CORE
Metadata, citation and similar papers at core.ac.uk
Provided by IUPUIScholarWorks

The Differentiation, Evaluation, and Application of Human Induced Pluripotent Stem Cell Derived Endothelial Cells

Yang Lin[1, 2†], Chang-Hyun Gil[1†], Mervin C. Yoder[1, 2*]

1. Department of Pediatrics, Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana, USA
2. Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA

Running title: **Human iPSC derived EC**

Submitted to ***Arteriosclerosis, Thrombosis, and Vascular Biology***

[†]These two authors contributed equally to this work.

*Correspondence: Mervin C. Yoder, MD

Wells Center for Pediatric Research

1044 W. Walnut Street, R4-W125

Indianapolis, IN 46202

Tel, +1-317-274-4738

E-mail, myoder@iu.edu

This is the author's manuscript of the article published in final edited form as:

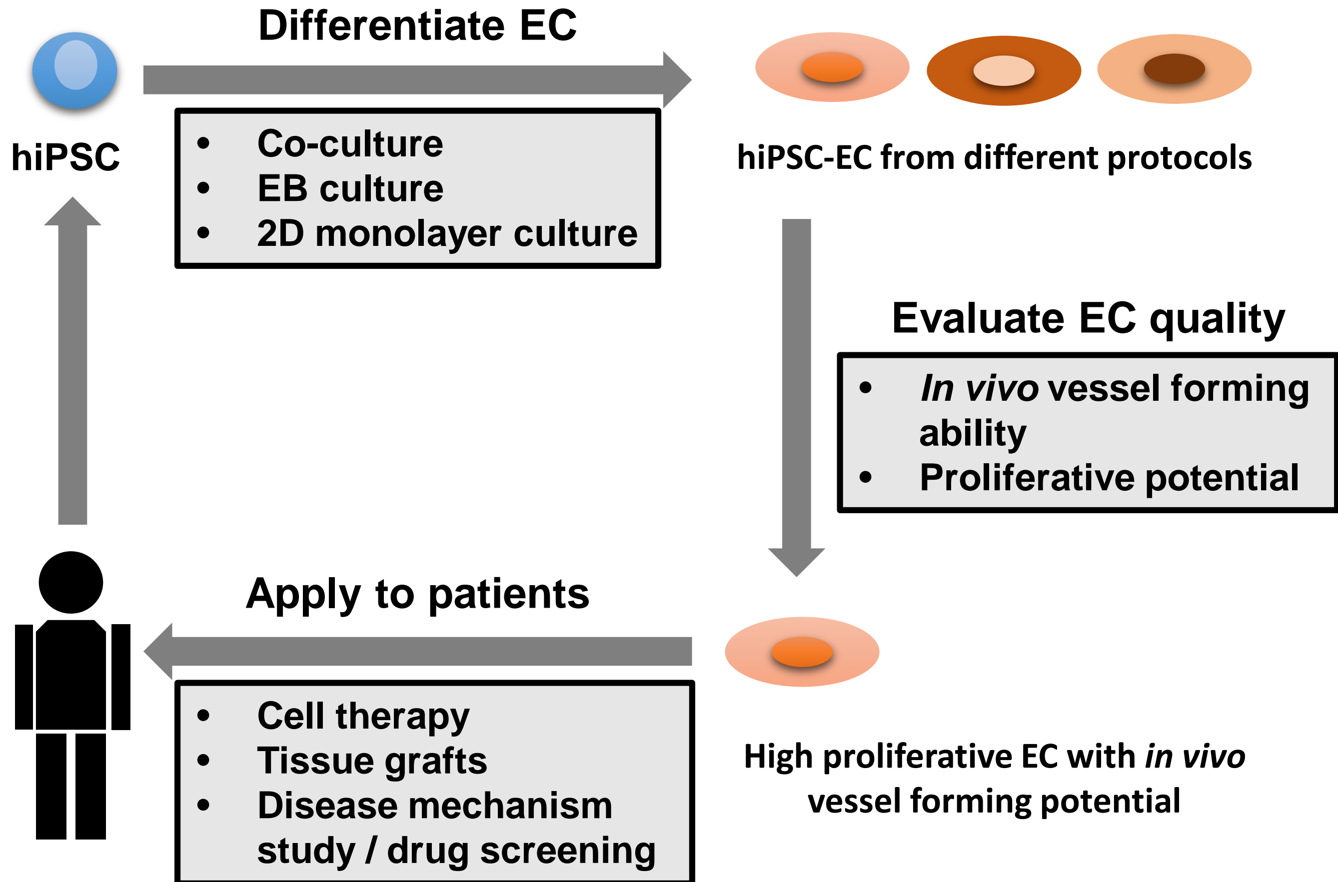
Lin, Y., Gil, C.-H., & Yoder, M. C. (2017). Differentiation, Evaluation, and Application of Human Induced Pluripotent Stem Cell–Derived Endothelial Cells. Arteriosclerosis, Thrombosis, and Vascular Biology, 37(11), 2014–2025. https://doi.org/10.1161/ATVBAHA.117.309962

Abstract

The emergence of induced pluripotent stem cell (iPSC) technology paves the way to generate large numbers of patient-specific endothelial cells (EC) that can be potentially delivered for regenerative medicine in patients with cardiovascular disease. In the last decade, numerous protocols that differentiate EC from iPSC have been developed by many groups. In this review we will discuss several common strategies that have been optimized for human iPSC-EC (hiPSC-EC) differentiation and subsequent studies that have evaluated the potential of hiPSC-EC as a cell therapy or as a tool in disease modeling. In addition, we will emphasize the importance of using *in vivo* vessel forming ability and *in vitro* clonogenic colony forming potential as a gold standard with which to evaluate the quality of hiPSC-EC derived from various protocols.

Key Words: cell therapy ■ differentiation ■ endothelial cell ■ endothelial colony forming cell ■ induced pluripotent stem cell

Graphic abstract



Nonstandard Abbreviations and Acronyms

CRISPR/Cas9	Clustered regularly-interspaced short palindromic repeats-associated protein 9
CVD	Cardiovascular disease
EB	Embryoid body
EC	Endothelial cell
ECFC	Endothelial colony forming cell
ESC	Embryonic stem cell
iPSC	Induced pluripotent stem cell
TALEN	Transcription activator-like effector nucleases

Introduction

Cardiovascular diseases (CVD), which are often triggered by endothelial dysfunction, represent one of the leading causes of mortality in the world. In 2015, over 17 million deaths, which represents 31% of global deaths, were caused by CVD.[1] The treatment of CVD through endothelial replacement is hindered by the lack of methods to isolate sufficient numbers of functionally normal autologous endothelial cells (EC) for transplantation and our limited understanding of how endogenous endothelial cells become dysfunctional during development of various cardiovascular diseases. The innovation of human induced pluripotent stem cells (hiPSC), cells that are reprogrammed from somatic cells to an embryonic stem cell (ESC)-like pluripotent state,[2, 3] presents an opportunity for us to generate large numbers of patient-specific EC that can be used for transplantation, drug screening, or studies to probe the mechanisms for endothelial dysfunction in certain disease states. Here we will retrospectively catalog and survey the methods developed by various groups to differentiate hiPSC to EC. We will focus on several common topics shared by all the papers, including the strategies for hiPSC-EC differentiation, the importance of functional and phenotypic testing of the hiPSC for quality control, and the potential applications of hiPSC-EC as a cell therapy for human subjects. Articles discussing human ESC derived EC derivation, PSC-EC differentiation from non-human species, and the development of other cell types through EC intermediates, like hemogenic endothelium, will not be covered here and discussions on these topics can be found elsewhere.[4-7]

Strategies for hiPSC-EC differentiation

Methods to differentiate EC from hiPSC can be divided into 3 general categories: 1) Stromal cell co-culture, 2) Embryoid body (EB) differentiation, and 3) Feeder-free monolayer differentiation (Figure 1).

The methods to differentiate EC from hiPSC by co-culture with stromal cells, usually murine bone marrow derived stromal cell lines like OP9 or M10B2, were mainly used in some of the earliest studies in the field.[8-11] This is an undirected differentiation strategy in which the frequency of differentiated EC is generally low and the EC produced were often mixed with other cell types including hematopoietic cells,[8] smooth muscle cells,[9, 10] and inevitably murine stromal cells. Thus this method is not ideal for the generation of a large number of EC for regenerative medicine applications.

