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ABOUT COVER

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AIMS AND SCOPE

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Advances in the differentiation of pluripotent stem cells into vascular cells

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Abstract

Blood vessels constitute a closed pipe system distributed throughout the body, transporting blood from the heart to other organs and delivering metabolic waste products back to the lungs and kidneys. Changes in blood vessels are related to many disorders like stroke, myocardial infarction, aneurysm, and diabetes, which are important causes of death worldwide. Translational research for new approaches to disease modeling and effective treatment is needed due to the huge socioeconomic burden on healthcare systems. Although mice or rats have been widely used, applying data from animal studies to human-specific vascular physiology and pathology is difficult. The rise of induced pluripotent stem cells (iPSCs) provides a reliable *in vitro* resource for disease modeling, regenerative medicine, and drug discovery because they carry all human genetic information and have the ability to directionally differentiate into any type of human cells. This review summarizes the latest progress from the establishment of iPSCs, the strategies for differentiating iPSCs into vascular cells, and the *in vivo* trans-plantation of these vascular derivatives. It also introduces the application of these technologies in disease modeling, drug screening, and regenerative medicine. Additionally, the application of high-tech tools, such as omics analysis and high-throughput sequencing, in this field is reviewed.

Key Words: Induced pluripotent stem cell; Blood vessels; Vascular organoids; Endothelial cells; Smooth muscle cells; Pericytes; Tissue engineering vascular graft

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Core Tip: Blood vessels play crucial physiological roles and are closely related to many human diseases. Although mouse or rats have been widely used in current biomedical studies, human specific-vascular bio- and patho-physiology are hardly to recapitulate because of the species differences between human and animals. The rise of induced pluripotent stem cells (iPSCs) provides a reliable method. Until now, iPSC technology and its differentiation into vascular cells or organoids provide valuable tools for studies of vascular diseases in the fields of disease modeling, drug development, regenerative medicine and gene manipulation.

INTRODUCTION

Induced pluripotent stem cells

Human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced PSCs (iPSCs), are characterized by unlimited proliferation and the ability to differentiate into three germ layers (ectoderm, mesoderm, and endoderm). ESCs are derived from the undifferentiated cell mass of human embryos, whereas iPSCs can be regarded as "artificial ESCs" obtained by reprogramming adult cells such as peripheral blood mononuclear cells, urine cells, skin fibroblasts, or hair keratinocytes with transcription factors[1]. Compared with ESCs, iPSCs overcome ethical issues such as embryo damage during ESC isolation, the limited cell source, and the immunogenicity of allogeneic transplantation. The availability of abundant cell sources has notably increased patients' psychological acceptance of sampling and transplantation, thereby enhancing promising clinical prospects in regenerative medicine, drug screening, disease modeling, and other related areas.

In 2006, Japanese scientists Takahashi and Yamanaka[2] discovered that when the genes related to stem cell pluripotency and self-renewal, including octamer-binding transcription factor 4 (*Oct4*), the sex-determining region Y-frame protein 2 (*Sox2*) gene, the proto-oncogene (*C-MYC*), and the epidermal zinc finger factor-related (*KLF4*) gene, were introduced into mouse fibroblasts by retroviruses, adult cells can be de-differentiated into cells with the potential for self-renewal and multidirectional differentiation. Such cells derived from somatic ones were called iPSCs. Subsequently, Takahashi *et al*[3] introduced these four pluripotent transcription factors into human skin fibroblasts to generate the first human iPSCs (hiPSCs) lines.

However, due to the existence of proto-oncogene *C-MYC* and the integration of retrovirus, this method has some limitations, such as the possibility of tumorigenesis, low induction efficiency, and poor ability of directed differentiation. To solve these problems, scientists performed further selection and optimization of transcription factors used for reprogramming. For example, in 2007, Yu *et al*[4] obtained iPSCs using different combinations of transcription factors (Oct4, Sox2, Nanog, and Lin28). In 2008, Kim *et al*[5] successfully reversed the pluripotency of neural stem cells using only Oct4 and Klf4. Subsequently, only transcription factors Oct3/4 have been considered indispensable, while Sox2, Klf4, and c-Myc have been considered substitutable[6]. Yu *et al*[7] obtained iPSCs by the non-integrated episomal method, providing an idea to avoid tumors caused by viral vectors. Anokye-Danso *et al*[8] demonstrated that expression of the miR302/367 cluster rapidly and efficiently reprograms somatic cells to an iPSC state without the need for exogenous transcription factors. Hou *et al*[9] used small-molecule compounds to reprogram mouse somatic cells into iPSCs. In 2017, Kim *et al*[10] showed that mechanical stretch stimulation could significantly increase the reprogramming efficiency. In the same year, Blanchard *et al*[11] discovered a new method of using antibodies to replace transcription factors during reprogramming, avoiding the potential risk of transcription factor introduction. In 2022, Guan *et al*[12], who created an intermediate plastic state that overcomes the barriers of human somatic cells to chemical stimulation due to a stable epigenome and reduced plasticity, successfully reprogrammed human somatic cells into pluripotent cells using exposure to small molecule chemical substances.

Until now, with research progress, multiple methods to reprogram various types of somatic cells into iPSCs have been developed[13], allowing targeted differentiation of iPSCs into various cells, tissues, and organs. Since then, enormous progress has been made in stem cell biology[14]. For example, iPSCs can generate vascular endothelial cells (VECs), vascular smooth muscle cells (VSMCs), and three-dimensional (3D) vascular organoids through a series of differentiation, providing new hope for disease modeling, drug development, and screening, as well as regenerative medicine research for vascular disorders.

Different methods of inducing differentiation

The induced differentiation of iPSCs into blood vessels is basically divided into two types: 2D cell differentiation and 3D vascular organoid differentiation.

2D CELL DIFFERENTIATION

iPSCs can be differentiated into VECs and mural cells. These differentiation protocols can be mainly divided into two categories: The embryoid body (EB) method and the monolayer cell induction method (Figure 1). Figure 1 provides a concise summary of various methods used to differentiate iPSCs into 2D vascular cells. The inner and outer rings represent differentiation toward VECs and mural cells, respectively, while the key steps of these modeling strategies are depicted in the schematic drawing.

