Advanced Drug Delivery Reviews 140 (2019) 68–77

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Advanced *in vitro* models of vascular biology: Human induced pluripotent stem cells and organ-on-chip technology

Amy Cochrane [a,1], Hugo J. Albers [b,c,1], Robert Passier [a,c], Christine L. Mummery [a,c], Albert van den Berg [b], Valeria V. Orlova [a,2], Andries D. van der Meer [c,*,2]

[a] Department of Anatomy and Embryology, Leiden University Medical Center, the Netherlands
[b] BIOS Lab-on-a-Chip group, University of Twente, the Netherlands
[c] Applied Stem Cell Technologies group, University of Twente, the Netherlands

ARTICLE INFO

Article history:
Received 23 February 2018
Received in revised form 11 June 2018
Accepted 12 June 2018
Available online 23 June 2018

Keywords:
Vascular
Endothelial
Induced pluripotent stem cells
Organs-on-chips
Microfluidics

ABSTRACT

The vascular system is one of the first to develop during embryogenesis and is essential for all organs and tissues in our body to develop and function. It has many essential roles including controlling the absorption, distribution and excretion of compounds and therefore determines the pharmacokinetics of drugs and therapeutics. Vascular homeostasis is under tight physiological control which is essential for maintaining tissues in a healthy state. Consequently, disruption of vascular homeostasis plays an integral role in many disease processes, making cells of the vessel wall attractive targets for therapeutic intervention. Experimental models of blood vessels can therefore contribute significantly to drug development and aid in predicting the biological effects of new drug entities. The increasing availability of human induced pluripotent stem cells (hiPSC) derived from healthy individuals and patients have accelerated advances in developing experimental *in vitro* models of the vasculature: human endothelial cells (ECs), pericytes and vascular smooth muscle cells (VSMCs), can now be generated with high efficiency from hiPSC and used in 'microfluidic chips' (also known as 'organ-on-chip' technology) as a basis for *in vitro* models of blood vessels. These near physiological scaffolds allow the controlled integration of fluid flow and three-dimensional (3D) co-cultures with perivascular cells to mimic tissue- or organ-level physiology and dysfunction *in vitro*. Here, we review recent multidisciplinary developments in these advanced experimental models of blood vessels that combine hiPSC with microfluidic organ-on-chip technology. We provide examples of their utility in various research areas and discuss steps necessary for further integration in biomedical applications so that they can be contribute effectively to the evaluation and development of new drugs and other therapeutics as well as personalized (patient-specific) treatments.

Contents

* Corresponding author.
E-mail address: andries.vandermeer@utwente.nl (A.D. van der Meer).
[1] Shared first authorship.
[2] Shared last authorship.

https://doi.org/10.1016/j.addr.2018.06.007

1. Introduction

Blood vessels have a primary role in promoting the proper exchange of oxygen and nutrients in tissues across the body. In addition, the vasculature also plays key roles in the regulation of blood pressure *via* maintenance of vascular tone, the response to injury by regulating inflammation and hemostasis, and active transport of compounds, including therapeutics, into and out of organs. Importantly, even though the vascular system consists of a single continuous network, there are great differences between blood vessels depending on whether they are part of the arterial, microcirculatory or venous vascular beds, and the specific organ or tissue with which they interact. Given their wide array of biological functions and presence throughout the body, it is no surprise that blood vessels play an integral role in many diseases. Some diseases are primarily of a vascular nature such as coronary artery disease [1] and brain aneurysms [2], while other diseases, like diabetes and cancer, have mechanisms or complications that are directly or indirectly related to vascular dysfunction [3, 4].

Experimental models of blood vessels are of great importance for understanding vascular physiology and pathophysiology. They are already being used to provide new insights into vascular function, to elucidate details of molecular signaling pathways as well as to serve as a platform for the development of new therapeutics against vascular targets. Multiple *in vivo* and *ex vivo* models of blood vessels have historically contributed significantly to our understanding of vascular biology. *In vivo* experiments on transgenic animal models such as fish, rodents, pigs and primates have been particularly informative in understanding complex vascular physiology in different tissues of interest and to determine systemic effects on the body mediated by the vasculature. Experimental *ex vivo* models are also sometimes used and based on primary human tissue, but given its limited availability (except in the case of medical "waste tissue" such as human umbilical vein), animal tissues or human cell lines have been used more extensively. However, there are many limitations of current *in vivo* and *ex vivo* models, not least ethical implications in tissue procurement and differences between animal and human vascular physiology which make it challenging to translate data from *in vivo* animal models to humans and make accurate predictions of human responses to drugs and disease. Most experimental *in vitro* cell culture models of blood vessels tend to be relatively simple and in 2D, focusing on understanding molecular mechanisms of cellular biology rather than integrated, complex blood vessel function even though they can be based on human vascular cells. However, the key advantages of *in vitro* models are their standardized, well-controlled nature, and the possibility to systematically study human cells under a wide range of experimental conditions [5] although it is challenging in these *in vitro* studies to fully emulate the tension, flow, and cellular interactions with extracellular matrix (ECM) and supporting cells *in vivo*. Observations may thus be inaccurate representations of vascular physiology.

The most commonly used *in vitro* models of blood vessels are often grouped into three classes: monolayer, Transwell, and pseudo-capillary cell cultures. Monolayer cultures of vascular endothelial cells (ECs) or mural cells like pericytes and vascular smooth muscle cells (VSMCs) are typically used to evaluate molecular or cellular responses (morphology, proliferation, damage, migration, barrier function and contraction) to treatments with soluble factors or fluid flow. In Transwell cell cultures, vascular cell layers are grown on semi-permeable membranes which allow basic transport properties, barrier function and cell migration to be measured in the absence or presence of controlled gradients of solutes. Pseudo-capillary culture is achieved by seeding ECs on soft hydrogels and analyzing the structure of microvascular capillary-like networks that they form.

Recently, there has been a strong interest in the development of more advanced *in vitro* models of blood vessels that also allow formation of the blood vessel lumen. One driver of this research is the discovery that hiPSC can differentiate to vascular cells with fairly authentic functionality [6] (Fig. 1). The second main driver is the emergence of microfluidic organ-on-chip technology [7, 8] and its application in setting up smaller, yet more complex and dynamic *in vitro* models of blood vessels. Together, these developments are leading to *in vitro*

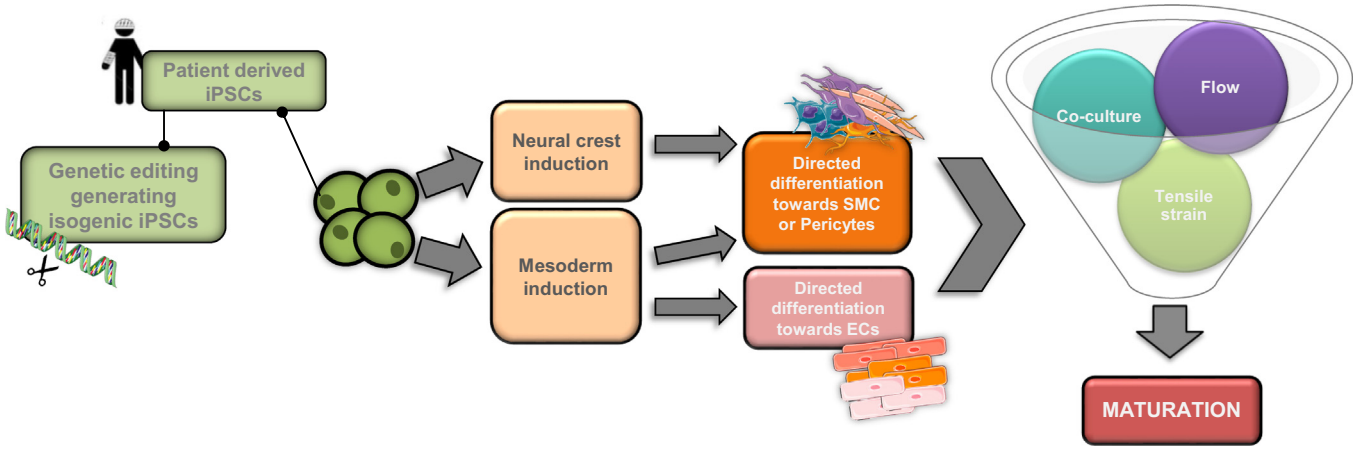

Fig. 1. Schematic summarizing methods to differentiate vascular cells from iPSCs. iPSCs are derived from human somatic cells. These can be generated from patients and furthermore, genetic editing techniques can revert the genetic mutation to create 'healthy' matched isogenic control cell lines. These cells are then directed towards the germ line in which the vascular cell of interest is known to derive from. Here, directed differentiation towards ECs, VSMCs or Pericytes is achieved through addition of defined factors. Vascular cell maturity can then be attained by cell co-culture, fluid flow and/or stretching and regulation of tensile strain.

models of blood vessels that capture not just the basic molecular and cell biological aspects of vascular tissue, but that also exhibit more advanced vascular physiology and morphology. It is expected that such advanced models will have a significant impact on the development of new therapeutic drugs. In the next sections, we review how hiPSC-derived vascular cells and microfluidic organ-on-chip technology are being used to model human vasculature. We then describe the efforts towards integrating hiPSC-derived cells properly into microfluidic chips, and finally discuss the opportunities, challenges and future impact of advanced *in vitro* models of blood vessels in drug development and understanding disease.

2. Human iPSC-derived vascular cells

Healthy vasculature is dependent on its key cellular components interacting and functioning in perfect synchrony. The cellular components are ECs, pericytes, VSMCs and surrounding cells in the tissues and the ECM. ECs form a monolayer which works as a selective barrier for oxygen and nutrient delivery to the tissues. In the healthy state, ECs possess anti-coagulative, anti-adhesive and anti-inflammatory properties which are critical for the proper distribution of viscous blood. Injury of the endothelium precedes many critical events that occur in atherosclerosis and inflammation, such as platelet aggregation, proliferation of VSMCs and infiltration of inflammatory cells. Pericytes and VSMCs play important roles in stabilizing EC tubes and regulating vascular tone. More recent studies indicated that pericytes in particular are very important for the induction of tissue-specific characteristics of ECs, maintenance of barrier function and regulation of inflammatory responses [9, 10]. Therefore, approaches that specifically utilize drugs that normalize or restore vascular function *via* targeting ECs, pericytes and/or VSMCs is potentially a route to therapy for many diseases.

To date, it has been difficult to perform drug screening on primary patient-derived vascular cells due to their limited availability and batch-to-batch variability has compromised making robust conclusions. The exciting discovery of hiPSC in 2007 [6] for which the Nobel Prize was awarded in 2012, now represents a renewable source of vascular (and other) cells without the ethical issues associated with the use of the earlier embryonic stem cells and the limited ability of adult stem cells to form cells of blood vessels.

Multiple protocols for generation of ECs, pericytes and VSMCs from hiPSC have been described and reviewed elsewhere [11, 12], so will not be covered here.