EB methods rely on the spontaneous differentiation of aggregated hiPSC in the context of a self-assembled three dimensional (3D) structure. The development of many cell lineages in EB seems to recapitulate the progression of early embryonic development. Thus, the differentiation of EB is also not a fully controlled process and single step long term (10-12 days) EB differentiation method usually gives rise to a low EC yield.[12-22] Many researchers differentiate EB for a shorter period (3-6 days), usually in the presence of added growth factors that promote mesoderm and/or endothelial lineage specification including BMP4, Activin A, bFGF, and VEGF. At this stage, mesoderm and immature endothelial cells are robustly induced. These cells, together with other differentiated cell types from EB,[23-34] or enriched by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) via the use of monoclonal antibodies that identify the expression of mesodermal and/or endothelial cell surface markers like CD309 (KDR),[35-40] CD34,[37] and CD31 (PECAM-1),[35] are then expanded/enriched in monolayer culture conditions that favors the growth of EC. Some protocols developed using this strategy have differentiated functional EC from human hiPSC that exhibited the ability to form *in vivo* vessels.[24, 25, 29, 34, 36, 38]

The final general strategy is to culture a monolayer of hiPSC on a matrix coated culture plate and treat them with different molecules or growth factors in a timed fashion to dictate the progressive differentiation from PSC to mesoderm and finally towards the EC lineage. To date,

the protocols that have led to some of the highest EC yields were developed using this general strategy.[25, 41, 42] Most protocols using this strategy can be divided into a mesoderm differentiation phase and an endothelial differentiation phase. Some protocols will change the culture medium and growth factors between the mesoderm and endothelial stages, but without disturbing the cultured cells,[25, 41-62] while others purify the mesoderm cells or immature EC through detection of cells expressing KDR,[54, 63-65] CD34,[66-68] MESP1,[69] or CD31[67] between these two phases. Once purified the cell populations are further optimized to generate homogenous EC preparations.

Manipulating the signaling pathways that specify mesoderm differentiation from the hiPSC is common to these monolayer differentiation systems. Thus, growth factors like Activin A, BMP4, and bFGF are commonly used. GSK-3 inhibitors like CHIR99021 and BIO are also frequently added to the medium during this phase to promote the canonical Wnt signaling pathway which stimulates the differentiation of the hiPSC derived mesoderm towards the endothelial lineage,[25, 30, 37, 43, 44, 47, 48, 50, 55, 56, 59, 60, 62, 63, 66, 67, 69] although the effectiveness of GSK-3 inhibition in enhancing EC specification has been questioned.[63] Endothelial lineage cells are expanded in the second phase. VEGF is the most common growth factor that is used during this phase. Inhibition of the TGF-β signaling pathway has been reported to be crucial for promoting the endothelial specification from mesoderm cells and the maintenance of EC (to avoid endothelial to mesenchymal transition) in some protocols.[30] Thus SB431542, an inhibitor of TGF-β signaling pathway, has often been included in many protocols to maintain and expand EC in the second phase.[30, 43, 44, 49, 51, 57, 58, 61]

To further improve the differentiation efficiency of EC from hiPSC, a deeper understanding of the development of the EC lineage is required. Researchers are trying to discover new signaling pathways or develop novel culture conditions to improved EC differentiation. Park *et al.*[68] have reported that inhibition of the MEK/ERK pathway by addition of the inhibitor PD98059 during the mesoderm induction stage increased the production of EC. However, in Lian *et al.*'s study,[66]

addition of the MEK inhibitor PD0325901 decreased the production of $CD31^{+}CD34^{+}$ EC. Sahara *et al.*[25] reported that inhibition of the Notch signaling pathway during the late phase of EC differentiation, either by transcript knockdown or by addition of the γ-secretase inhibitor DAPT, led to a higher efficiency of EC production. Liang *et al.*,[51] showed that miR-495 could promote the production of EC from hiPSC and improve the angiogenic potential, as well as, the *in vivo* vessel forming ability of hiPSC-EC via interacting with vascular endothelial zinc finger 1. Cochrane and colleagues reported that the RNA binding protein Quaking isoform 5 stabilizes STAT3 and activates the expression of KDR, thus promoting EC differentiation and angiogenesis functions.[36] In addition, cyclic adenosine monophosphate (cAMP) has also been added in the EC phase of differentiation to enhance EC enrichment.[54, 64] Zhang *et al.*[70] promoted the differentiation of hiPSC into EC in a 3D porous fibrin scaffold and 45% of hiPSC were differentiated to EC. They reasoned that the higher EC differentiation efficiency was due to the 3D surface tension provided by the scaffold.[70] Recently, Zhang *et al.*[69] suspended hiPSC derived $MESP1^{+}$ mesodermal cells in a 3D biodegradable gelatin based scaffold and observed more enrichment of the $CD31^{+}$ EC population than from hiPSC-derived mesoderm cells grown in 2D cultures.

Because hiPSC-EC are differentiated *in vitro*, they are not exposed to the fluid flow, pulsatile pressure, or specific tissue microenvironments that impact endogenous EC differentiation *in vivo* and thus, hiPSC-EC display an indeterminate phenotype; neither committed fully to an arterial or a venous fate.[29] Rufaihah *et al.*[29] discovered that hiPSC-EC derived using the EB differentiation method possess heterogeneous arterial, venous and lymphatic marker expression and they could be directed to differentiate toward the arterial,[29, 31, 71] lymphatic[29, 72] or venous[29] EC lineages through the manipulation of culture conditions. HiPSC-EC have also been induced in various culture conditions that mimic specific tissue microenvironments to induce tissue specific EC phenotypes. The most extensively studied tissue specific hiPSC-EC phenotype is brain microvascular EC (BEC).[64, 73-85] Corneal-[86] and choroidal-[87] like EC have also been

differentiated from hiPSC. However, at this time none of these *in vitro* induced tissue specific EC has been validated extensively *in vivo*. Thus, whether these cells genuinely represent their *in vivo* counterparts and how long can they keep their tissue-specific phenotype *in vivo* remains unknown.

Quality controls for hiPSC-EC

While EC differentiated from hiPSC through numerous methods exhibit some common traits including, expression of a panel of EC cell surface markers, *in vitro* 2D capillary-like tube forming potential, and ability to uptake low-density lipoprotein (LDL) (Figure 2), these properties alone may be insufficient to assess the function of EC to generate blood vessels *in vivo*. Indeed, many cell types other than EC, like some myeloid lineage hematopoietic cells, can also express EC markers and exhibit tube formation and LDL binding phenotypes *in vitro*[88, 89], whereas only bona fide EC form stable, functional blood vessel networks that inosculate with host vasculature upon transplantation.[88] Thus, *in vivo* vessel forming potential should be a necessary assay of EC derived from hiPSC (Figure 2). One commonly used *in vivo* vessel forming assay is to implant cells within a matrix and deliver the matrix into the subcutaneous space or prepared tissue. In this assay, donor test EC are loaded into a 3D matrix comprised of collagen,[41, 42] Matrigel,[16, 19, 24, 25, 29, 34, 36, 38, 40, 68, 90] fibrinogen,[47] or a degradable hydrogel[61] for transplantation. The matrices can be retrieved from the animals at different time points post-implantation to assess the extent of donor derived vessels, determination of whether or not the donor-derived vessels have inosculated with the host vasculature, and the length of time that the donor cells persist. This method is reproducible (once preliminary studies are completed to optimize the matrix composition, donor cell number, and strain of mice implanted and attention paid to ongoing reagent quality control), straightforward to perform, and thus, is suitable as a standard test to compare the quality of hiPSC-EC derived from different methods of production. Other more invasive methods to assess

endothelial functions of hiPSC-EC have also been tested including, experimental hind limb ischemia,[22-24, 42, 68, 69] myocardial infarction,[24, 25, 51] wound healing[16, 65, 72] and retinopathy[34, 42] models. Though these models are important for testing the therapeutic potential of hiPSC-EC, these interventional models are influenced by a host of variables including animal strain, sex, age, skill of the technicians, and whether or not the host animals are wild type or carry transgenic modifications. Moreover, in these models, the beneficial effect of the donor cell populations can be conveyed via indirect mechanisms in addition to effects derived from the direct formation of donor derived vessels.[89] For example, cell types like hematopoietic cells and mesenchymal lineage cells, which are common co-products of many hiPSC-EC differentiation protocols, can also improve blood perfusion by indirect means via secreting angiocrine factors or stabilizing newly formed vessels.[41, 89, 91] However, these non-EC cell types do not integrate into newly-formed blood vessels.[89, 92, 93] Thus, in their potential clinical applications, whether they can provide long-term protection to the blood vessels to the hosts, especially those who have EC with compromised proliferative potential,[42] is questionable. For this reason, whenever these animal disease models are used for testing the function of hiPSC-EC, it is necessary to validate the long-term contribution of donor EC to the host blood vessels. Finally, inclusion of a standard source of primary human EC, such as human umbilical cord venous endothelial cells (HUVEC) or umbilical cord blood endothelial colony forming cells (ECFC) as a control for comparison to the test hiPSC-EC population in the implant assay provides important information as to how similar the test EC are functioning *in vivo*. Recently, *in vitro* 3D vasculogenesis/angiogenesis models that mimic the vessel forming process *in vivo* have been applied to test hiPSC-EC.[34, 48, 57, 61, 62, 94, 95] In these studies, hiPSC-EC are embedded into 3D matrix made with fibrin[61], collagen,[34, 48], hyaluronic acid hydrogel,[57] or poly(ethylene glycol) (PEG) hydrogels[62, 94, 95] under static conditions[34, 48, 57, 61, 62, 94, 95] or flow conditions in microfluidic chips[95] and the hiPSC-EC self-assembled into lumenized blood vessel-like structures.[34, 48, 57, 61, 62, 94, 95] Transcriptome analyses showed that the gene expression pattern of hiPSC-EC cultured in such conditions are more consistent with that of EC during the *in*

vivo vasculogenesis/angiogenesis process compared to cells cultured *in vitro* under static conditions.[62, 95] Thus, *in vitro* 3D tube forming assays can be used for pre-screening of functional hiPSC-EC before *in vivo* testing (Figure 2).

