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Advances in Microfluidic Platforms for Analyzing and Regulating Human Pluripotent Stem Cells

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Abstract

Microfluidic devices employ submillimeter length scale control of flow to achieve high-resolution spatial and temporal control over the microenvironment, providing powerful tools to elucidate mechanisms of human pluripotent stem cell (hPSC) regulation and to elicit desired hPSC fates. In addition, microfluidics allow control of paracrine and juxtacrine signaling, thereby enabling fabrication of microphysiological systems comprised of multiple cell types organized into organs-on-a-chip. Microfluidic cell culture systems can also be integrated with actuators and sensors, permitting construction of high-density arrays of cell-based biosensors for screening applications. This review describes recent advances in using microfluidics to understand mechanisms by which the microenvironment regulates hPSC fates and applications of microfluidics to realize the potential of hPSCs for *in vitro* modeling and screening applications.

Introduction

Human pluripotent stem cells (hPSCs) possess unlimited expansion capacity and the ability to differentiate to somatic cells in all germ lineages. Hence, hPSCs provide vast opportunities for modeling human development and disease, assessing the effects of drugs and other compounds on human cells and tissues, and enabling cell-based regenerative medicine. Realizing the potential of hPSCs in these applications will require the ability to control differentiation of hPSCs to desired cell types, which in turn necessitates a fundamental understanding of mechanisms that regulate hPSC fates.

Microfluidic systems, defined as devices that manipulate fluids at the sub-millimeter length scale, can be constructed to provide high resolution spatial and temporal regulation over the stem cell microenvironment. As shown in Figure 1, microfluidic platforms enable precise manipulation of the microenvironment to deliver soluble factors to cells [1–3], construct well-defined gradients in soluble or immobilized cues [4], and dynamically alter the application of mechanical signals to cultured cells [5]. Microfluidic systems have also

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advanced hPSC applications in cell separations [6,7], biosensing [8], and high-throughput screening [9] by integrating fluid handling with cell culture.

This concise review will highlight important advances in the past two years where microfluidic devices have been employed to elucidate basic mechanisms of hPSC regulation or have utilized microfluidic devices to construct platforms for using hPSC-derived cells in separation, biosensing, and screening applications. In light of this significant recent progress, the potential of microfluidics to further advance stem cell science and engineering will also be discussed.

Microfluidic control of the stem cell microenvironment and cell co-culture

hPSCs continually monitor signals from their microenvironment, including soluble factors, extracellular matrix, cell-cell contact, and biophysical cues, and then integrate this information to make discrete fate choices such as self-renewal or differentiation [10–14]. Difficulty in precisely regulating the stem cell microenvironment, through both space and time, has limited advancement of our understanding of how the microenvironment affects hPSC fate. Several recent studies have used microfluidic devices to systematically present cues to hPSCs and unravel mechanisms of microenvironmental regulation of hPSC fates.

In the past few years, enabling advances in culturing and differentiating hPSCs in microfluidic devices have been reported. For example, polydimethylsiloxane (PDMS) micro-chamber arrays were constructed to identify ECM proteins capable of maintaining hPSCs in an undifferentiated, pluripotent state [15]. In this system, laminin and fibronectin were identified to better maintain hPSC cultures in a defined culture medium in PDMS microchannels than collagen or gelatin. Also, a microfluidic trap was designed to control embryoid body (EB) formation from human embryonic stem cells (hESCs), to confine the EBs to the trap, and to facilitate gas/nutrient exchange thereby allowing cell differentiation in the EBs [5]. EBs were able to be maintained for up to five days and each aggregate could be controlled independently from other aggregates in the same microfluidic chamber array, thus the differentiation process of each aggregate could be monitored individually. Additionally, incorporation of small wells in a microfluidic channel enabled clonal expansion of induced pluripotent stem cell (iPSC) colonies from singularized cells [16].

These advances in hPSC culture in microfluidic devices enable mechanistic studies of hPSC self-renewal and differentiation that could not be realized in traditional Petri dish or flask culture systems. For example, by dynamically controlling spatial and temporal gradients of morphogens Wnt3a, Activin A, BMP4 and corresponding inhibitors in a microbio-reactor, Cimetta *et al.* [4] screened effects of these morphogen gradients on differentiation trajectories of individual EBs. Their study showed that spatial differentiation in EBs responded non-linearly to linear morphogen concentration gradients.