2.1. Human iPSC-derived endothelial cells

Most of the current protocols for deriving of ECs are based on 2D monolayer differentiation. These differentiation protocols all first direct hiPSC towards mesoderm specification (by adding a combination of Activin A, Bone Morphogenetic Protein-4 (BMP4), Basic Fibroblast Growth Factor (bFGF)) and induce EC specification by addition of vascular endothelial growth factor (VEGF). Other components such as transforming growth factor β (TGFβ) or NOTCH inhibitors [13–15] have been added to increase EC differentiation efficiency. Alternatively, ECs can also be generated from hiPSC by simply overexpressing the core transcription factors ETV2 and GATA2 [16].

ECs display significant heterogeneity across tissue types and efforts to derive tissue-specific ECs has been the subject of many studies. Recently, a protocol for co-differentiation of ECs and cardiomyocytes from cardiac mesoderm has been developed that essentially includes induction of cardiac mesoderm followed by WNT inhibition and VEGF supplementation [17]. Importantly, WNT inhibition was also shown to support EC specification in the mouse heart [18, 19]. In recent work by Thompson and colleagues [20], EC differentiation was directed towards the arterial lineage and these cells were shown to have angiogenic benefits in mouse models of myocardial infarction. The authors found that in addition to standard EC-inducing factors, such as bFGF, VEGF and the TGFβ inhibitor SB431542, supplementation with the NOTCH agonist (RESV) and inositol monophosphatase inhibitor (L690) increased expression of arterial markers, such as CXCR4, EFNB2, GJA4, NRP1 and NOTCH ligands and receptors (DLL4/JAG1 and NOTCH1/4) in hiPSC-ECs and induced down-regulation of venous markers (eg NR2F2). Functionally these arterial-like ECs exhibited increased nitric oxide production, reduced leukocyte binding and improved responses to shear stress [20]. Recently, functionality of hiPSC-derived ECs was compared with primary ECs [21]. This study showed that barrier function and inflammatory responses were consistent in hiPSC-derived ECs across independent batches of cells but that they also showed some differences, such as higher barrier function and lower inflammatory responses when compared to the more widely used primary cell type, human umbilical vein endothelial cells (HUVEC). Two further studies demonstrated that exposure of hiPSC-ECs to high, arterial-like shear stress (1–2 Pa) further promoted acquisition of arterial-like characteristics and that overexpression of the RNA-binding protein QKI-5 directed EC differentiation from hiPSCs towards an arterial fate [22–24]. At present, venous and lymphatic specification of hiPSC-ECs remains a challenge with only a limited number of studies showing acquisition of some venous or lymphatic EC-associated markers; however, markers alone cannot truly confirm their identity [25]. Therefore, multiparameter approaches that integrate vascular bed-specific hemodynamic and mechanical parameters in addition to biochemical cues are required for the development of mature and functional phenotypes in hiPSC-ECs (Fig. 1) [26–28].

2.2. Human iPSC-derived vascular smooth muscle cells and pericytes

Significant progress towards differentiation of VSMCs and pericytes from hiPSC has been made recently. The primary difficulty has been that both of these cell types have different developmental origins, that are not limited to mesoderm; they can also originate from the neural crest. They are subsequently recruited to the developing EC tubes from the surrounding tissues, and their identity and functionality are largely dictated by the location in the vascular bed *in vivo*. This makes it difficult to distinguish pericytes/VSMCs from other cell types that are present in differentiating cultures due to the lack of cell type-specific surface markers. With regard to VSMC differentiation, protocols have been developed to induce these cell types from different mesoderm lineages, such as lateral plate and cardiac (*via* epicardial state) mesoderm, paraxial mesoderm, ectoderm and neural crest (Fig. 1) [14, 29–32]. Most protocols utilize 2D monolayer differentiation methods towards the germ-lineage of interest following supplementation with Platelet-Derived Growth Factor-BB (PDGF-BB) and TGFβ. Essentially, VSMCs differentiated using a combination of PDGF-BB and TGFβ remain proliferative and are indistinguishable from each other based on surface marker expression. Functionally, mesoderm-derived VSMCs can be distinguished from ectoderm/neural crest cells based on their phenotypic appearances; ectoderm-derived cells having more defined epithelial cell-like morphology and lack growth inhibitory responses to TGFβ. Maturation of these cells is evidenced by the acquisition of a more contractile phenotype and can be achieved by removal of PDGF-BB and either supplementation with TGFβ in the presence of low or high serum concentrations, although this varies across different labs with no clear consensus at present on how best to do this. Maturation of hiPSC-derived VSMCs can be further enhanced upon administration of tensile strain (cyclic uniaxial or circumferential) [33, 34] which increases the deposition of elastin protein, cell alignment and improves calcium and contractile responses. Comparison of co-cultures of primary ECs and primary VSMCs with hiPSC-ECs and hiPSC-VSMCs recently showed that in both cases, similar responses to atheroprotective or atheroprone hemodynamic cues were observed [35]. Interestingly, upon culture in atheroprotective hemodynamic conditions, genes associated with vascular maturation (specifically; EC markers KLF4, NOS3 and ASS1 and VSMC markers MYH11 and DES) were up-regulated in hiPSC-derived

ECs and VSMCs. Overall, responses to stimuli and relative marker expression was quite different in hiPSC-derived cells compared to primary cells. However, the responses of hiPSC-derived cells were greater when cultured in advanced inflammatory conditions consisting of atheroprone hemodynamic cues with additional Tumor Necrosis Factor-α (TNFα) and oxidized low-density lipoprotein (LDL).

Protocols to differentiate pericytes in 2D cultures have been developed [13, 31, 36, 37]. However, it has proven very difficult to establish whether these cells do in fact recapitulate true pericyte-like properties, or are simply synthetic VSMCs. Recent work by two independent groups demonstrated that pericytes can be distinguished from non-pericytes by a combination of surface markers, such as high expression of NG2, CD146, CD73 and CD44 [31, 36], or alternatively sorting of CD146-^{high}CD73^{high} cells. RNA-sequencing analysis of hiPSC-derived pericytes further confirmed close similarity with primary placenta, retina and brain pericytes [31] and adipose tissue-derived pericytes [36].

2.3. Patient hiPSC-derived vascular cells

Recently, patient-specific hiPSC-derived ECs and VSMCs were shown to be invaluable for human disease modeling applications. hiPSCs have been derived from patients suffering from fibrodysplasia ossificans progressiva (FOP) [38, 39], aortic valve calcification, pulmonary arterial hypertension [40] and Marfan syndrome [41]. In addition to known mutations, disease-causing genetic variants were recently examined using this technology [42], which allowed identification of novel biological mechanisms. Combined with genome editing techniques, such as CRISPR/Cas9, mutations of interest can be corrected providing unique opportunities to study human diseases using genetically matched control lines (isogenic cell lines; Fig. 1). Accurate comparison using isogenic lines can further expand our knowledge on genotype/phenotype association for more precise and personalized drug discovery. Moreover, better understanding of the disease-causing mechanisms allows for the discovery of new approaches for treatment or prevention.

3. Organ-on-chip technology for vascular biology

Over the past decade, many micro-engineered cell culture devices, or 'microfluidic chips', have been developed that allow *in vitro* studies of human physiology of various organ and tissue types. Typically, the microfluidic chips consist of sub-millimeter rectangular culture chambers which are connected to each other *via* semi-permeable barriers or micro-channels. The culture chambers either contain culture medium or 3D hydrogel, and cells are cultured on the walls of the chamber, on hydrogel surfaces, or inside the 3D hydrogel. The culture chambers in the microfluidic chips can be actively perfused at controlled flow rates and they can sometimes be actively deformed and stretched. Finally, the chips can contain integrated sensors or can be connected to external measurements set-ups, including microscopes, in order to monitor the cells cultured inside them.

The development of microfluidic chips that contain multiple cell types have enabled *in vitro* studies of processes that are typically associated with organ-level physiology. The ultimate goal of recreating more of the key aspects of organ-level physiology 'on-chip' has led to the coinage of the term 'organs-on-chips' (or sometimes 'microphysiological systems') to refer to this new class of *in vitro* models. Organ-on-chip technology has also been used to capture key physiological aspects of vascular biology in systems that allow 3D network culture, that include shear stress [27, 43] from fluid flow and that allow measurements of functional vascular output in an environment that is more biologically accurate than in conventional 2D static systems. In the following sections, an overview is given of advanced *in vitro* models in which organ-on-chip technology has been applied to mimic key aspects of vascular physiology.

3.1. Vascular barrier function in chips

ECs form barriers that control passage of cells and molecules from the blood to the surrounding tissue and *vice versa*. These barriers are tissue-type dependent; for example, nutrients are reduced to essential building blocks in the gastrointestinal tract and absorbed into the local vasculature, hormones and drugs are selectively passed at the blood-brain barrier (BBB), and pulmonary microvascular cells allow for the transport of oxygen into the blood and carbon dioxide into the alveoli.

Several organ-on-chip models in which ECs are co-cultured with other cells have been developed [44]. These models have been used to test EC barrier function by directly measuring diffusion or migration of tracers [45–48] and cells, [46, 47, 49–53] by measuring the transendothelial electrical resistance (TEER) [46, 54–56] or by performing junction-specific staining [46, 55–57]. For example, when co-cultures of ECs and pulmonary epithelial cells inside lung-on-a-chip devices (Fig. 2A) were stimulated with the inflammatory cytokine TNF-α, neutrophil transmigration could be induced [46]. When human brain-derived ECs in a BBB-on-a-chip (Fig. 2B) were stimulated with shear stress, increased barrier function was evident in TEER measurements with integrated electrodes [55, 56]. Furthermore, barrier function of brain-derived ECs was also affected by co-culture in a 3D BBB-on-a-chip with pericytes or astrocytes (Fig. 2C) as measured by microscopic tracking of a fluorescent dextran tracer molecule [45]. Finally, when ECs in microfluidic devices were exposed to a vasotoxic therapeutic or to hypoxic conditions, increases in permeability were detected by quantification of gaps in cell-cell junctions [57].

3.2. Regulation of blood flow, hemostasis and thrombosis

Microfluidic assays have been crucial in recapitulating and unraveling the complex and dynamic cellular and extracellular interactions [58–63] seen in thrombosis. For example, using the platelet adhesion molecule; von Willebrand Factor (vWF)-coated spherical microbeads in a high shear rate microfluidic environment, the role of shear rate in discoid platelet aggregation was elucidated [64]. Perfusion of platelet-free plasma through microfluidic channels with severe stenotic geometries showed the unfolding and deposition of vWF onto collagen due to an increase in shear rate [65]. Addition of ECs to these microfluidic channels with stenotic geometries demonstrated that platelet aggregation occurs after the apex of the stenosis as a result of endothelial vWF release (Fig. 2D) [50, 66]. In addition, the role of vessel wall inflammation on platelet aggregation has been studied by direct stimulation of ECs in microfluidic channels with TNF-α [67], and even tissue-level interactions were demonstrated in an alveolus-on-a-chip system, where an endotoxin only instigated thrombosis when applied to a co-culture of epithelium and endothelium [68].