Another property of EC that is often neglected in assessing the quality of the EC derived from the various differentiation protocols is a quantitative measure of the proliferative potential of the hiPSC-EC (Figure 2). Evidence from both human and other species has shown that vascular EC are heterogeneous with respect to proliferative potential. Over the past decade, it has been reported that among the EC derived from blood vessels or within the bloodstream, some EC display the potential to clonally propagate *in vitro*, self-renew *in vivo*, and give rise to more EC during vessel growth or regeneration.[96-103] Those EC identified by their potential to form endothelial colonies *in vitro*[96-102] have been called outgrowth EC or endothelial colony forming cells (ECFC).[98, 104] A single high proliferative potential ECFC can give rise to a colony that contains over 10,000 EC progeny, which may include more progenitors with equal or less proliferative potential,[97, 98] while more mature EC may not be capable of dividing or giving rise to only a few progeny. Indeed, hiPSC-EC derived from many protocols have displayed restricted proliferative potential and either undergo senescence within 5-8 passages or undergo endothelial to mesenchymal transition (EMT). To date, only a few studies have compared the proliferative potential of different hiPSC-EC fractions and tried to enrich the population of hiPSC-EC with high proliferative potential.[25, 41, 42] Two studies have compared the colony forming potential of different EC fractions at a single cell level.[25, 42] Prasain *et al.*[42] reported that after 12 days of hiPSC differentiation, EC could be discriminated into 2 groups based upon the expression of Neuropilin1 (NRP1), a co-receptor of VEGF and CD31. Only NRP1^{+}CD31^{+} hiPSC-EC gave rise to ECFC that contained high proliferative potential when plated in a single cell assay and showed overwhelming superior *in vivo* vessel forming potential after transplantation than the NRP1^{-}CD31^{+} population.[42] Using this culture strategy, hiPSC-EC derived from this protocol can be propagated for up to 18

passages and the hiPSC:EC ratio reached 1:100,000,000 within an 2-3 month period.[42] In the same year, Sahara *et al*. reported[25] that among all hiPSC-EC populations, $CD34^{+}CD14^{-}$ EC displayed the highest single cell EC colony forming potential and *in vitro* tube-forming ability. EC derived from this population also exhibited *in vivo* vessel forming potential in Matrigel plug and myocardial infarction models.[25] In addition, Samuel *et al*.[41] discovered and reported, that $CD34^{+}NRP1^{+}KDR^{+}$ EC displayed the highest proliferative potential among all hiPSC-EC tested in a specific protocol. EC from this fraction could be expanded for 15 passages and showed long-term *in vivo* vessel forming potential.[41] However, unlike hiPSC-EC derived from the Prasain[42] or Sahara[25] methods, hiPSC-EC derived from the Samuel *et al*.[41] protocol could not form *in vivo* vessels alone without the co-transplantation of mesenchymal cells.[41] Thus, whether these cell populations represent a similar cell type among all three reports is still elusive. Further efforts to specifically enrich and expand high proliferative ECFC, or an equivalent population, gain a deeper understanding of the mechanisms of EC self-renewal and proliferation, develop new culture strategies to maintain high proliferative ECFC fractions, and to compare and contrast specific ECFC populations derived from hiPSC with EC present in the vasculature of human subjects will be important areas for investigation.

The source of pluripotent cells and hiPSC-EC phenotype

While hiPSC have similar properties and differentiation potential with EC, evidence has emerged that hiPSC possess slightly different gene expression and epigenetic patterns due to their gain or loss of epigenetic memory from their somatic sourced material during the reprogramming process.[105-108] Thus, to safely apply hiPSC-EC for cell therapy or mechanistic studies, it is crucial to understand the differences among EC derived from hESC and those hiPSC derived from various somatic cell origins.

Many studies that differentiated EC from hiPSC line have also tested their protocols with ESC lines and some researchers compared the phenotype and function of EC derived from both sources. With a few exceptions,[27] most authors have agreed that hiPSC-EC generally exhibit comparable EC phenotypes and functions with ESC derived EC.[19, 20, 22, 24, 25, 40, 42-44, 59, 65] Three studies compared the transcriptome between multiple ESC-EC and hiPSC-EC lines differentiated using the EB differentiation method and found that gene expression profiles between ESC-EC and hiPSC-EC showed some variance but, overall were very similar.[19, 34, 40]

To date, most differentiation protocols have focused on the use of hiPSC derived from fibroblasts, a mature cell type. Some studies compared the EC differentiation potential from hiPSC derived from fibroblasts and other cell sources, include some immature cell populations like cord blood[34] or peripheral blood[109] mononuclear cells, bone marrow mesenchymal stem cells (BM-MSC)[33] and dental pulp cells,[24] or EC like human umbilical cord vein endothelial cells (HUVEC)[17, 28] and cord blood ECFC.[43, 44] The conclusions from those studies have provided multiple different interpretations of the importance of the source of cells with respect to derivation of hiPSC-EC. While some reports suggest immature cells or EC derived iPSC have a higher tendency to differentiate to EC when compared with fibroblast derived iPSC.[28, 33, 34] others showed similar or highly variable differentiation efficiency between these two groups.[24, 43, 44] Obviously, more studies are needed to answer this question.

The applications of hiPSC-EC

EC derived from patient-specific hiPSC can provide a nearly limitless source of autologous cells for various applications against CVD. The most direct application of hiPSC-EC would be to provide these cells as a therapy to replace injured or dysfunctional EC present in the systemic vasculature. Indeed, in some cases, such as patients suffering critical limb ischemia (CLI), evidence has been presented that there is loss of the microvasculature and within isolated blood

vascular EC from these patients, high proliferative ECFC are completely absent.[42] It has been shown that hiPSC-EC from various protocols form functional blood vessels upon transplantation in a 3D gel matrix[16, 19, 24, 25, 29, 34, 36, 38, 40-42, 47, 61, 68, 90] or into zebrafish embryos,[43, 44] suggesting that hiPSC-EC display potential to treat CVD by directly forming vessels and improving blood perfusion. Some other cell types, like mesenchymal and hematopoietic lineage cells, can also promote blood vessel growth and improve blood perfusion through indirect mechanisms in these animal models[41, 88, 90]. However, these non-EC cell types generally do not integrate into the newly formed blood vessels as long-term components,[89, 92, 93] thus as a potential cell therapy, repetitive delivery of these cells might be necessary. Likewise, though some recent evidence show that extracellular vesicles, like microvesicles and exosomes from different cell source could also promote angiogenesis,[110, 111] their short-half life and low encytosis rate are potential hurdles for their clinical applications.[110] In addition, all in-direct pro-angiogenic effects from these non-EC cell types or extracellular vesicles rely on the communication between these cells/vesicles and local EC in host vessels. Thus for those patients who suffer from diminished EC proliferative potential, like CLI patients,[42] the therapeutic benefits of non-EC agents are unlikely to be effective. In contrast, hiPSC-EC have the potential to form stabilized functional vessels and thus provide long-lasting support to the patients' blood vessel system. Indeed, although to date, no clinical trail using hiPSC-EC on human CVD patients has been performed, the therapeutic potential of hiPSC-EC has already been tested in many pre-clinical animal studies including hind limb ischemia,[22-24, 42, 68, 69] myocardial infarction,[24, 25, 51] retinopathy,[34, 42] carotid artery injury[26] and dermal wound healing models.[16, 65, 72] In those studies, *in situ* administration of hiPSC-EC improved the recovery of model animals after injury and most reports[16, 22-25, 34, 42, 51, 68, 69, 72] highlighted significant levels of integration of donor cells into the host vasculature after transplantation. Thus, patient subjects suffering from peripheral artery disease, delayed wound healing, acute and chronic heart failure, stroke, and certain forms of retinopathy that are associated with endothelial dysfunction and vascular dropout are prospective target groups to benefit from this form of cell therapy (Figure 3).

Other than direct administration of EC suspensions, hiPSC-EC can also be mixed with other cell types in a 3D scaffold to assemble transplantable tissue grafts. Donor EC formed blood vessels can promote the connection of a graft into the host vascular system thus improving the survival of grafts after transplantation. To date, liver,[46] cardiac[10, 45, 53, 112] and skin grafts[50] have been developed *in vitro* and transplanted into injured animals. In all these cases, the engrafted animals showed improved recovery and inosculated donor vessels.[10, 45, 46, 50, 53, 112] In addition, Lu *et al*.[39] have reported that decellularized murine heart scaffolds could be recellularized with hiPSC derived multipotent cardiovascular progenitors to engineer heart tissue with contractile function (Figure 3). Other than those tissue grafts, miniaturized organoids that mimics the structure and function of *in vivo* organs including blood vessel,[113] kidney,[114, 115] liver,[116] cornea,[117] and retina,[118] in 3D culture have also been constructed using hiPSC-EC and other cell types. These organoids can be potentially used for drug screening, disease study, or cell therapy as units of an artificial tissue graft.

