus, it is important to identify an efficient and robust differentiation approach that can increase the differentiation ratio of target cells, produce stronger functions in cells, generate more complete structural organoids, or be reproduced in different cell lines or in other laboratories. Currently, there are great differences in these experimental schemes. Differentiation efficiency[6] and stability are impacted by whether the method is based on an embryoid body (EB) or a two-dimensional (2D) or 3D system, or if single or multiple cell co-cultures are used.

In this review article, we combine the experiences of our laboratory with a summary of existing mainstream approaches involving hPSC differentiation, with the goal of providing a reference and time-saving guide for future experimental design.

**DIFFERENTIATION INDUCTION FROM hPSCs**

***EB-based differentiation system***

EB has been a very common model of *in vitro* hPSC differentiation for more than 50 years[7]. The EB-based method is widely used to differentiate majority of cell lineages from the three germ layers (Table 1) and has an obvious advantage in improving the differentiation efficiency of some cells[8-10], such as hematopoietic progenitors[11] and melanocytes[12]. Combined with suspension bioreactor technology, this advantage can be further amplified for large-scale production[13]. Additionally, EB formation provides an excellent way to assess and manipulate developmental potential[7]. Differentiation predictions can be made in the early stage of EB to predict which germ layer hPSC is likely to differentiate into, which can save on the cost for subsequent differentiation and indirectly improve differentiation efficiency. For example, Spalt like transcription factor 3 (*SALL3)* expression in EB indicates a high probability of differentiating into the ectoderm and a low chance of differentiating into the mesoderm/endoderm[14]. Our study also confirmed these findings, and we found that iPSC lines that expressed higher levels of *SALL3* on day 7 of EB formation showed greater potential for melanocyte differentiation[15]. Additionally, miR-371-3 plays both a predictive and functional role in neurogenic differentiation[16], and the low expression of fibroblast growth factor 1 (commonly known as *FGF-1)*, ras homolog family member U (commonly known as *RHOU)*, and thymidine phosphorylase (commonly known as *TYMP)* genes are associated with low hepatic differentiation[17], which can be used to predict the differentiation potential in early EB. Therefore, EB-based differentiation systems not only help to increase the percentage of target cells, but also contribute to the prediction of differentiation potentials in the early stage, which improves efficiency directly and indirectly, respectively.

***Matrigel-mediated system***

Matrigel, a natural extracellular matrix, is widely used in hPSC maintenance and can also be used in 2D and 3D hPSC differentiation (Table 1). During 2D differentiation, the culture vessels are first coated with Matrigel, followed by single cell or cell cluster inoculation. The role of Matrigel in 2D is adherence of cells or cell clumps to a culture vessel. Furthermore, the major component of Matrigel is laminin, which promotes the formation of a rigid neuroepithelial structure[18]. Laminin-positive basement membranes are crucial for continuous epithelial integrity[19]. A massive volume increase of the human neocortex results from expansion of the cortical area and the related emergence of extensive cortical folding[20], which is thought to be due to the increase of the proliferative potential of neural progenitors (NPs)[21]. As this study shows, two human ESC lines were differentiated into NPs in Matrigel-coated 2D adherent culture. Jaenisch and his colleagues constituted human cerebral organoids in an EB-based 3D system, which displayed markedly increased outgrowth of neuroepithelial tissue surrounding ventricle-like structures[21]. Other desired cells can also be differentiated in a Matrigel-coated 2D culture system such as hepatocytes[22], hepatic stellate cells[23], intestinal epithelium[24,25], mesenchymal cells[26], cardiomyocytes (CMs)[27], monocytes, and macrophages[28]. Thus, the Matrigel-based 2D culture approach is a basic method for the directed induced differentiation of hPSCs.

Matrigel can also be used for 3D differentiation of hPSCs. In addition to coating the substrate, the Matrigel-based 3D construct is formed by adding mixed Matrigel and special differentiation medium[29] in the hPSC differentiation process, resulting in differentiation in the solution of a suspended system. A 3D differentiation system provides enough space for establishing an organoid, and promotes cellular communication and interaction among cells compared to a 2D approach. Currently, many target cell lineages or tissues can be differentiated in this way including the brain[30], retinae[31,32], intestinal organoids[33,34], and heart[35]. Interestingly, after adding Matrigel, retinal induction cells increase by up to 30%-70% of the total cells in the low cell adhesion plate[18]. Because this gel promotes the epithelialization of hPSCs toward retinal differentiation, researchers have tried to use 3D Matrigel methods for differentiating hPSCs. Epithelialized cysts are obtained by floating culture clumps of Matrigel/hESCs and the subsequent floating culture results in self-formation of retinal organoids[31]. This includes patterned neuroretina, ciliary margin, and retinal pigment epithelium, which autonomously generates stratified retinal tissues, comprising photoreceptors with ultrastructure of outer segments in long-term culture. This system has been validated in two lines of human hPSCs[31]. Clearly, the use of Matrigel is common in 2D or 3D differentiation of hPSCs into target cells. However, the Matrigel-embedded 3D differentiation system has distinct advantages in self-organizing and generating organoids with a more complete structure when compared to a 2D culture.