Gradients in chemical cues generated in microfluidic devices also enable quantitative assessment of phenotypes in stem cells and their progeny. For example, hPSC-derived endothelial cells (hPSC-ECs) were cultured in stable, linear gradients of vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 α (SDF1 α) to identify mechanisms of chemotactic regulation [17]. hPSC-ECs migrated toward VEGF gradients but not SDF1 α .

gradients, consistent with *in vivo* experiments demonstrating a lack of hPSC-EC homing to sites of ischemic injury. A microfluidic system was also employed to screen heart morphogenic cues that elicit chemotaxis and haptotaxis of hPSC-derived cardiomyocytes (hPSC-CMs) [18]. Gradients of immobilized fibronectin elicited haptotaxis, mediated by α_5 and α_v integrins, while hPSC-CMs migrated toward Wnt5a gradients in a Frizzled-dependent manner. Microfluidic chemotaxis assays are also useful in comparing cells differentiated from disease model iPSCs vs. control iPSCs. For example, a recent study showed that neural progenitors differentiated from schizophrenia iPSCs exhibited reduced migration in a microfluidic channel compared to neural progenitors differentiated from control iPSCs [19]. In future studies, microfluidic devices may prove useful in discovery of mechanisms that regulate these differences in motility.

Microfluidic systems also enable mechanistic analysis of autocrine, paracrine, and physical interactions between cells. Traditional co-culture systems are typically limited by a lack of precise control over cell localization and soluble factor exchange. However, microfluidics permit micron-scale spatial organization of cells and intercellular communication by modulating flow between cells in culture. For example, Moledina *et al.* used fluid flow in a microfluidic channel to control accumulation of cell-secreted factors and demonstrated that autocrine and paracrine factors activates STAT3 signaling in murine ESCs [20]. Using a PDMS-glass branched microchannel, van der Meer *et al.* [1] showed co-culture of human endothelial cells and embryonic stem cell-derived pericytes induced organization of vascular structures in a TGF β -dependent manner. Inhibition of TGF β signaling led to disorganized endothelial cell-cell contacts and endothelial cell-pericyte interactions. Co-culture of hESC-derived Schwann cells with hESC-derived neurons in microfluidic channels led to axonal wrapping by the Schwann cells [21]. Warmflash *et al.* [22] recently cultured hPSCs on micropatterned substrates to recapitulate early embryonic spatial patterning. Upon addition of BMP4, gradients of morphogens generated by the cells induced colony differentiation to an outer trophectoderm, a mesoderm ring, and an inner ectoderm. While this study did not employ microfluidics, the combination of micropatterned substrates and microfluidics may permit better control over cell-cell signaling during morphogenesis.

Fluid flow can deliver nutrients and signaling factors to cells in microfluidic devices, but it also exposes the cells to laminar shear stresses which could aberrantly influence cell fate. A shear stress of 0.6 dyn/cm² was able to pattern laminar fluid flows to spatially deliver soluble factors in a microchannel containing hESCs without affecting proliferation or differentiation compared to hESCs cultured under static conditions [2]. Another study of the effects of flow on hESC culture in bioreactors reported an optimum, intermediate flow rate that overcame nutrient depletion but did not inhibit proliferation or detach the hESCs from the substrate [23]. In this optimal flow regime, hESCs proliferated at a similar rate as hESCs cultured under static conditions. Yoshimitsu *et al.* demonstrated that perfusion, which generated a shear stress of approximately 0.01 dyn/cm², was sufficient to overcome nutrient limitations in a microfluidic channel without damaging or detaching iPSCs [15].

Microfluidic flow allows precise placement of cells in culture. For example, Suri *et al.* developed a microfluidic trap to pattern and fuse two different types of EBs, one differentiated in the presence of BMP4 and the other in the absence of BMP4 [24]. The

fused EBs exhibited spatial induction of mesoderm differentiation reminiscent of primitive streak formation, providing a system to study early embryonic morphogenesis *in vitro*. Lee *et al.* [25] designed microchannels that allowed axon, but not dendrite, migration in a culture of hPSCs differentiating to neural lineages, demonstrating how physical patterns in a microfluidic device can spatially organize cell pattern formation. Flow can also be used to characterize mechanically-induced phenotypes of hPSC-derived cells. Belair *et al.* used a microfluidic device to probe alignment of iPSC-derived endothelial cells in response to shear flow [26].

