CELL & GENE THERAPY INSIGHTS

AUTOMATION OF CELL AND GENE THERAPY MANUFACTURING: FROM VEIN TO VEIN

SPOTLIGHT

EXPERT INSIGHT

Benefits of automation for pluripotent stem cell therapies, disease modeling & drug discovery

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Pluripotent stem cells show much promise for cellular therapies, drug discovery and disease modeling. There are a number of challenges involved in the culture of pluripotent stem cells to increase the scale of stem cell culture for such applications. Automated processes may prove beneficial in improving consistency and scalability of culture systems for better therapeutic and disease modeling outcomes.

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PLURIPOTENT STEM CELL THERAPY

The first report demonstrating the derivation of pluripotent human embryonic stem cells (hESCs) was published in 1998 [1]. This along with a subsequent study showed that hESCs could be maintained indefinitely and differentiated into the three germ layers that give rise to all cells found in the human body [2]. Essentially, the ability to have a limitless source of cells for transplantation brought with it the hope that hESCs would ultimately be of benefit for transplantation and the repair or replacement of damaged or diseased tissues. However, the subsequent findings by Shinya Yamanaka, which led to the generation of induced pluripotent stem cells (iPSCs) from adult somatic cells, meant that potentially patients could be treated with autologous cells [3]. The translation of pluripotent stem cells from the bench to clinical use is in its infancy and has been a slow yet cautious process. This is largely due to careful consideration of the potential for undifferentiated stem cells to form teratomas *in vivo* [2] and lack of efficient differentiation/purification strategies for many cell types. However, there are several published clinical trials [4–8] and others underway listed at clinicaltrials.gov that have generated much excitement and the field as a whole is watching with the hope

that the cell transplantations do not exacerbate disease pathologies, and/or cause adverse effects particularly, teratomas.

In order to be utilized for cellular therapies, the stem cells need to be maintained at the utmost high quality with rigorous characterization and preferably the use of fully defined and xeno-free components to maximize control of culture systems and minimize risk for patients. Already good manufacturing process (GMP) compliant pluripotent stem cell lines have been derived [9–12], a critical step towards human therapies. Aside from the other regulatory requirements under GMP framework, the most important aspects of any attempts towards use of hESCs or iPSCs therapeutically are:

- Using non-integrating technologies for iPSC generation, maintenance of undifferentiated cells
- Controlled and robust differentiation to achieve the desired cell types for transplant
- Purification of differentiated cells/removal of residual pluripotent cells to avoid risk of teratoma formation
- Maintenance of a normal karyotype

The basis for such characterization is available through the protocols implemented by Lanza and colleagues for hESC-derived retinal pigment epithelial (RPE) cells [5–7] and Takahashi and colleagues, who have recently reported the first safe human transplantation of autologous iPSC-derived cells, again RPE cells [4]. So far only small patient numbers have been assessed in clinical trials and the question remains of how to scale the cell culture systems to supply the demand for the potential millions needing such treatments.

This question of high scale culture was, in part, answered by Yamanaka and colleagues. Their work on human leukocyte antigens (HLA) across the Japanese population revealed that 90% of the population could be treated with just 140 different HLA types and therefore 140 different lines [13]. Strikingly, it was described that one donor could cover almost 20% of the population. If similar trends are observed in other populations then the overall number of cell lines required to treat the majority of the global population will be substantially reduced. The establishment of PSC banks will be instrumental for both clinical and pre-clinical research across the wider scientific and medical community [14,15]. However, there are a number of characteristics of human PSCs, largely surrounding the heterogeneity of both the cells and technical procedures that will need to be controlled for in order to reliably produce end-stage products for transplantation. Incorporation of automated stem cell culture systems into workflows for production of clinical grade cell lines may be able to meet some of these needs. Daniszewski *et al* provides a detailed review of all automated systems currently in use for stem cell culture [16].