***3D suspension system***

During hPSC differentiation, there are many decisions in creating a 3D floating state such as a non- or ultra-low attachment plate, microwell plate, and suspended bioreactors. At present, a variety of cell lineages have been generated by using 3D suspension system such as eye[36-38], skin[39], brain[40-43], liver[44,45], heart[46,47] and blood[11]. For example, during the 3D differentiation process, the authors generated iPSC-derived fully functional hepatocyte-like organoids in gene expression, protein secretion, and biotransformation[48]. Likewise, iPSC-derived platelets can be harvested by using a 3D differentiation system, and it is very similar to human platelets in terms of both ultrastructural features and *in vivo* and *in vitro* functional characterizations[49]. Thus, the 3D differentiation system can produce cells with ideal functions. The yield of differentiated cells is also important. The omni-well-array culture platform can produce massive and miniaturized iPSC-derived liver buds on a clinically relevant large scale (> 108)[50]. Hama *et al*[13] designed a protocol that generated > 90% hiPSC-derived CMs that yielded on average 72 million cells per 100 mL in a 3D bioreactor. These results show that the yield from the 3D suspension system is remarkable in contrast to the 2D system. To test the reproducibility of the CM 3D differentiation protocol, a previous study compared biologically independent experiments with various passage numbers of iPSCs, and found minor inter-experimental variations[13]. Overall, the 3D differentiation culture appears to have advantages in differentiation efficiency and stability over the 2D system. This indicates that the 3D differentiation method is optimal when hPSCs differentiation experiments are conducted.

***Multiple cells co-culture system***

Each organ has a variety of cell components with a certain structure and its own specific functions. Because of the communication and interaction among cells, co-culturing with different supportive and tissue-constructive cells has been become attractive. The benefits of co-culturing multiple cells are that they can facilitate communication and interaction among different cells, enhance the hPSCs differentiation efficiency[51], and better simulate the environment *in vivo*. It can bring surprises when used in a co-culture system to self-organize and generate an organoid. For example, to recapitulate hepatitis B virus-host interactions in liver organoids, Nie *et al*[52] co-cultured iPSC endoderm cells, human umbilical cord vein endothelial cells (HUVECs), and human bone marrow mesenchymal stem cells to form liver organoids in a 3D microwell plate, which exhibits stronger hepatic functions than iPSC-derived hepatic like cells. Furthermore, the co-culture pattern also has a higher differentiation yield[48] and organoids with more complex functions[53]. There are co-culture combinations in other studies such as co-culturing hPSC-derived neurons and astrocytes[54]; co-culturing iPSC-derived hepatic parenchymal and non-parenchymal cells[55]; co-culturing hiPSC-derived retinal pigment epithelium and retinal organoids[32]; and co-culturing HUVECs, hESC-derived MSCs, and hESC-derived cardiac progenitor cells[35]. Human PSC-derived organoids with multiple cell components have a complete structure and sturdy function similar to a human organ, which may provide an alternative source for organ transplantation. Therefore, the 3D culture method is a better choice for organoid generation.

***Transcription factor-directed differentiation***

Transcription factors (TFs) play an important role in pluripotent stem cell induction and transdifferentiation[56]. Recently, they have been used to differentiate hPSCs into desired cells or tissues such as neural[57], liver[58,59], and cardiac muscle[60,61]. A growing body of TF-directed differentiation method of hPSCs has demonstrated that efficient cell fate is reprogrammed *via* forced expression of single or multiple TFs[62]. Sun *et al*[63] used the technique to design a single-step protocol for forebrain GABAergic neuron differentiation, which could generate cells similar to rodent cortical interneurons with > 80% efficiency, and the target cells showed mature functional properties within 6-8 wk. By contrast, other process takes as long as 30 wk[64]. The TF-mediated method can differentiate hPSCs into terminal cells directly, and the experimental procedure is relatively brief.

**LIMITATION AND CHOICE**

Current methods for hPSC differentiation described above have various limitations. 2D differentiation culturing is performed on the surface of the culture vessel and the limited contact area limits the yield of the target cells. Furthermore, all structural components of organoids cannot be generated[37,65]. Without 3D contact with Matrigel, Lowe *et al*[31] reported that most cells died and the few surviving cells formed solid cell masses on 2D culturing. Most 3D culture methods involve various intermediate stages requiring varying combinations of recombinant factors and small molecules[63], thus rendering the method cumbersome to repeat. Although TF-mediated methods improve the differentiation efficiency of hPSCs, numerous tools for TF transfection, including plasmids and viruses, have led to the integration of exogenetic genes[66] into the target cells, thus presenting a remote prospects for their clinical application[56]. In this situation, EB-based 3D culture systems allow for large-scale directional differentiation of hPSCs, and the co-culture method seems to constitute highly functional organoids *in vitro* to compensate for organ transplantation insufficiency.

**CONCLUSION**

Although only a few articles have compared the differences between 2D and 3D differentiation, it can be concluded that 3D system with EB has obvious advantages for hPSC differentiation compared to 2D culture. The details of the differentiation approaches are shown in the “cultural approaches” of Table 1. Regarding future studies, there are some key recommendations. First, the ability of EB not only can scale up culture systems and differentiation, but also predict the fate of hPSCs differentiation for reducing unnecessary waste. Second, the 3D differentiation system also has significant improvement in differentiation efficiency, and 3D space is necessary for organoid formation. Finally, it is a promising and challenging task that co-cultures multiple kinds of cells, supportive, structured, vascularized and further neurovascularized for organoid organization in 3D suspension system. Simply put, an EB-based 3D differentiation culture system is an efficient and powerful choice for hPSCs to meet the demand in clinical applications and basic research.

