

Human-on-chip for therapy development and fundamental science

Camilla Luni^{1,2}, Elena Serena^{1,2} and Nicola Elvassore^{1,2}

Organ-on-chip systems integrate microfluidic technology and living cells to study human physiology and pathophysiology. These human *in vitro* models are promising substitutes for animal testing, and their small scale enables precise control of culture conditions and high-throughput experiments, which would not be economically sustainable on a macroscopic level. Multiple sources of biological material are used in the development of organ-on-chips, from biopsies to stem cells. Each source has its own peculiarities and technical requirements for integration into microfluidic chips, and is suitable for specific applications. While a biopsy is the tissue of choice for the biomimetic response to ageing, induced pluripotent stem cells hold great promise for the study of genetic-related disease pathogenesis, and primary cultures can fill the gap.

Addresses

¹ Department of Industrial Engineering, University of Padova,
Via Marzolo 9, 35131 Padova, Italy

² Venetian Institute of Molecular Medicine, Via Orus 2, 35129 Padova,
Italy

Corresponding author: Elvassore, Nicola (nicola.elvassore@unipd.it)

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Introduction

The majority of researchers test their biological hypotheses on primary cultures in conventional Petri dishes, focusing their attention to the biological response of the cells, without tight control of the biological context surrounding them. In contrast, pharmaceutical companies conventionally test drugs on animals, but a high percentage of lead compounds fail during the clinical trial phase [1]. The linking concept between these two approaches is given by the human organ-on-chip that will soon revolutionize the way researchers perform their investigations and pharmaceutical companies test their drugs [2]. The term ‘chip’ refers to the design principles and microfabrication techniques used. The term ‘organ’ derives from the tailoring of microenvironment architecture inspired by organ-level functions *in vivo* [3].

The final goal of organ-on-chips is related to mimicking human normal and pathological physiology *in vitro* within systems that have high measurement accessibility. Basic science and drug development will greatly benefit from human functional organ-on-chips, both in terms of reliability of results and costs. Human *in vitro* models are promising substitutes for animal testing, and their small scale will enable high-throughput experiments not economically sustainable on a macroscopic level, also giving insight into heterogeneity of the human population. Microfluidics offers unprecedented control over culture conditions, and this technology is currently in a mature state to provide stimuli such as chemical gradients [4], spatial homogeneity [5], time-dependent biochemical stimulations, and substrate mechanical properties [6].

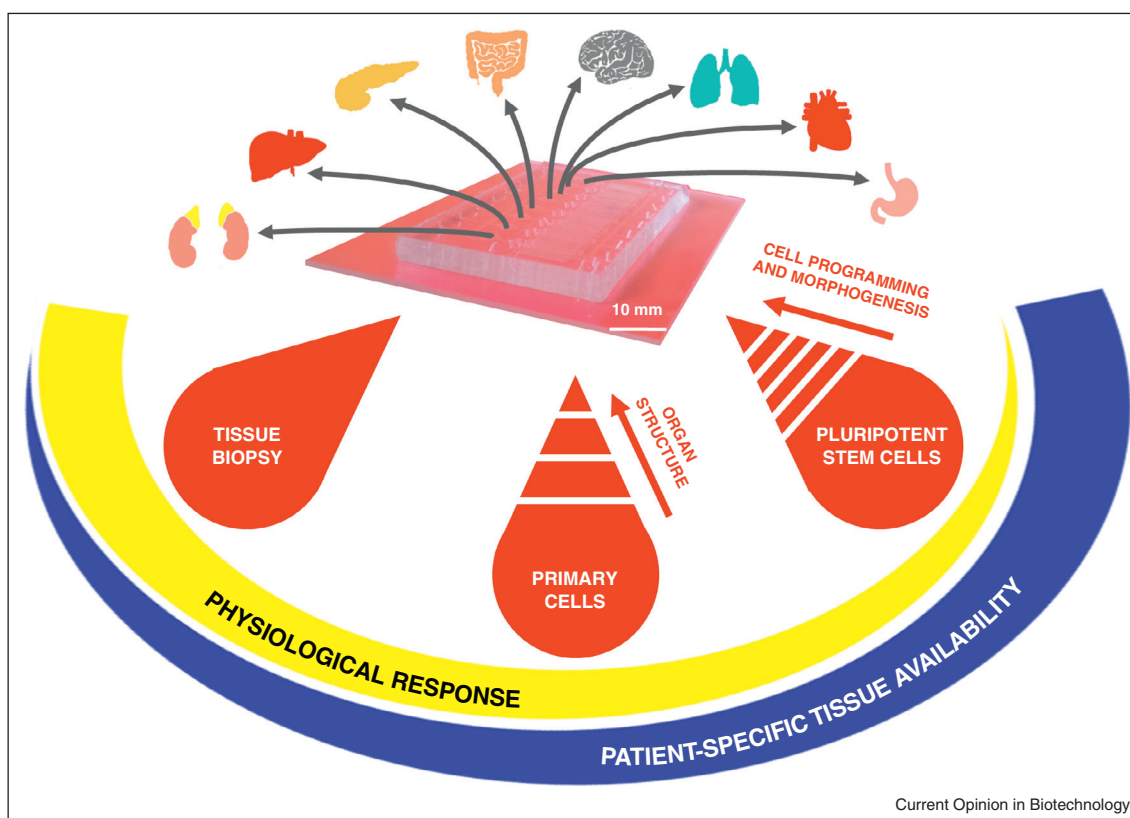
Here, we will review recent progresses in single-organ-on-chip or multi-organ-on-chip technology. We analyze the development of these systems starting from three main sources of human biological material: ex vivo tissues, primary cells, and pluripotent stem cells. We will discuss the up-to-date different performance of these sources in terms of availability and ability to recapitulate *in vivo* physiology and pathophysiology (Figure 1).