REDUCING THE IMPACT OF PLURIPOTENT STEM CELL HETEROGENEITY WITH AUTOMATION

PSCs grow as colonies during *in vitro* culture. The colonies have a defined border and the cellular growth is largely even with cells showing a high nuclear-to-cytoplasmic ratio. With each culture, there is a

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heterogeneous mixture of cells with different growth and differentiation characteristics that can be identified by their surface marker expression levels [17]. Coupling these together with distinct genetic traits that affect proliferation and differentiation efficiencies across cell lines [18], it is clear that a cost-effective method of consistently growing hESCs and iPSCs in a precise fashion will be difficult. Adding in operator variability, both in skill and experience will add further inconsistency to the undifferentiated cultures and subsequent differentiation protocols. In order to control such processes, there are a number of systems currently available that are capable of culturing PSCs ([19–23], reviewed in [16]). If implemented across multiple nodes with careful control of reagents in use, at least operator variability could theoretically be kept to a minimum.

Furthermore, automated imaging systems and colony pickers may be useful in terms of determining passaging time points and colony selection in a more objective manner [24,25], especially for gene editing applications where ability to automate clonal selection is crucial.

There can also be significant variation in stem cell differentiation due to both the genetic background of the cells [18] and more simple aspects of cell culture such as methodologies used (suspension vs adherent, growth factor vs small molecule) or even the confluency of stem cell cultures. Here there are two options: one is to use a standard protocol for all lines and have a specified acceptable range for differentiation efficiency or secondly use automated approaches to optimize conditions for each line to maximise the differentiation efficiency. Currently there is no consensus on which approach would be optimal , and the best approach will probably depend on the end cell-type. Careful consideration also needs to be made for purification of the desired cell types as no procedure exists which gives pure populations of cells, without either genetic modification to include selectable markers (which would be undesirable for therapy) or further *in vitro* manipulations and/or cell sorting. In terms of RPE cells currently in trial, the differentiated stem cell progenies were manually excised based upon their unique pigmented and cobblestone morphology [4]. Additionally, RPE cells can be expanded in culture [26,27], which allows for generation of largely pure populations of cells for transplantation. Again these could be automated through image recognition and automated excision of desired cell morphologies. However, not all cell types will be amenable to such manipulations, which means that automated systems should also include some form of flow activated or magnetic cell sorting to select for differentiated cell surface markers and to remove cells still expressing PSC markers [28].

AUTOMATION OF SUSPENSION BIOREACTORS FOR INCREASED SCALE & ORGANOID CULTURE SYSTEMS

While 2D cell culture systems have been used in the field of biomedical research for over a hundred years [29], they do not, however, truly reflect the architecture and function of a tissue *in vivo* [30]. These drawbacks have shifted interest of the

scientific community towards 3D systems. Since the initial report of a 3D model in 1956 [31], much progress has been made to improve the feasibility of generating organotypic structures. This has resulted in development of several platforms that may be used in 3D research, including: (I) scaffold-free approach; (II) synthetic scaffolds (e.g., [32]); (III) naturally derived matrices (e.g., [33]); (IV) hydrogels (reviewed in [34]); and (V) an emerging field of microfluidics (reviewed in [35]). The scaffold-free method relies on an intrinsic ability of cells to self-organize into aggregates, known as spheroids or organoids, while in scaffold-based methods, cells of interest are seeded onto prepared scaffolds followed by aggregate formation. Both techniques hold much promise not only for improving our understanding of the physiological behavior of tissues under *in vitro* conditions, but also of the drug development process [36]. Nevertheless, several issues must be addressed before 3D culture systems can be widely implemented in the industry.

Firstly, organoids provide a fair approximation of cellular functions [37]. However, more accurate simulation of *in vivo* conditions will require incorporation of biomechanical cues like vibration [38,39] or shear force [40] in order to detect typical cellular responses, i.e., gene expression patterns characteristic for the tissue of interest.

Secondly, early stages of organoid formation require substantial manual manipulation of skilled personnel. This raise concerns about feasibility and reproducibility of the method at the same time precluding its use in a large-scale setting. With automation, it will be possible to uniformly generate and mature organoids indistinguishable in structure and cellular physiology. This is paramount for high-throughput screening to guarantee that observed differences are the consequence of drug activity and not culture conditions. Moreover, adaptation of automated 3D approach by the pharmaceutical industry would significantly reduce costs of drug development. Using R&D productivity model, it is estimated that 66% and 30% of newly developed drugs attrite in Phase 2 and 3 clinical trials respectively, leading to major loss of time and resources [41]. Thus, development of an automated system for efficacy and toxicity testing at early stages is highly desirable, as this would allow implementation of the 'fail early, fail cheaply' framework [42]. This could also potentially lead to a vital reduction of animal testing in preclinical studies [43]. Although several automated microbioreactor- [44–46] and microtiter plate- based [47–49] platforms have been developed, they do require further optimization to meet industrial requirements.