These examples, summarized in Table 1, illustrate how microfluidic platforms permit systematic and/or multiplexed experiments to identify and optimize molecular regulation of hPSC differentiation by the stem cell microenvironment.

Human pluripotent stem cell separations, biosensing, and high-throughput screening

As a result of their ability to precisely apply forces, microfluidic devices are well-suited to label-free cell sorting applications. For example, by applying shear flow to a heterogeneous mixture of cells in a reprogramming culture, Singh *et al.* utilized the differences in adhesion strength between iPSCs and somatic cells to isolate iPSC clones from unprogrammed cells [6]. Optical tweezers have also been integrated into a microfluidic chip to provide multidimensional control over cell movement. In a proof-of-concept study, Wang *et al.* demonstrated automated sorting of GFP-expressing hESCs from GFP negative hESCs with 90% recovery and 90% purity [7]. Acoustic waves, which require lower power density than optical traps, have also been incorporated into microfluidic devices to facilitate spatial handling of cells [27]. Size-based sorting of cells in inertial microfluidic channels [28,29] and affinity-based separations using capture antibodies or lectins immobilized in a microchannel [30,31] will likely have applications in sorting and purifying cells differentiated from hPSCs. Compared to fluorescence activated cell sorting (FACS), microfluidic devices can separate cells based on differences in physical properties such as adhesion, size, and shape. Microfluidic-based separations can also be performed on very small cell populations or scaled up via parallelization.

Microfluidic cell culture can also be integrated with cellular and molecular analysis technologies. Reporter cell lines and on-chip fluorescence detection are a complimentary tool to microfluidics in screening applications, allowing dynamic assessment of reporter gene expression or enzyme activity to microenvironmental cues. For example, hPSC lines expressing GFP under control of an artificial *OCT4/POU5F1* promoter sequence were used to screen for conditions that induce hPSC differentiation in a microbioreactor [8]. Gene expression [32,33] and secretome [34] analysis has been appropriately scaled and integrated with microfluidic cell culture and will likely prove valuable in assessing effects of cues applied to hPSCs in a microfluidic device.

While microfluidic culture is not easily scaled up to produce large numbers of cells, it is amenable to scale-out for multiplexed culture applications such as high throughput screening. The low volume of microfluidic devices is also an advantage when handling

small samples and expensive reagents. Titmarsh *et al.* [35] developed a continuously-connected flow array that applied exogenous soluble cues and circulated endogenous paracrine factors. Using this device, they determined that Wnt signaling-induced primitive streak differentiation in hESCs required accumulation of endogenous factors or FGF-2 supplementation. In a subsequent study, they combined the microbioreactor array with a pluripotency reporter line to screen the effects of exogenous soluble factors and paracrine cues on hESC self-renewal [36]. Results of this screen suggest that intrinsic paracrine factors repress pluripotency. Microfluidic platforms have also been used to screen for self-renewal factors in hematopoietic stem cells [37] and proliferation and differentiation cues in mesenchymal stem cells [38]. As microfluidic technology matures, these high throughput screens will likely complement hypothesis-directed approaches to develop and optimize hPSC differentiation processes.

Microphysiological systems

The precise liquid handling of microfluidic devices enables construction of integrated microphysiological systems consisting of cells and tissues organized to mimic and study organ and organism level interactions. These “organs-on-a-chip” (Figure 2) also facilitate real-time monitoring of drug effects on interconnected networks of cells and tissues differentiated from hPSCs, and can be used to predict effects of these drugs on the human body [39]. To date, several proofs-of-concept organs-on-a-chip systems using cells differentiated from hPSCs have been described, including heart-on-a-chip [40,41]. Wang *et al.* developed a microchannel culture system containing embedded cantilevers to measure forces exerted by cardiac microtissues [42]. They used these heart-on-a-chip platforms to demonstrate that cardiomyocytes differentiated from Barth Syndrome iPSCs exerted less stress than cardiomyocytes differentiated from control iPSCs [41]. Numerous other hPSC-derived microfluidic microphysiological systems are currently under development to enable predictive drug toxicology [43].