A limitation of the simplest microfluidic models of thrombosis is that they have been established in channels with rectangular cross-sections that have non-physiological edge-effects [58]. Therefore, multiple groups have tried to better recapitulate the pathology by designing and fabricating round channel geometries [69–73]. For example, circular lumens in ECM lined with ECs and perfused with whole blood could be created by soft lithography [69] and 3D bioprinting [71], while polymethyl methacrylate (PMMA) optical fiber-molds for polydimethylsiloxane soft lithography have been used to create microchannels with circular geometries resembling aneurysms, stenoses or bifurcations [70]. Microchannels with truly defined 3D geometries based on computed tomography angiography (CTA) data have also been fabricated by 3D printing and used in thrombosis studies (Fig. 2F) [72].

Hemostasis is the arrest of blood flow, and a number of studies have applied microfluidic assays to study its mechanism. For example, an extracorporeal device, with collagen-coated microfluidic channels that mimic the shear rates in occluded arterioles, was connected to an

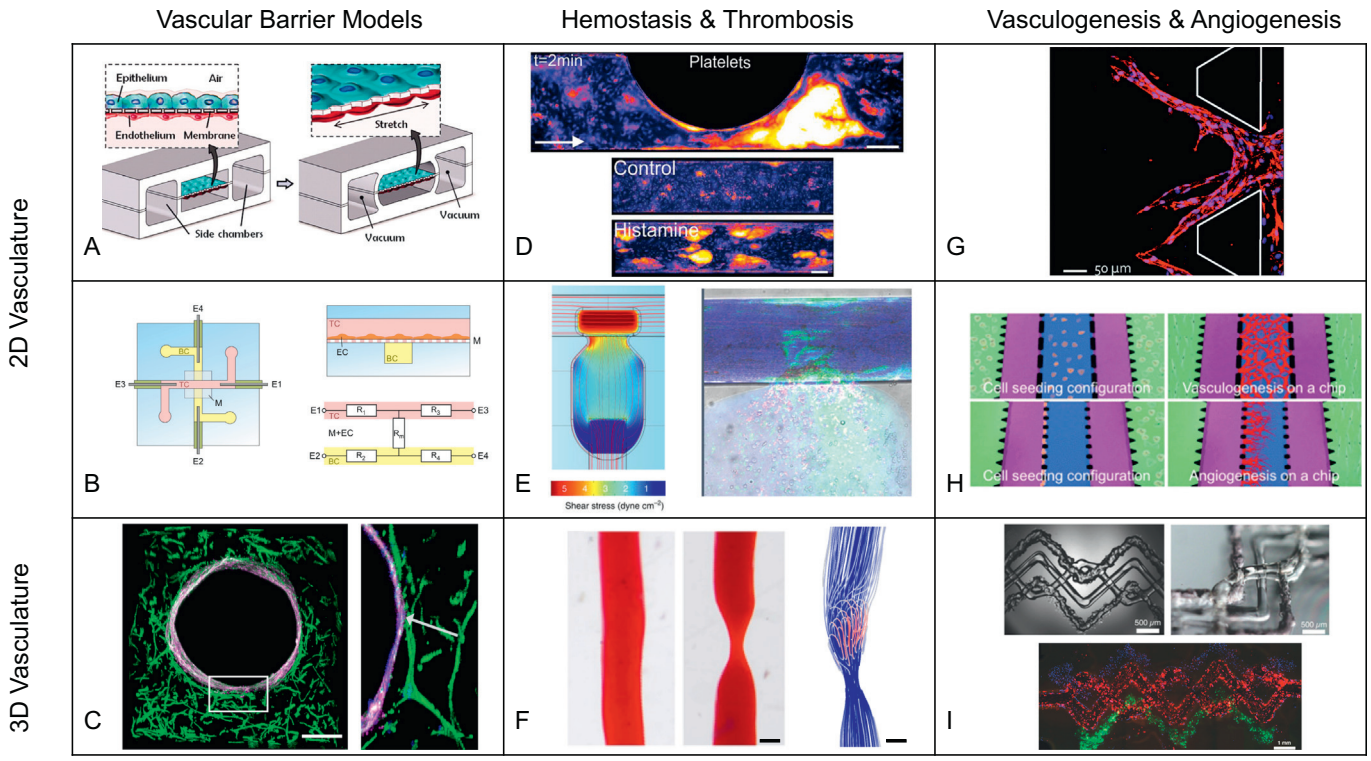

Fig. 2. Overview of vascular models for barrier functionality, hemostasis, thrombosis, vasculogenesis and angiogenesis in both 2D and 3D geometries. (A) Lung-on-a-chip device with two channels lined with epithelial and endothelial cells which are separated by a stretching porous membrane [46]. (B) A BBB device with two perpendicular channels separated by a porous membrane and platinum electrodes for TEER measurements [56]. (C) Cross-section of 3D blood-brain barrier chip lined with ECs (purple) and astrocytes in the surrounding gel (green). Scale bar, 200 μm [45]. (D) Platelet aggregation in endothelialized devices, platelets have been stained using $DiOC_6$. Scale bar, 100 μm [66]. (E) Computational fluid dynamics (CFD) of shear stress after actuating an integrated valve, fluorescence microscopy image of ECs (blue), platelets (red) and fibrinogen (green) [77]. (F) Healthy and stenotic microfluidic channels based on CTA data filled with red food dye. CFD data of streamlines showing recirculation. Scale bar, 200 μm [72]. (G) Angiogenesis assay in collagen scaffold, nuclei (blue) and actin filaments (red) were stained. Scale bar, 50 μm [85]. (H) Vasculogenesis and angiogenesis assay for co-cultures of ECs and fibroblasts [47]. (I) Bright field images of 3D printed constructs. Fluorescence microscopy images of channels lined with various cell types: fibroblasts (blue, green), ECs (red). Scale bars: 500 μm (top), 1 mm (bottom) [92]. Reprinted and adapted with permission from: Huh et al. [46], Van der Helm et al. [56], Herland et al. [45], Westein et al. [66], Sakurai et al. [77], Costa et al. [72], Kim et al. [85], Kim et al. [47] and Kolesky et al. [92].

anaesthetized pig. Here, clotting times in the presence and absence of anti-platelet therapeutics were determined [74]. Hemostasis in response to vascular injury could be mimicked in microfluidic devices by using a perpendicular channel that is either coated with tissue factor and collagen or filled with a collagen scaffold [75, 76]. A recent study demonstrated a microfluidic model of vascular injury by actuating a pneumatic valve in a chip with ECs and flowing blood (Fig. 2E) [77].

3.3. Vasculogenesis, angiogenesis and vessel morphology

Vasculogenesis and angiogenesis refer to the formation of blood vessel networks *de novo* and by vessel sprouting from established vasculature, respectively. Both processes involve controlled breakdown of ECM and are directed by a large array of growth factors. Microfluidics allows control over patterned ECM geometries and growth factor gradients [78, 79].

A typical approach to study vessel formation in microfluidic devices is to pattern 3D matrices such as collagen I, fibrin, Matrigel and alginate in specific areas of the chip [53, 80–86]. Most of these assays use rows of pillars to pin the matrix in a region between two channels and allow ECs and perivascular cells to form a microvascular network (Fig. 2G) either by mixing the cells with the matrix or by promoting angiogenic sprouting from one of the two channels [47]. In such devices, the role of growth factors and interstitial flow in microvascular network formation can be studied systematically [48]. Moreover, when establishing co-cultures of ECs with other cells (Fig. 2H), tissue-level interactions like tumor-induced angiogenesis and tumor cell extravasation can be studied [51–53, 87].

Due to the limitation of artificial 2D structures like pillars and flat microchannels in these devices, there has been a focus on chips with fully engineered 3D patterns. For example, complex growth factor gradients in meandering geometries [88], 3D lumens fabricated by injection molding, viscous fingering, microneedles, 3D-printed sacrificial carbohydrate-glass and 3D-printed ECM (Fig. 2I) [69, 89–92]. The 3D lumens can be lined with ECs and surrounded by cell-laden ECM. Studying ECs in this 3D system allows the underlying physiology to be further elucidated, for example by highlighting a new role for established vascular mechanisms such as NOTCH signaling in ECs [93, 94].

4. Integration of iPSC-derived vascular cells in organ-on-chip systems

The first *in vitro* models based on hPSC-derived vascular tissue cultured in microfluidic chips are now being reported, along with studies on primary vascular cells. Notably, these systems demonstrate that hiPSC-derived vascular cells can form lumenized vessel structures which can be perfused by fluid. In one study, human iPSC-derived ECs cultured in a custom hydrogel inside a microfluidic device generated a 3D capillary network that was stable for at least 14 days [95]. In another study, primary human ECs were mixed with human embryonic stem cell-derived pericytes inside microfluidic channels, resulting in self-organized 3D structures that displayed close interaction between pericytes and the endothelium. Moreover, the morphology of the structures could be altered by interfering with TGFβ signaling and this condition can be used to mimic disease conditions, such as for example seen in the genetic disorder hereditary hemorrhagic telangiectasia (HHT) in which patients have vascular malformations [96]. Hutchinson-Gilford progeria syndrome (HGPS) is associated with premature aging that affects all cells in the body, including the vascular system due to increases in mechanical stress upon continued pulsatile blood flow. In a study by

Truskey and colleagues [97], 3D vascular constructs were generated with the use of HGPS patient derived-VMSCs. These constructs provide the potential to study the disease pathophysiology and overcome the current limitations faced from the use of murine and 2D culture. Further studies using a microfluidic organ-on-a-chip based approach was also used to re-create an *in vitro* model of HGPS using hiPSC-derived VSMCs [98]. Interestingly, hiPSC-derived VSMCs from HGPS patients cultured in microfluidic channels with continuous pulsatile stretch and relaxation stimuli showed increased DNA damage responses, senescence markers and inflammatory cytokines. Furthermore, this aging phenotype was reversed upon administration of statin (lovastatin); a drug that has been currently exploited in combination with other statins as therapeutics for life extension of HGPS patients.

Testing drugs for potential embryonic toxicity is another promising application of hiPSC-derived vascular cells in microfluidic systems [24]. In this regard, the embryonic-like phenotype of hiPSC-ECs is advantageous due to increased sensitivity to toxic compounds when compared to primary (adult) ECs. hiPSC-ECs cultured in customized tubes made of polydimethylsiloxane (PDMS) showed increased inflammatory responses upon exposure to the known cytotoxic agent (7-cyclo), that has been previously found to exhibit anti-angiogenic properties in Xenopus and zebrafish models [99, 100]. Mechanistically, hiPSC-ECs were found to exhibit increased sensitivity due to higher VEGF Receptor 2 (VEGFR2) expression levels which is one of the targets of 7-cyclo in embryonic ECs.