Patient-specific hiPSC-EC can also be potentially used for studying disease mechanisms or drug screening to find new molecules to modulate pathways contributing to endothelial dysfunction in a patient and disease specific fashion. Compared to animal models, models using patient derived hiPSC-EC may provide more human-relevant information on disease mechanisms or drug responses (Figure 3). Recently, the pool of CVD patient specific hiPSC lines is expanding rapidly. Disease specific hiPSC-EC have been generated and studied to model BMPR2 mutation caused pulmonary arterial hypertension,[119-121] Moyamoya disease,[56, 58] fibrodysplasia ossificans progressiva,[35] Huntington's Disease,[78] Kawasaki disease,[109] type I diabetes,[41, 57, 122] atrial or ventricular septal defects, pulmonary valve stenosis, cardiomyopathy,[123] calcified aortic valve disease (CAVD),[124] and Hemophilia A (HA).[11] We have summarized above-mentioned disease studies that have characterized disease patient hiPSC derived EC in Table 1. For more information about patients derived hiPSC lines, please check International Stem Cell Registry

(https://www.umassmed.edu/iscr) or CIRM human pluripotent stem cell registry (https://catalog.coriell.org/1/CIRM).

Along with the development of advanced gene editing tools such as transcription activator-like effector nucleases (TALEN) and CRISPR/Cas9, more and more new strategies using targeted gene correction to treat or study cardiovascular related diseases have been developed. To understand the effect of BMPR2 mutations in EC, Gu *et al.*[121] compared EC differentiated from familial pulmonary arterial hypertension (FPAH) patient derived hiPSC, normal control patient derived hiPSC, unaffected BMPR2 mutation carrier patient derived hiPSC, and CRISPR/Cas9 rescued FPAH patient derived hiPSC. EC differentiated from FPAH patient derived hiPSC with BMPR2 mutations possessed impaired EC functions and survival, while unaffected carrier patient hiPSC or BMPR2 rescued FPAH patient hiPSC derived EC were relatively normal, suggesting additional BMPR2 modifiers play a role for the protection of unaffected carriers from FPAH phenotype.[121] Theodoris *et al.*[124] compared gene expression and the epigenetic landscape between EC derived from CAVD patient iPSC with *NOTCH 1* mutation and TALEN rescued isogenic iPSC and discovered *NOTCH1* haploinsufficiency caused epigenetic changes that lead to the up-regulation of osteogenesis gene expression in CAVD patient EC.[124] Yang *et al.*[125] surveyed the genome of patients with BAV and identified *GATA4* as a target of mutation in these patients.[125] EC differentiated from CRISPR/Cas9 induced *GATA4* targeted mutation hiPSC were then used to confirm this mutation is associated with impaired endothelial to mesenchymal transition.[125] Wu *et al.*[11] have recently generated hiPSC lines from a patient with HA caused by a factor eight (FVIII) gene intron 22 inversion and used TALEN to correct this specific mutation. Corrected HA patient specific hiPSC-EC restored FVIII transcription and secretion.[11]

As a platform for high-throughput drug screening and toxicity testing, hiPSC-EC can potentially be used to generate personalized therapeutic strategies. Recently, Vazao *et al.* developed a method to screen drugs that could potentially affect embryonic vasculature

development and identified two drugs, fluphenazine and pyrrolopyrimidine, that could decrease EC viability and disrupted EC network formation.[126] Sharma *et al*. generated hiPSC-EC as well as cardiomyocytes and fibroblasts from 11 healthy patients and 2 cancer patient to test the cardiotoxicity effect of 21 FDA approved anti-cancer tyrosine kinase inhibitors and developed a "cardiac safety index" for those drugs.[127] To better mimic *in vivo* environments in which EC constantly undergo shear stress from blood flow and often interact with other local cell types, new microfluidic 3-D platforms like "organ-on-a chip" have been developed and tested for drug screening on various *in vitro* organ mimics.[128, 129] Recently, Wang *et al*. developed an *in vitro* BBB microfluidic model using hiPSC-EC and validated the potential of this approach for screening drugs that affect EC permeability.[130] As more sophisticated *in vitro* vascularized organ models are developed, it can be expected that pre-clinical drug development process for CVD can be greatly shortened.

Challenges for the applications of hiPSC-EC as cell therapy

IPSC technology has only been developing as a discipline for a decade. Though hiPSC has been extensively applied for *in vitro* mechanistic studies, their application as cell therapy still face numerous practical problems, like low reprogramming efficiency,[131] genetic instability,[132-134] and potential of teratoma formation from undifferentiated iPSC.[135] To date, only one clinical trial has been completed in human subjects, using hiPSC derived retinal pigment epithelial cells,[136] and the safety and effectiveness of hiPSC therapy are still under evaluation. In general, application of hiPSC derived cells Cell therapy for CVD will require a large number of EC to replace the dysfunctional EC present in specific tissues and organs. However, most hiPSC-EC differentiation protocols that have been developed so far generate low EC yields and only produce mature EC with limited proliferative potential. Scaling-up the starting hiPSC cell number can partially solve

this problem but may not be practical or cost-efficient. Recently several groups have started to focus on differentiating hiPSC into EC precursors that display high proliferative potential.[25, 41, 42] It can be predicted that along with a better understanding of the mechanisms of EC lineage specification and proliferation, more efficient proliferative EC differentiation strategies should be developed to get clinical relevant numbers of hiPSC-EC for cell therapies.

The second limitation for current hiPSC-EC studies is the lack of a commonly-accepted standard to evaluate the quality of hiPSC-EC derived from various protocols to assess their potential for clinical use. While the route of delivery and the dose of cells to be used need to be determined case-by-case for each therapy, a basic set of quality control requirements for a hiPSC-EC therapeutic should be discussed and evidence based. However, many *in vitro* assays that are frequently used to define the quality of the hiPSC-EC, like CD31 expression, LDL binding, or 2-D Matrigel network formation, cannot definitively predict the ability of the EC to form vessels *in vivo*.[88, 89] Thus, to evaluate the quality of a hiPSC-EC product, some form of testing to validate *in vivo* vessel formation in animal models is recommended.

The next challenge for the practical utilization of hiPSC-EC is developing differentiation protocols that mimic the heterogeneous nature and tissue specificity of vascular EC. It is well known that arterial, venous, lymphatic and capillary EC show different patterns of gene expression and function.[137-139] Additionally, EC from each organ vascular bed share some common features, but also show unique gene expression signatures.[140] While most hiPSC-EC are not fully committed to any specific EC phenotype, several studies have attempted to induce hiPSC-EC to express tissue specific functions[64, 73-87] or arterial/venous/lymphatic EC phenotypes.[29, 31, 71, 72] For example, *in vitro* cultured ECFC, which mostly form capillary EC after transplantation, also possess the ability to differentiate into arterial EC *in vivo* when constitutively activated NOTCH expressing stromal cells are co-implanted.[141] The local tissue microenvironment has been reported to "educate" murine ESC derived EC to adapt the gene expression pattern of tissue

specific EC upon transplantation.[140] Thus, some EC possess the capacity to adapt to local environmental cues *in vivo* and further work to develop similar responsive hiPSC-EC are underway.

Finally, although autologous patient derived hiPSC-EC are promising for the treatment of chronic CVD, the process of hiPSC induction and EC differentiation can take several months to complete, which makes their potential for treating acute injuries unlikely. Some investigators are directly reprogramming somatic cells into EC without a pluripotent intermediate.[142-144] At present it is unclear if the reprogrammed EC possess high proliferative potential. As an alternative strategy, some investigators are proposing to use banked allogeneic haplotype-matched hiPSC to produce cell products to diminish the potential for immunologic detection and elimination.[145] Other strategies include development of hiPSC lines that are genome edited to make universal donor cells that lack major histocompatibility antigens I and II that should be immunologically well tolerated by the host unmatched patients.[146-148] These strategies will be helpful in overcoming some of the practical issues for production of banks of hiPSC derived EC for cell therapy.

Conclusions

In the past decade, numerous methods to differentiate EC from hiPSC, have been developed and the potential of applying hiPSC-EC in regenerative medicine approaches have been tested in various animal models of human disease. In addition, many patient-specific hiPSC lines have been generated and EC differentiated from these lines have been used for disease mechanism exploration and discovery. The accumulating knowledge on hiPSC-EC differentiation, functional evaluation and potential application will benefit the development of new strategies against CVD.

Acknowledgments

We would like to thank Tiffany Lewallen for her expert administrative assistance in preparation of the article.

Sources of Funding

This work was supported in part by funds from Riley Children's Foundation.

Disclosures

The authors have no competing financial interests to disclose.