Regulatory facts

A critical gap still needs to be filled between pre-clinical toxicology and patient response during clinical trials. The percentage of failure in the drug discovery pipeline is still very high, especially at late stages, due to reduced efficacy or emerging toxicity, and research expenses are not compensated by yielding new products [1]. More predictive models and assays are necessary to increase the success rate, anticipating the failure of bad lead compounds before clinical trials.

Multiple governmental authorities are thus opening to innovative tools for drug screening, in particular to *in vitro* cell-based or tissue-based models reproducing human physiology and, in perspective, pathophysiology. Fundamental requirements for these systems are flexibility, reliability, economic affordability, and accessibility to a wide scientific community. For instance, the European Commission is funding ‘The body on a chip’ project within the 7th Framework program, while a partnership between National Institute of Health (NIH), Food and Drug Administration (FDA) and Defense Advanced Research Projects Agency (DARPA) has been recently established for the development of an *in vitro* human multi-tissue platform able to reproduce specific aspects of

Figure 1



Cell sources for organ-on-chip development. Fresh tissue biopsies currently best mimic *in vitro* the *in vivo* physiology, but lack wide availability for collection from human patients. Primary cells, although also derived by biopsies, can be maintained in culture for longer periods of time, currently allowing more thorough studies; however, the 3D architecture of *in vivo* organs need to be reconstructed for development of representative functional assays. Pluripotent stem cells include different cell types derived from embryos and embryo-related tissues or obtained by reprogramming of adult somatic cells (human induced pluripotent stem cells, hiPS). hiPS represent a potentially unlimited human cell source to use, as they are derived from patients by minimally invasive procedures. Currently iPS technology is still in an immature phase, but rapid progresses are performed to differentiate these cells and, in perspective, build fully functional human organ-on-chips.

in vivo physiology, ideally including circulatory, endocrine, gastrointestinal, immune, integumentary, musculoskeletal, nervous, reproductive, respiratory, and urinary systems.

Development of organ-on-chip from ex vivo tissue culture

The most faithful *in vitro* representation of the *in vivo* physiological response is still given by a fresh biopsy, which preserves the original tissue architecture with all cell types present in their functional arrangement. This *in vitro* model is indispensable for studying environment-related and age-related diseases whose pathogenesis is still unknown.

A microfluidic device for long-term ex vivo culture should provide the necessary stimuli of the microenvironment surrounding the extracted tissue *in vivo* in order to maintain its functionality *in vitro*. For example, perfusion is generally needed to simulate hemodynamics and avoid

cell necrosis in the inner parts of a three-dimensional (3D) biopsy. Most works used animal biopsies for ex vivo microfluidic cultures, but few human studies have also been documented [7–10]. Although the availability of human biopsies is certainly limited, the current restraint for ex vivo organ culture is the ability to preserve the tissue in culture for long term, which prevents the development of long-term assays.