In the EB method, iPSCs are first formed into embryoid bodies, stimulating the development of embryos, which can be further directed to differentiate into derivatives of blood vessels. The advantage of this method is that it simulates the physiological process of embryonic development and is closer to the physiological state of the human body. However, during initial induction, various growth factors can only act on peripherical cells of the EB, and cells in the central part might not differentiate completely. The induced adult cells might be a mixture of three germ layer sources, and the subsequent fluorescence-activated cell sorting (FACS) might also reduce the vitality of target cells. These limitations have led to a focus on using monolayers of extracellular matrix (ECM) proteins such as collagen IV and gelatin, resulting in the development of monolayer cell methods that induce iPSCs first into the mesoderm and then into specific cell types. This approach is fast and effective. However, this protocol requires the combined action of multiple cytokines, which are expensive, and the animals that developed exogenous cytokines might limit their further clinical application.

VECS

In 2000, Yamashita *et al*[15] found that Flk1+ cells derived from ESCs can differentiate into endothelial cells and mural cells. In 3D culture, vascular cells derived from Flk1+ cells can be organized into vascular structures composed of endodermal tubes supported by mural cells. Thus, Flk1+ cells can act as vascular progenitor cells to form mature blood vessels. Based on this, Narazaki *et al*[16] induced iPSCs into vascular progenitor cells for the first time. The iPSCs were first induced into mesoderm cells by placing original iPSCs on collagen IV-coated plates with differentiation medium (DM), including basal cell culture medium plus 10% fetal bovine serum and 5×10^{-5} mol/L 2-mercaptoethanol. Flk1, the earliest marker of differentiated endothelial and mural cells[15], was assessed. VESCs were then induced from Flk1+ cells in the medium containing human vascular endothelial growth factor (VEGF)165 and 8-bromo-cAMP[17]. VECs derived from this differentiation could form vascular-like structures and robustly and stably express CD31 or spinal muscular atrophy (SMA). Moreover, CD31$^+$ and SMA$^+$ cells could connect with each other and further differentiate into arterioles and venules.

In another study, Choi *et al*[18] co-cultured iPSCs from fibroblasts with mouse bone marrow stromal cell line OP9 and successfully differentiated iPSCs into CD34$^+$CD31$^+$CD43$^-$ endothelial cells. In 2011, Li *et al*[19] obtained endothelial cells from iPSCs differentiation by the EB method. This differentiation was promoted by the presence of VEGF165 and basic fibroblast growth factor (bFGF) in type I collagen. This method differs from previous protocols that usually involved isolating vascular lineage cells from spontaneously differentiated cells through EB formation or co-culture with mouse mesenchymal cells[20,21]. In contrast, Park *et al*[22] directly differentiated hiPSCs into cells of the mesodermal lineage by treating them with PD98059 and bone morphogenetic protein 4 (BMP4), which regulated the MEK/ERK and BMP4 signaling pathways. Later, Di Bernardini *et al*[23] discovered that microRNA-21 mediates the differentiation of iPSCs into ECs in the presence of VEGF, discovering a new signaling pathway that can be adjusted. In the same year, Lian *et al*[24] used glycogen synthase kinase 3 (GSK3) inhibitor CHIR99021 to activate the WNT signal in iPSCs, which then differentiated into CD34$^+$CD31$^+$ cells in the absence of exogenous FGF and VEGF. Subsequent differentiation assays showed that these CD34$^+$CD31$^+$ endothelial progenitor cells can further differentiate into functional ECs. Sahara *et al*[25] found that early administration of BMP4 and GSK3 inhibitors, alongside treatment with VEGF165 and inhibition of the Notch signaling pathway, resulted in rapid and efficient differentiation of hiPSCs into endothelial lineage cells. Using flow cytometry, they identified a fixed population of progenitor cells in this lineage, VEC$^+$CD31$^+$CD34$^+$CD14$^-$KDR^{high} endothelial progenitor cells, which showed higher angiogenesis and clonal proliferation potential in endothelial lineage cells. Moreover, Elcheva *et al*[26] successfully induced ESCs from hiPSCs by overexpressing mRNA modified with selected transcription factors such as ETV2/GATA2 or GATA2/TAL1/LMO2. In 2017, Zhang *et al*[27] found that in addition to the standard endothelial cell–inducing factors such as FGF, VEGF, and transforming growth factor (TGF)-β inhibitor SB431542, supplementation with NOTCH agonists (RESV) and inositol mono-phosphatase inhibitors (L690) increased the expression of arterial markers of hiPSCs-ECs and induced downregulation of venous markers. This resulted in the differentiation of hiPSCs into arterial-like endothelial cells, which showed increased nitric oxide production, decreased leukocyte binding, and improved response to shear stress. Other studies also showed that hiPSC-ECs exposed to high arterial-like shear stress promote the acquisition of arterial characteristics, and overexpression of the RNA binding protein QKI-5 guides the differentiation of hiPSCs into arterial ECs[28-30].
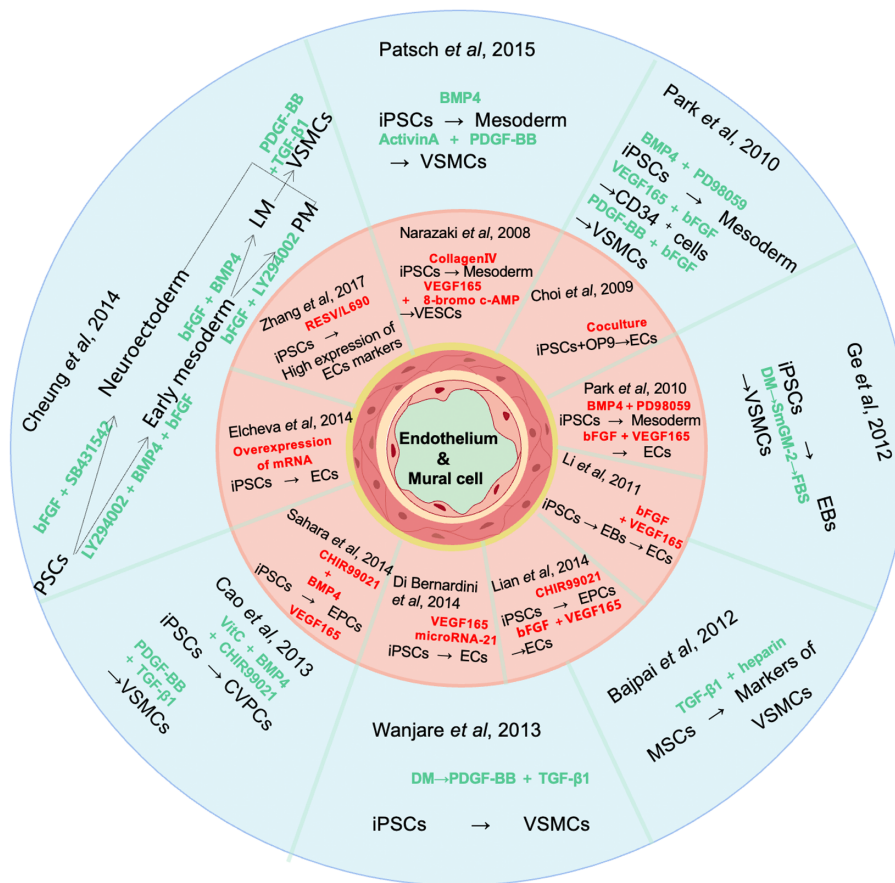
Figure 1 Modeling methods for endothelial cells and mural cells. Several methods for differentiating induced pluripotent cell into endothelial cells and mural cells have been developed. The procedures of these modeling strategies are presented here as a schematic drawing. The inner ring shows the modeling strategies for endothelial cells, while the outer ring shows those for mural cells. The complementary factors and differentiation nodes are the focus of this graph. VEGF: Vascular endothelial growth factor; TGF: Transforming growth factor; iPSC: Induced pluripotent cell; VSMC: Vascular smooth muscle cell; EB: Embryoid body; MSC: Mesenchymal stem cell; DM: Differentiation medium; CVPC: Cardiovascular progenitor cell; LM: Lateral mesoderm; PM: Paraxial mesoderm; VESC: Vascular endothelial stem cells; EC: Endothelial cell; EPC: Endothelial progenitor cell; BMP4: Bone morphogenetic protein 4; PDGF-BB: Platelet-derived growth factor-BB; FBS: Fetal bovine serum; bFGF: Basic fibroblast growth factor.