5. Future perspectives

5.1. Characterization and quality control of hiPSC-derived vascular cells

Although hiPSC-derived vascular cells are now increasingly used as *in vitro* models of human blood vessels, there are still many challenges to their wide implementation in drug discovery in disease. For instance, despite the emergence of the variety of robust differentiation protocols there has been little cross-comparison of the derivative cells and protocols across independent labs. Consensus on standardized quality control assays and phenotypic profile would be of value. Interaction with other cells also needs further investigation; for example, hematopoietic cells can increase angiogenesis and they may even express some EC markers so be mistaken for true ECs unless tested for their ability to form hollow (lumenized) vascular structures when embedded in hydrogels in a microfluidic system. More stringent functional assessment of hiPSC-EC identity has been recently proposed by Yoder and colleagues and outlined in their recent review [11]. There is some consensus on differentiating hiPSC-ECs but there is a greater difficulty in the differentiation and definition of VSMC and pericytes because of the lack of specific cell markers or functional assays to confirm phenotypes: expression of surface or intracellular markers is not always correlated with cell identity. Pericytes and VSMCs are recruited to newly formed EC tubes and the vascular cells mature further, which largely defines their phenotype and functionality. For these reasons, it would be interesting to consider other ways of assessing vascular cell functionality, for example by introducing standardized assays for measuring cell contractility and intracellular calcium responses (for VSMCs), as well as *in vitro* and *in vivo* vascular tube stabilization and contraction assays. Open resources on cross-comparison of published transcriptome datasets would also certainly benefit the field, and some initiatives have been established, such as the bioinformatics resource on stem cells (www.stemformatics.com).

The question also remains whether hiPSC-derived vascular cells can actually acquire mature characteristics in engineered microfluidic devices similar to those of primary cells. This is a widely recognized problem for all hiPSC derivative [101]. Unanimity on defined and standardized markers and functional assays to confirm EC and pericyte/VSMCs lineage specificity is necessary to provide cell lines that are validated as being acceptable mimics for patient-specific therapeutic research.

5.2. Standardization and robustness of organ-on-chip models

Most of the microfluidic *in vitro* models described in this review are currently only available to researchers and collaborators of groups that developed them. Fortunately, commercial activity in the field of organs-on-chips is changing this, with various basic systems now being commercially available [102]. Commercialization of organ-on-chip technology manufacture is beginning to take shape but those developing this technology should take into account that future adoption by end-users will depend strongly on proper standardization. For example, designing systems with inlets, outlets and chip lay-outs that are compatible with multi-pipettes, pipetting robots and automated imaging systems, and avoiding manual fabrication of devices using PDMS-based soft lithography. PDMS is well known for its selective adsorption of drugs and medicinal entities [103]. Focus should be on large-scale fabrication and manufacturing processes based on materials like cyclic olefin copolymer (COC), polystyrene and glass [104, 105]. Moreover, technology developers need to demonstrate robustness and unique added value to end-users such as. showing dose-response data for well-known experimental stimuli and by comparing this data to measurements obtained with other *in vitro* techniques and human *in vivo* data. Such comparisons can only be achieved by developing objective, quantitative endpoints, as recently carried out with measurements of TEER that allow comparison between microfluidic chips with other *in vitro* assays [106], and with measurements of pro-inflammatory cytokines in microfluidic BBB chips [45] that allow future comparison between biomarker candidates in chips and clinical samples.

5.3. Advanced in vitro patient-specific disease modeling

Disease modeling using primary cells derived from patients provides essential insight into the underlying pathology. Importantly, this provides a great advantage over the use of animal models which often don't fully encapsulate human physiology and response to stimuli. Unfortunately, acquisition of patient tissue is often difficult and sometimes impossible. Moreover, the primary cells derived from the patient tissue are difficult to maintain long-term in culture. Therefore, a major advantage in using hiPSC-derived vascular cells is that they can serve as a robust source of patient-specific cells. However, genetic and ethnic background may impact responses of hiPSC-derived vascular cells to drugs and disease. In fact, even apparently "healthy controls" may have certain genetic predisposition towards some diseases that manifests only under some lifestyle conditions or later in life. Furthermore, donor age and reprogramming followed by *in vitro* expansion can introduce genomic changes [107]. Therefore, careful choice of "healthy" control lines requires extensive analysis of genomic integrity, single nuclear polymorphism (SNP) arrays or whole genome sequencing. Genetically corrected isogenic controls are becoming a standard in modeling of monogenic diseases. Polygenic diseases are still difficult to model with hiPSCs, as this requires larger cohorts of hiPSCs from affected and unaffected individuals. However, despite being challenging, progress had been made in unexpected fields, such as modeling of psychiatric disorders with hiPSC-derived neuronal cells [108–110]. Similar studies on genetically complex cardiovascular diseases are expected using comparable approaches and selective patient inclusion. Besides genetic background, environmental factors, age and lifestyle impact disease phenotypes and drug responses. Reprogramming itself results in reversing the "biological clocks" of the cells [111–113] but may be associated with retention of "epigenetic memory" of the tissue of origin [114, 115]. For late-onset diseases approaches to re-induce an aged phenotype in the cells of interest are being investigated; these include overexpression of progerin (a mutation that causes premature aging in humans) or pharmacological inhibition of telomerase [112, 113].

Furthermore, vascular bed specificity of cardiovascular diseases, such as atherosclerosis, deep vein thrombosis, vascular dementia, or

organ-specific failure-associated vascular dysfunction requires careful assessment of developmental origin of the cells of interest and recapitulation of tissue-specific characteristics of the differentiated cells. For instance, ECs in the vasculature are exposed to a variable fluid shear stress depending on the arterial, venous or capillary location. Tissue microenvironment is another essential driver in EC maturation during embryogenesis. Therefore, it is essential to develop culture systems that mimic as closely as possible the *in vivo* microenvironment.

5.4. Advanced in vitro models in drug development

Patient-specific vascular *in vitro* models may have a significant impact on drug development, with potential applications in various phases of the drug development process: (1) disease modeling and target discovery, (2) high-throughput compound screening, (3) lead optimization and preclinical studies of pharmacokinetic properties and toxicity and (4) patient-specific studies for stratification of the population in terms of efficacy or toxicity [116]. Application of the assays in these phases of drug development enhance the chances of new drugs being effective in trial in humans. At the same time, it places different demands on future development of the biology and technology, with some phases mainly requiring assay automation and scaling, with others mainly requiring clear disease phenotypes. In order to navigate these application-driven requirements, engineers and cell biologists will ideally actively interact with the key stakeholders in drug development, from biomedical scientists and hi-tech companies to pharmaceutical industry and regulatory agencies [117, 118]. The active involvement of stakeholders and end-users, even in early technology development, will be instrumental in maximizing the impact of advanced *in vitro* models of vascular biology in drug development in the coming years.

5.5. Outlook

The development of advanced *in vitro* models for vascular biology based on organ-on-chip technology and hiPSC-derived cells may be recent, but the first results are very promising.

Many vascular diseases develop as a result of multiple cell type dysfunction, genetic or environmental factors. Organs-on-chips will have to become increasingly complex to capture these intricate and multifactorial aspects of vascular pathophysiology. Challenging as this may be, there is a strong sentiment in the field that by step-wise, controlled engineering of disease-related stimuli, organs-on-chips will exhibit disease phenotypes that are ever more realistic [119–121]. Eventually, the systematic inclusion of disease-related factors and tissues will result in an *in vitro* model that consist of multiple connected organs-on-chips that will be able to capture multi-organ or systemic aspects of vascular pathophysiology [122, 123]. The advancements in the generation of patient specific iPSC types, combined with patient data in biobanks and genetic studies, as well as microfluidic technology can provide a platform for the development of *in vitro* systems to truly mimic complex disease phenotypes.

The high level of variables in animal models, along with the genetic and physiological inter-species differences, brings into question the use of *in vivo* studies as a translational tool in drug development for human disease and therapy. With advanced *in vitro* technology being developed rapidly, animal models may be reduced or eventually replaced with autologous human 3D *in vitro* systems in early drug development.

With standardized quality control in both the development of differentiation and maturation protocols for hiPSC-derived vascular cells and in the development of microfluidic technology, *in vitro* disease modeling for the understanding of disease origin, processes, biomarkers and drug responses is becoming achievable to in order to provide patient-relevant data (Fig. 3).

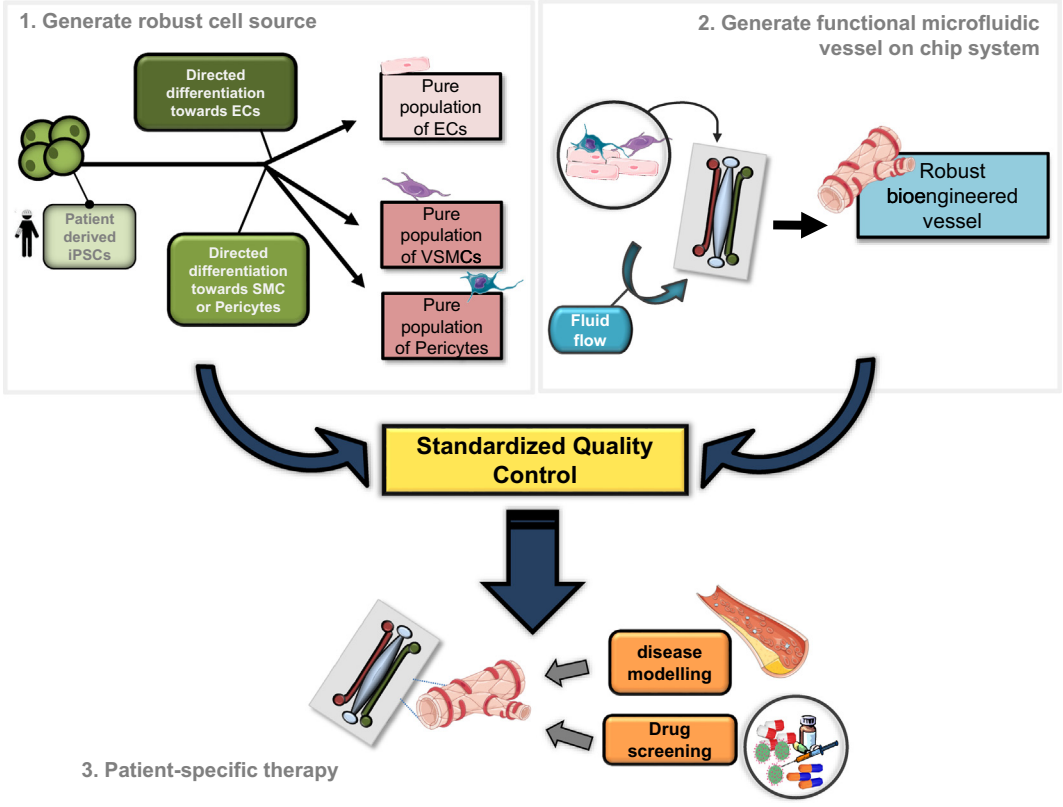

Fig. 3. Schematic summarizing iPSC based Vessel-on-Chip technologies. (1) It is essential to start with robust cell source with defined and standardized differentiation protocols and quality control assays to confirm cell phenotypes (2) advanced microfluidic model possessing the required functional tests where cells can be cultured to generate 3D vasculature (3) robust platform for functional tests such as drug screening, disease modeling.