Microfluidic cultures of pancreatic islets of Langerhans mainly focused on islet characterization before transplantation and lasted up to 48 hours [7,11,12]. Sankar *et al.* have focused on understanding why *in vitro* systems are still inadequate for long-term cultures, specifically looking at the role played by endothelial cells; however, also their experiments lasted only 48 hours [13].

Besides cell–cell communication, interactions with the extra-cellular matrix (ECM) were investigated. Van Midwoud *et al.* were able to prolong the microfluidic culture of

liver slices isolated from Wistar rats from 24 to 72 hours, by embedding a liver slice in a Matrigel matrix [14,15].

Taking advantage of the versatility and high potential of microfluidic technology, mechanical properties of microvessels [16] and electrophysiology of brain slices [17,18,19**] and heart tissue [10] were performed.

Overall, current organ-on-chips integrating ex vivo tissues are more similar to short-term functional assay devices than to culture systems. Measurements are performed within few hours after tissue extraction, and functionality is rapidly compromised soon after they are taken. For example, Cheah et al. maintained the rat heart tissue with regular contractions for 5 hours [10].

Development of organ-on-chip from primary cells

After cell lines, primary cells represent the most common cell source used to develop organ-on-chip systems. Like ex vivo tissues, they are derived from biopsies, and several protocols have been developed to keep them alive in culture for weeks after isolation. Although they can undergo phenotypic changes with time during expansion *in vitro*, they still show higher availability. Despite single-cell phenotype resembles the *in vivo* one, the lack of tissue architecture and hierarchy compromises the cellular functional response compared to *in vivo* at tissue and organ level.

Different strategies were pursued to reproduce in culture the critical aspects necessary to mimic cell interplay with the surrounding microenvironment *in vivo*. The lung-on-chip model successfully integrated epithelial and endothelial cells on either side of a membrane, within a device also mimicking the mechanical stress due to respiration [20]. Other systems were developed using membranes as semi-permeable surfaces that, besides giving mechanical support, also modulate molecular transfer across them, for example simulating kidney proximal tube [21**], gastro-intestinal tract [22], and neurovascular unit [23].

Cell density and cell–cell direct contact between like or non-like cells were topographically promoted in two-dimensional (2D) culture systems by microfluidic device geometry [24], micropillar-induced compartmentalization [25], laser cell deposition [26], dielectrophoretic flow [27*], and in 3D systems by cell encapsulation in hydrogel matrix [28,29]. Interactions between cells of different types normally coexisting *in vivo* are very important for organ-on-chip functionality and *in vitro* disease modeling, as demonstrated by multiple examples, such as the reconstruction of hepatic lobules by a topographically organized co-culture of hepatocytes and hepatic stellate cells [29], the angiogenesis reproduced on chip by co-culturing endothelial cells with pericytes [30**], and co-cultures of

neurons with glial cells to *in vitro* reproduce synaptic structures [31] or with astrocytes for modeling amyotrophic lateral sclerosis [32*].

Development of organ-on-chip from pluripotent stem cells

The development of organ-on-chip cultures starting from pluripotent stem cells is still in its infancy. Its progress parallels our understanding of organogenesis during embryo development. Most of the published works using microfluidic devices have focused on culture of embryonic stem cells, either human or murine, for studying the experimental conditions that preserve self-renewal or induce early differentiation. Figallo *et al.* described the first miniaturized system for human embryonic stem cells (hES) that were cultured for 4 days [33]. Few works demonstrated the pluripotent stem cell ability to early differentiate within a microfluidic system towards multiple lineages [33–39]. In particular, Wan *et al.* performed cardiogenesis of mES-derived embryo bodies (EB) in a microfluidic device, and were able to obtain contracting cardiomyocytes expressing α -myosin heavy chain (α -MHC) after 48–72 hours [35].

The control of cell phenotype under perfusion is not an easy task as, depending on medium flow rate, different regimes are obtained, such as insufficient exogenous factor delivery, wash-out of cell-secreted factors, and detrimental shear stress. Furthermore, besides flow rate magnitude, different intermitted flow strategies can be pursued increasing the number of experimental conditions to explore. Playing on perfusion, Przybyla *et al.* studied the minimal conditions sufficient to preserve mES self-renewal, stressing the importance of the ECM remodeling [40**]. Following the same principles, Moledina *et al.* used medium perfusion to modulate LIF pathway in mES microfluidic cultures [41].