Thirdly, use of scaffolds may lead to another set of problems. Adoption of human- or animal-derived scaffolds would force testing for infectious diseases to avoid contamination of the culture. Moreover, reproducibility of results may also be an issue, particularly when animal components are used [36]. Finally, materials used for scaffold construction may need further chemical modifications, so that cells can attach [50], while at the same time scaffolds may also interact with compounds added to the culture, for example new drugs [51]. Thus before the exact scaffold is used in 3D culture, it would have to be

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thoroughly tested to ensure compatibility with the application, what could ultimately lead to switching to scaffold-free systems [36].

Additionally, in terms of iPSC culture systems, there are many reports of scalable bioreactor and suspension culture systems in the literature. There is evidence that mathematical modeling could be used to further enhance the reproducibility of said systems (reviewed in [52]). If modeling, monitoring and automated harvesting and cell maintenance/differentiation could be successfully combined and implemented, those would be superior systems for stem cell manufacturing. Furthermore, current trends towards organoid-based culture systems for generating tissues for disease modeling and potentially therapeutic applications should be readily amenable to suspension culture platforms. Though ultimately these processes would be more suited for bulk production of cells, rather than maintenance and differentiation of high numbers of cell lines that can be achieved in automated 2D culture platforms.

AUTOMATION FOR DISEASE MODELING & DRUG DISCOVERY

One of the major applications of PSC research with more short-to-medium term outcomes is using disease-specific cell lines for understanding disease pathophysiology and drug discovery. There are many examples in the literature whereby iPSCs are derived from those with genetic diseases, such as long QT syndrome [53]. It is now possible to derive cell-types affected in many diseases, creating *in vitro* disease models and using these to identify novel genes, pathways and pharmaceutical interventions. Together the models and screening may allow for prevention or treatment of pathologies where transplantation of cells may not be suitable due to the destructive environment caused by the disease state. It is also feasible that pharmaceutical treatments identified through disease modeling may reduce the requirement for cell transplantation.

Multiple groups are already using automated platforms for such research. This includes initiatives such as New York Stem Cell Foundation (NYSCF), I-Stem or AUTOSTEM consortiums that aim to utilize automation to facilitate generation of large repositories of human ESCs and iPSCs but also high-throughput screening processes for development of new molecules that could potentially be used in clinical trials [54]. Moreover, numerous smaller groups are also employing robotics for differentiating stem cells into specific cell types, for example retinal cells [21], neurons [55], cardiomyocytes [56] or pancreatic islet cells [55].

The use of robotics gives the unprecedented ability to significantly increase the number of samples processed in parallel time without the need of employing a cohort of highly trained and experienced staff. It also eliminates variation introduced by human error as samples undergo treatment the same way every time they are processed. Moreover, it may decrease the analysis time and the volume of reagents used to run the experiment that will undoubtedly have a positive impact on the cost–effectiveness of the process. Having reduced variation and higher throughput could also benefit

analyses of cells through next-generation sequencing and for studies of diseases with genetic risk factors where larger numbers of iPSC lines and their derivatives are required for analyses [57]. There is also the prospect of integrating medium-to-high throughput functional assays in the automation workflow via systems, for example automated patch clamping [58,59], and 'lab-on-a-chip' technologies [60,61] that could be useful for both drug assessment and identification of disease-related phenotypes.

Automation will most likely become a necessity for stem cell therapies at scale. It would be most ideal if the same system could be adopted globally to ensure reproducibility across multiple sites. However, due to various factors, including commercial and regional regulatory factors, it is more likely that separate systems will be adopted in different areas and within different networks. Therefore, it will be up to the broader scientific and clinical communities to ensure minimum standards relating to pluripotent stem cell therapies are devised to maximize safety for patients.