Outlook

Because of their ability to control the stem cell microenvironment with high spatial and temporal precision, microfluidic systems have enabled a better understanding of biochemical and biophysical regulation of hPSC fates. Pioneering efforts using microfluidic technologies for realizing the *in vitro* modeling and drug screening potential of hPSCs in high-throughput screening and microphysiological system platforms have also been reported in the past several years. Currently, most microfluidic platforms are custom-designed and manufactured for a particular application. Broader implementation of these powerful systems will require greater access of standardized microfluidic culture systems for the general stem cell research community. Given the demand for low-cost, reproducible, high-density, high-resolution hPSC culture systems, the role of microfluidic devices in hPSC culture is expected to increase dramatically in the near future. We expect drug screening in human tissues in microfluidic arrays and drug evaluation in microphysiologic systems to be routine components of the drug development pipeline in the near future.

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References and recommended reading

Papers of particular interest, published within the past two years, have been highlighted as:

• of special interest

•• of outstanding interest

1. van der Meer AD, Orlova VV, ten Dijke P, van den Berg A, Mummery CL. Three-dimensional co-cultures of human endothelial cells and embryonic stem cell-derived pericytes inside a microfluidic device. *Lab on a Chip*. 2013; 13:3562–3568. [PubMed: 23702711] • Co-culture of hESC-derived pericytes with primary human endothelial cells in a microfluidic channel induced vascular structure formation in a TGF β -dependent manner.
2. Villa-Diaz LG, Torisawa Y-s, Uchida T, Ding J, Nogueira-de-Souza NC, O'Shea KS, Takayama S, Smith GD. Microfluidic culture of single human embryonic stem cell colonies. *Lab on a Chip*. 2009; 9:1749–1755. [PubMed: 19495459]
3. Chingozha L, Zhan M, Zhu C, Lu H. A generalizable, tunable microfluidic platform for delivering fast temporally varying chemical signals to probe single-cell response dynamics. *Anal Chem*. 2014; 86:10138–10147. [PubMed: 25254360]
4. Cimetta E, Sirabella D, Yeager K, Davidson K, Simon J, Moon RT, Vunjak-Novakovic G. Microfluidic bioreactor for dynamic regulation of early mesodermal commitment in human pluripotent stem cells. *Lab on a chip*. 2013; 13:355–364. [PubMed: 23232509] • A screen of linear spatial gradients of morphogens applied to EBs in a microfluidic channel revealed a nonlinear response in differentiation fates
5. Khoury M, Bransky A, Korin N, Konak LC, Enikolopov G, Tzchori I, Levenberg S. A microfluidic traps system supporting prolonged culture of human embryonic stem cells aggregates. *Biomedical microdevices*. 2010; 12:1001–1008. [PubMed: 20665114]
6. Singh A, Suri S, Lee T, Chilton JM, Cooke MT, Chen W, Fu J, Stice SL, Lu H, McDevitt TC, et al. Adhesion strength-based, label-free isolation of human pluripotent stem cells. *Nat Meth*. 2013; 10:438–444. •• This study applied shear stress to heterogeneous mixtures of somatic cells undergoing reprogramming to iPSCs. Fully-reprogrammed iPSCs were separated from partially reprogrammed cells based on differential adhesion.
7. Wang X, Chen S, Kong M, Wang Z, Costa KD, Li RA, Sun D. Enhanced cell sorting and manipulation with combined optical tweezer and microfluidic chip technologies. *Lab on a Chip*. 2011; 11:3656–3662. [PubMed: 21918752]
8. Ovchinnikov DA, Titmarsh DM, Fortuna PR, Hidalgo A, Alharbi S, Whitworth DJ, Cooper-White JJ, Wolvetang EJ. Transgenic human ES and iPS reporter cell lines for identification and selection of pluripotent stem cells in vitro. *Stem cell research*. 2014; 13:251–261. [PubMed: 25108530]
9. ForáYu ZT. An integrated microfluidic culture device for quantitative analysis of human embryonic stem cells. *Lab on a Chip*. 2009; 9:555–563. [PubMed: 19190791]
10. Hazeltine LB, Selekmán JA, Palecek SP. Engineering the human pluripotent stem cell microenvironment to direct cell fate. *Biotechnology advances*. 2013; 31:1002–1019. [PubMed: 23510904]
11. Paik I, Scurr DJ, Morris B, Hall G, Denning C, Alexander MR, Shakesheff KM, Dixon JE. Rapid micropatterning of cell lines and human pluripotent stem cells on elastomeric membranes. *Biotechnology and bioengineering*. 2012; 109:2630–2641. [PubMed: 22511037]
12. Satoh T, Sugiura S, Sumaru K, Ozaki S, Gomi S, Kurakazu T, Oshima Y, Kanamori T. Inverting microwell array chip for the cultivation of human induced pluripotent stem cells with controlled aggregate size and geometrical arrangement. *Biomicrofluidics*. 2014; 8:024112. [PubMed: 24803961]