Mural cells (VSMCs and pericytes)

Previous studies showed that type IV collagen[31], retinoic acid[32,33], and growth factors platelet-derived growth factor-BB (PDGF-BB)[34,35] and TGF-β1[36-38] were involved in inducing the differentiation of VSMCs. In 2012, Ge *et al*[39] rapidly induced iPSCs into VSMCs by the method of EB formation according to the previously established human ESCs (hESCs) scheme. The brief process was as follows. First, iPSCs were differentiated into EBs and cultured in suspension for 6 d. EBs were transferred to gelatin-coated dishes and cultured with fresh DM for another 6 d. The cells were digested and placed on Matrigel-coated dishes, followed by culture in VSMCs growth culture medium (SmGM-2) for 1 wk. Then, the cells were transferred to gelatin-coated dishes and cultured in a serum-containing medium for 5 d. This method can induce VSMCs with up to 95% of calponin-positive cells without sorting by flow cytometry, being the simplest EB method to obtain high-purity VSMCs.

Wanjare *et al*[40] inoculated iPSCs onto plates coated with type IV collagen and cultured them in DM for 6 d. The differentiated cells were then reseeded on type IV collagen-coated plates, and 10 ng/mL PDGF-BB and 1 ng/mL TGF-β1 were added for final VSMCs differentiation. In this method, the derivative of synthetic VSMCs and contractility VSMCs were generated by changing the concentration of serum, PDGF-BB, and TGF-β1 in DM. Some studies also focused on the effects of intrinsic substrate properties (such as the elastic modulus and cross-linking density of hydrogels in a 3D static and dynamic environment) on the phenotypic transformation between synthetic and contractile types of human mural cells derived from hiPSC-derived organoids (ODMCs). They demonstrated that the phenotypic plasticity of ODMCs in response to 2D biological and 3D biological mechanical stimuli is equivalent to that of primary human VSMCs[41].

The main difficulty in studying the differentiation of iPSCs into VSMCs and pericytes is that they have different developmental origins, which are not confined to the mesoderm but can also originate from the neural crest. In 2014, Cheung *et al*[42] successfully induced iPSCs into neuroectoderm, lateral plate mesoderm, and paraxial mesoderm and then induced differentiation of cells from these different germinal layers to VSMCs by optimizing different combinations of small-molecule compounds. Thus, directional induction of VSMCs from different germinal sources was achieved. Afterward, Patsch *et al*[43] established a fast and efficient induction scheme using monolayer cell induction. First, cells were differentiated into mesodermal cells using WNT signaling pathway inhibitor BMP4; then, activin-A and PDGF-BB

were used for targeted differentiation of mesoderm cells. The entire procedure took 6 d to complete, and the induced cells were of high purity.