References

1. World health organization. Who web site. Http://www.Who.Int/. Accessed june 30,. 2017
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861-872
3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663-676
4. Chen T, Wang F, Wu M, Wang ZZ. Development of hematopoietic stem and progenitor cells from human pluripotent stem cells. *Journal of cellular biochemistry*. 2015;116:1179-1189
5. Yoder MC. Differentiation of pluripotent stem cells into endothelial cells. *Current opinion in hematology*. 2015;22:252-257
6. Lacaud G, Kouskoff V. Hemangioblast, hemogenic endothelium, and primitive versus definitive hematopoiesis. *Exp Hematol*. 2017;49:19-24
7. Slukvin, II. Generating human hematopoietic stem cells in vitro -exploring endothelial to hematopoietic transition as a portal for stemness acquisition. *FEBS Lett*. 2016;590:4126-4143
8. Choi KD, Yu J, Smuga-Otto K, Salvagiotto G, Rehrauer W, Vodyanik M, Thomson J, Slukvin I. Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. *Stem Cells*. 2009;27:559-567
9. Taura D, Sone M, Homma K, Oyamada N, Takahashi K, Tamura N, Yamanaka S, Nakao K. Induction and isolation of vascular cells from human induced pluripotent stem cells--brief report. *Arterioscler Thromb Vasc Biol*. 2009;29:1100-1103
10. Ye L, Chang YH, Xiong Q, et al. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. *Cell Stem Cell*. 2014;15:750-761
11. Wu Y, Hu Z, Li Z, Pang J, Feng M, Hu X, Wang X, Lin-Peng S, Liu B, Chen F, Wu L, Liang D. In situ genetic correction of f8 intron 22 inversion in hemophilia a patient-specific ipscs. *Sci Rep*. 2016;6:18865
12. Adams WJ, Zhang Y, Cloutier J, Kuchimanchi P, Newton G, Sehrawat S, Aird WC, Mayadas TN, Luscinskas FW, Garcia-Cardena G. Functional vascular endothelium derived from human induced pluripotent stem cells. *Stem Cell Reports*. 2013;1:105-113
13. Bai H, Xie YL, Gao YX, Cheng T, Wang ZZ. The balance of positive and negative effects of tgf-beta signaling regulates the development of hematopoietic and endothelial progenitors in human pluripotent stem cells. *Stem Cells Dev*. 2013;22:2765-2776
14. Drowley L, Koonce C, Peel S, Jonebring A, Plowright AT, Kattman SJ, Andersson H, Anson B, Swanson BJ, Wang QD, Brolen G. Human induced pluripotent stem cell-derived cardiac progenitor cells in phenotypic screening: A transforming growth factor-beta type 1 receptor kinase inhibitor induces efficient cardiac differentiation. *Stem Cells Transl Med*. 2016;5:164-174
15. Faye PA, Vedrenne N, De la Cruz-Morcillo MA, Barrot CC, Richard L, Bourthoumieu S, Sturtz F, Funalot B, Lia AS, Battu S. New method for sorting endothelial and neural progenitors from human induced pluripotent stem cells by sedimentation field flow fractionation. *Anal Chem*. 2016;88:6696-6702
16. Kim KL, Song SH, Choi KS, Suh W. Cooperation of endothelial and smooth muscle cells derived from human induced pluripotent stem cells enhances neovascularization in dermal wounds. *Tissue Eng Part A*. 2013;19:2478-2485

17. Lagarkova MA, Shutova MV, Bogomazova AN, Vassina EM, Glazov EA, Zhang P, Rizvanov AA, Chestkov IV, Kiselev SL. Induction of pluripotency in human endothelial cells resets epigenetic profile on genome scale. *Cell Cycle*. 2010;9:937-946
18. Lee JB, Werbowetski-Ogilvie TE, Lee JH, McIntyre BA, Schnerch A, Hong SH, Park IH, Daley GQ, Bernstein ID, Bhatia M. Notch-hes1 signaling axis controls hemato-endothelial fate decisions of human embryonic and induced pluripotent stem cells. *Blood*. 2013;122:1162-1173
19. Li Z, Hu S, Ghosh Z, Han Z, Wu JC. Functional characterization and expression profiling of human induced pluripotent stem cell- and embryonic stem cell-derived endothelial cells. *Stem Cells Dev*. 2011;20:1701-1710
20. Park TS, Zimmerlin L, Zambidis ET. Efficient and simultaneous generation of hematopoietic and vascular progenitors from human induced pluripotent stem cells. *Cytometry A*. 2013;83:114-126
21. Wang Y, Zhang WY, Hu S, et al. Genome editing of human embryonic stem cells and induced pluripotent stem cells with zinc finger nucleases for cellular imaging. *Circ Res*. 2012;111:1494-1503
22. Lai WH, Ho JC, Chan YC, Ng JH, Au KW, Wong LY, Siu CW, Tse HF. Attenuation of hind-limb ischemia in mice with endothelial-like cells derived from different sources of human stem cells. *PLoS One*. 2013;8:e57876
23. Rufaihah AJ, Huang NF, Jame S, Lee JC, Nguyen HN, Byers B, De A, Okogbaa J, Rollins M, Reijo-Pera R, Gambhir SS, Cooke JP. Endothelial cells derived from human ipscs increase capillary density and improve perfusion in a mouse model of peripheral arterial disease. *Arterioscler Thromb Vasc Biol*. 2011;31:e72-79
24. Yoo CH, Na HJ, Lee DS, Heo SC, An Y, Cha J, Choi C, Kim JH, Park JC, Cho YS. Endothelial progenitor cells from human dental pulp-derived ips cells as a therapeutic target for ischemic vascular diseases. *Biomaterials*. 2013;34:8149-8160
25. Sahara M, Hansson EM, Wernet O, Lui KO, Spater D, Chien KR. Manipulation of a vegf-notch signaling circuit drives formation of functional vascular endothelial progenitors from human pluripotent stem cells. *Cell Res*. 2014;24:820-841
26. Giordano S, Zhao X, Xing D, Hage F, Oparil S, Cooke JP, Lee J, Nakayama KH, Huang NF, Chen YF. Targeted delivery of human ips-ecs overexpressing il-8 receptors inhibits neointimal and inflammatory responses to vascular injury in the rat. *Am J Physiol Heart Circ Physiol*. 2016;310:H705-715
27. Feng Q, Lu SJ, Klimanskaya I, Gomes I, Kim D, Chung Y, Honig GR, Kim KS, Lanza R. Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. *Stem Cells*. 2010;28:704-712
28. Hu S, Zhao MT, Jahanbani F, Shao NY, Lee WH, Chen H, Snyder MP, Wu JC. Effects of cellular origin on differentiation of human induced pluripotent stem cell-derived endothelial cells. *JCI Insight*. 2016;1
29. Rufaihah AJ, Huang NF, Kim J, Herold J, Volz KS, Park TS, Lee JC, Zambidis ET, Reijo-Pera R, Cooke JP. Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity. *Am J Transl Res*. 2013;5:21-35
30. James D, Nam HS, Seandel M, Nolan D, Janovitz T, Tomishima M, Studer L, Lee G, Lyden D, Benezra R, Zaninovic N, Rosenwaks Z, Rabbany SY, Rafii S. Expansion and maintenance of human embryonic stem cell-derived endothelial cells by tgfbeta inhibition is id1 dependent. *Nat Biotechnol*. 2010;28:161-166
31. Sivarapatna A, Ghaedi M, Le AV, Mendez JJ, Qyang Y, Niklason LE. Arterial specification of endothelial cells derived from human induced pluripotent stem cells in a biomimetic flow bioreactor. *Biomaterials*. 2015;53:621-633

32. Huang NF, Dewi RE, Okogbaa J, Lee JC, Jalilrufaihah A, Heilshorn SC, Cooke JP. Chemotaxis of human induced pluripotent stem cell-derived endothelial cells. *Am J Transl Res*. 2013;5:510-520
33. Xu Y, Liu L, Zhang L, Fu S, Hu Y, Wang Y, Fu H, Wu K, Xiao H, Liu S, Yu X, Zheng W, Feng B, Huang H. Efficient commitment to functional cd34+ progenitor cells from human bone marrow mesenchymal stem-cell-derived induced pluripotent stem cells. *PLoS One*. 2012;7:e34321
34. Park TS, Bhutto I, Zimmerlin L, et al. Vascular progenitors from cord blood-derived induced pluripotent stem cells possess augmented capacity for regenerating ischemic retinal vasculature. *Circulation*. 2014;129:359-372
35. Barruet E, Morales BM, Lwin W, White MP, Theodoris CV, Kim H, Urrutia A, Wong SA, Srivastava D, Hsiao EC. The acvr1 r206h mutation found in fibrodysplasia ossificans progressiva increases human induced pluripotent stem cell-derived endothelial cell formation and collagen production through bmp-mediated smad1/5/8 signaling. *Stem Cell Res Ther*. 2016;7:115
36. Cochrane A, Kelaini S, Tsifaki M, et al. Quaking is a key regulator of endothelial cell differentiation, neovascularization, and angiogenesis. *Stem Cells*. 2017;35:952-966
37. Costa M, Sourris K, Lim SM, Yu QC, Hirst CE, Parkington HC, Jokubaitis VJ, Dear AE, Liu HB, Micallef SJ, Koutsis K, Elefanty AG, Stanley EG. Derivation of endothelial cells from human embryonic stem cells in fully defined medium enables identification of lysophosphatidic acid and platelet activating factor as regulators of enos localization. *Stem Cell Res*. 2013;10:103-117
38. Lin B, Kim J, Li Y, Pan H, Carvajal-Vergara X, Salama G, Cheng T, Li Y, Lo CW, Yang L. High-purity enrichment of functional cardiovascular cells from human ips cells. *Cardiovasc Res*. 2012;95:327-335
39. Lu TY, Lin B, Kim J, Sullivan M, Tobita K, Salama G, Yang L. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. *Nat Commun*. 2013;4:2307
40. White MP, Rufaihah AJ, Liu L, Ghebremariam YT, Ivey KN, Cooke JP, Srivastava D. Limited gene expression variation in human embryonic stem cell and induced pluripotent stem cell-derived endothelial cells. *Stem Cells*. 2013;31:92-103
41. Samuel R, Daheron L, Liao S, Vardam T, Kamoun WS, Batista A, Buecker C, Schafer R, Han X, Au P, Scadden DT, Duda DG, Fukumura D, Jain RK. Generation of functionally competent and durable engineered blood vessels from human induced pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2013;110:12774-12779
42. Prasain N, Lee MR, Vemula S, et al. Differentiation of human pluripotent stem cells to cells similar to cord-blood endothelial colony-forming cells. *Nat Biotechnol*. 2014;32:1151-1157
43. Orlova VV, Drabsch Y, Freund C, Petrus-Reurer S, van den Hil FE, Muenthaisong S, Dijke PT, Mummery CL. Functionality of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts. *Arterioscler Thromb Vasc Biol*. 2014;34:177-186
44. Orlova VV, van den Hil FE, Petrus-Reurer S, Drabsch Y, Ten Dijke P, Mummery CL. Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. *Nat Protoc*. 2014;9:1514-1531
45. Masumoto H, Ikuno T, Takeda M, Fukushima H, Marui A, Katayama S, Shimizu T, Ikeda T, Okano T, Sakata R, Yamashita JK. Human ips cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. *Sci Rep*. 2014;4:6716
46. Du C, Narayanan K, Leong MF, Wan AC. Induced pluripotent stem cell-derived hepatocytes and endothelial cells in multi-component hydrogel fibers for liver tissue engineering. *Biomaterials*. 2014;35:6006-6014