13. Diana XY, Marchetto MC, Gage FH. Therapeutic translation of iPSCs for treating neurological disease. *Cell Stem Cell*. 2013; 12:678–688. [PubMed: 23746977]
14. Kawada J, Kimura H, Akutsu H, Sakai Y, Fujii T. Spatiotemporally controlled delivery of soluble factors for stem cell differentiation. *Lab on a Chip*. 2012; 12:4508–4515. [PubMed: 22968416]
15. Yoshimitsu R, Hattori K, Sugiura S, Kondo Y, Yamada R, Tachikawa S, Satoh T, Kurisaki A, Ohnuma K, Asashima M. Microfluidic perfusion culture of human induced pluripotent stem cells under fully defined culture conditions. *Biotechnology and bioengineering*. 2014; 111:937–947. [PubMed: 24222619]
16. Matsumura T, Tatsumi K, Noda Y, Nakanishi N, Okonogi A, Hirano K, Li L, Osumi T, Tada T, Kotera H. Single-cell cloning and expansion of human induced pluripotent stem cells by a microfluidic culture device. *Biochem Biophys Res Commun*. 2014; 453:131–137. [PubMed: 25264198]
17. Huang NF, Dewi RE, Okogbaa J, Lee JC, Rufaihah A, Heilshorn SC, Cooke JP. Chemotaxis of human induced pluripotent stem cell-derived endothelial cells. *American journal of translational research*. 2013; 5:510. [PubMed: 23977410]
18. Moyes KW, Sip CG, Obenza W, Yang E, Horst C, Welikson RE, Hauschka SD, Folch A, Laflamme MA. Human embryonic stem cell-derived cardiomyocytes migrate in response to gradients of fibronectin and Wnt5a. *Stem cells and development*. 2013; 22:2315–2325. [PubMed: 23517131] • • hPSC-derived cardiomyocytes exhibited a chemotactic response to Wnt5a gradients established in a microfluidic device and a haptotactic response to fibronectin gradients.
19. Brennand, K.; Savas, J.; Kim, Y.; Tran, N.; Simone, A.; Hashimoto-Torii, K.; Beaumont, K.; Kim, H.; Topol, A.; Ladrán, I. *Molecular psychiatry*. 2014. Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia.
20. Moledina F, Clarke G, Oskoei A, Onishi K, Günther A, Zandstra PW. Predictive microfluidic control of regulatory ligand trajectories in individual pluripotent cells. *Proceedings of the National Academy of Sciences*. 2012; 109:3264–3269.
21. Ziegler L, Grigoryan S, Yang I, Thakor N, Goldstein R. Efficient Generation of Schwann Cells from Human Embryonic Stem Cell-Derived Neurospheres. *Stem Cell Reviews and Reports*. 2011; 7:394–403. [PubMed: 21052870]
22. Warmflash A, Sorre B, Etoc F, Siggia ED, Brivanlou AH. A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. *Nature Methods*. 2014; 11:847–854. [PubMed: 24973948]
23. Titmarsh D, Hidalgo A, Turner J, Wolvetang E, Cooper - White J. Optimization of flowrate for expansion of human embryonic stem cells in perfusion microbioreactors. *Biotechnology and bioengineering*. 2011; 108:2894–2904. [PubMed: 21732331]
24. Suri S, Singh A, Nguyen AH, Bratt-Leal AM, McDevitt TC, Lu H. Microfluidic-based patterning of embryonic stem cells for in vitro development studies. *Lab on a Chip*. 2013; 13:4617–4624. [PubMed: 24113509]
25. Lee N, Park JW, Kim HJ, Yeon JH, Kwon J, Ko JJ, Oh S-H, Kim HS, Kim A, Han BS, et al. Monitoring the Differentiation and Migration Patterns of Neural Cells Derived from Human Embryonic Stem Cells Using a Microfluidic Culture System. *Molecules and Cells*. 2014; 37:497–502. [PubMed: 24938227]
26. Belair DG, Whisler JA, Valdez J, Velazquez J, Molenda JA, Vickerman V, Lewis R, Daigh C, Hansen TD, Mann DA, et al. Human Vascular Tissue Models Formed from Human Induced Pluripotent Stem Cell Derived Endothelial Cells. *Stem Cell Rev*. 2014
27. Ding XY, Lin SCS, Kiraly B, Yue HJ, Li SX, Chiang IK, Shi JJ, Benkovic SJ, Huang TJ. On-chip manipulation of single microparticles, cells, and organisms using surface acoustic waves. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:11105–11109. [PubMed: 22733731]
28. Lee WC, Shi H, Poon Z, Nyan LM, Kaushik T, Shivashankar GV, Chan JKY, Lim CT, Han J, Van Vliet KJ. Multivariate biophysical markers predictive of mesenchymal stromal cell multipotency. *Proceedings of the National Academy of Sciences*. 2014; 111:E4409–E4418.