Acknowledgements

The authors acknowledge the funding received from the Dutch Science Foundation (NWO) under the Gravitation Grant 'NOCI' Program (Grant no. 024.003.001), the European Research Council (ERC) under the Advanced Grant 'STEMCARDIOVASC' Program (Grant no. 323182) of prof. Mummery and 'VESCEL' Program (Grant no. 669768) of prof. Van den Berg, the University of Twente Strategic Research Orientation 'Organs-on-Chips' of Andries van der Meer.

References

[1] P. Libby, P. Theroux, Pathophysiology of coronary artery disease, Circulation 111 (2005) 3481–3488, https://doi.org/10.1161/CIRCULATIONAHA.105.537878.
[2] N. Chalouhi, M.S. Ali, P.M. Jabbour, S.I. Tjoumakaris, L.F. Gonzalez, R.H. Rosenwasser, W.J. Koch, A.S. Dumont, Biology of intracranial aneurysms: role of inflammation, J. Cereb. Blood Flow Metab. 32 (2012) 1659–1676, https://doi.org/10.1038/jcbfm.2012.84.
[3] F. Paneni, J.A. Beckman, M.A. Creager, F. Cosentino, Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part i, Eur. Heart J. 34 (2013) 2436–2446, https://doi.org/10.1093/eurheartj/eht149.
[4] D. Fukumura, R.K. Jain, Tumor microvasculature and microenvironment: targets for anti-angiogenesis and normalization, Microvasc. Res. 74 (2007) 72–84, https://doi.org/10.1016/j.mvr.2007.05.003.
[5] K.H. Benam, S. Dauth, B. Hassell, A. Herland, A. Jain, K.-J. Jang, K. Karalis, H.J. Kim, L. Macqueen, R. Mahmoodian, S. Musah, Y. Torisawa, A.D. van der Meer, R. Villenave, M. Yadid, K.K. Parker, D.E. Ingber, Engineered in vitro disease models, Annu. Rev. Pathol. Mech. Dis. 10 (2015) 195–262, https://doi.org/10.1146/annurev-pathol-012414-040418.
[6] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 107 (2007) 861–872, https://doi.org/10.1016/j.cell.2007.11.019.
[7] M.W. van der Helm, A.D. van der Meer, J.C.T. Eijkel, A. van den Berg, L.I. Segerink, Microfluidic organ-on-chip technology for blood-brain barrier research, Tissue Barriers 4 (2016)https://doi.org/10.1080/21688370.2016.1142493.
[8] D. Huh, Y. Torisawa, G.A. Hamilton, H.J. Kim, D.E. Ingber, Microengineered physiological biomimicry: organs-on-chips, Lab Chip 12 (2012) 2156, https://doi.org/10.1039/c2lc40089h.
[9] J. Rustenhoven, D. Jansson, L.C. Smyth, M. Dragunow, Brain pericytes as mediators of neuroinflammation, Trends Pharmacol. Sci. 38 (2017) 291–304, https://doi.org/10.1016/j.tips.2016.12.001.
[10] S. Ogura, K. Kurata, Y. Hattori, H. Takase, T. Ishiguro-Oonuma, Y. Hwang, S. Ahn, I. Park, W. Ikeda, S. Kusuhara, Y. Fukushima, H. Nara, H. Sakai, T. Fujiwara, J. Matsushita, M. Ema, M. Hirashima, T. Minami, M. Shibuya, N. Takakura, P. Kim, T. Miyata, Y. Ogura, A. Uemura, Sustained inflammation after pericyte depletion induces irreversible blood-retina barrier breakdown, JCI Insight. 2 (2017)https://doi.org/10.1172/jci.insight.90905.
[11] Y. Lin, C.-H. Gil, M.C. Yoder, Differentiation, evaluation, and application of human induced pluripotent stem cell–derived endothelial cells, Arterioscler. Thromb. Vasc. Biol. 37 (2017) 2014–2025, https://doi.org/10.1161/ATVBAHA.117.309962.
[12] S. Sinha, D. Iyer, A. Granata, Embryonic origins of human vascular smooth muscle cells: implications for in vitro modeling and clinical application, Cell. Mol. Life Sci. 71 (2014) 2271–2288, https://doi.org/10.1007/s00018-013-1554-3.
[13] V.V. Orlova, Y. Drabsch, C. Freund, S. Petrus-Reurer, F.E. Van Den Hil, S. Muenthaisong, P. Ten Dijke, C.L. Mummery, Functionality of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 177–186, https://doi.org/10.1161/ATVBAHA.113.302598.
[14] C. Patsch, L. Challet-Meylan, E.C. Thoma, E. Urich, T. Heckel, J.F. O'Sullivan, S.J. Grainger, F.G. Kapp, L. Sun, K. Christensen, Y. Xia, M.H.C. Florido, W. He, W. Pan, M. Prummer, C.R. Warren, R. Jakob-Roetne, U. Certa, R. Jagasia, P.O. Freskgard, I. Adatto, D. Kling, P. Huang, L.I. Zon, E.L. Chaikof, R.E. Gerszten, M. Graf, R. Iacone, C.A. Cowan, Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells, Nat. Cell Biol. 17 (2015) 994–1003, https://doi.org/10.1038/ncb3205.
[15] M. Sahara, E.M. Hansson, O. Wernet, K.O. Lui, D. Später, K.R. Chien, Manipulation of a VEGF-Notch signaling circuit drives formation of functional vascular endothelial progenitors from human pluripotent stem cells, Cell Res. 24 (2014) 820–841, https://doi.org/10.1038/cr.2014.59.
[16] I. Elcheva, V. Brok-Volchanskaya, A. Kumar, P. Liu, J.H. Lee, L. Tong, M. Vodyanik, S. Swanson, R. Stewart, M. Kyba, E. Yakubov, J. Cooke, J.A. Thomson, I. Slukvin, Direct induction of haematoendothelial programs in human pluripotent stem cells by transcriptional regulators, Nat. Commun. 5 (2014)https://doi.org/10.1038/ncomms5372.
[17] N.J. Palpant, L. Pabon, M. Roberts, B. Hadland, D. Jones, C. Jones, R.T. Moon, W.L. Ruzzo, I. Bernstein, Y. Zheng, C.E. Murry, Inhibition of -catenin signaling respecifies anterior-like endothelium into beating human cardiomyocytes, Development 142 (2015) 3198–3209, https://doi.org/10.1242/dev.117010.
[18] D.E. Reichman, L. Park, L. Man, D. Redmond, K. Chao, R.P. Harvey, M.M. Taketo, Z. Rosenwaks, D. James, Wnt inhibition promotes vascular specification of embryonic cardiac progenitors, Development 145 (2018), dev159905. https://doi.org/10.1242/dev.159905.
[19] E. Giacomelli, M. Bellin, V.V. Orlova, C.L. Mummery, Co-differentiation of human pluripotent stem cells-derived cardiomyocytes and endothelial cells from cardiac mesoderm provides a three-dimensional model of cardiac microtissue, Curr. Protoc. Hum. Genet. (2017) 21.9.1–21.9.22, https://doi.org/10.1002/cphg.46.
[20] J. Zhang, L.-F. Chu, Z. Hou, M.P. Schwartz, T. Hacker, V. Vickerman, S. Swanson, N. Leng, B.K. Nguyen, A. Elwell, J. Bolin, M.E. Brown, R. Stewart, W.J. Burlingham, W.L. Murphy, J.A. Thomson, Functional characterization of human pluripotent stem cell-derived arterial endothelial cells, Proc. Natl. Acad. Sci. 201702295 (2017)https://doi.org/10.1073/pnas.1702295114.
[21] O.V. Halaidych, C. Freund, F. van den Hil, D.C.F. Salvatori, M. Riminucci, C.L. Mummery, V.V. Orlova, Inflammatory responses and barrier function of endothelial cells derived from human induced pluripotent stem cells, Stem Cell Reports 10 (2018) 1642–1656, https://doi.org/10.1016/j.stemcr.2018.03.012.
[22] A. Cochrane, S. Kelaini, M. Tsifaki, J. Bojdo, M. Vilà-González, D. Drehmer, R. Caines, C. Magee, M. Eleftheriadou, Y. Hu, D. Grieve, A.W. Stitt, L. Zeng, Q. Xu, A. Margariti, Quaking is a key regulator of endothelial cell differentiation, neovascularization, and angiogenesis, Stem Cells 35 (2017) 952–966, https://doi.org/10.1002/stem.2594.
[23] A. Sivarapatna, M. Ghaedi, A.V. Le, J.J. Mendez, Y. Qyang, L.E. Niklason, Arterial specification of endothelial cells derived from human induced pluripotent stem cells in a biomimetic flow bioreactor, Biomaterials 53 (2015) 621–633, https://doi.org/10.1016/j.biomaterials.2015.02.121.
[24] H. Vazão, S. Rosa, T. Barata, R. Costa, P.R. Pitrez, I. Honório, M.R. de Vries, D. Papatsenko, R. Benedito, D. Saris, A. Khademhosseini, P.H.A. Quax, C.F. Pereira, N. Mercader, H. Fernandes, L. Ferreira, High-throughput identification of small molecules that affect human embryonic vascular development, Proc. Natl. Acad. Sci. 114 (2017) E3022–E3031, https://doi.org/10.1073/pnas.1617451114.
[25] G. Sriram, J.Y. Tan, I. Islam, A.J. Rufaihah, T. Cao, Efficient differentiation of human embryonic stem cells to arterial and venous endothelial cells under feeder- and serum-free conditions, Stem Cell Res Ther 6 (2015)https://doi.org/10.1186/s13287-015-0260-5.
[26] J. Ando, K. Yamamoto, Vascular mechanobiology - endothelial cell responses to fluid shear stress, Circ. J. 73 (2009) 1983–1992, https://doi.org/10.1253/circj.CJ-09-0583.
[27] R. Sinha, S. Le Gac, N. Verdonschot, A. Van Den Berg, B. Koopman, J. Rouwkema, Endothelial cell alignment as a result of anisotropic strain and flow induced shear stress combinations, Sci. Rep. 6 (2016) 1–12, https://doi.org/10.1038/srep29510.
[28] R. Ohtani-Kaneko, K. Sato, A. Tsutiya, Y. Nakagawa, K. Hashizume, H. Tazawa, Characterisation of human induced pluripotent stem cell-derived endothelial cells under shear stress using an easy-to-use microfluidic cell culture system, Biomed. Microdevices 19 (2017)https://doi.org/10.1007/s10544-017-0229-5.
[29] C. Cheung, A.S. Bernardo, M.W.B. Trotter, R.A. Pedersen, S. Sinha, Generation of human vascular smooth muscle subtypes provides insight into embryological origin–dependent disease susceptibility, Nat. Biotechnol. 30 (2012) 165–173, https://doi.org/10.1038/nbt.2107.
[30] C. Cheung, Y.T. Goh, J. Zhang, C. Wu, E. Guccione, Modeling cerebrovascular pathophysiology in amyloid-beta metabolism using neural-crest-derived smooth muscle cells, Cell Rep. 9 (2014) 391–401, https://doi.org/10.1016/j.celrep.2014.08.065.
[31] A. Kumar, S.S. D'Souza, O.V. Moskvin, H. Toh, B. Wang, J. Zhang, S. Swanson, L.W. Guo, J.A. Thomson, I.I. Slukvin, Specification and diversification of pericytes and smooth muscle cells from mesenchymoangioblasts, Cell Rep. 19 (2017) 1902–1916, https://doi.org/10.1016/j.celrep.2017.05.019.
[32] M. Wanjare, F. Kuo, S. Gerecht, Derivation and maturation of synthetic and contractile vascular smooth muscle cells from human pluripotent stem cells, Cardiovasc. Res. 97 (2013) 321–330, https://doi.org/10.1093/cvr/cvs315.
[33] M. Wanjare, N. Agarwal, S. Gerecht, Biomechanical strain induces elastin and collagen production in human pluripotent stem cell-derived vascular smooth muscle cells, Am. J. Physiol. - Cell Physiol. 309 (2015) C271–C281, https://doi.org/10.1152/ajpcell.00366.2014.
[34] J.H. Eoh, N. Shen, J.A. Burke, S. Hinderer, Z. Xia, K. Schenke-Layland, S. Gerecht, Enhanced elastin synthesis and maturation in human vascular smooth muscle tissue derived from induced-pluripotent stem cells, Acta Biomater. 52 (2017) 49–59, https://doi.org/10.1016/j.actbio.2017.01.083.
[35] M.S. Collado, B.K. Cole, R.A. Figler, M. Lawson, D. Manka, M.B. Simmers, S. Hoang, F. Serrano, B.R. Blackman, S. Sinha, B.R. Wamhoff, Exposure of induced pluripotent stem cell-derived vascular endothelial and smooth muscle cells in coculture to hemodynamics induces primary vascular cell-like phenotypes, Stem Cells Transl. Med. 6 (2017) 1673–1683, https://doi.org/10.1002/sctm.17-0004.
[36] C.J. Chin, S. Li, M. Corselli, D. Casero, Y. Zhu, C. Bin He, R. Hardy, B. Péault, G.M. Crooks, Transcriptionally and functionally distinct mesenchymal subpopulations are generated from human pluripotent stem cells, Stem Cell Reports (2018) https://doi.org/10.1016/j.stemcr.2017.12.005.
[37] A. Dar, H. Domev, O. Ben-Yosef, M. Tzukerman, N. Zeevi-Levin, A. Novak, I. Germanguz, M. Amit, J. Itskovitz-Eldor, Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb, Circulation 125 (2012) 87–99, https://doi.org/10.1161/CIRCULATIONAHA.111.048264.
[38] J. Cai, V.V. Orlova, X. Cai, E.M.W. Eekhoff, K. Zhang, D. Pei, G. Pan, C.L. Mummery, P. Ten Dijke, Induced pluripotent stem cells to model human fibrodysplasia ossificans progressiva, Stem Cell Reports 5 (2015) 963–970, https://doi.org/10.1016/j.stemcr.2015.10.020.
[39] K. Hino, M. Ikeya, K. Horigome, Y. Matsumoto, H. Ebise, M. Nishio, K. Sekiguchi, M. Shibata, S. Nagata, S. Matsuda, J. Toguchida, Neofunction of ACVR1 in fibrodysplasia ossificans progressiva, Proc. Natl. Acad. Sci. 112 (2015) 15438–15443, https://doi.org/10.1073/pnas.1510540112.
[40] M. Gu, N.Y. Shao, S. Sa, D. Li, V. Termglinchan, M. Ameen, I. Karakikes, G. Sosa, F. Grubert, J. Lee, A. Cao, S. Taylor, Y. Ma, Z. Zhao, J. Chappell, R. Hamid, E.D. Austin,