47. Patsch C, Challet-Meylan L, Thoma EC, et al. Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. *Nat Cell Biol*. 2015;17:994-1003
48. Palpant NJ, Pabon L, Friedman CE, Roberts M, Hadland B, Zaunbrecher RJ, Bernstein I, Zheng Y, Murry CE. Generating high-purity cardiac and endothelial derivatives from patterned mesoderm using human pluripotent stem cells. *Nat Protoc*. 2017;12:15-31
49. Wang L, Xiang M, Liu Y, Sun N, Lu M, Shi Y, Wang X, Meng D, Chen S, Qin J. Human induced pluripotent stem cells derived endothelial cells mimicking vascular inflammatory response under flow. *Biomicrofluidics*. 2016;10:014106
50. Abaci HE, Guo Z, Coffman A, Gillette B, Lee WH, Sia SK, Christiano AM. Human skin constructs with spatially controlled vasculature using primary and ipsc-derived endothelial cells. *Adv Healthc Mater*. 2016;5:1800-1807
51. Liang J, Huang W, Cai W, Wang L, Guo L, Paul C, Yu XY, Wang Y. Inhibition of microrna-495 enhances therapeutic angiogenesis of human induced pluripotent stem cells. *Stem Cells*. 2017;35:337-350
52. Yang D, Wang J, Xiao M, Zhou T, Shi X. Role of mir-155 in controlling hif-1alpha level and promoting endothelial cell maturation. *Sci Rep*. 2016;6:35316
53. Masumoto H, Nakane T, Tinney JP, Yuan F, Ye F, Kowalski WJ, Minakata K, Sakata R, Yamashita JK, Keller BB. The myocardial regenerative potential of three-dimensional engineered cardiac tissues composed of multiple human ips cell-derived cardiovascular cell lineages. *Sci Rep*. 2016;6:29933
54. Ikuno T, Masumoto H, Yamamizu K, Yoshioka M, Minakata K, Ikeda T, Sakata R, Yamashita JK. Efficient and robust differentiation of endothelial cells from human induced pluripotent stem cells via lineage control with vegf and cyclic amp. *PLoS One*. 2017;12:e0173271
55. Giacomelli E, Bellin M, Sala L, van Meer BJ, Tertoolen LG, Orlova VV, Mummery CL. Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Development*. 2017;144:1008-1017
56. Hitomi T, Habu T, Kobayashi H, et al. Downregulation of securin by the variant rnf213 r4810k (rs112735431, g>a) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients. *Biochem Biophys Res Commun*. 2013;438:13-19
57. Chan XY, Black R, Dickerman K, Federico J, Levesque M, Mumm J, Gerecht S. Three-dimensional vascular network assembly from diabetic patient-derived induced pluripotent stem cells. *Arterioscler Thromb Vasc Biol*. 2015;35:2677-2685
58. Hamauchi S, Shichinohe H, Uchino H, Yamaguchi S, Nakayama N, Kazumata K, Osanai T, Abumiya T, Houkin K, Era T. Cellular functions and gene and protein expression profiles in endothelial cells derived from moyamoya disease-specific ips cells. *PLoS One*. 2016;11:e0163561
59. Homma K, Sone M, Taura D, Yamahara K, Suzuki Y, Takahashi K, Sonoyama T, Inuzuka M, Fukunaga Y, Tamura N, Itoh H, Yamanaka S, Nakao K. Sirt1 plays an important role in mediating greater functionality of human es/ips-derived vascular endothelial cells. *Atherosclerosis*. 2010;212:42-47
60. Cao N, Liang H, Huang J, Wang J, Chen Y, Chen Z, Yang HT. Highly efficient induction and long-term maintenance of multipotent cardiovascular progenitors from human pluripotent stem cells under defined conditions. *Cell Res*. 2013;23:1119-1132
61. Kusuma S, Shen YI, Hanjaya-Putra D, Mali P, Cheng L, Gerecht S. Self-organized vascular networks from human pluripotent stem cells in a synthetic matrix. *Proc Natl Acad Sci U S A*. 2013;110:12601-12606
62. Zhang J, Schwartz MP, Hou Z, Bai Y, Ardalani H, Swanson S, Steill J, Ruotti V, Elwell A, Nguyen BK, Bolin J, Stewart R, Thomson JA, Murphy WL. A genome-wide analysis of

human pluripotent stem cell-derived endothelial cells in 2d or 3d culture. *Stem Cell Reports*. 2017;8:907-918
63. Glaser DE, Turner WS, Madfis N, Wong L, Zamora J, White N, Reyes S, Burns AB, Gopinathan A, McCloskey KE. Multifactorial optimizations for directing endothelial fate from stem cells. *PLoS One*. 2016;11:e0166663
64. Yamamizu K, Iwasaki M, Takakubo H, Sakamoto T, Ikuno T, Miyoshi M, Kondo T, Nakao Y, Nakagawa M, Inoue H, Yamashita JK. In vitro modeling of blood-brain barrier with human ipsc-derived endothelial cells, pericytes, neurons, and astrocytes via notch signaling. *Stem Cell Reports*. 2017;8:634-647
65. Azhdari M, Baghaban-Eslaminejad M, Baharvand H, Aghdami N. Therapeutic potential of human-induced pluripotent stem cell-derived endothelial cells in a bleomycin-induced scleroderma mouse model. *Stem Cell Res*. 2013;10:288-300
66. Lian X, Bao X, Al-Ahmad A, Liu J, Wu Y, Dong W, Dunn KK, Shusta EV, Palecek SP. Efficient differentiation of human pluripotent stem cells to endothelial progenitors via small-molecule activation of wnt signaling. *Stem Cell Reports*. 2014;3:804-816
67. Bao X, Lian X, Palecek SP. Directed endothelial progenitor differentiation from human pluripotent stem cells via wnt activation under defined conditions. *Methods Mol Biol*. 2016;1481:183-196
68. Park SW, Jun Koh Y, Jeon J, Cho YH, Jang MJ, Kang Y, Kim MJ, Choi C, Sook Cho Y, Chung HM, Koh GY, Han YM. Efficient differentiation of human pluripotent stem cells into functional cd34+ progenitor cells by combined modulation of the mek/erk and bmp4 signaling pathways. *Blood*. 2010;116:5762-5772
69. Zhang F, Wang L, Li Y, Liu W, Duan F, Huang R, Chen X, Chang SC, Du Y, Na J. Optimizing mesoderm progenitor selection and three-dimensional microniche culture allows highly efficient endothelial differentiation and ischemic tissue repair from human pluripotent stem cells. *Stem Cell Res Ther*. 2017;8:6
70. Zhang S, Dutton JR, Su L, Zhang J, Ye L. The influence of a spatiotemporal 3d environment on endothelial cell differentiation of human induced pluripotent stem cells. *Biomaterials*. 2014;35:3786-3793
71. Zhang J, Chu LF, Hou Z, et al. Functional characterization of human pluripotent stem cell-derived arterial endothelial cells. *Proc Natl Acad Sci U S A*. 2017;114:E6072-E6078
72. Lee SJ, Park C, Lee JY, Kim S, Kwon PJ, Kim W, Jeon YH, Lee E, Yoon YS. Generation of pure lymphatic endothelial cells from human pluripotent stem cells and their therapeutic effects on wound repair. *Sci Rep*. 2015;5:11019
73. Lippmann ES, Azarin SM, Kay JE, Nessler RA, Wilson HK, Al-Ahmad A, Palecek SP, Shusta EV. Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. *Nat Biotechnol*. 2012;30:783-791
74. Patel R, Alahmad AJ. Growth-factor reduced matrigel source influences stem cell derived brain microvascular endothelial cell barrier properties. *Fluids Barriers CNS*. 2016;13:6
75. Hollmann EK, Bailey AK, Potharazu AV, Neely MD, Bowman AB, Lippmann ES. Accelerated differentiation of human induced pluripotent stem cells to blood-brain barrier endothelial cells. *Fluids Barriers CNS*. 2017;14:9
76. Patel R, Page S, Al-Ahmad AJ. Isogenic blood-brain barrier models based on patient-derived stem cells display inter-individual differences in cell maturation and functionality. *J Neurochem*. 2017
77. Lippmann ES, Al-Ahmad A, Azarin SM, Palecek SP, Shusta EV. A retinoic acid-enhanced, multicellular human blood-brain barrier model derived from stem cell sources. *Sci Rep*. 2014;4:4160