TRANSLATION INSIGHT

Although transferring culture of human stem cells into automated systems opens up new possibilities for researchers and clinical translation, one needs to keep in mind that the process of transition is challenging. Firstly, considering cost of the robotic platform, purchasing one will generate a significant financial investment. Secondly, labware, for example conductive sterile tips and reagents, required to run the experiments may be more expensive when compared to manual processes. Thirdly, maintenance contracts ensuring reliable operation of the equipment may also represent a financial constraint. Fourthly, expenses and time required for training future operators and experiment optimisation cannot be omitted either. Moving stem cell biology towards automation is as exciting as challenging, however, ongoing technological progress will offer scientists more flexible and user-friendly machines facilitating their research.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

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REFERENCES

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282(5391): 1145–7.
2. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat. Biotechnol.* 2000; 18(4): 399–404.
3. Takahashi K, Tanabe K, Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131(5): 861–72.
4. Mandai M, Watanabe A, Kurimoto Y *et al.* Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *N. Engl. J. Med.* 2017; 376(11): 1038–46.
5. Schwartz SD, Tan G, Hosseini H, Nagiel A. Subretinal Transplantation of Embryonic Stem Cell-Derived Retinal Pigment Epithelium for the Treatment of Macular Degeneration: An Assessment at 4 Years. *Invest. Ophthalmol. Vis. Sci.* 2016; 57(5): ORSFc1-9.
6. Schwartz SD, Regillo CD, Lam BL *et al.* Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet* 2015; 385(9967): 509–16.
7. Schwartz SD, Hubschman JP, Heilwell G *et al.* Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet* 2012; 379(9817): 713–20.
8. Song WK, Park KM, Kim HJ *et al.* Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in Asian patients. *Stem Cell Rep.* 2015; 4(5): 860–72.
9. Crook JM, Peura TT, Kravets L *et al.* The generation of six clinical-grade human embryonic stem cell lines. *Cell Stem Cell.* 2007; 1(5): 490–4.
10. Ye J, Bates N, Soteriou D *et al.* High quality clinical grade human embryonic stem cell lines derived from fresh discarded embryos. *Stem Cell Res. Ther.* 2017; 8(1): 128.
11. Baghbaderani BA, Tian X, Neo BH *et al.* cGMP-Manufactured Human Induced Pluripotent Stem Cells Are Available for Pre-clinical and Clinical Applications. *Stem Cell Rep.* 2015; 5(4): 647–59.
12. Tannenbaum SE, Turetsky TT, Singer O *et al.* Derivation of xeno-free and GMP-grade human embryonic stem cells--platforms for future clinical applications. *PLoS One* 2012; 7(6): e35325.
13. Okita K, Matsumura Y, Sato Y, *et al.* A more efficient method to generate integration-free human iPS cells. *Nat. Methods* 2011; 8(5): 409–12.
14. Turner M, Leslie S, Martin NG *et al.* Toward the development of a global induced pluripotent stem cell library. *Cell Stem Cell* 2013; 13(4): 382–4.
15. Andrews PW, Baker D, Benvinisty N *et al.* Points to consider in the development of seed stocks of pluripotent stem cells for clinical applications: International Stem Cell Banking Initiative (ISCBI). *Regen. Med.* 2015; 10(2 Suppl.): 1–44.
16. Daniszewski M, Crombie DE, Henderson R *et al.* Automated Cell Culture Systems and Their Applications to Human Pluripotent Stem Cell Studies. *SLAS Technol.* 2017; 2472630317712220.
17. Kolle G, Ho M, Zhou Q *et al.* Identification of human embryonic stem cell surface markers by combined membrane-polysome translation state array analysis and immunotranscriptional profiling. *Stem Cells* 2009; 27(10): 2446–56.
18. Kilpinen H, Goncalves A, Leha A *et al.* Common genetic variation drives molecular heterogeneity in human iPSCs. *Nature* 2017; 546(7658): 370–375.
19. Archibald PR, Chandra A, Thomas D *et al.* Comparability of automated human induced pluripotent stem cell culture: a pilot study. *Bioprocess Biosyst. Eng.* 2016; 39(12): 1847–58.
20. Conway MK, Gerger MJ, Balay EE *et al.* Scalable 96-well Plate Based iPSC Culture and Production Using a Robotic Liquid Handling System. *J. Vis. Exp.* 2015(99): e52755.
21. Crombie DE, Daniszewski M, Liang HH *et al.* Development of a Modular Automated System for Maintenance and Differentiation of Adherent Human Pluripotent Stem Cells. *SLAS Discov.* 2017: 2472555217696797.
22. Paull D, Sevilla A, Zhou H *et al.* Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells. *Nat. Methods* 2015; 12(9): 885–92.
23. Yang W, Liu Y, Slovik KJ *et al.* Generation of iPSCs as a Pooled Culture Using Magnetic Activated Cell Sorting of Newly Reprogrammed Cells. *PLoS One* 2015; 10(8): e0134995.
24. Schneider A, Spitkovsky D, Riess P *et al.* "The good into the pot, the bad into the crop!"--a new technology to free stem cells from feeder cells. *PLoS One* 2008; 3(11): e3788.
25. Haupt S, Grutzner J, Thier MC, *et al.* Automated selection and harvesting of pluripotent stem cell colonies. *Biotechnol. Appl. Biochem.* 2012; 59(2): 77–87.