Furthermore, some researchers targeted intermediate cell populations such as cardiac progenitor cells and mesenchymal stem cells (MSCs). These intermediate cell populations derived from hPSCs, including hESCs and hiPSCs, play a significant role in the derivation of hPSCs to VSMCs. Park *et al*[22] demonstrated that CD34+ progenitor cells are an important source of VSMCs. Mesodermal cell lines were obtained from hiPSCs treated with PD98059 (a MEK/ERK pathway inhibitor) and BMP4, followed by VEGF165 and bFGF treatment for 6 d to obtain CD34+ cells. VSMCs were cultured in endothelial cell growth medium-2 (EGM-2) medium supplemented with PDGF-BB and bFGF for 15-21 d. Bajpai *et al*[44] generated VSMCs from an intermediate stage of MSCs using soluble signaling and ECM molecules. MSCs were treated with TGF-β1 (10 ng/mL) and heparin (30 mg/mL). After 5 d, MSCs expressed markers of VSMC differentiation, such as myosin heavy chain, α-SMA, calponin, and calmodulin. Zhang *et al*[45] cultured iPSC-MSCs in EGM-2 medium containing sphingylcholine (5 mmol/L) and TGF-β1 (2 ng/mL). After 3 wk, 50%-60% of the differentiated cells expressed VSMC markers, such as α-SMA and calponin 1. The cells also exhibited a spindle-like morphology and could contract under phenol treatment. Cao *et al*[46] developed an effective method for generating populations of early cardiovascular progenitor cells (CVPCs) from hiPSCs, key early developmental pathways involved in human cardiovascular norms and CVPCs self-renewal are modulated using a chemo-definition system containing bone BMP4, GSK3 inhibitors CHIR99021, and ascorbic acid. CVPCs were differentiated into VSMCs after 12 d of stimulation with 10 ng/mL PDGF-BB and 2 ng/mL TGF-β1. Additionally, CVPCs can also differentiate into other cardiovascular cells (such as cardiac muscle cells and endothelial cells) with high purity. Further studies found that tensile strain (cyclic uniaxial or circumferential) can increase elastin deposition and cell alignment and improve contractile responses, which further promote the maturation of hiPSC-derived VSMCs[47,48].

Co-differentiation and co-culture of VECs and VSMCs or pericytes

Kusuma *et al*[49] induced the co-differentiation of hPSC to generate early vascular cells (EVCs), which can mature into endothelial cells and pericytes. These cells can self-assemble to form microvascular networks in an engineered matrix. Monolayer cultures were employed without the need for specific differentiation-inducing feeder layers, EB formation, or sorting. Briefly, researchers developed a stepwise vascular lineage differentiation, in which EVCs were cultured in a medium supplemented with TGF-β inhibitor SB431542 and high VEGF concentrations to eliminate the pericyte-mediated inhibition of endothelial cell growth that often occurs when pericytes and endothelial cells are co-cultured[50]. VEcad+ cells were induced early during differentiation to ensure endothelial maturation. EVCs obtained in this way were highly purified (> 95%) for CD105 and CD146 expression, which are surface antigens common to ECs and pericytes. This population, composed of CD105+CD146+VEcad+ and CD105+CD146+ PDGFRβ+ subtypes, contained the cells required for microvascular construction and formed a network within collagen and hyaluronic acid (HA)-based hydrogel systems. This integrative approach leverages the inherent self-assembly ability of derived two-cell populations to create microvessels in a deliverable matrix, which has tremendous implications for vessel construction and regenerative medicine.

Orlova *et al*[51] established 2D co-culture of hPSC-derived ECs and pericytes. This protocol overcomes the shortcomings of previous efforts to standardize the culture and differentiation conditions of hPSCs by not using fully defined reagents, often leading to difficult-to-replicate results or low and difficult-to-scale up differentiation efficiency. In this protocol, mesodermal differentiation of hPSC was induced by BMP4, activin-A, GSK3β inhibitor CHIR99021, and VEGF. Mesenchymal cells were observed on the third day of differentiation. The mesoderm-inducing factor was removed and replaced with a standard medium supplemented with VEGF and TGF-β inhibitor SB431542 to support EC proliferation. On day 10, ECs were isolated using anti-CD31 antibody-coupled magnetic beads. If necessary, pericytes could be obtained from CD31- cells while ECs were isolated. Subsequently, ECs and pericytes could be co-cultured. However, ECs would first adhere to the substrate and form EC islands surrounded by pericytes, which can organize into vascular tube-like structures mimicking the formation of primary vascular plexuses. This co-culture approach allows the study of endothelial network formation and endothelial cell-pericyte interactions. For example, if the disease-affected vascular cell type is unknown, healthy donor or patient-derived ECs can be co-cultured with pericytes from a healthy or affected individual.