29. Warkiani ME, Guan GF, Luan KB, Lee WC, Bhagat AAS, Chaudhuri PK, Tan DSW, Lim WT, Lee SC, Chen PCY, et al. Slanted spiral microfluidics for the ultra-fast, label-free isolation of circulating tumor cells. *Lab on a Chip*. 2014; 14:128–137. [PubMed: 23949794]
30. Liu Y, Germain T, Pappas D. Microfluidic antibody arrays for simultaneous cell separation and stimulus. *Analytical and Bioanalytical Chemistry*. 2014; 406:7867–7873. [PubMed: 25354890]
31. Vickers DAL, Chory EJ, Murthy SK. Separation of two phenotypically similar cell types via a single common marker in microfluidic channels. *Lab on a Chip*. 2012; 12:3399–3407. [PubMed: 22782544]
32. White AK, VanInsberghe M, Petriv I, Hamidi M, Sikorski D, Marra MA, Piret J, Aparicio S, Hansen CL. High-throughput microfluidic single-cell RT-qPCR. *Proceedings of the National Academy of Sciences*. 2011; 108:13999–14004.
33. Narsinh KH, Sun N, Sanchez-Freire V, Lee AS, Almeida P, Hu S, Jan T, Wilson KD, Leong D, Rosenberg J. Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. *The Journal of clinical investigation*. 2011; 121:1217. [PubMed: 21317531]
34. Lu Y, Chen JJ, Mu LY, Xue Q, Wu Y, Wu PH, Li J, Vortmeyer AO, Miller-Jensen K, Wirtz D, et al. High-Throughput Secretomic Analysis of Single Cells to Assess Functional Cellular Heterogeneity. *Analytical Chemistry*. 2013; 85:2548–2556. [PubMed: 23339603]
35. Titmarsh DM, Hudson JE, Hidalgo A, Elefanti AG, Stanley EG, Wolvetang EJ, Cooper-White JJ. Microbioreactor arrays for full factorial screening of exogenous and paracrine factors in human embryonic stem cell differentiation. *PloS one*. 2012; 7:e52405. [PubMed: 23300662]
36. Titmarsh DM, Ovchinnikov DA, Wolvetang EJ, Cooper - White JJ. Full factorial screening of human embryonic stem cell maintenance with multiplexed microbioreactor arrays. *Biotechnology journal*. 2013; 8:822–834. [PubMed: 23813764] • The authors employed a pluripotency reporter hESC line in a microbioreactor array to identify soluble and paracrine factors that regulate self-renewal.
37. Lecault V, VanInsberghe M, Sekulovic S, Knapp DJHF, Wohrer S, Bowden W, Viel F, McLaughlin T, Jarandehi A, Miller M, et al. High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. *Nat Meth*. 2011; 8:581–586.
38. Gomez-Sjoberg R, Leyrat AA, Pirone DM, Chen CS, Quake SR. Versatile, fully automated, microfluidic cell culture system. *Anal Chem*. 2007; 79:8557–8563. [PubMed: 17953452]
39. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. *Nat Biotech*. 2014; 32:760–772.
40. Mathur A, Loskill P, Hong S, Lee JY, Marcus SG, Dumont L, Conklin BR, Willenbring H, Lee LP, Healy KE. Human induced pluripotent stem cell-based microphysiological tissue models of myocardium and liver for drug development. *Stem Cell Research & Therapy*. 2013; 4
41. Wang G, McCain ML, Yang L, He A, Pasqualini FS, Agarwal A, Yuan H, Jiang D, Zhang D, Zangi L, et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. *Nat Med*. 2014; 20:616–623. [PubMed: 24813252] • • This study used a mechanosensing heart-on-a-chip to determine that cardiomyocytes differentiated from Barth syndrome iPSCs exert less contractile stress than cardiomyocytes differentiated from control iPSCs.
42. Agarwal A, Goss JA, Cho A, McCain ML, Parker KK. Microfluidic heart on a chip for higher throughput pharmacological studies. *Lab Chip*. 2013; 13:3599–3608. [PubMed: 23807141]
43. Sutherland ML, Fabre KM, Tagle DA. The National Institutes of Health Microphysiological Systems Program focuses on a critical challenge in the drug discovery pipeline. *Stem Cell Res Ther*. 2013; 4(Suppl 1):I1. [PubMed: 24565163]