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**REFERENCES**

1 **Croze RH**, Clegg DO. Differentiation of pluripotent stem cells into retinal pigmented epithelium. *Dev Ophthalmol* 2014; **53**: 81-96 [PMID: 24732763 DOI: 10.1159/000357361]

2 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]

3 **Studer L**. Derivation of dopaminergic neurons from pluripotent stem cells. *Prog Brain Res* 2012; **200**: 243-263 [PMID: 23195422 DOI: 10.1016/B978-0-444-59575-1.00011-9]

4 **Bock C**, Kiskinis E, Verstappen G, Gu H, Boulting G, Smith ZD, Ziller M, Croft GF, Amoroso MW, Oakley DH, Gnirke A, Eggan K, Meissner A. Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 2011; **144**: 439-452 [PMID: 21295703 DOI: 10.1016/j.cell.2010.12.032]

5 **Liu LP**, Zheng YW. Predicting differentiation potential of human pluripotent stem cells: Possibilities and challenges. *World J Stem Cells* 2019; **11**: 375-382 [PMID: 31396366 DOI: 10.4252/wjsc.v11.i7.375]

6 **Ohta S**, Imaizumi Y, Okada Y, Akamatsu W, Kuwahara R, Ohyama M, Amagai M, Matsuzaki Y, Yamanaka S, Okano H, Kawakami Y. Generation of human melanocytes from induced pluripotent stem cells. *PLoS One* 2011; **6**: e16182 [PMID: 21249204 DOI: 10.1371/journal.pone.0016182]

7 **Brickman JM**, Serup P. Properties of embryoid bodies. *Wiley Interdiscip Rev Dev Biol* 2017; **6**: [PMID: 27911036 DOI: 10.1002/wdev.259]

8 **Hirschhaeuser F**, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. *J Biotechnol* 2010; **148**: 3-15 [PMID: 20097238 DOI: 10.1016/j.jbiotec.2010.01.012]

9 **Ungrin MD**, Joshi C, Nica A, Bauwens C, Zandstra PW. Reproducible, ultra high-throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. *PLoS One* 2008; **3**: e1565 [PMID: 18270562 DOI: 10.1371/journal.pone.0001565]

10 **Guo NN**, Liu LP, Zheng YW, Li YM. Inducing human induced pluripotent stem cell differentiation through embryoid bodies: A practical and stable approach. *World J Stem Cells* 2020; **12**: 25-34 [PMID: 32110273 DOI: 10.4252/wjsc.v12.i1.25]

11 **Buchrieser J**, James W, Moore MD. Human Induced Pluripotent Stem Cell-Derived Macrophages Share Ontogeny with MYB-Independent Tissue-Resident Macrophages. *Stem Cell Reports* 2017; **8**: 334-345 [PMID: 28111278 DOI: 10.1016/j.stemcr.2016.12.020]

12 **Liu LP**, Li YM, Guo NN, Li S, Ma X, Zhang YX, Gao Y, Huang JL, Zheng DX, Wang LY, Xu H, Hui L, Zheng YW. Therapeutic Potential of Patient iPSC-Derived iMelanocytes in Autologous Transplantation. *Cell Rep* 2019; **27**: 455-466.e5 [PMID: 30970249 DOI: 10.1016/j.celrep.2019.03.046]

13 **Hamad S**, Derichsweiler D, Papadopoulos S, Nguemo F, Šarić T, Sachinidis A, Brockmeier K, Hescheler J, Boukens BJ, Pfannkuche K. Generation of human induced pluripotent stem cell-derived cardiomyocytes in 2D monolayer and scalable 3D suspension bioreactor cultures with reduced batch-to-batch variations. *Theranostics* 2019; **9**: 7222-7238 [PMID: 31695764 DOI: 10.7150/thno.32058]

14 **Kuroda T**, Yasuda S, Tachi S, Matsuyama S, Kusakawa S, Tano K, Miura T, Matsuyama A, Sato Y. SALL3 expression balance underlies lineage biases in human induced pluripotent stem cell differentiation. *Nat Commun* 2019; **10**: 2175 [PMID: 31092818 DOI: 10.1038/s41467-019-09511-4]

15 **Guo NN**, Liu LP, Zhang YX, Cai YT, Guo Y, Zheng YW, Li YM. Early prediction of the differentiation potential during the formation of human iPSC-derived embryoid bodies. *Biochem Biophys Res Commun* 2019; **516**: 673-679 [PMID: 31248595 DOI: 10.1016/j.bbrc.2019.06.081]

16 **Kim H**, Lee G, Ganat Y, Papapetrou EP, Lipchina I, Socci ND, Sadelain M, Studer L. miR-371-3 expression predicts neural differentiation propensity in human pluripotent stem cells. *Cell Stem Cell* 2011; **8**: 695-706 [PMID: 21624813 DOI: 10.1016/j.stem.2011.04.002]

17 **Yanagihara K**, Liu Y, Kanie K, Takayama K, Kokunugi M, Hirata M, Fukuda T, Suga M, Nikawa H, Mizuguchi H, Kato R, Furue MK. Prediction of Differentiation Tendency Toward Hepatocytes from Gene Expression in Undifferentiated Human Pluripotent Stem Cells. *Stem Cells Dev* 2016; **25**: 1884-1897 [PMID: 27733097 DOI: 10.1089/scd.2016.0099]

18 **Eiraku M**, Sasai Y. Mouse embryonic stem cell culture for generation of three-dimensional retinal and cortical tissues. *Nat Protoc* 2011; **7**: 69-79 [PMID: 22179593 DOI: 10.1038/nprot.2011.429]

19 **Fujiwara H**, Hayashi Y, Sanzen N, Kobayashi R, Weber CN, Emoto T, Futaki S, Niwa H, Murray P, Edgar D, Sekiguchi K. Regulation of mesodermal differentiation of mouse embryonic stem cells by basement membranes. *J Biol Chem* 2007; **282**: 29701-29711 [PMID: 17690109 DOI: 10.1074/jbc.M611452200]