These works show that, although the field is growing rapidly, the development of an organ-on-chip from pluripotent stem cells still requires achieving a better understanding of the micro-environmental cues that promote a more differentiated phenotype. In parallel, methods for cell purification within the microfluidic platform are being developed, based on simple differential strength of adhesion [42] or differential attachment to lectin-coated microfluidic channels [43].

Multi-organ *in vitro* models

The development of single organ-on-chip systems highlighted the importance of interactions between cells of different types within the same functional unit of the organ and between cell and ECM. A further level of complexity that is currently under study is the interplay between tissues physically separated *in vivo* but whose circulation-mediated interaction could be important for each other functionality. In perspective,

this patient-on-chip *in vitro* model will represent the link between pre-clinical and clinical studies.

Preliminary microfluidic platforms have already been developed giving proof of concept of the patient-on-chip importance for functional studies [9,14,44], for investigating the effect of anti-cancer drugs [45,46] and cytoprotective agents [47], and for testing drug toxicity [8].

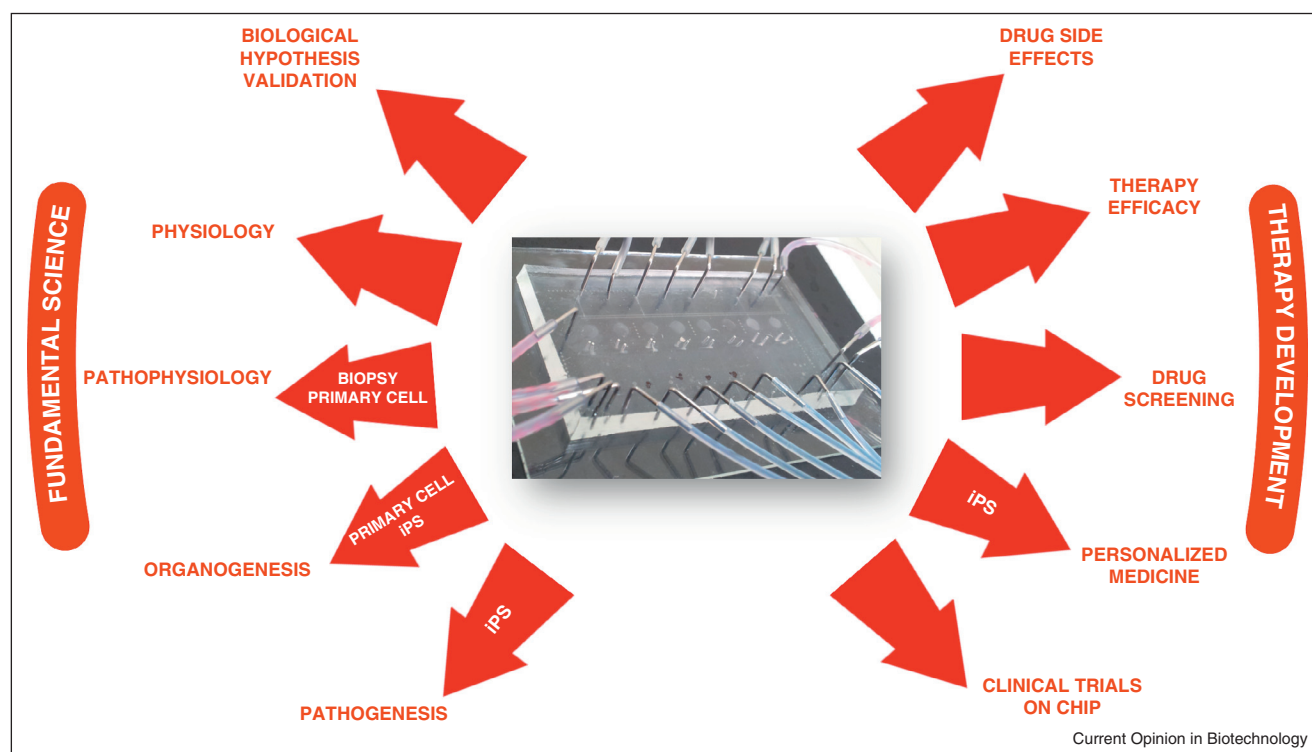
Wagner *et al.* cultured human skin biopsies in a microfluidic device that also integrated human primary hepatocytes [8]. This two-organ system was maintained in culture for up to 28 days, but with high mortality. The same system was also adapted for including skin and hair follicles for a prolonged period of time [9]. Instead, van Midwoud *et al.* integrated intestinal and liver slices, isolated from the same rat, into a microfluidic device where inlet and outlet could be connected in multiple ways in order to study tissue cross-effects [48]. Although measurements were taken after only few hours of incubation, some interplay between the two tissues emerged under bile chenodeoxycholic acid stimulation. Other studies integrated multiple tissues in microfluidic systems using representative cell lines [44–46].