3D VASCULAR ORGANOIDS

In 2019, Wimmer *et al*[52] reported a method for direct differentiation of hiPSCs into self-assembling vascular organoids. hiPSC aggregates were first generated and subsequently induced for mesoderm differentiation by WNT activation (CHIR99021) and BMP4 stimulation. These aggregates were directly differentiated and grew into sprouting blood vessels in 3D collagen I-Matrigel gel driven by VEGF165 and bFGF in the presence of serum. The endothelial tubes formed lumens that interacted tightly with pericytes and were covered by a continuous basement membrane. Differentiated vascular organoids could be transplanted as a whole into the renal capsule of immunocompromised mice, entered the mouse vasculature, formed intact human blood vessels (endothelial and mural cells), and stabilized for up to 60 months (end of observation). The vascular organoids were 3D, recapitulating not only the endothelial network and endothelial-pericyte interactions but also vascular lumen formation and basement membrane deposition, which is significantly different from previously published protocols (Figure 2). Figure 2 compares several main strategies for constructing 3D vascular organoids and endothelial-pericyte co-culture. Both approaches simultaneously generate various types of vascular cells, with certain differences and similarities in the main procedures and the small-molecule compounds used.
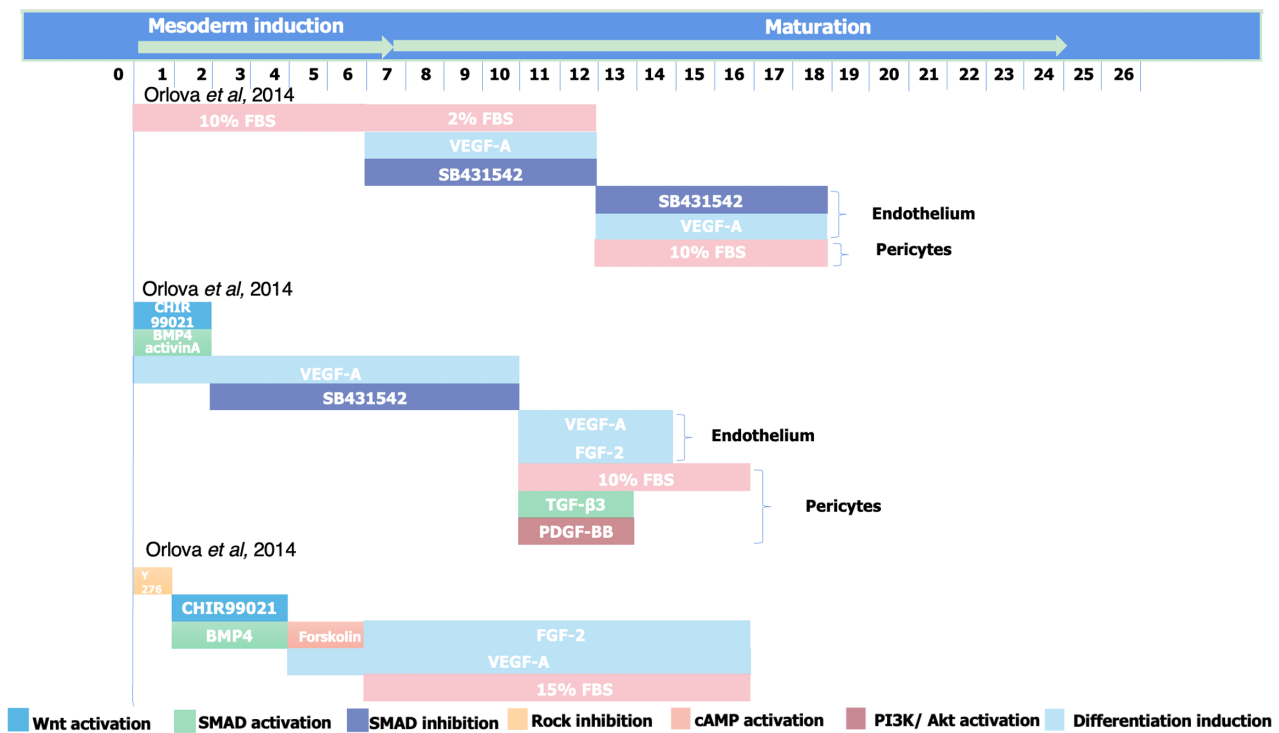
Figure 2 Methods for modeling three-dimensional vascular organoid or pericyte-endothelium co-culture. Both co-differentiation or co-culture of endothelium and pericyte and three-dimensional vascular organoids can model different cell types of vascular cells at the same time. This figure shows the main procedures of these methods, revealing the similarities and differences in the order of activation and inhibition of key pathways related to differentiation. FBS: Foetal bovine serum; VEGF: Vascular endothelial growth factor; BMP4: Bone morphogenetic protein 4; FGF-2: Fibroblast growth factor 2; TGF: Transforming growth factor; PDGF-BB: Platelet-derived growth factor-BB.

Although the blood vessel organoids constructed using this approach have demonstrated irreplaceable advantages in disease modeling, the developmental process of single cell types and their structural differences (arterial *vs* venous endothelial cells) are still unknown. Nikolova *et al*[53] solved this problem by comprehensive single-cell transcriptome analysis of vascular organoids at different developmental stages. After the vascular lineage induction, PDGFRβ+ pericytes were detected on day 3, while CD31+ endothelial cells appeared on day 4, indicating that pericyte development preceded endothelial cell maturation. The transcriptome characteristics of endothelial cells in organoids are dynamic, similar to the development *in vivo*. On the 4th d of induction, a transient up-regulation of endothelial cell fate transcription factor ETV2 was observed in a small cell population, followed by the up-regulation of common endothelial cell markers. Until the 14th d of induction, ECs showed the same transcriptional characteristics as arterial ECs. Subsequently, venous endothelial cell markers were expressed on the 21st d. The reason for such conversion is not clear.

In the current methods of generating blood vessel organoids, Matrigel is employed as the ECM for vascular sprouting. Matrigel is often used as a matrix for culturing various cell types because it is rich in ECM proteins such as type IV collagen, laminin, and nestin. However, the exact composition can vary between different production batches. To solve this problem, Schmidt *et al*[54] proposed a vascular organoid induction protocol without Matrigel. They used a 96-well plate coated with conical agarose to aggregate iPSCs for subsequent organoid culture. This study confirms that 3D vascular structures can be obtained in organoids even without the use of highly variable matrices and the long-term effects of angiogenic factors. This reflects the intrinsic ability of cells to self-organize in the appropriate tissue environment.

CO-TRANSPLANTATION OF VECS AND PERICYTES

In a previous study, Kusuma *et al*[49] used a synthetic HA matrix to allow the transplantation of a multicellular (ECs and pericytes) network into mice. These cells were differentiated from EVCs (defined as CD105+CD146+) and subsequently embedded into host vessels. This finding brings new inspiration for the application of iPSC-derived vascular cells in regenerative medicine. However, this protocol has limited application due to difficult functional demonstration of human blood vessels, as it generates chimeric blood vessels of human and mouse vascular cells. In 2013, Samuel *et al*[55] reported a method for generating functional and durable engineered blood vessels from hiPSCs by co-transplanting hiPSC-derived ECs with mouse or hPSC-derived mesenchymal progenitor cells (MPCs), resulting in the generation of functional blood vessels *in vivo*. The researchers initially optimized selection markers and a culture system to efficiently expand hiPSC-derived EPCs and assessed the vasculogenic capacity of these cells. Subsequently, they induced MPCs and confirmed their ability to support vasculogenesis when co-implanted with hiPSC-ECs *in vivo*. However, in this study, hPSC-derived

MSCs only maintained the human endothelial network for about 28 d, limiting the study of their long-term effects on the intact human vascular system. Ren *et al*[56] generated functional pulmonary blood vessels by repopulating the vascular compartments of decellularized rat and human lung scaffolds with ECs and pericytes derived from iPSCs. In this study, cells were delivered to a lung scaffold by co-seeding and a two-stage culture protocol with endothelial and pericytes. Similar to the organization of natural vasculature, ECs form an interconnected network, and pericytes adhere individually around the network. The endothelial cell coverage of the regenerated rat lung was about 75% of that of the native human lung, and a continuous polarized vascular lumen was formed and perfused 3 d after transplantation.