20 **Sun T**, Hevner RF. Growth and folding of the mammalian cerebral cortex: from molecules to malformations. *Nat Rev Neurosci* 2014; **15**: 217-232 [PMID: 24646670 DOI: 10.1038/nrn3707]

21 **Li Y**, Muffat J, Omer A, Bosch I, Lancaster MA, Sur M, Gehrke L, Knoblich JA, Jaenisch R. Induction of Expansion and Folding in Human Cerebral Organoids. *Cell Stem Cell* 2017; **20**: 385-396.e3 [PMID: 28041895 DOI: 10.1016/j.stem.2016.11.017]

22 **Calabrese D**, Roma G, Bergling S, Carbone W, Mele V, Nuciforo S, Fofana I, Campana B, Szkolnicka D, Hay DC, Tchorz J, Bouwmeester T, Wieland S, Heim MH. Liver biopsy derived induced pluripotent stem cells provide unlimited supply for the generation of hepatocyte-like cells. *PLoS One* 2019; **14**: e0221762 [PMID: 31465481 DOI: 10.1371/journal.pone.0221762]

23 **Coll M**, Perea L, Boon R, Leite SB, Vallverdú J, Mannaerts I, Smout A, El Taghdouini A, Blaya D, Rodrigo-Torres D, Graupera I, Aguilar-Bravo B, Chesne C, Najimi M, Sokal E, Lozano JJ, van Grunsven LA, Verfaillie CM, Sancho-Bru P. Generation of Hepatic Stellate Cells from Human Pluripotent Stem Cells Enables In Vitro Modeling of Liver Fibrosis. *Cell Stem Cell* 2018; **23**: 101-113.e7 [PMID: 30049452 DOI: 10.1016/j.stem.2018.05.027]

24 **Múnera JO**, Sundaram N, Rankin SA, Hill D, Watson C, Mahe M, Vallance JE, Shroyer NF, Sinagoga KL, Zarzoso-Lacoste A, Hudson JR, Howell JC, Chatuvedi P, Spence JR, Shannon JM, Zorn AM, Helmrath MA, Wells JM. Differentiation of Human Pluripotent Stem Cells into Colonic Organoids via Transient Activation of BMP Signaling. *Cell Stem Cell* 2017; **21**: 51-64.e6 [PMID: 28648364 DOI: 10.1016/j.stem.2017.05.020]

25 **Mithal A**, Capilla A, Heinze D, Berical A, Villacorta-Martin C, Vedaie M, Jacob A, Abo K, Szymaniak A, Peasley M, Stuffer A, Mahoney J, Kotton DN, Hawkins F, Mostoslavsky G. Generation of mesenchyme free intestinal organoids from human induced pluripotent stem cells. *Nat Commun* 2020; **11**: 215 [PMID: 31924806 DOI: 10.1038/s41467-019-13916-6]

26 **Chin CJ**, Li S, Corselli M, Casero D, Zhu Y, He CB, Hardy R, Péault B, Crooks GM. Transcriptionally and Functionally Distinct Mesenchymal Subpopulations Are Generated from Human Pluripotent Stem Cells. *Stem Cell Reports* 2018; **10**: 436-446 [PMID: 29307583 DOI: 10.1016/j.stemcr.2017.12.005]

27 **Mills RJ**, Parker BL, Quaife-Ryan GA, Voges HK, Needham EJ, Bornot A, Ding M, Andersson H, Polla M, Elliott DA, Drowley L, Clausen M, Plowright AT, Barrett IP, Wang QD, James DE, Porrello ER, Hudson JE. Drug Screening in Human PSC-Cardiac Organoids Identifies Pro-proliferative Compounds Acting via the Mevalonate Pathway. *Cell Stem Cell* 2019; **24**: 895-907.e6 [PMID: 30930147 DOI: 10.1016/j.stem.2019.03.009]

28 **Cao X**, Yakala GK, van den Hil FE, Cochrane A, Mummery CL, Orlova VV. Differentiation and Functional Comparison of Monocytes and Macrophages from hiPSCs with Peripheral Blood Derivatives. *Stem Cell Reports* 2019; **12**: 1282-1297 [PMID: 31189095 DOI: 10.1016/j.stemcr.2019.05.003]

29 **Lee GY**, Kenny PA, Lee EH, Bissell MJ. Three-dimensional culture models of normal and malignant breast epithelial cells. *Nat Methods* 2007; **4**: 359-365 [PMID: 17396127 DOI: 10.1038/nmeth1015]

30 **Kim H**, Park HJ, Choi H, Chang Y, Park H, Shin J, Kim J, Lengner CJ, Lee YK, Kim J. Modeling G2019S-LRRK2 Sporadic Parkinson's Disease in 3D Midbrain Organoids. *Stem Cell Reports* 2019; **12**: 518-531 [PMID: 30799274 DOI: 10.1016/j.stemcr.2019.01.020]

31 **Lowe A**, Harris R, Bhansali P, Cvekl A, Liu W. Intercellular Adhesion-Dependent Cell Survival and ROCK-Regulated Actomyosin-Driven Forces Mediate Self-Formation of a Retinal Organoid. *Stem Cell Reports* 2016; **6**: 743-756 [PMID: 27132890 DOI: 10.1016/j.stemcr.2016.03.011]

32 **Akhtar T**, Xie H, Khan MI, Zhao H, Bao J, Zhang M, Xue T. Accelerated photoreceptor differentiation of hiPSC-derived retinal organoids by contact co-culture with retinal pigment epithelium. *Stem Cell Res* 2019; **39**: 101491 [PMID: 31326746 DOI: 10.1016/j.scr.2019.101491]