An interesting aspect raised by Zhang *et al.* is the importance of defining a culture medium suitable for cells from different tissues [44]. They developed a common culture medium enriched in the components specific for each cell type. However, medium composition will need further optimization as a high concentration of signaling molecules could potentially hinder the detection of tissue interplay.

Perspectives and conclusions

The peculiarity of each cell source analyzed above implies different uses in biomedical applications (Figure 2). Biopsies and primary cells are the reference biological material for the study of environment-related and age-related diseases because preserve their cellular *in vivo* phenotype. However, 3D biopsies are more difficult to handle and currently fail to sustain a long-term *in vitro* model. Primary cells are instead easier to maintain in culture, but need to be topologically organized for preserving their *in vivo* functionality. Bioprinting technologies are particularly interesting in this respect, promising to shape both cells and ECM in structurally organized organ-like architectures [49].

Figure 2



Applications of organ-on-chip technology. The two main fields of application of human *in vitro* models include fundamental science studies and therapy development. Each purpose has specific requirements as for the cell source used. For example, studies of organogenesis and pathogenesis strongly rely on iPS technology and primary cell derivation of 3D tissue structures; on the contrary, the study of pathophysiology of diseases related to ageing or environmental conditions require biopsy-derived biological material. While all biological material is suitable for most of drug testing enquires, the development of personalized medicine, in terms of drugs combination and dosages, is strongly based on hiPS technology.

The scarce availability of human biopsies limits their possible inclusion in potentially standard microfluidic platforms for biological studies and therapeutic development. An organ such as liver, which is key for toxicological studies, is not routinely accessible for biopsy extraction, especially from healthy donors. Embryonic-like pluripotent stem cells represent instead a potentially inexhaustible source of human cells, able to differentiate into any tissue of the body. The discovery that adult somatic cells could be reprogrammed to the state of induced pluripotent stem cells (iPS) has revolutionized the approach to human biology [50]. iPS can be derived from skin biopsies, but also from less invasive sources, such as blood [51] and urine [52], paving the way for personalized therapy design with ad hoc optimization of drug combinations and dosages. Our ability to program pluripotent stem cells into mature phenotypes is still challenging. However, techniques for controlling cell fate by direct intervention on cell transcriptional regulation, such as miRNA or transcription factor delivery, can be integrated within microfluidic devices [53,54].