HIPSC-DERIVED VASCULAR CELLS AND ORGAN-ON-A-CHIP TECHNOLOGY

Researchers developed a number of micro-engineered cell culture devices known as "microfluidic chips" to study the physiology of organs and tissues *in vitro*. These microfluidic chips consist of submillimeter rectangular culture chambers containing medium or 3D hydrogel. The tissue arrangement observed in living organs can be reconstructed in these chips to study physiology in organ-specific contexts and develop specialized *in vitro* disease models[57].

Organ-on-a-chip technology has been applied to simulate key aspects of vascular physiology. In one study, primary human ECs were mixed with hESC-derived pericytes within microfluidic channels to generate self-organizing 3D structures with close interaction between pericytes and ECs[58]. They injected a mixture of human umbilical vein endothelial cells, hESCs-derived pericytes, and rat tail collagen I into a polydimethylsiloxane microfluidic channel. After 12 h, these cells self-organized into a single, long tube resembling a blood vessel that conformed to the contours of the channel. In another study, hiPSC-derived ECs cultured in a custom hydrogel inside a microfluidic device generated a 3D capillary network that was stable for at least 14 d[59]. In a study conducted in 2021[60], researchers developed a 3D multicellular blood vessel chip model entirely based on hiPSCs, in which hiPSC-derived vascular cells self-organized to form stable microvascular networks in a fibrin hydrogel microenvironment. Functional studies demonstrated that engineered blood vessels can respond to vasoactive stimulation as expected.

HIPSC-DERIVED VASCULAR CELLS AND TISSUE-ENGINEERED VASCULAR GRAFTS

Several studies explored the use of hiPSCs-derived vascular cells to create hierarchically organized 3D scaffold blood vessels[61-64]. Functional vascular cells such as VSMCs, ECs, and mesenchymal cells or their precursor cells derived from hiPSCs were implanted into polymeric scaffolds in a bioreactor for vascular tissue growth to generate tissue-engineered blood vessels. However, these studies had some limitations, namely the lack of a combination of hiPSC-ECs and hiPSC-MCs on *in vitro* 3D scaffolds to construct complex human perfusion vessel models. To address this problem, Meijer *et al* [65] used ECs and mural cells derived from vascular organoids to grow electrospinning polycaprolactone double urea 3D vascular scaffolds in solution and form naturally stratified vascular tissues similar to human primary vascular cells, eliminating the need to establish and maintain two independent hiPSC differentiated cultures.

Over the past decades, numerous efforts have been made to develop *in-vitro* vascular models based on the human body. The most valuable human vascular models based on iPSC technology are introduced above. The complexity and the ability to faithfully represent *in vivo* processes of these human-based vascular models are increasing. However, at the same time, the ease of construction and scalability usually decrease (Figure 3).

After more than 10 years of development, iPSC technology has played an increasingly important role in the fields of disease modeling, drug development, regenerative medicine, and gene manipulation[14]. iPSC technology and its differentiation into vascular cells also provide valuable tools for researchers in the study of vascular diseases.

DISEASE MODELING

There are several difficult issues to overcome in the study of the pathophysiology of vascular diseases. Patient-specific iPSC disease models have many unique advantages compared with traditional disease models[14,66]. First, patient-specific iPSC disease models can more realistically and comprehensively reflect the pathology of the disease compared to transgenic animal models because they carry all the genetic information of the patient. Second, the process of iPSCs differentiating into adult cells can mimic the embryonic differentiation and development of adult cells, which can be used to study the evolution of various disease stages and then to find the best time window for intervention. Third, iPSCs can be expanded indefinitely to provide sufficient specimens for the study of rare diseases and can differentiate into various adult cells, which can provide sufficient raw materials for the study of VSMCs, which are difficult to culture *in vitro*. Fourth, iPSCs can be directed to differentiate into cells of any specific origin, which can solve the problem that vascular tissues come from different germinal layers and can specifically simulate the growth of vascular tissues at any location, being more conducive to the establishment of special disease models. These features allowed researchers to establish various vascular disease models using iPSC technology, providing new ideas for the study of vascular diseases. Additionally, the development of high-tech tools such as next-generation sequencing (NGS), bioprinting, and biomaterial development has begun a new era in medicine. When iPSC technology, with its advantages of simplicity and cost-effectiveness in generating *in vitro* cell models, is combined with these cutting-edge technologies, it becomes an excellent choice for constructing models of rare diseases, including rare single-gene vascular diseases. Until now, researchers have
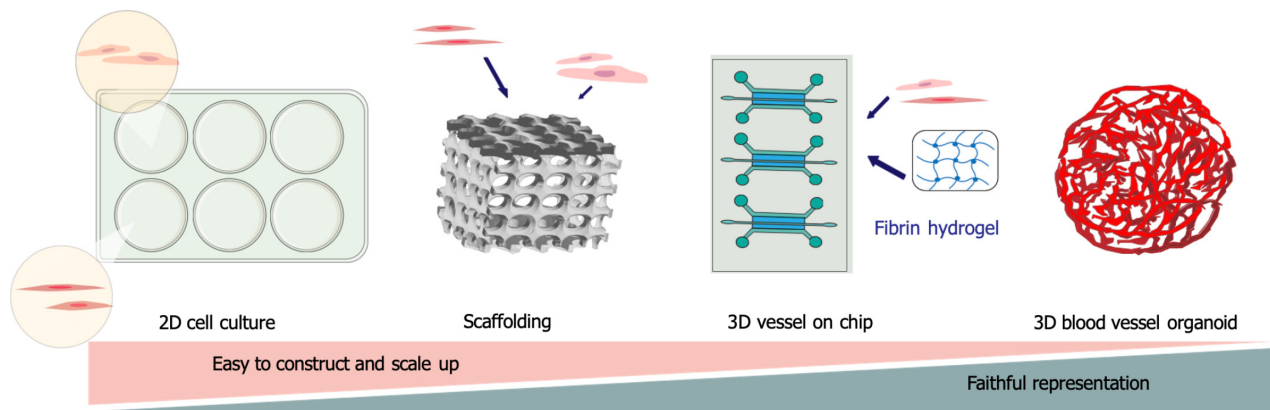
Figure 3 Summary of induced pluripotent cells-derived vascular derivatives. Various human vascular models based on induced pluripotent cell technology have been developed, including two-dimensional vascular cell culture, tissue-engineered vascular grafts, vascular organ chips, and three-dimensional vascular organoid construction. Alongside the increasing complexity and the ability to faithfully represent the *in vivo* process, the ease of handling and scalability of these human-based vascular models usually decrease. 3D: Three-dimensional.