J.D. Gold, J.C. Wu, M.P. Snyder, M. Rabinovitch, Patient-specific iPSC-derived endothelial cells uncover pathways that protect against pulmonary hypertension in BMPR2 mutation carriers, Cell Stem Cell 20 (2017) 490–504.e5, https://doi.org/10.1016/j.stem.2016.08.019.
[41] A. Granata, F. Serrano, W.G. Bernard, M. McNamara, L. Low, P. Sastry, S. Sinha, An iPSC-derived vascular model of Marfan syndrome identifies key mediators of smooth muscle cell death, Nat. Genet. 49 (2017) 97–109, https://doi.org/10.1038/ng.3723.
[42] R.M. Gupta, J. Hadaya, A. Trehan, S.M. Zekavat, C. Roselli, D. Klarin, C.A. Emdin, C.R.E. Hilvering, V. Bianchi, C. Mueller, A.V. Khera, R.J.H. Ryan, J.M. Engreitz, R. Issner, N. Shoresh, C.B. Epstein, W. de Laat, J.D. Brown, R.B. Schnabel, B.E. Bernstein, S. Kathiresan, A genetic variant associated with five vascular diseases is a distal regulator of Endothelin-1 gene expression, Cell 170 (2017) 522–533.e15, https://doi.org/10.1016/j.cell.2017.06.049.
[43] B. Zohar, Y. Blinder, D.J. Mooney, S. Levenberg, Flow-induced vascular network formation and maturation in three-dimensional engineered tissue, ACS Biomater. Sci. Eng. 4 (2017) 1265–1271 , acsbiomaterials.7b00025 https://doi.org/10.1021/acsbiomaterials.7b00025.
[44] S. Kim, W. Kim, S. Lim, J. Jeon, Vasculature-on-a-chip for in vitro disease models, Bioengineering 4 (2017) 8, https://doi.org/10.3390/bioengineering4010008.
[45] A. Herland, A.D. Van Der Meer, E.A. FitzGerald, T.E. Park, J.J.F. Sleeboom, D.E. Ingber, Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip, PLoS One 11 (2016) 1–21, https://doi.org/10.1371/journal.pone.0150360.
[46] D. Huh, B.D. Matthews, A. Mammoto, M. Montoya-Zavala, H.Y. Hsin, D.E. Ingber, Reconstituting organ-level lung functions on a chip, Science 328 (2010) 1662–1668, https://doi.org/10.1126/science.1188302.
[47] S. Kim, H. Lee, M. Chung, N.L. Jeon, Engineering of functional, perfusable 3D microvascular networks on a chip, Lab Chip 13 (2013) 1489, https://doi.org/10.1039/c3lc41320a.
[48] S. Kim, M. Chung, J. Ahn, S. Lee, N.L. Jeon, Interstitial flow regulates the angiogenic response and phenotype of endothelial cells in a 3D culture model, Lab Chip 16 (2016) 4189–4199, https://doi.org/10.1039/C6LC00910G.
[49] H. Cho, J.H. Seo, K.H.K. Wong, Y. Terasaki, J. Park, K. Bong, K. Arai, E.H. Lo, D. Irimia, Three-dimensional blood-brain barrier model for in vitro studies of neurovascular pathology, Sci. Rep. 5 (2015), 15222. https://doi.org/10.1038/srep15222.
[50] N.V. Menon, H.M. Tay, S.N. Wee, K.H.H. Li, H.W. Hou, Micro-engineered perfusable 3D vasculatures for cardiovascular diseases, Lab Chip 17 (2017) 2960–2968, https://doi.org/10.1039/C7LC00607A.
[51] J.S. Jeon, S. Bersini, M. Gilardi, G. Dubini, J.L. Charest, M. Moretti, R.D. Kamm, J.S. Jeon, S. Bersini, M. Gilardi, G. Dubini, J.L. Charest, M. Moretti, Correction for Jeon et al., Human 3D vascularized organotypic microfluidic assays to study breast cancer cell extravasation, Proc. Natl. Acad. Sci. 112 (2015) , E818-E818 https://doi.org/10.1073/pnas.1501426112.
[52] I.K. Zervantonakis, S.K. Hughes-Alford, J.L. Charest, J.S. Condeelis, F.B. Gertler, R.D. Kamm, Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function, Proc. Natl. Acad. Sci. 109 (2012) 13515–13520, https://doi.org/10.1073/pnas.1210182109.
[53] S. Chung, R. Sudo, P.J. Mack, C.-R. Wan, V. Vickerman, R.D. Kamm, Cell migration into scaffolds under co-culture conditions in a microfluidic platform, Lab Chip 9 (2009) 269–275, https://doi.org/10.1039/B807585A.
[54] H.J. Kim, H. Li, J.J. Collins, D.E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, Proc. Natl. Acad. Sci. 113 (2016) E7–E15, https://doi.org/10.1073/pnas.1522193112.
[55] L.M. Griep, F. Wolbers, B. De Wagenaar, P.M. Ter Braak, B.B. Weksler, I.A. Romero, P.O. Couraud, I. Vermes, A.D. Van Der Meer, A. Van Den Berg, BBB on CHIP: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function, Biomed. Microdevices 15 (2013) 145–150, https://doi.org/10.1007/s10544-012-9699-7.
[56] M.W. van der Helm, M. Odijk, J.P. Frimat, A.D. van der Meer, J.C.T. Eijkel, A. van den Berg, L.I. Segerink, Direct quantification of transendothelial electrical resistance in organs-on-chips, Biosens. Bioelectron. 85 (2016) 924–929, https://doi.org/10.1016/j.bios.2016.06.014.
[57] H.E. Abaci, Y.I. Shen, S. Tan, S. Gerecht, Recapitulating physiological and pathological shear stress and oxygen to model vasculature in health and disease, Sci. Rep. 4 (2014) 1–9, https://doi.org/10.1038/srep04951.
[58] E. Westein, S. De Witt, M. Lamers, J.M.E.M. Cosemans, J.W.M. Heemskerk, Monitoring in vitro thrombus formation with novel microfluidic devices, Platelets 23 (2012) 501–509, https://doi.org/10.3109/09537104.2012.709653.
[59] R. Van Kruchten, J.M.E.M. Cosemans, J.W.M. Heemskerk, Measurement of whole blood thrombus formation using parallel-plate flow chambers a practical guide, Platelets 23 (2012) 229–242, https://doi.org/10.3109/09537104.2011.630848.
[60] D.M. Coenen, T.G. Mastenbroek, J.M.E.M. Cosemans, Platelet interaction with activated endothelium: mechanistic insights from microfluidics, Blood 130 (2017) 2819–2828, https://doi.org/10.1182/blood-2017-04-780825.
[61] N.K.R. Pandian, R.G. Mannino, W.A. Lam, A. Jain, Thrombosis-on-a-chip: prospective impact of microphysiological models of vascular thrombosis, Curr. Opin. Biomed. Eng. 5 (2018) 29–34, https://doi.org/10.1016/j.cobme.2017.12.001.
[62] S. Zhu, B.A. Herbig, R. Li, T.V. Colace, R.W. Muthard, K.B. Neeves, S.L. Diamond, In microfluidico: recreating in vivo hemodynamics using miniaturized devices, Biorheology 52 (2015) 303–318, https://doi.org/10.3233/BIR-15065.
[63] R.G. Mannino, N.K. Pandian, A. Jain, W.A. Lam, Engineering "Endothelialized" microfluidics for investigating vascular and hematologic processes using non-traditional fabrication techniques, Curr. Opin. Biomed. Eng. 5 (2017) 13–20, https://doi.org/10.1016/j.cobme.2017.11.006.