To fully appreciate the potential of human *in vitro* models as tools for performing clinical trials on chip, as a bridge between conventional cell cultures and standard clinical trials procedures (without passing through animal models), correlations need to be established between *in vitro* measurements and traditional *in vivo* parameters, such as drug permanence time in a tissue or disposal rate.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Engle SJ, Puppala D: **Integrating human pluripotent stem cells into drug development.** *Cell Stem Cell* 2013, **12**:669-677.
2. Huh D, Hamilton GA, Ingber DE: **From 3D cell culture to organs-on-chips.** *Trends Cell Biol* 2011, **21**:745-754.
3. van der Meer AD, van den Berg A: **Organs-on-chips: breaking the in vitro impasse.** *Integr Biol* 2012, **4**:461-470.
4. Weibel DB, Whitesides GM: **Applications of microfluidics in chemical biology.** *Curr Opin Chem Biol* 2006, **10**:584-591.
5. Luni C, Feldman HC, Pozzobon M, De Coppi P, Meinhart CD, Elvassore N: **Microliter-bioreactor array with buoyancy-driven stirring for human hematopoietic stem cell culture.** *Biomicrofluidics* 2010, **4**:034105.
6. Schwarz US, Bischofs IB: **Physical determinants of cell organization in soft media.** *Med Eng Phys* 2005, **27**:763-772.
7. Mohammed JS, Wang Y, Harvat TA, Oberholzer J, Eddington DT: **Microfluidic device for multimodal characterization of pancreatic islets.** *Lab Chip* 2009, **9**:97-106.
8. Wagner I, Materne E-M, Brincker S, Süßbier U, Frädich C, Busek M, Sonntag F, Sakharov DA, Trushkin EV, Tonevitsky AG et al.: **A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture.** *Lab Chip* 2013 <http://dx.doi.org/10.1039/C3LC50234A>.
9. Ataç B, Wagner I, Horland R, Lauster R, Marx U, Tonevitsky AG, Azar RP, Lindner G: **Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion.** *Lab Chip* 2013 <http://dx.doi.org/10.1039/c3lc50227a>.
10. Cheah L-T, Dou Y-H, Seymour A-ML, Dyer CE, Haswell SJ, Wadhawan JD, Greenman J: **Microfluidic perfusion system for maintaining viable heart tissue with real-time electrochemical monitoring of reactive oxygen species.** *Lab Chip* 2010, **10**:2720-2726.
11. Wang Y, Lo JF, Mendoza-Elias JE, Adewola AF, Harvat TA, Kinzer KP, Lee D, Qi M, Eddington DT, Oberholzer J: **Application of microfluidic technology to pancreatic islet research: first decade of endeavor.** *Bioanalysis* 2010, **2**:1729-1744.
12. Dishinger JF, Reid KR, Kennedy RT: **Quantitative monitoring of insulin secretion from single islets of Langerhans in parallel on a microfluidic chip.** *Anal Chem* 2009, **81**: 3119-3127.
13. Sankar KS, Green BJ, Crocker AR, Verity JE, Altamentova SM, Rocheleau JV: **Culturing pancreatic islets in microfluidic flow enhances morphology of the associated endothelial cells.** *PLoS ONE* 2011, **6**:e24904.
14. Van Midwoud PM, Groothuis GMM, Merema MT, Verpoorte E: **Microfluidic biochip for the perfusion of precision-cut rat liver slices for metabolism and toxicology studies.** *Biotechnol Bioeng* 2010, **105**:184-194.
15. Van Midwoud PM, Merema MT, Verweij N, Groothuis GMM, Verpoorte E: **Hydrogel embedding of precision-cut liver slices in a microfluidic device improves drug metabolic activity.** *Biotechnol Bioeng* 2011, **108**:1404-1412.
16. Günther A, Yasotharan S, Vagaon A, Lochovsky C, Pinto S, Yang J, Lau C, Voigtlaender-Bolz J, Bolz S-S: **A microfluidic platform for probing small artery structure and function.** *Lab Chip* 2010, **10**:2341-2349.
17. Huang Y, Williams JC, Johnson SM: **Brain slice on a chip: opportunities and challenges of applying microfluidic technology to intact tissues.** *Lab Chip* 2012, **12**: 2103-2117.
18. Mauleon G, Fall CP, Eddington DT: **Precise spatial and temporal control of oxygen within in vitro brain slices via microfluidic gas channels.** *PLoS ONE* 2012, **7**:e43309.
19. Scott A, Weir K, Easton C, Huynh W, Moody WJ, Folch A: **A microfluidic microelectrode array for simultaneous electrophysiology, chemical stimulation, and imaging of brain slices.** *Lab Chip* 2013, **13**:527-535.
- The authors developed a microfluidic multi-electrode array for the study of neural circuits, compatible with high-resolution fluorescence microscopy, and combining multi-site electrical recording and multisite focal chemical stimulation.
20. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE: **Reconstituting organ-level lung functions on a chip.** *Science* 2010, **328**:1662-1666.
21. Jang K-J, Mehr AP, Hamilton GA, McPartlin LA, Chung S, Suh K-Y, Ingber DE: **Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment.** *Integr Biol* 2013 <http://dx.doi.org/10.1039/C3IB40049B>.
- The authors developed a membrane-based kidney-on-chip system starting from primary human kidney proximal tubular epithelial cells. Besides verifying its functional response they performed preliminary studies of nephrotoxicity.
22. Ramadan Q, Jafarpourchekab H, Huang C, Silacci P, Carrara S, Koklū G, Ghaye J, Ramsden J, Ruffert C, Vergeres G et al.: **NutriChip: nutrition analysis meets microfluidics.** *Lab Chip* 2012, **13**:196-203.
23. Achyuta AKH, Conway AJ, Crouse RB, Bannister EC, Lee RN, Katnik CP, Behensky AA, Cuevas J, Sundaram SS: **A modular approach to create a neurovascular unit-on-a-chip.** *Lab Chip* 2013, **13**:542-553.