constructed numerous vascular disease models using iPSC technology, which are discussed below.

2D vascular cells models

The induction of 2D vascular cells, including ECs and SMCs, from patient-specific iPSCs is a crucial step in constructing patient-specific disease models. For instance, VECs induced from iPSCs have been successfully utilized to investigate the pathophysiological mechanisms of familial pulmonary arterial hypertension (FPAH). Researchers discovered that compared to ECs derived from healthy control iPSCs, ECs derived from patients with BMP receptor 2 mutations exhibit reduced adhesion, migration, and angiogenesis potential. This study played a critical role in identifying, evaluating, and developing alternative treatment options for FPAH patients[67]. In another study, researchers utilized SMCs derived from iPSCs to investigate the pathological mechanism of aortic aneurysm (AA) in Marfan syndrome. The model successfully replicated the phenotype observed in the aorta of Marfan syndrome patients, aiding the identification of new therapeutic targets (such as p38 and Kruppel-like factor 4) and offering an innovative human platform for testing new drugs[68].

Vascular cells co-culture model

Using a co-culture model of iPSC-derived ECs and mural cells, researchers investigated the role of the ECM at the junction of blood and brain in the pathological progression of COL4A1/A2 mutation-related cerebral small vessel disease. This study found that the mutation can induce mural cell apoptosis, migration defects, ECM remodeling, and transcriptome changes. Importantly, these mural cell defects have a detrimental effect on endothelial cell tight junctions through a paracrine pathway. The COL4A1/A2 mutant model also expresses high levels of matrix metalloproteinases. Inhibition of matrix metalloproteinase activity can partially prevent ECM abnormalities and mural cell phenotype changes[69].

Organs-on-chips model

AA is a potentially fatal disease characterized by aortic dilatation that can only be treated by surgery or endovascular procedures. The underlying mechanism of AA is still unclear due to the heterogeneity of the segmental aorta and the limitations of existing disease models, resulting in insufficient investigation into early preventive treatment. Liu *et al*[70] established a comprehensive lineage-specific VSMCs-on-a-chip model using hiPSCs to generate cell lineages representing different segments of the aorta. They integrated this organ chip model with high-tech tools, such as transcriptomics, and conducted many experiments, including RNA sequencing, real-time reverse transcriptase-polymerase chain reaction, immunofluorescence, western blotting, and FACS analyses, to study the heterogeneity of the response of the segmental aorta to tensile stress and drug testing. These differences might be associated with the distinct transcription profiles of different lineage-specific VSMCs under tension stress. Furthermore, the organ chip exhibited contractile physiology and perfect fluid coordination, making it conducive to drug testing and demonstrating heterogeneous segmental aortic responses. This model has been evaluated as a novel and appropriate complement to animal models of AA for identifying differential physiological and pharmacological responses in different parts of the aorta. The system has the potential for future disease modeling, drug testing, and personalized treatment for patients with AA.

3D organoids model

3D vascular organoids also offer unique advantages over traditional 2D cell culture models in the study of vascular diseases. These vascular organoids, derived from patients, closely resemble natural blood vessels, containing important vascular cell types such as mural cells and ECs and retaining patient-specific metabolic memory. This feature enables blood vessel organoids to better simulate natural pathophysiological responses. In 2019, Wimmer *et al*[71] constructed a model of diabetic vasculopathy using vascular organoid technology. In another study, researchers constructed a vascular

organoid model of CADASIL, offering a new opportunity to explore gene therapy strategies[72]. In a study investigating the mechanisms of endothelial injury and coagulation disorders caused by severe acute respiratory syndrome coronavirus 2[73], human vascular organoids derived from iPSCs were used to model endothelial damage resulting from viral infection. Longitudinal serum proteomics analysis identified abnormal complement patterns driven by the amplification cycle regulated by complement factors B and D (CFB and CFD) in critically ill patients. This aberrant complement pattern initiated endothelial damage, neutrophil activation, and organoid-derived human blood vessel-specific thrombosis, as confirmed by *in vivo* imaging. These results highlight the role of the alternative complement pathway in exacerbating endothelial injury and inflammation and suggest the potential of targeted therapy for CFD in severe virus-induced inflammatory thrombosis outcomes.

These models not only help us understand the pathological mechanisms of vascular diseases and study the common molecular mechanisms shared between rare and common vascular diseases but also provide potential treatment targets and new avenues for studying vascular diseases.

DRUG DEVELOPMENT

hiPSCs offer more advantages than animal models in clinical testing for new drug development and cytotoxicity studies. iPSCs disease models can be used for drug screening, not only to achieve individualized drug treatment but also for drug toxicity screening to improve the safety of clinical drug trials. Moreover, the unlimited expansion capacity and the ability to simulate all stages of embryonic development provide endless samples for high-throughput drug screening to develop new drugs and identify highly efficient drugs.