78. Lim RG, Quan C, Reyes-Ortiz AM, et al. Huntington's disease ipsc-derived brain microvascular endothelial cells reveal wnt-mediated angiogenic and blood-brain barrier deficits. *Cell Rep*. 2017;19:1365-1377
79. Wilson HK, Canfield SG, Hjortness MK, Palecek SP, Shusta EV. Exploring the effects of cell seeding density on the differentiation of human pluripotent stem cells to brain microvascular endothelial cells. *Fluids Barriers CNS*. 2015;12:13
80. Minami H, Tashiro K, Okada A, Hirata N, Yamaguchi T, Takayama K, Mizuguchi H, Kawabata K. Generation of brain microvascular endothelial-like cells from human induced pluripotent stem cells by co-culture with c6 glioma cells. *PLoS One*. 2015;10:e0128890
81. Page S, Munsell A, Al-Ahmad AJ. Cerebral hypoxia/ischemia selectively disrupts tight junctions complexes in stem cell-derived human brain microvascular endothelial cells. *Fluids Barriers CNS*. 2016;13:16
82. Stebbins MJ, Wilson HK, Canfield SG, Qian T, Palecek SP, Shusta EV. Differentiation and characterization of human pluripotent stem cell-derived brain microvascular endothelial cells. *Methods*. 2016;101:93-102
83. Katt ME, Xu ZS, Gerecht S, Searson PC. Human brain microvascular endothelial cells derived from the bc1 ips cell line exhibit a blood-brain barrier phenotype. *PLoS One*. 2016;11:e0152105
84. Canfield SG, Stebbins MJ, Morales BS, Asai SW, Vatine GD, Svendsen CN, Palecek SP, Shusta EV. An isogenic blood-brain barrier model comprising brain endothelial cells, astrocytes, and neurons derived from human induced pluripotent stem cells. *J Neurochem*. 2017;140:874-888
85. Appelt-Menzel A, Cubukova A, Gunther K, Edenhofer F, Piontek J, Krause G, Stuber T, Walles H, Neuhaus W, Metzger M. Establishment of a human blood-brain barrier co-culture model mimicking the neurovascular unit using induced pluri- and multipotent stem cells. *Stem Cell Reports*. 2017;8:894-906
86. Zhao JJ, Afshari NA. Generation of human corneal endothelial cells via in vitro ocular lineage restriction of pluripotent stem cells. *Invest Ophthalmol Vis Sci*. 2016;57:6878-6884
87. Songstad AE, Worthington KS, Chirco KR, Giacalone JC, Whitmore SS, Anfinson KR, Ochoa D, Cranston CM, Riker MJ, Neiman M, Stone EM, Mullins RF, Tucker BA. Connective tissue growth factor promotes efficient generation of human induced pluripotent stem cell-derived choroidal endothelium. *Stem Cells Transl Med*. 2017;6:1533-1546
88. Medina RJ, Barber CL, Sabatier F, Dignat-George F, Melero-Martin JM, Khosrotehrani K, Ohneda O, Randi AM, Chan JKY, Yamaguchi T, Van Hinsbergh VWM, Yoder MC, Stitt AW. Endothelial progenitors: A consensus statement on nomenclature. *Stem Cells Transl Med*. 2017;6:1316-1320
89. Yoder MC. Endothelial progenitor cell: A blood cell by many other names may serve similar functions. *J Mol Med (Berl)*. 2013;91:285-295
90. Wu YT, Yu IS, Tsai KJ, Shih CY, Hwang SM, Su IJ, Chiang PM. Defining minimum essential factors to derive highly pure human endothelial cells from ips/es cells in an animal substance-free system. *Sci Rep*. 2015;5:9718
91. Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, Lambrichts I. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. *Pharmacol Ther*. 2014;143:181-196
92. Huang NF, Li S. Mesenchymal stem cells for vascular regeneration. *Regenerative medicine*. 2008;3:877-892
93. Asahara T, Kawamoto A, Masuda H. Concise review: Circulating endothelial progenitor cells for vascular medicine. *Stem cells*. 2011;29:1650-1655

94. Belair DG, Schwartz MP, Knudsen T, Murphy WL. Human ipsc-derived endothelial cell sprouting assay in synthetic hydrogel arrays. *Acta Biomater*. 2016;39:12-24
95. Zanotelli MR, Ardalani H, Zhang J, et al. Stable engineered vascular networks from human induced pluripotent stem cell-derived endothelial cells cultured in synthetic hydrogels. *Acta Biomater*. 2016;35:32-41
96. Alphonse RS, Vadivel A, Zhong S, McConaghy S, Ohls R, Yoder MC, Thebaud B. The isolation and culture of endothelial colony-forming cells from human and rat lungs. *Nat Protoc*. 2015;10:1697-1708
97. Huang L, Harkenrider M, Thompson M, Zeng P, Tanaka H, Gilley D, Ingram DA, Bonanno JA, Yoder MC. A hierarchy of endothelial colony-forming cell activity displayed by bovine corneal endothelial cells. *Invest Ophthalmol Vis Sci*. 2010;51:3943-3949
98. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood*. 2005;105:2783-2786
99. Javed MJ, Mead LE, Prater D, Bessler WK, Foster D, Case J, Goebel WS, Yoder MC, Haneline LS, Ingram DA. Endothelial colony forming cells and mesenchymal stem cells are enriched at different gestational ages in human umbilical cord blood. *Pediatr Res*. 2008;64:68-73
100. Naito H, Kidoya H, Sakimoto S, Wakabayashi T, Takakura N. Identification and characterization of a resident vascular stem/progenitor cell population in preexisting blood vessels. *EMBO J*. 2012;31:842-855
101. Naito H, Wakabayashi T, Kidoya H, Muramatsu F, Takara K, Eino D, Yamane K, Iba T, Takakura N. Endothelial side population cells contribute to tumor angiogenesis and antiangiogenic drug resistance. *Cancer Res*. 2016;76:3200-3210
102. Shelley WC, Leapley AC, Huang L, Critser PJ, Zeng P, Prater D, Ingram DA, Tarantal AF, Yoder MC. Changes in the frequency and in vivo vessel-forming ability of rhesus monkey circulating endothelial colony-forming cells across the lifespan (birth to aged). *Pediatr Res*. 2012;71:156-161
103. Ramasamy SK, Kusumbe AP, Wang L, Adams RH. Endothelial notch activity promotes angiogenesis and osteogenesis in bone. *Nature*. 2014;507:376-380
104. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest*. 2000;105:71-77
105. Chin MH, Mason MJ, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 2009;5:111-123
106. Guenther MG, Frampton GM, Soldner F, Hockemeyer D, Mitalipova M, Jaenisch R, Young RA. Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell*. 2010;7:249-257
107. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, Huo H, Loh YH, Aryee MJ, Lensch MW, Li H, Collins JJ, Feinberg AP, Daley GQ. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat Biotechnol*. 2011;29:1117-1119
108. Ma H, Morey R, O'Neil RC, et al. Abnormalities in human pluripotent cells due to reprogramming mechanisms. *Nature*. 2014;511:177-183
109. Ikeda K, Mizoro Y, Ameku T, et al. Transcriptional analysis of intravenous immunoglobulin resistance in kawasaki disease using an induced pluripotent stem cell disease model. *Circ J*. 2016;81:110-118
110. Kholia S, Ranghino A, Garnieri P, Lopatina T, Deregibus MC, Rispoli P, Brizzi MF, Camussi G. Extracellular vesicles as new players in angiogenesis. *Vascular pharmacology*. 2016;86:64-70
111. Zhang J, Guan J, Niu X, Hu G, Guo S, Li Q, Xie Z, Zhang C, Wang Y. Exosomes released from human induced pluripotent stem cells-derived mscs facilitate cutaneous