24. Park JW, Kim HJ, Kang MW, Jeon NL: **Advances in microfluidics-based experimental methods for neuroscience research.** *Lab Chip* 2013, **13**:509-521.
25. Dinh N-D, Chiang Y-Y, Hardelauf H, Baumann J, Jackson E, Waide S, Sissnaise J, Frimat J-P, Thriel C van, Janasek D *et al.*: **Microfluidic construction of minimalistic neuronal co-cultures.** *Lab Chip* 2013, **13**:1402-1412.
26. Pirlo RK, Sweeney AJ, Ringeisen BR, Kindy M, Gao BZ: **Biochip/laser cell deposition system to assess polarized axonal growth from single neurons and neuron/glia pairs in microchannels with novel asymmetrical geometries.** *Biomicrofluidics* 2011, **5**:013408.
27. Ho C-T, Lin R-Z, Chen R-J, Chin C-K, Gong S-E, Chang H-Y, Peng H-L, Hsu L, Yew T-R, Chang S-F *et al.*: **Liver-cell patterning lab chip: mimicking the morphology of liver lobule tissue.** *Lab Chip* 2013 <http://dx.doi.org/10.1039/C3LC50402F>.
The authors developed a liver lobule system precisely patterning hepatic and endothelial cells by dielectrophoresis and verified the effect of the structured co-culture on hepatic metabolism.
28. Goral VN, Yuen PK: **Microfluidic platforms for hepatocyte cell culture: new technologies and applications.** *Ann Biomed Eng* 2012, **40**:1244-1254.
29. Lee S-A, No DY, Kang E, Ju J, Kim D-S, Lee S: **Spheroid-based three-dimensional liver-on-a-chip to investigate hepatocyte-hepatic stellate cell interactions and flow effects.** *Lab Chip* 2013 <http://dx.doi.org/10.1039/C3LC50197C>.
30. Meer AD, van der, Orlova VV, Dijke P, ten, Berg A, van den, Mummery CL: **Three-dimensional co-cultures of human endothelial cells and embryonic stem cell-derived pericytes inside a microfluidic device.** *Lab Chip* 2013 <http://dx.doi.org/10.1039/C3LC50435B>.
The authors developed a process of angiogenesis-on-chip combining human umbilical vein endothelial cells and embryonic stem cell-derived pericytes. They verified that the co-culture self-assembles into 3D vascular-like structures, strongly dependent on TGF- β signaling.
31. Shi M, Majumdar D, Gao Y, Brewer BM, Goodwin CR, McLean JA, Li D, Webb DJ: **Glia co-culture with neurons in microfluidic platforms promotes the formation and stabilization of synaptic contacts.** *Lab Chip* 2013, **13**:3008-3021.
32. Kunze A, Lengacher S, Dirren E, Aebischer P, Magistretti PJ, Renaud P: **Astrocyte-neuron co-culture on microchips based on the model of SOD mutation to mimic ALS.** *Integr Biol* 2013 <http://dx.doi.org/10.1039/C3IB40022K>.
The authors validated a microfluidic system for astrocyte-neuron co-culture, with a special focus on cell-cell interactions with and without direct cell contact.
33. Figallo E, Cannizzaro C, Gerecht S, Burdick JA, Langer R, Elvassore N, Vunjak-Novakovic G: **Micro-bioreactor array for controlling cellular microenvironments.** *Lab Chip* 2007, **7**:710-719.
34. Lee JM, Kim J, Kang E, Lee S-H, Chung BG: **An integrated microfluidic culture device to regulate endothelial cell differentiation from embryonic stem cells.** *Electrophoresis* 2011, **32**:3133-3137.
35. Wan C, Chung S, Kamm RD: **Differentiation of embryonic stem cells into cardiomyocytes in a compliant microfluidic system.** *Ann Biomed Eng* 2011, **39**:1840-1847.
36. Kamei K, Guo S, Yu ZTF, Takahashi H, Gschweng E, Suh C, Wang X, Tang J, McLaughlin J, Witte ON *et al.*: **An integrated microfluidic culture device for quantitative analysis of human embryonic stem cells.** *Lab Chip* 2009, **9**:555-563.