26. Lidgerwood GE, Lim SY, Crombie DE *et al.* Defined Medium Conditions for the Induction and Expansion of Human Pluripotent Stem Cell-Derived Retinal Pigment Epithelium. *Stem Cell Rev.* 2016; 12(2): 179–88.
27. Klimanskaya I, Hipp J, Rezai KA, West M, Atala A, Lanza R. Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. *Cloning Stem Cells* 2004; 6(3): 217–45.
28. Tang C, Lee AS, Volkmer JP *et al.* An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. *Nat. Biotechnol.* 2011; 29(9): 829–34.
29. Harrison RG. The outgrowth of the nerve fiber as a mode of protoplasmic movement. *J. Exp. Zool.* 1910; 9(4): 787–846.
30. Greek R, Menache A. Systematic reviews of animal models: methodology versus epistemology. *Int. J. Med. Sci.* 2013; 10(3): 206–21.
31. Ehrmann RL, Gey GO. The growth of cells on a transparent gel of reconstituted rat-tail collagen. *J. Natl Cancer Inst.* 1956; 16(6): 1375–403.
32. Girard YK, Wang C, Ravi S *et al.* A 3D fibrous scaffold inducing tumoroids: a platform for anticancer drug development. *PLoS One* 2013; 8(10): e75345.
33. Modulevsky DJ, Lefebvre C, Haase K, Al-Rekabi Z, Pelling AE. Apple derived cellulose scaffolds for 3D mammalian cell culture. *PLoS One* 2014; 9(5): e97835.
34. Tibbitt MW, Anseth KS, Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol. Bioeng.* 2009; 103(4): 655–63.
35. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. *Nat. Biotechnol.* 2014; 32(8): 760–72.
36. Rimann M, Graf-Hausner U. Synthetic 3D multicellular systems for drug development. *Curr. Opin. Biotechnol.* 2012; 23(5): 803–9.
37. Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R. The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. *J. Biomol. Screen.* 2004; 9(4): 273–85.
38. Tomei AA, Boschetti F, Gervaso F, Swartz MA. 3D collagen cultures under well-defined dynamic strain: a novel strain device with a porous elastomeric support. *Biotechnol. Bioeng.* 2009; 103(1): 217–25.
39. Wolchok JC, Brokopp C, Underwood CJ, Tresco PA. The effect of bioreactor induced vibrational stimulation on extracellular matrix production from human derived fibroblasts. *Biomaterials* 2009; 30(3): 327–35.
40. Mathes SH, Wohlwend L, Uebersax L *et al.* A bioreactor test system to mimic the biological and mechanical environment of oral soft tissues and to evaluate substitutes for connective tissue grafts. *Biotechnol. Bioeng.* 2010; 107(6): 1029–39.
41. Paul SM, Mytelka DS, Dunwiddie CT *et al.* How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* 2010; 9(3): 203–14.
42. L. S, Drug discovery today: Fail early, fail cheaply - improving drug discovery output with early AMDE/Tox tests. Frost & Sullivan Market Insight, 2008.
43. Friedrich J, Seidel C, Ebner R, Kunz-Schughart LA. Spheroid-based drug screen: considerations and practical approach. *Nat. Protoc.* 2009; 4(3): 309–24.
44. Hsu WT, Aulakh RP, Traul DL, Yuk IH. Advanced microscale bioreactor system: a representative scale-down model for bench-top bioreactors. *Cytotechnology* 2012; 64(6): 667–78.
45. Rameez S, Mostafa SS, Miller C, Shukla AA. High-throughput miniaturized bioreactors for cell culture process development: reproducibility, scalability, and control. *Biotechnol. Prog.* 2014; 30(3): 718–27.
46. Puskeiler R, Kaufmann K, Weuster-Botz D. Development, parallelization, and automation of a gas-inducing milliliter-scale bioreactor for high-throughput bioprocess design (HTBD). *Biotechnol. Bioeng.* 2005; 89(5): 512–23.
47. Hallborn J, Carlsson R. Automated screening procedure for high-throughput generation of antibody fragments. Biotechniques 2002; Suppl: 30–7.
48. Tung YC, Hsiao AY, Allen SG, Torisawa YS, Ho M, Takayama S. High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array. *Analyst.* 2011; 136(3): 473–8.
49. Markert S, Joeris K, Establishment of a fully automated microtiter plate-based system for suspension cell culture and its application for enhanced process optimization. *Biotechnol. Bioeng.* 2017; 114(1): 113–21.
50. Bott K, Upton Z, Schrobback K *et al.* The effect of matrix characteristics on fibroblast proliferation in 3D gels. *Biomaterials* 2010; 31(32): 8454–64.
51. Nugraha B, Hong X, Mo X *et al.* Galactosylated cellulosic sponge for multi-well drug safety testing. *Biomaterials* 2011; 32(29): 6982–94.
52. Galvanauskas V, Grincas V, Simutis R, Kagawa Y, Kino-Oka M, Current state and perspectives in modeling and control of human pluripotent