33 **Miura S**, Suzuki A. Generation of Mouse and Human Organoid-Forming Intestinal Progenitor Cells by Direct Lineage Reprogramming. *Cell Stem Cell* 2017; **21**: 456-471.e5 [PMID: 28943029 DOI: 10.1016/j.stem.2017.08.020]

34 **Nadkarni RR**, Abed S, Cox BJ, Bhatia S, Lau JT, Surette MG, Draper JS. Functional Enterospheres Derived In Vitro from Human Pluripotent Stem Cells. *Stem Cell Reports* 2017; **9**: 897-912 [PMID: 28867347 DOI: 10.1016/j.stemcr.2017.07.024]

35 **Varzideh F**, Pahlavan S, Ansari H, Halvaei M, Kostin S, Feiz MS, Latifi H, Aghdami N, Braun T, Baharvand H. Human cardiomyocytes undergo enhanced maturation in embryonic stem cell-derived organoid transplants. *Biomaterials* 2019; **192**: 537-550 [PMID: 30529872 DOI: 10.1016/j.biomaterials.2018.11.033]

36 **Parfitt DA**, Lane A, Ramsden CM, Carr AJ, Munro PM, Jovanovic K, Schwarz N, Kanuga N, Muthiah MN, Hull S, Gallo JM, da Cruz L, Moore AT, Hardcastle AJ, Coffey PJ, Cheetham ME. Identification and Correction of Mechanisms Underlying Inherited Blindness in Human iPSC-Derived Optic Cups. *Cell Stem Cell* 2016; **18**: 769-781 [PMID: 27151457 DOI: 10.1016/j.stem.2016.03.021]

37 **Deng WL**, Gao ML, Lei XL, Lv JN, Zhao H, He KW, Xia XX, Li LY, Chen YC, Li YP, Pan D, Xue T, Jin ZB. Gene Correction Reverses Ciliopathy and Photoreceptor Loss in iPSC-Derived Retinal Organoids from Retinitis Pigmentosa Patients. *Stem Cell Reports* 2018; **10**: 1267-1281 [PMID: 29526738 DOI: 10.1016/j.stemcr.2018.02.003]

38 **Völkner M**, Zschätzsch M, Rostovskaya M, Overall RW, Busskamp V, Anastassiadis K, Karl MO. Retinal Organoids from Pluripotent Stem Cells Efficiently Recapitulate Retinogenesis. *Stem Cell Reports* 2016; **6**: 525-538 [PMID: 27050948 DOI: 10.1016/j.stemcr.2016.03.001]

39 **Lee J**, Bӧscke R, Tang PC, Hartman BH, Heller S, Koehler KR. Hair Follicle Development in Mouse Pluripotent Stem Cell-Derived Skin Organoids. *Cell Rep* 2018; **22**: 242-254 [PMID: 29298425 DOI: 10.1016/j.celrep.2017.12.007]

40 **Bershteyn M**, Nowakowski TJ, Pollen AA, Di Lullo E, Nene A, Wynshaw-Boris A, Kriegstein AR. Human iPSC-Derived Cerebral Organoids Model Cellular Features of Lissencephaly and Reveal Prolonged Mitosis of Outer Radial Glia. *Cell Stem Cell* 2017; **20**: 435-449.e4 [PMID: 28111201 DOI: 10.1016/j.stem.2016.12.007]

41 **Iefremova V**, Manikakis G, Krefft O, Jabali A, Weynans K, Wilkens R, Marsoner F, Brändl B, Müller FJ, Koch P, Ladewig J. An Organoid-Based Model of Cortical Development Identifies Non-Cell-Autonomous Defects in Wnt Signaling Contributing to Miller-Dieker Syndrome. *Cell Rep* 2017; **19**: 50-59 [PMID: 28380362 DOI: 10.1016/j.celrep.2017.03.047]

42 **Linkous A**, Balamatsias D, Snuderl M, Edwards L, Miyaguchi K, Milner T, Reich B, Cohen-Gould L, Storaska A, Nakayama Y, Schenkein E, Singhania R, Cirigliano S, Magdeldin T, Lin Y, Nanjangud G, Chadalavada K, Pisapia D, Liston C, Fine HA. Modeling Patient-Derived Glioblastoma with Cerebral Organoids. *Cell Rep* 2019; **26**: 3203-3211.e5 [PMID: 30893594 DOI: 10.1016/j.celrep.2019.02.063]

43 **Xiang Y**, Tanaka Y, Cakir B, Patterson B, Kim KY, Sun P, Kang YJ, Zhong M, Liu X, Patra P, Lee SH, Weissman SM, Park IH. hESC-Derived Thalamic Organoids Form Reciprocal Projections When Fused with Cortical Organoids. *Cell Stem Cell* 2019; **24**: 487-497.e7 [PMID: 30799279 DOI: 10.1016/j.stem.2018.12.015]

44 **Akbari S**, Sevinç GG, Ersoy N, Basak O, Kaplan K, Sevinç K, Ozel E, Sengun B, Enustun E, Ozcimen B, Bagriyanik A, Arslan N, Önder TT, Erdal E. Robust, Long-Term Culture of Endoderm-Derived Hepatic Organoids for Disease Modeling. *Stem Cell Reports* 2019; **13**: 627-641 [PMID: 31522975 DOI: 10.1016/j.stemcr.2019.08.007]