37. Fung W-T, Beyzavi A, Abgrall P, Nguyen N-T, Li H-Y: **Microfluidic platform for controlling the differentiation of embryoid bodies.** *Lab Chip* 2009, **9**:2591-2595.
38. Blagovic K, Kim LY, Voldman J: **Microfluidic perfusion for regulating diffusible signaling in stem cells.** *PLoS ONE* 2011, **6**:e22892.
39. Kawada J, Kimura H, Akutsu H, Sakai Y, Fujii T: **Spatiotemporally controlled delivery of soluble factors for stem cell differentiation.** *Lab Chip* 2012, **12**:4508-4515.
40. Przybyla LM, Voldman J: **Attenuation of extrinsic signaling reveals the importance of matrix remodeling on maintenance of embryonic stem cell self-renewal.** *Proc Natl Acad Sci USA* 2012, **109**:835-840.
The authors used a microfluidic perfused system as a means to regulate the concentration of cell-secreted autocrine and paracrine factors for studying mES self-renewal and differentiation.
41. Moledina F, Clarke G, Oskooei A, Onishi K, Günther A, Zandstra PW: **Predictive microfluidic control of regulatory ligand trajectories in individual pluripotent cells.** *Proc Natl Acad Sci USA* 2012 <http://dx.doi.org/10.1073/pnas.1111478109>.
42. 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 Methods* 2013, **10**:438-444.
43. Vickers DAL, Kulik M, Hincapie M, Hancock WS, Dalton S, Murthy SK: **Lectin-functionalized microchannels for characterizing pluripotent cells and early differentiation.** *Biomicrofluidics* 2012, **6**:024122.
44. Zhang C, Zhao Z, Abdul Rahim NA, van Noort D, Yu H: **Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments.** *Lab Chip* 2009, **9**:3185-3192.
45. Tatosian DA, Shuler ML: **A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers.** *Biotechnol Bioeng* 2009, **103**:187-198.
46. Imura Y, Sato K, Yoshimura E: **Micro total bioassay system for ingested substances: assessment of intestinal absorption, hepatic metabolism, and bioactivity.** *Anal Chem* 2010, **82**:9983-9988.
47. Snyder JE, Hamid Q, Wang C, Chang R, Emami K, Wu H, Sun W: **Bioprinting cell-laden matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip.** *Biofabrication* 2011, **3**:034112.
48. Van Midwoud PM, Merema MT, Verpoorte E, Groothuis GMM: **A microfluidic approach for in vitro assessment of interorgan interactions in drug metabolism using intestinal and liver slices.** *Lab Chip* 2010, **10**:2778-2786.
49. Derby B: **Printing and prototyping of tissues and scaffolds.** *Science* 2012, **338**:921-926.
50. 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.
51. Staerk J, Dawlaty MM, Gao Q, Maetzel D, Hanna J, Sommer CA, Mostoslavsky G, Jaenisch R: **Reprogramming of peripheral blood cells to induced pluripotent stem cells.** *Cell Stem Cell* 2010, **7**:20-24.
52. Zhou T, Benda C, Dunzinger S, Huang Y, Ho JC, Yang J, Wang Y, Zhang Y, Zhuang Q, Li Y *et al.*: **Generation of human induced pluripotent stem cells from urine samples.** *Nat Protoc* 2012, **7**:2080-2089.
53. Lüningschrör P, Hauser S, Kaltschmidt B, Kaltschmidt C: **MicroRNAs in pluripotency, reprogramming and cell fate induction.** *Biochim Biophys Acta Bba - Mol Cell Res* 2013, **1833**:1894-1903.
54. Luni C, Michielin F, Barzon L, Calabrò V, Elvassore N: **Stochastic model-assisted development of efficient low-dose viral transduction in microfluidics.** *Biophys J* 2013, **104**:934-942.