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

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A B S T R A C T

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.

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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 cellular interactions with extracellular matrix (ECM) and supporting cells in vivo. Observations may thus be inaccurate representations of vascular physiology.

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 into 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 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 ET2V 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 isoinost monophosphatase inhibitor (L6890) increased expression of arterial markers, such as CXC4, FENB2, GJ4, NRP1 and NOTCH ligands and receptors (DLL4/JAG1 and NOTCH1/4) in hiPSC-ECs and induced down-regulation of venous markers (e.g. NRG2F2). 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 QKX5-directed EC differentiation from hiPSC 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, multi-parameter 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 tube 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 (cyclical 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 AS1 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 NC2, CD146, CD73 and CD44 [31, 36], or alternatively sorting of CD146highCD73high 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...
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 elements in hypoxic conditions, increases in permeability were detected by quantification of gaps in cell-cell junctions [57].

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

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 perpendicularly separated channels, 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 DiOC6. 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 fibroblasts (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]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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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 alveolar-on-a-chip system, where an endothoxin 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 polyethylene glycol (PEG) or 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 arteries, was connected to an anaesthetic pig. Here, clotting times in the presence and absence of antiplatelet 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 iPSC-derived vascular tissue cultured in microfluidic chips are now being reported, along with studies on primary vascular cells. Notably, these systems demonstrate that iPSC-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 TGF-β 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 VMSCs [98]. Interestingly, hiPSC-derived VMSCs 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 iPSC-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 background may impact responses of hiPSC-derived vascular cells to drugs and disease. In fact, even apparently “healthy controls” may

**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.
have certain genetic predisposition towards some diseases that manifest 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 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 in order to provide patient-relevant data (Fig. 3).

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