45 **Nie YZ**, Zheng YW, Ogawa M, Miyagi E, Taniguchi H. Human liver organoids generated with single donor-derived multiple cells rescue mice from acute liver failure. *Stem Cell Res Ther* 2018; **9**: 5 [PMID: 29321049 DOI: 10.1186/s13287-017-0749-1]

46 **Chauveau S**, Anyukhovsky EP, Ben-Ari M, Naor S, Jiang YP, Danilo P Jr, Rahim T, Burke S, Qiu X, Potapova IA, Doronin SV, Brink PR, Binah O, Cohen IS, Rosen MR. Induced Pluripotent Stem Cell-Derived Cardiomyocytes Provide In Vivo Biological Pacemaker Function. *Circ Arrhythm Electrophysiol* 2017; **10**: e004508 [PMID: 28500172 DOI: 10.1161/CIRCEP.116.004508]

47 **Hoque A**, Sivakumaran P, Bond ST, Ling NXY, Kong AM, Scott JW, Bandara N, Hernández D, Liu GS, Wong RCB, Ryan MT, Hausenloy DJ, Kemp BE, Oakhill JS, Drew BG, Pébay A, Lim SY. Mitochondrial fission protein Drp1 inhibition promotes cardiac mesodermal differentiation of human pluripotent stem cells. *Cell Death Discov* 2018; **4**: 39 [PMID: 29531836 DOI: 10.1038/s41420-018-0042-9]

48 **Pettinato G**, Lehoux S, Ramanathan R, Salem MM, He LX, Muse O, Flaumenhaft R, Thompson MT, Rouse EA, Cummings RD, Wen X, Fisher RA. Generation of fully functional hepatocyte-like organoids from human induced pluripotent stem cells mixed with Endothelial Cells. *Sci Rep* 2019; **9**: 8920 [PMID: 31222080 DOI: 10.1038/s41598-019-45514-3]

49 **Feng Q**, Shabrani N, Thon JN, Huo H, Thiel A, Machlus KR, Kim K, Brooks J, Li F, Luo C, Kimbrel EA, Wang J, Kim KS, Italiano J, Cho J, Lu SJ, Lanza R. Scalable generation of universal platelets from human induced pluripotent stem cells. *Stem Cell Reports* 2014; **3**: 817-831 [PMID: 25418726 DOI: 10.1016/j.stemcr.2014.09.010]

50 **Takebe T**, Sekine K, Kimura M, Yoshizawa E, Ayano S, Koido M, Funayama S, Nakanishi N, Hisai T, Kobayashi T, Kasai T, Kitada R, Mori A, Ayabe H, Ejiri Y, Amimoto N, Yamazaki Y, Ogawa S, Ishikawa M, Kiyota Y, Sato Y, Nozawa K, Okamoto S, Ueno Y, Taniguchi H. Massive and Reproducible Production of Liver Buds Entirely from Human Pluripotent Stem Cells. *Cell Rep* 2017; **21**: 2661-2670 [PMID: 29212014 DOI: 10.1016/j.celrep.2017.11.005]

51 **Wang LY**, Liu LP, Ge JY, Yuan YY, Sun LL, Xu H, Huang PY, Hui LJ, Isoda H, Ohkohchi N, Li YM, Zheng YW. A Multiple-Cell Microenvironment in a 3-Dimensional System Enhances Direct Cellular Reprogramming Into Hepatic Organoids. *Transplant Proc* 2018; **50**: 2864-2867 [PMID: 30401413 DOI: 10.1016/j.transproceed.2018.03.076]

52 **Nie YZ**, Zheng YW, Miyakawa K, Murata S, Zhang RR, Sekine K, Ueno Y, Takebe T, Wakita T, Ryo A, Taniguchi H. Recapitulation of hepatitis B virus-host interactions in liver organoids from human induced pluripotent stem cells. *EBioMedicine* 2018; **35**: 114-123 [PMID: 30120080 DOI: 10.1016/j.ebiom.2018.08.014]

53 **Furuya K**, Zheng YW, Sako D, Iwasaki K, Zheng DX, Ge JY, Liu LP, Furuta T, Akimoto K, Yagi H, Hamada H, Isoda H, Oda T, Ohkohchi N. Enhanced hepatic differentiation in the subpopulation of human amniotic stem cells under 3D multicellular microenvironment. *World J Stem Cells* 2019; **11**: 705-721 [PMID: 31616545 DOI: 10.4252/wjsc.v11.i9.705]

54 **Krencik R**, Seo K, van Asperen JV, Basu N, Cvetkovic C, Barlas S, Chen R, Ludwig C, Wang C, Ward ME, Gan L, Horner PJ, Rowitch DH, Ullian EM. Systematic Three-Dimensional Coculture Rapidly Recapitulates Interactions between Human Neurons and Astrocytes. *Stem Cell Reports* 2017; **9**: 1745-1753 [PMID: 29198827 DOI: 10.1016/j.stemcr.2017.10.026]

55 **Koui Y**, Kido T, Ito T, Oyama H, Chen SW, Katou Y, Shirahige K, Miyajima A. An In Vitro Human Liver Model by iPSC-Derived Parenchymal and Non-parenchymal Cells. *Stem Cell Reports* 2017; **9**: 490-498 [PMID: 28757162 DOI: 10.1016/j.stemcr.2017.06.010]

56 **Ulasov AV**, Rosenkranz AA, Sobolev AS. Transcription factors: Time to deliver. *J Control Release* 2018; **269**: 24-35 [PMID: 29113792 DOI: 10.1016/j.jconrel.2017.11.004]