REGENERATIVE MEDICINE AND PRECISION MEDICINE

In regenerative medicine, stem cells can be used to promote endogenous regenerative repair or replace damaged tissues after cell transplantation. Many experiments confirmed the feasibility of iPSCs-based cell therapy. iPSC has been used as a renewable source of vascular cells, especially VSMCs. Some studies showed that iPSC-derived VSMCs secrete substances such as VEGF when encapsulated in collagen scaffolds, promoting wound healing[74].

iPSCs disease model can be used for precision medicine research. Using CRISPR and other gene editing technologies to correct defective genes at the iPSC level can not only further verify the relationship between gene defects and disease occurrence but also provide opportunities for achieving precision treatment. For example, scientists established a CADASIL iPSC model and corrected NOTCH3 mutation using the double AAV split-ABEmax system. This provides a valuable model for studying pathogenesis and developing clinical treatment strategies[72]. Moreover, Song *et al*[75] used CRISPR/Cas9 to establish a patient-specific syngeneic iPSC control, differentiating it into ECs, thereby eliminating differences in genetic background and other aspects. Then, they studied the pathological mechanism of Fabry disease and demonstrated phenotypic abnormalities in FD iPSC-derived ECs (FD-ECs), including intracellular Gb3 accumulation, autophagy flux damage, and production of reactive oxygen species. These abnormalities were rescued in isogenic control iPSC-derived ECs (corrected FD-ECs).

CONCLUSION

iPSCs have become a revolutionary tool in regenerative medicine, with great potential in disease modeling, drug screening, and cell therapy. However, there are still some problems and challenges that need to be addressed. Many researchers aim to overcome these existing obstacles and expand the scope and application of iPSCs and their derivatives.

Moral and regulatory issues

Although iPSCs have addressed numerous ethical concerns associated with ESCs and offer significant potential for applications, research related to them has still sparked many ethical debates. For example, the transplantation of patient-derived iPSCs and their derived cells or organoid products into non-human animals to observe *in vivo* phenotypes has raised ethical concerns regarding the creation of chimeric organisms. Chimeric research falls under the category of animal research, and many animal studies are harmful, fatal, and non-therapeutic experiments conducted on vulnerable groups lacking the capacity to provide consent, aiming to benefit less vulnerable groups. Furthermore, many people are concerned that chimeric research raises questions about playing the role of God: When scientists use human cells to create non-human animals, they are essentially creating new creatures and crossing traditional species boundaries. In this process, scientists might be perceived as violating the inherent dignity of human beings by creating and destroying new forms of life for their own purposes[76]. Thus, balancing scientific progress and ethics remains a complex issue that cannot be ignored. It necessitates sustained dialogue and joint efforts from all parties. Furthermore, the establishment of a regulatory framework is necessary to ensure the safe and reliable use of PSCs as a powerful tool in scientific research and clinical applications.

Challenges and limitations

There are still numerous challenges in the study of iPSCs and their derived vascular cells or vascular organoids. The first consideration is the efficiency and safety of the reprogramming techniques used to generate iPSCs. Some studies found that current reprogramming methods might result in genetic abnormalities and epigenetic changes that can impact cell behavior[77]. Furthermore, the tumorigenicity of iPSCs makes their medical safety controversial. Local microenvironment changes resulting from the transplantation of iPSCs and their derivatives might also induce carcinogenic changes in host cells[78]. Moreover, iPSCs and their derivatives have inherent limitations. The differentiation of iPSCs into 2D vascular cells is valuable for creating patient-specific *in vitro* models to study the pathophysiology of individual blood vessel cell types. However, these approaches cannot reflect the interactions among different cell types or the response of blood vessels to varying blood flow parameters. Tissue-engineered vascular grafts, which are more complex vascular models with a certain integrity, lack the ability to fully represent all developmental stages and might overlook crucial intermediate cell types. Although 3D vascular organoids encompass various cell types, they only partially simulate the function and structure of blood vessels, making it challenging to completely replicate the complexity of real blood vessels. Additionally, they can only maintain activity and function for a limited duration. Maintaining long-term stability and fully reflecting the interaction and overall effect between different organs of the body are difficult. Moreover, the cost of establishing and maintaining iPSC technology is high, and the requirements for experimental conditions and technology are strict. Difficulties in expanding the scale of production also cannot be ignored.

Prospects and opportunities

While there are still some challenging problems associated with iPSC technology that need further resolution, recognizing that opportunities and challenges always go hand in hand is important. Personalized medicine is gaining momentum, and the development of NGS technology has significantly enhanced our ability to identify genes with pathogenic changes in rare diseases[79,80]. Once pathogenic gene mutations are identified in patients, it becomes possible to generate iPSCs and their induced 2D vascular cells, vascular organoids, organ chips, and tissue-engineered vascular grafts. These *in vitro* models can be utilized as patient-specific disease models to facilitate further studies of pathological mechanisms and provide a platform for high-throughput drug screening. The therapeutic potential of iPSCs is also receiving significant attention. Currently, the therapeutic effectiveness of transplanting iPSCs and their derived cells has been validated in several clinical and animal studies. The use of gene editing to correct such cells for the treatment of diseases shows promise as a personalized treatment approach. Combined with the continuous advancement of cutting-edge technologies such as bioprinting and nanotechnology in biomaterial development, their integration with iPSC technology offers new hope for the study of rare vascular diseases.

The study of hiPSCs and their derivatives has consistently been a focal point in medical research. Given the substantial social and economic burden of vascular diseases, researchers have been dedicated to developing more effective models for transformative research. The differentiation of iPSCs into vascular cells represents an undeniable cutting-edge technology that holds great promise for improving the lives of patients with vascular diseases. It paves the way for advancements in vascular regeneration and repair.

FOOTNOTES

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Author contributions: Jiao YC wrote the manuscript and designed the figures; Wang YX and Liu WZ collected the references; Xu JW provided the input in writing the paper; Zhao YY modified the manuscript; Yan CZ and Liu FC designed the manuscript and approved the final manuscript for publication; and all authors read and approved the final manuscript. Yan CZ and Liu FC contributed equally to this work. The choice of these researchers as co-corresponding authors acknowledges and respects this equal contribution, while recognizing the spirit of teamwork and collaboration of this study.

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