[64] W.S. Nesbitt, E. Westein, F.J. Tovar-Lopez, E. Tolouei, A. Mitchell, J. Fu, J. Carberry, A. Fouras, S.P. Jackson, A shear gradient-dependent platelet aggregation mechanism drives thrombus formation, Nat. Med. 15 (2009) 665–673 (doi:nm.1955 [pii] \r10.1038/nm.1955).
[65] T.V. Colace, S.L. Diamond, Direct observation of von Willebrand factor elongation and fiber formation on collagen during acute whole blood exposure to pathological flow, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 105–113, https://doi.org/10.1161/ATVBAHA.112.300522.
[66] E. Westein, A.D. van der Meer, M.J.E. Kuijpers, J.-P. Frimat, A. van den Berg, J.W.M. Heemskerk, Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 1357–1362, https://doi.org/10.1073/pnas.1209905110.
[67] A. Jain, A.D. van der Meer, A.L. Papa, R. Barrile, A. Lai, B.L. Schlechter, M.A. Otieno, C.S. Louden, G.A. Hamilton, A.D. Michelson, A.L. Frelinger, D.E. Ingber, Assessment of whole blood thrombosis in a microfluidic device lined by fixed human endothelium, Biomed. Microdevices 18 (2016) 1–7, https://doi.org/10.1007/s10544-016-0095-6.
[68] A. Jain, R. Barrile, A.D. van der Meer, A. Mammoto, T. Mammoto, K. De Ceunynck, O. Aisiku, M.A. Otieno, C.S. Louden, G.A. Hamilton, R. Flaumenhaft, D.E. Ingber, Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics, Clin. Pharmacol. Ther. 103 (2018) 332–340, https://doi.org/10.1002/cpt.742.
[69] Y. Zheng, J. Chen, M. Craven, N.W. Choi, S. Totorica, A. Diaz-Santana, P. Kermani, B. Hempstead, C. Fischbach-Teschl, J.A. López, A.D. Stroock, In vitro microvessels for the study of angiogenesis and thrombosis, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 9342–9347, https://doi.org/10.1073/pnas.1201240109.
[70] R.G. Mannino, D.R. Myers, B. Ahn, Y. Wang, Margo Rollins, H. Gole, A.S. Lin, R.E. Guldberg, D.P. Giddens, L.H. Timmins, W.A. Lam, Do-it-yourself in vitro vasculature that recapitulates in vivo geometries for investigating endothelial-blood cell interactions, Sci. Rep. 5 (2015), 12401. https://doi.org/10.1038/srep12401.
[71] Y.S. Zhang, F. Davoudi, P. Walch, A. Manbachi, X. Luo, V. Dell'Erba, A.K. Miri, H. Albadawi, A. Arneri, X. Li, X. Wang, M.R. Dokmeci, A. Khademhosseini, R. Oklu, Bioprinted thrombosis-on-a-chip, Lab Chip 16 (2016) 4097–4105, https://doi.org/10.1039/C6LC00380J.
[72] P.F. Costa, H.J. Albers, J.E.A. Linssen, H.H.T. Middelkamp, L. van der Hout, R. Passier, A. van den Berg, J. Malda, A.D. van der Meer, Mimicking arterial thrombosis in a 3D-printed microfluidic in vitro vascular model based on computed tomography angiography data, Lab Chip 17 (2017)https://doi.org/10.1039/C7LC00202E.
[73] V. van Duinen, S.J. Trietsch, J. Joore, P. Vulto, T. Hankemeier, Microfluidic 3D cell culture: from tools to tissue models, Curr. Opin. Biotechnol. 35 (2015) 118–126, https://doi.org/10.1016/j.copbio.2015.05.002.
[74] A. Jain, A. Graveline, A. Waterhouse, A. Vernet, R. Flaumenhaft, D.E. Ingber, A shear gradient-activated microfluidic device for automated monitoring of whole blood haemostasis and platelet function, Nat. Commun. 7 (2016)https://doi.org/10.1038/ncomms10176.
[75] R.W. Muthard, S.L. Diamond, Side view thrombosis microfluidic device with controllable wall shear rate and transthrombus pressure gradient, Lab Chip 13 (2013) 1883–1891, https://doi.org/10.1039/c3lc41332b.
[76] R.M. Schoeman, K. Rana, N. Danes, M. Lehmann, J.A. Di Paola, A.L. Fogelson, K. Leiderman, K.B. Neeves, A microfluidic model of hemostasis sensitive to platelet function and coagulation, Cell. Mol. Bioeng. 10 (2017) 3–15, https://doi.org/10.1007/s12195-016-0469-0.
[77] Y. Sakurai, E.T. Hardy, B. Ahn, R. Tran, M.E. Fay, J.C. Ciciliano, R.G. Mannino, D.R. Myers, Y. Qiu, M.A. Carden, W.H. Baldwin, S.L. Meeks, G.E. Gilbert, S.M. Jobe, W.A. Lam, A microengineered vascularized bleeding model that integrates the principal components of hemostasis, Nat. Commun. 9 (1) (2018)https://doi.org/10.1038/s41467-018-02990-x.
[78] J. Rouwkema, A. Khademhosseini, Vascularization and angiogenesis in tissue engineering: beyond creating static networks, Trends Biotechnol. 34 (2016) 733–745, https://doi.org/10.1016/j.tibtech.2016.03.002.
[79] S. Chung, R. Sudo, V. Vickerman, I.K. Zervantonakis, R.D. Kamm, Microfluidic platforms for studies of angiogenesis, cell migration, and cell-cell interactions: sixth international bio-fluid mechanics symposium and workshop March 28–30, 2008 Pasadena, California, Ann. Biomed. Eng. 38 (2010) 1164–1177, https://doi.org/10.1007/s10439-010-9899-3.
[80] R. Sudo, S. Chung, I.K. Zervantonakis, V. Vickerman, Y. Toshimitsu, L.G. Griffith, R.D. Kamm, Transport-mediated angiogenesis in 3D epithelial coculture, FASEB J. 23 (2009) 2155–2164, https://doi.org/10.1096/fj.08-122820.
[81] V. Vickerman, J. Blundo, S. Chung, R. Kamm, Design, fabrication and implementation of a novel multi-parameter control microfluidic platform for three-dimensional cell culture and real-time imaging, Lab Chip 8 (2008) 1468, https://doi.org/10.1039/b802395f.
[82] Y. Shin, J.S. Jeon, S. Han, G.-S. Jung, S. Shin, S.-H. Lee, R. Sudo, R.D. Kamm, S. Chung, In vitro 3D collective sprouting angiogenesis under orchestrated ANG-1 and VEGF gradients, Lab Chip 11 (2011) 2175, https://doi.org/10.1039/c1lc20039a.
[83] G.S. Jeong, S. Han, Y. Shin, G.H. Kwon, R.D. Kamm, S.H. Lee, S. Chung, Sprouting angiogenesis under a chemical gradient regulated by interactions with an endothelial monolayer in a microfluidic platform, Anal. Chem. 83 (2011) 8454–8459, https://doi.org/10.1021/ac202170e.
[84] C.P. Huang, J. Lu, H. Seon, A.P. Lee, L.A. Flanagan, H.-Y. Kim, A.J. Putnam, N.L. Jeon, Engineering microscale cellular niches for three-dimensional multicellular cocultures, Lab Chip 9 (2009) 1740, https://doi.org/10.1039/b818401a.
[85] C. Kim, J. Kasuya, J. Jeon, S. Chung, R.D. Kamm, A quantitative microfluidic angiogenesis screen for studying anti-angiogenic therapeutic drugs, Lab Chip 15 (2015) 301–310, https://doi.org/10.1039/C4LC00866A.