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stem cell expansion processes in stirred-tank bioreactors. *Biotechnol. Prog.* 2017; 33(2): 355–64.

53. Moretti A, Bellin M, Welling A *et al.* Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N. Engl. J. Med.* 2010; 363(15): 1397–409.
54. McKernan R, Watt FM. What is the point of large-scale collections of human induced pluripotent stem cells? *Nat. Biotechnol.* 2013; 31(10): 875–7.
55. Konagaya S, Ando T, Yamauchi T, Suemori H, Iwata H. Long-term maintenance of human induced pluripotent stem cells by automated cell culture system. *Sci. Rep.* 2015; 5: 16647.
56. Kowalski MP, Yoder A, Liu L, Pajak L. Controlling embryonic stem cell growth and differentiation by automation: enhanced and more reliable differentiation for drug discovery. *J. Biomol. Screen.* 2012; 17(9): 1171–9.
57. Pashos EE, Park Y, Wang X *et al.* Large, Diverse Population Cohorts of hiPSCs and Derived Hepatocyte-like Cells Reveal Functional Genetic Variation at Blood Lipid-Associated Loci. *Cell Stem Cell* 2017; 20(4): 558–570.e10.
58. Rajamohan D, Kalra S, Duc Hoang M *et al.* Automated Electrophysiological and Pharmacological Evaluation of Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Stem Cells Dev.* 2016; 25(6): 439–52.
59. Franz D, Olsen HL, Klink O, Gimsa J. Automated and manual patch clamp data of human induced pluripotent stem cell-derived dopaminergic neurons. *Sci. Data* 2017; 4: 170056.
60. Tatarova Z, Abbuehl JP, Maerkl S, Huelsken J. Microfluidic co-culture platform to quantify chemotaxis of primary stem cells. *Lab Chip* 2016; 16(10): 1934–45.
61. Agarwal A, Goss JA, Cho A, McCain ML, Parker KK. Microfluidic heart on a chip for higher throughput pharmacological studies. *Lab Chip* 2013; 13(18): 3599–608.

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