57 **Pang ZP**, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Südhof TC, Wernig M. Induction of human neuronal cells by defined transcription factors. *Nature* 2011; **476**: 220-223 [PMID: 21617644 DOI: 10.1038/nature10202]

58 **Tomizawa M**, Shinozaki F, Motoyoshi Y, Sugiyama T, Yamamoto S, Ishige N. Transcription Factors and Medium Suitable for Initiating the Differentiation of Human-Induced Pluripotent Stem Cells to the Hepatocyte Lineage. *J Cell Biochem* 2016; **117**: 2001-2009 [PMID: 26773721 DOI: 10.1002/jcb.25494]

59 **Takayama K**, Inamura M, Kawabata K, Sugawara M, Kikuchi K, Higuchi M, Nagamoto Y, Watanabe H, Tashiro K, Sakurai F, Hayakawa T, Furue MK, Mizuguchi H. Generation of metabolically functioning hepatocytes from human pluripotent stem cells by FOXA2 and HNF1α transduction. *J Hepatol* 2012; **57**: 628-636 [PMID: 22659344 DOI: 10.1016/j.jhep.2012.04.038]

60 **Kwon C**, Qian L, Cheng P, Nigam V, Arnold J, Srivastava D. A regulatory pathway involving Notch1/beta-catenin/Isl1 determines cardiac progenitor cell fate. *Nat Cell Biol* 2009; **11**: 951-957 [PMID: 19620969 DOI: 10.1038/ncb1906]

61 **Bai F**, Ho Lim C, Jia J, Santostefano K, Simmons C, Kasahara H, Wu W, Terada N, Jin S. Directed Differentiation of Embryonic Stem Cells Into Cardiomyocytes by Bacterial Injection of Defined Transcription Factors. *Sci Rep* 2015; **5**: 15014 [PMID: 26449528 DOI: 10.1038/srep15014]

62 **Oh Y**, Jang J. Directed Differentiation of Pluripotent Stem Cells by Transcription Factors. *Mol Cells* 2019; **42**: 200-209 [PMID: 30884942 DOI: 10.14348/molcells.2019.2439]

63 **Sun AX**, Yuan Q, Tan S, Xiao Y, Wang D, Khoo AT, Sani L, Tran HD, Kim P, Chiew YS, Lee KJ, Yen YC, Ng HH, Lim B, Je HS. Direct Induction and Functional Maturation of Forebrain GABAergic Neurons from Human Pluripotent Stem Cells. *Cell Rep* 2016; **16**: 1942-1953 [PMID: 27498872 DOI: 10.1016/j.celrep.2016.07.035]

64 **Nicholas CR**, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D, Arnold CM, Chen YJ, Stanley EG, Elefanty AG, Sasai Y, Alvarez-Buylla A, Rubenstein JL, Kriegstein AR. Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell* 2013; **12**: 573-586 [PMID: 23642366 DOI: 10.1016/j.stem.2013.04.005]

65 **Osakada F**, Ikeda H, Sasai Y, Takahashi M. Stepwise differentiation of pluripotent stem cells into retinal cells. *Nat Protoc* 2009; **4**: 811-824 [PMID: 19444239 DOI: 10.1038/nprot.2009.51]

66 **Ge JY**, Zheng YW, Liu LP, Isoda H, Oda T. Impelling force and current challenges by chemicals in somatic cell reprogramming and expansion beyond hepatocytes. *World J Stem Cells* 2019; **11**: 650-665 [PMID: 31616541 DOI: 10.4252/wjsc.v11.i9.650]

67 **Sharma A**, McKeithan WL, Serrano R, Kitani T, Burridge PW, Del Álamo JC, Mercola M, Wu JC. Use of human induced pluripotent stem cell-derived cardiomyocytes to assess drug cardiotoxicity. *Nat Protoc* 2018; **13**: 3018-3041 [PMID: 30413796 DOI: 10.1038/s41596-018-0076-8]

68 **Nakajima-Takagi Y**, Osawa M, Oshima M, Takagi H, Miyagi S, Endoh M, Endo TA, Takayama N, Eto K, Toyoda T, Koseki H, Nakauchi H, Iwama A. Role of SOX17 in hematopoietic development from human embryonic stem cells. *Blood* 2013; **121**: 447-458 [PMID: 23169777 DOI: 10.1182/blood-2012-05-431403]

69 **Minagawa A**, Yoshikawa T, Yasukawa M, Hotta A, Kunitomo M, Iriguchi S, Takiguchi M, Kassai Y, Imai E, Yasui Y, Kawai Y, Zhang R, Uemura Y, Miyoshi H, Nakanishi M, Watanabe A, Hayashi A, Kawana K, Fujii T, Nakatsura T, Kaneko S. Enhancing T Cell Receptor Stability in Rejuvenated iPSC-Derived T Cells Improves Their Use in Cancer Immunotherapy. *Cell Stem Cell* 2018; **23**: 850-858.e4 [PMID: 30449714 DOI: 10.1016/j.stem.2018.10.005]