Figure 1.

Example applications of microfluidic devices in hPSC culture and differentiation. (A) The chemical environment in a microfluidic chamber can be dynamically regulated and used to establish stable gradients. This example shows integrated microchambers for parallel analysis of the effects of chemical compounds on hPSCs (Reprinted with permission from [15]). (B) Microfluidic devices facilitate the study of mechanisms of paracrine and juxtacrine signaling by enabling precise placement of cells and control over intercellular diffusible signaling. In this example, co-culture of hPSC-derived pericytes with endothelial cells stimulated assembly of a vascular-like structure (Reprinted with permission from [1]). (C) Shear forces applied by fluid flow in a microfluidic device can be harnessed for cell separation applications, such as this example which used shear to selectively detach fully reprogrammed iPSCs from a heterogeneous reprogramming mixture (Reprinted with permission from [6]). (D) Microbioreactors on a chip can also be employed for multiplexed screening for drugs that elicit hPSC self-renewal or differentiation (Reprinted with permission from [36]).

Figure 2.

Concept of microphysiological systems. hPSC-derived cells, representing distinct tissues and organs, are cultured in chambers connected by microfluidic channels. Flow between these channels can be controlled to assess organ and system responses to microenvironment perturbations, such as drug application.

Table 1

Microenvironment manipulation of hPSCs with microfluidic devices.

Cell behavior studied	Methods enabled by microfluidic culture	Findings	Ref.
Cell-cell communication	Co-culture of hPSC-derived pericytes with endothelial cells	TGF β stimulates organization of vascular structures	[1]
Differentiation	Gradients of Wnt3a, Activin A, BMP4 and corresponding inhibitors	Spatial differentiation in EBs responded non-linearly to linear growth factor gradients	[4]
Pluripotency	MicroChannel culture on different ECM substrates	Laminin and fibronectin better maintain pluripotency than collagen or gelatin in a microfluidic channel	[15]
Expansion and differentiation	Spatial segregation of EBs and monitoring of differentiation	Developed a method for high throughput clonal culture and differentiation of hPSCs	[16]
Migration	Generation of VEGF and SDF1 α gradients	hPSC-ECs migrate toward VEGF gradients but not SDF1 α gradients	[17]
Migration	Generation of Wnt5a gradients	hPSC-CMs migrate toward Wnt5a gradients in a Frizzled-dependent manner	[18]
Migration	Directed migration in microchannels containing microgrooves	NPCs differentiated from schizophrenia iPSCs exhibited reduced migration	[19]
Migration, cell-cell interactions	Monitoring neurite outgrowth in microchannels containing microgrooves	Microchannels can guide axon organization	[25]
Phenotype assessment	Application of shear stress	iPSC-derived ECs align in the direction of flow	[26]