[86] J.M. Chan, I.K. Zervantonakis, T. Rimchala, W.J. Polacheck, J. Whisler, R.D. Kamm, Engineering of in vitro 3D capillary beds by self-directed angiogenic sprouting, PLoS One 7 (2012) 1–11, https://doi.org/10.1371/journal.pone.0050582.
[87] Y. Zheng, Y. Sun, X. Yu, Y. Shao, P. Zhang, G. Dai, J. Fu, Angiogenesis in liquid tumors: an in vitro assay for leukemic-cell-induced bone marrow angiogenesis, Adv. Healthc. Mater. 5 (2016) 1014–1024, https://doi.org/10.1002/adhm.201501007.
[88] B.M. Baker, B. Trappmann, S.C. Stapleton, E. Toro, C.S. Chen, Microfluidics embedded within extracellular matrix to define vascular architectures and pattern diffusive gradients, Lab Chip 13 (2013) 3246, https://doi.org/10.1039/c3lc50493j.
[89] D.-H.T. Nguyen, S.C. Stapleton, M.T. Yang, S.S. Cha, C.K. Choi, P.A. Galie, C.S. Chen, Biomimetic model to reconstitute angiogenic sprouting morphogenesis in vitro, Proc. Natl. Acad. Sci. 110 (2013) 6712–6717, https://doi.org/10.1073/pnas.1221526110.
[90] L.L. Bischel, E.W.K. Young, B.R. Mader, D.J. Beebe, Tubeless microfluidic angiogenesis assay with three-dimensional endothelial-lined microvessels, Biomaterials 34 (2013) 1471–1477, https://doi.org/10.1016/j.biomaterials.2012.11.005.
[91] J.S. Miller, K.R. Stevens, M.T. Yang, B.M. Baker, D.-H.T. Nguyen, D.M. Cohen, E. Toro, A.A. Chen, P.A. Galie, X. Yu, R. Chaturvedi, S.N. Bhatia, C.S. Chen, Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues, Nat. Mater. 11 (2012) 768–774, https://doi.org/10.1038/nmat3357.
[92] D.B. Kolesky, R.L. Truby, A.S. Gladman, T.A. Busbee, K.A. Homan, J.A. Lewis, 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs, Adv. Mater. 26 (2014)https://doi.org/10.1002/adma.201305506.
[93] W.J. Polacheck, M.L. Kutys, J. Yang, J. Eyckmans, Y. Wu, H. Vasavada, K.K. Hirschi, C.S. Chen, A non-canonical Notch complex regulates adherens junctions and vascular barrier function, Nature 552 (2017) 258–262, https://doi.org/10.1038/nature24998.
[94] J.J. Mack, T.S. Mosqueiro, B.J. Archer, W.M. Jones, H. Sunshine, G.C. Faas, A. Briot, R.L. Aragón, T. Su, M.C. Romay, A.I. McDonald, C.H. Kuo, C.O. Lizama, T.F. Lane, A.C. Zovein, Y. Fang, E.J. Tarling, T.Q. De Aguiar Vallim, M. Navab, A.M. Fogelman, L.S. Bouchard, M.L. Iruela-Arispe, NOTCH1 is a mechanosensor in adult arteries, Nat. Commun. 8 (2017) 1–18, https://doi.org/10.1038/s41467-017-01741-8.
[95] Y.K. Kurokawa, R.T. Yin, M.R. Shang, V.S. Shirure, M.L. Moya, S.C. George, Human iPS-derived endothelial cells for 3D microphysiological systems, Tissue Eng. Part C Methods 23 (2017) 474–484 , ten.TEC.2017.0133 https://doi.org/10.1089/ten.TEC.2017.0133.
[96] A.D. van der Meer, V.V. Orlova, P. ten Dijke, A. van den Berg, C.L. Mummery, Three-dimensional co-cultures of human endothelial cells and embryonic stem cell-derived pericytes inside a microfluidic device, Lab Chip 13 (2013) 3562–3568, https://doi.org/10.1039/C3LC50435B.
[97] L. Atchison, H. Zhang, K. Cao, G.A. Truskey, A. Tissue Engineered, Blood vessel model of Hutchinson-Gilford progeria syndrome using human iPSC-derived smooth muscle cells, Sci. Rep. 7 (2017) 1–12, https://doi.org/10.1038/s41598-017-08632-4.
[98] J. Ribas, Y.S. Zhang, P.R. Pitrez, J. Leijten, M. Miscuglio, J. Rouwkema, M.R. Dokmeci, X. Nissan, L. Ferreira, A. Khademhosseini, Biomechanical strain exacerbates inflammation on a progeria-on-a-chip model, Small 13 (2017)https://doi.org/10.1002/smll.201603737.
[99] V.E. Gallardo, G.K. Varshney, M. Lee, S. Bupp, L. Xu, P. Shinn, N.P. Crawford, J. Inglese, S.M. Burgess, Phenotype-driven chemical screening in zebrafish for compounds that inhibit collective cell migration identifies multiple pathways potentially involved in metastatic invasion, Dis. Model. Mech. 8 (2015) 565–576, https://doi.org/10.1242/dmm.018689.
[100] R.E. Kälin, N.E. Bänziger-Tobler, M. Detmar, A.W. Brändli, An in vivo chemical library screen in *Xenopus tadpoles* reveals novel pathways involved in angiogenesis and lymphangiogenesis, Blood 114 (2009) 1110–1122, https://doi.org/10.1182/blood-2009-03-211771.
[101] C.C. Veerman, G. Kosmidis, C.L. Mummery, S. Casini, A.O. Verkerk, M. Bellin, Immaturity of human stem-cell-derived cardiomyocytes in culture: fatal flaw or soluble problem? Stem Cells Dev. 24 (2015) 1035–1052, https://doi.org/10.1089/scd.2014.0533.
[102] B. Zhang, M. Radisic, Organ-on-a-chip devices advance to market, Lab Chip 17 (2017) 2395–2420, https://doi.org/10.1039/C6LC01554A.
[103] B.J. van Meer, H. de Vries, K.S.A. Firth, J. van Weerd, L.G.J. Tertoolen, H.B.J. Karperien, P. Jonkheijm, C. Denning, A.P. IJzerman, C.L. Mummery, Small molecule absorption by PDMS in the context of drug response bioassays, Biochem. Biophys. Res. Commun. 482 (2017) 323–328, https://doi.org/10.1016/j.bbrc.2016.11.062.
[104] H. Becker, C. Gärtner, Polymer microfabrication technologies for microfluidic systems, Anal. Bioanal. Chem. 390 (2008) 89–111, https://doi.org/10.1007/s00216-007-1692-2.
[105] E. Berthier, E.W.K. Young, D. Beebe, Engineers are from PDMS-land, Biologists are from Polystyrenia, Lab Chip 12 (2012) 1224, https://doi.org/10.1039/c2lc20982a.
[106] M. Odijk, A.D. van der Meer, D. Levner, H.J. Kim, M.W. van der Helm, L.I. Segerink, J.-P. Frimat, G.A. Hamilton, D.E. Ingber, A. van den Berg, Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems, Lab Chip 15 (2015) 745–752, https://doi.org/10.1039/C4LC01219D.
[107] A. Gore, Z. Li, H.-L. Fung, J.E. Young, S. Agarwal, J. Antosiewicz-Bourget, I. Canto, A. Giorgetti, M.A. Israel, E. Kiskinis, J.-H. Lee, Y.-H. Loh, P.D. Manos, N. Montserrat, A.D. Panopoulos, S. Ruiz, M.L. Wilbert, J. Yu, E.F. Kirkness, J.C.I. Belmonte, D.J. Rossi, J.A. Thomson, K. Eggan, G.Q. Daley, L.S.B. Goldstein, K. Zhang, Somatic coding mutations in human induced pluripotent stem cells, Nature 471 (2011) 63–67, https://doi.org/10.1038/nature09805.
[108] F.M. de Vrij, C.G. Bouwkamp, N. Gunhanlar, G. Shpak, B. Lendemeijer, M. Baghdadi, S. Gopalakrishna, M. Ghazvini, T.M. Li, M. Quadri, S. Olgiati, G.J. Breedveld, M. Coesmans, E. Mientjes, T. de Wit, F.W. Verheijen, H.B. Beverloo, D. Cohen, R.M. Kok, P.R. Bakker, A. Nijburg, A.T. Spijker, P.M.J. Haffmans, E. Hoencamp, V. Bergink, J.A. Vorstman, T. Wu, L.M. Olde Loohuis, N. Amin, C.D. Langen, A. Hofman, W.J. Hoogendijk, C.M. van Duijn, M.A. Ikram, M.W. Vernooij, H. Tiemeier, A.G. Uitterlinden, Y. Elgersma, B. Distel, J. Gribnau, T. White, V. Bonifati, S.A. Kushner, Candidate CSPG4 mutations and induced pluripotent stem cell modeling implicate oligodendrocyte progenitor cell dysfunction in familial schizophrenia, Mol. Psychiatry 1–15 (2018)https://doi.org/10.1038/s41380-017-0004-2.
[109] G.E. Hoffman, B.J. Hartley, E. Flaherty, I. Ladran, P. Gochman, D.M. Ruderfer, E.A. Stahl, J. Rapoport, P. Sklar, K.J. Brennand, Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons are concordant with post-mortem adult brains, Nat. Commun. 8 (2017)https://doi.org/10.1038/s41467-017-02330-5.
[110] Z. Wen, H.N. Nguyen, Z. Guo, M.A. Lalli, X. Wang, Y. Su, N.S. Kim, K.J. Yoon, J. Shin, C. Zhang, G. Makri, D. Nauen, H. Yu, E. Guzman, C.H. Chiang, N. Yoritomo, K. Kaibuchi, J. Zou, K.M. Christian, L. Cheng, C.A. Ross, R.L. Margolis, G. Chen, K.S. Kosik, H. Song, G.L. Ming, Synaptic dysregulation in a human iPS cell model of mental disorders, Nature 515 (2014) 414–418, https://doi.org/10.1038/nature13716.
[111] D. Cornacchia, L. Studer, Back and forth in time: directing age in iPSC-derived lineages, Brain Res. 1656 (2017) 14–26, https://doi.org/10.1016/j.brainres.2015.11.013.
[112] J.D. Miller, Y.M. Ganat, S. Kishinevsky, R.L. Bowman, B. Liu, E.Y. Tu, P.K. Mandal, E. Vera, J.W. Shim, S. Kriks, T. Taldone, N. Fusaki, M.J. Tomishima, D. Krainc, T.A. Milner, D.J. Rossi, L. Studer, Human iPSC-based modeling of late-onset disease via progerin-induced aging, Cell Stem Cell 13 (2013) 691–705, https://doi.org/10.1016/j.stem.2013.11.006.
[113] E. Vera, N. Bosco, L. Studer, Generating late-onset human iPSC-based disease models by inducing neuronal age-related phenotypes through telomerase manipulation, Cell Rep. 17 (2016) 1184–1192, https://doi.org/10.1016/j.celrep.2016.09.062.
[114] M.S. Roost, R.C. Slieker, M. Bialecka, L. Van Iperen, M.M. Gomes Fernandes, N. He, H.E.D. Suchiman, K. Szuhai, F. Carlotti, E.J.P. De Koning, C.L. Mummery, B.T. Heijmans, S.M. Chuva De Sousa Lopes, DNA methylation and transcriptional trajectories during human development and reprogramming of isogenic pluripotent stem cells, Nat. Commun. 8 (2017)https://doi.org/10.1038/s41467-017-01077-3.
[115] M. Nishizawa, K. Chonabayashi, M. Nomura, A. Tanaka, M. Nakamura, A. Inagaki, M. Nishikawa, I. Takei, A. Oishi, K. Tanabe, M. Ohnuki, H. Yokota, M. Koyanagi-Aoi, K. Okita, A. Watanabe, A. Takaori-Kondo, S. Yamanaka, Y. Yoshida, Epigenetic variation between human induced pluripotent stem cell lines is an Indicator of differentiation capacity, Cell Stem Cell 19 (2016) 341–354, https://doi.org/10.1016/j.stem.2016.06.019.
[116] E.W. Esch, A. Bahinski, D. Huh, Organs-on-chips at the frontiers of drug discovery, Nat. Rev. Drug Discov. 14 (2015) 248–260, https://doi.org/10.1038/nrd4539.
[117] H.H.T. Middelkamp, A.D. van der Meer, J.M. Hummel, D.F. Stamatialis, C.L. Mummery, R. Passier, M.J. IJzerman, Organs-on-chips in drug development: the importance of involving stakeholders in early health technology assessment, Appl. Vitr. Toxicol. 2 (2016) 74–81, https://doi.org/10.1089/aivt.2015.0029.
[118] A. Bahinski, R. Horland, D. Huh, C. Mummery, D.A. Tagle, T. MacGill, The promise and potential of "Organs-on-Chips" as preclinical models, Appl. Vitr. Toxicol. 1 (2015) 235–242, https://doi.org/10.1089/aivt.2015.29002.rtl.
[119] A.D. van der Meer, A. van den Berg, Organs-on-chips: breaking the in vitro impasse, Integr. Biol. 4 (2012) 461, https://doi.org/10.1039/c2ib00176d.
[120] D.E. Ingber, Developmentally inspired human "organs on chips", Development 10–13 (2018)https://doi.org/10.1242/dev.156125.
[121] D.E. Ingber, Reverse engineering human pathophysiology with organs-on-chips, Cell 164 (2016) 1105–1109, https://doi.org/10.1016/j.cell.2016.02.049.
[122] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, Trends Cell Biol. 21 (2011) 745–754, https://doi.org/10.1016/j.tcb.2011.09.005.
[123] M.B. Esch, T.L. King, M.L. Shuler, The role of body-on-a-chip devices in drug and toxicity studies, Annu. Rev. Biomed. Eng. 13 (2011) 55–72, https://doi.org/10.1146/annurev-bioeng-071910-124629.