**Footnotes**

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**Table 1 Summary of current approaches for human pluripotent stem cells differential direction into targeted cells or tissues**

|  |  |  |  |
| --- | --- | --- | --- |
| **Targeted cells or tissues** | **Cultural approaches** | **Check points of differential status** | **Ref.** |
| **EB formation** | **2D or 3D** | **2D Surface or 3D system** | **Multiple cells co-culture** | **Gene expression** | **Protein level** | **Under *in vitro* or *in vivo*** |
| Neural progenitor | - | 2D | Matrigel | - | *PAX6*, *TBR2* | - | *In vitro* | [21] |
| Brain | EB | 3D | Low attachment plate, Matrigel | - | *PAX6, SOX2, FOXG1, TBR2, ARHGAP11B* | Nestin | *In vitro* | [21] |
| Midbrain | EB | 3D | Matrigel | - | *SOX1, VMAT2, TH, NURR1, DAT, GIRK2, PITX3, AADC, ANLN, FAH, MBP, GLA35ST1, ACSL1, CLDN11, CHAT, MAPT, GFAP, S100B, ALDH1L1* | - | *In vitro* | [30] |
| Brain | - | 3D | Microwell, bioreactor | Neuronal, astrocyte | *-* | - | *In vitro* | [54] |
| Retinae | - | 3D | Matrigel | - | *SIX3, PAX6, RAX, OTX2, VSX2, PRKCZ, MITF* | TJP1, LAMB1, RHO, OPN1LW/OPN1MW, OPN1SW | *In vitro* | [31] |
| Retinae | EB | 3D | Low attachment plate | - | *BRN3B, PAX6, RAX, SIX3, LHX2, CHX10, OTX2* | RHO, PKCα, Arl13b, OPN1SW, OPN1MW | *In vitro* | [37] |
| Retinae | - | 3D | Matrigel | RPE, retinal organoid | *CRX, RCVRN, NRL, GNAT1, RHO, CHX10, OPN1LW/MW, OTX2, RLBP1, PROX1* | - | *In vitro* | [32] |
| Melanocyte | EB | 3D | Microwell, low attachment plate | - | *MITF, PAX3, SOX10, KIT, TYR, TYRP, DCT, PEML* | Melanin | *In vivo* | [12] |
| Hepatic stellate cells | - | 2D | Matrigel  | - | *NCAM, KDR, PDGFRα, P75NTR, ALCAM, ACTA2, COL1α1, LRAT, RELN, PCDH7, PDGFRβ, SYP, GFAP, PPARγ, NGF, α-SMA* | Desmin, PDGFRβ, P75NTR, ALCAM, PDGFRα, CD73, KDR, NCAM | *In vitro* | [23] |
| Liver | EB | 3D | Microwell | iPSC endoderm cell, HUVEC, BM-MSC | *ALB, G6PC, CYP2C9, CYP2C19, CYP3A4, CYP3A7* | CYP3A4, ALB, Urea, NTCP | *In vitro* | [52] |
| Liver | - | 3D | Microwell | iPSC-tHE, iPSC-EC, iPSC-STM | *TBX3, ADRA1B* | AFP, ALB, Complement factor H, Coagulation factor VIII, Transferrin, AAT | *In vivo* | [50] |
| Intestinal  | - | 3D | Matrigel | - | *KLF5, ECAD, SOX9, KI67* | Villin | *In vivo* | [33] |
| Entersphere  | - | 3D | Matrigel  | Pan-epithelium cell, HLF, HUVEC | *SOX9, CK20, CDX2, NNKX2.1, LGR5, OLFM4, TACSTD2, VIL1, APOA1, FABP2* | E-cadherin, Cytokeratin18, α-SMA | *In vitro* | [34] |
| Cardiomyocyte | - | 2D | Matrigel | - | *-* | TNNT2, ACTN2 | *In vitro* | [67] |
| Cardiomyocyte | EB | 3D | Low attachment plate | - | *TBX5, NKX2.5, GATA4* | TNNT2, TNNI3, MYH6, MYL7 | *In vitro* | [47] |
| Cardiomyocyte | EB | 3D | Suspension bioreactor | - | *-* | TNNT2, α-Actinin, MLC-2v, MLC-2a | *In vitro* | [13] |
| Heart  | EB | 3D | Matrigel | hESC-CPC, hESC-MSC, HUVEC | *KDR, MESP1, NKX2.5, TBX5, GATA4, ISL1, PDGFR-α, MEF2C, CD90, CD73, CD105, CD44, CD31, cTNT, β-MHC, MLC2v, KCNA4, KCNJ2, KCNH2* | - | *In vivo* | [35] |
| Hematopoietic cell | EB | 3D | - | - | *RUNX1, SCL/TAL1* | CD34, CD43, CD45 | *In vitro* | [68] |
| T Cell | - | 2D | - | - | *TRA, TRB, RAG1, RAG2*  | CD8ab, LMP2, TCR, TCRab-CD3 | *In vivo* | [69] |
| Macrophage | EB | 3D | Low attachment plate | - | *MAF, CSFR1, FLT3, CCR2* | CD14, CD45, CD11b, CD16, TNF-α | *In vitro* | [11] |
| Liver sinusoidal endothelial cell | EB | 3D | Low-cluster plate | - | *CD31, CDH5, CD34, F8, STAB2, LYVE1, FLK1, FLT4, FCGR2B* | - | *In vitro* | [55] |
| Platelet | - | 3D | Ultra-low attachment plate  | - | *CD41a, CD13, CD42b, CD31, CD34, CD43, CD41b* | Thrombospondin4, Platelet factor 4 | *In vivo* | [49] |
| Mesenchymal cell | - | 2D | Matrigel | - | *CD146, CD73, CD140a, CD90, CD105, CD44, PDGFRβ, CSPG4, NES, LEPR, ADRB2, KITLG, IGFBP2, TNC, CXCL12, ADRB3* | - | *In vitro* | [26] |

-: None; EB: Embryoid body; 2D: Two-dimensional; 3D: Three-dimensional; RPE: retinal pigment epithelium; iPSCs: induced pluripotent stem cells; HUVECs: human umbilical cord vein endothelial cells; BM-MSC: Bone marrow mesenchymal stem cell.