wound healing by promoting collagen synthesis and angiogenesis. *Journal of translational medicine*. 2015;13:49
112. Gao L, Kupfer ME, Jung JP, Yang L, Zhang P, Da Sie Y, Tran Q, Ajeti V, Freeman BT, Fast VG, Campagnola PJ, Ogle BM, Zhang J. Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. *Circ Res*. 2017;120:1318-1325
113. Collado MS, Cole BK, Figler RA, Lawson M, Manka D, Simmers MB, Hoang S, Serrano F, Blackman BR, Sinha S, Wamhoff BR. 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*. 2017;6:1673-1683
114. Takasato M, Little MH. Making a kidney organoid using the directed differentiation of human pluripotent stem cells. *Methods Mol Biol*. 2017;1597:195-206
115. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, Parton RG, Wolvetang EJ, Roost MS, Lopes SM, Little MH. Kidney organoids from human ips cells contain multiple lineages and model human nephrogenesis. *Nature*. 2016;536:238
116. Asai A, Aihara E, Watson C, Mourya R, Mizuochi T, Shivakumar P, Phelan K, Mayhew C, Helmrath M, Takebe T, Wells J, Bezerra JA. Paracrine signals regulate human liver organoid maturation from induced pluripotent stem cells. *Development*. 2017;144:1056-1064
117. Foster JW, Wahlin K, Adams SM, Birk DE, Zack DJ, Chakravarti S. Cornea organoids from human induced pluripotent stem cells. *Sci Rep*. 2017;7:41286
118. Fields MA, Bowrey HE, Gong J, Moreira EF, Cai H, Del Priore LV. Extracellular matrix nitration alters growth factor release and activates bioactive complement in human retinal pigment epithelial cells. *PLoS One*. 2017;12:e0177763
119. West JD, Austin ED, Gaskill C, et al. Identification of a common wnt-associated genetic signature across multiple cell types in pulmonary arterial hypertension. *Am J Physiol Cell Physiol*. 2014;307:C415-430
120. Sa S, Gu M, Chappell J, et al. Induced pluripotent stem cell model of pulmonary arterial hypertension reveals novel gene expression and patient specificity. *Am J Respir Crit Care Med*. 2017;195:930-941
121. Gu M, Shao NY, Sa S, et al. Patient-specific ipsc-derived endothelial cells uncover pathways that protect against pulmonary hypertension in bmpr2 mutation carriers. *Cell Stem Cell*. 2017;20:490-504 e495
122. Carcamo-Orive I, Huang NF, Quertermous T, Knowles JW. Induced pluripotent stem cell-derived endothelial cells in insulin resistance and metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2017
123. Ang YS, Rivas RN, Ribeiro AJ, et al. Disease model of gata4 mutation reveals transcription factor cooperativity in human cardiogenesis. *Cell*. 2016;167:1734-1749 e1722
124. Theodoris CV, Li M, White MP, Liu L, He D, Pollard KS, Bruneau BG, Srivastava D. Human disease modeling reveals integrated transcriptional and epigenetic mechanisms of notch1 haploinsufficiency. *Cell*. 2015;160:1072-1086
125. Yang B, Zhou W, Jiao J, et al. Protein-altering and regulatory genetic variants near gata4 implicated in bicuspid aortic valve. *Nat Commun*. 2017;8:15481
126. Vazao H, Rosa S, Barata T, et al. High-throughput identification of small molecules that affect human embryonic vascular development. *Proc Natl Acad Sci U S A*. 2017;114:E3022-E3031
127. Sharma A, Burridge PW, McKeithan WL, et al. High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Sci Transl Med*. 2017;9

128. Skardal A, Murphy SV, Devarasetty M, et al. Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Sci Rep*. 2017;7:8837
129. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. *Nat Biotechnol*. 2014;32:760-772
130. Wang YI, Abaci HE, Shuler ML. Microfluidic blood-brain barrier model provides in vivo-like barrier properties for drug permeability screening. *Biotechnol Bioeng*. 2017;114:184-194
131. Schlaeger TM, Daheron L, Brickler TR, et al. A comparison of non-integrating reprogramming methods. *Nat Biotechnol*. 2015;33:58-63
132. Hussein SM, Batada NN, Vuoristo S, et al. Copy number variation and selection during reprogramming to pluripotency. *Nature*. 2011;471:58-62
133. Laurent LC, Ulitsky I, Slavin I, et al. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human escs and ipscs during reprogramming and time in culture. *Cell Stem Cell*. 2011;8:106-118
134. Lister R, Pelizzola M, Kida YS, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011;471:68-73
135. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer*. 2011;11:268-277
136. Trounson A, DeWitt ND. Pluripotent stem cells progressing to the clinic. *Nat Rev Mol Cell Biol*. 2016;17:194-200
137. dela Paz NG, D'Amore PA. Arterial versus venous endothelial cells. *Cell Tissue Res*. 2009;335:5-16
138. Richardson MR, Lai X, Witzmann FA, Yoder MC. Venous and arterial endothelial proteomics: Mining for markers and mechanisms of endothelial diversity. *Expert Rev Proteomics*. 2010;7:823-831
139. Swift MR, Weinstein BM. Arterial-venous specification during development. *Circ Res*. 2009;104:576-588
140. Nolan DJ, Ginsberg M, Israely E, et al. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell*. 2013;26:204-219
141. Kim H, Huang L, Critser PJ, Yang Z, Chan RJ, Wang L, Carlesso N, Voytik-Harbin SL, Bernstein ID, Yoder MC. Notch ligand delta-like 1 promotes in vivo vasculogenesis in human cord blood-derived endothelial colony forming cells. *Cytotherapy*. 2015;17:579-592
142. .Morita R, Suzuki M, Kasahara H, Shimizu N, Shichita T, Sekiya T, Kimura A, Sasaki K, Yasukawa H, Yoshimura A. Ets transcription factor etv2 directly converts human fibroblasts into functional endothelial cells. *Proc Natl Acad Sci U S A*. 2015;112:160-165
143. Li J, Huang NF, Zou J, Laurent TJ, Lee JC, Okogbaa J, Cooke JP, Ding S. Conversion of human fibroblasts to functional endothelial cells by defined factors. *Arterioscler Thromb Vasc Biol*. 2013;33:1366-1375
144. Margariti A, Winkler B, Karamariti E, Zampetaki A, Tsai TN, Baban D, Ragoussis J, Huang Y, Han JD, Zeng L, Hu Y, Xu Q. Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels. *Proc Natl Acad Sci U S A*. 2012;109:13793-13798
145. Gourraud PA, Gilson L, Girard M, Peschanski M. The role of human leukocyte antigen matching in the development of multiethnic "haplobank" of induced pluripotent stem cell lines. *Stem Cells*. 2012;30:180-186
146. Abrahimi P, Qin L, Chang WG, Bothwell AL, Tellides G, Saltzman WM, Pober JS. Blocking mhc class ii on human endothelium mitigates acute rejection. *JCI Insight*. 2016;1
147. Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, Prunkard D, Colunga AG, Hanafi LA, Clegg DO, Turtle C, Russell DW. Hla-e-expressing pluripotent

stem cells escape allogeneic responses and lysis by nk cells. *Nat Biotechnol*. 2017;35:765-772

148. Pick M, Ronen D, Yanuka O, Benvenisty N. Reprogramming of the mhc-i and its regulation by nfkappab in human-induced pluripotent stem cells. *Stem Cells*. 2012;30:2700-2708

Highlights

- Numerous protocols have been developed to differentiate hiPSC into EC using stromal cell co-culture, embryoid body (3D) culture, or 2-D monolayer culture strategies.
- The *in vivo* blood vessel forming potential and *in vitro* clonal proliferative potential should be assayed as a quality control measure before using hiPSC-EC for cell therapy.
- HiPSC-EC can be employed in patients with cardiovascular disease through direct cell administration, implantation of engineered tissue grafts, or as models for studying the disease mechanisms causing EC dysfunction, or for drug screening.

Table Legend

Table 1. List of publications using patient derived hiPSC-EC for disease study

Figure Legends

Figure 1. General strategies for hiPSC-EC differentiation.

Figure 2. General methods to evaluate the quality of hiPSC-EC.

Figure 3. Potential applications of hiPSC-EC.

Table 1. List of publications using patient derived hiPSC-EC for disease study

Disease	Mutation	EC Phenotype	Year	Authors	Reference
Moyamoya disease	RNF213 R4810K polymorphism	-In vitro tube formation↓ -Securin expression↓	2013	Hitomi *et al.*	56
Pulmonary arterial hypertension (PAH)	BMPR2	-Differentiation↓ -LDL uptake↓ -In vitro tube formation↓ -Wnt signaling↑	2014	West *et al.*	119
Type I diabetes (T1D)	-	-Differentiation no effect	2015	Chan *et al.*	57
Calcified aortic valve disease (CAVD)	NOTCH1 haploinsufficiency	-Epigenetic architecture change - pro-osteogenic genes↑ -Inflammatory genes↑	2015	Theodoris *et al.*	124
Hemophilia A (HA)	*FVIII* intron 22 inversion	-FVIII secretion↓ -FVIII activity↓	2016	Wu *et al.*	11
Fibrodysplasia ossificans progressiva (FOP)	ACVR1 R206H	-BMP4 independent differentiation -Fibroblast related gene expression↑ -BMP4 induced SMAD1/5/8 phosphorylation↑	2016	Barruet *et al.*	35
Moyamoya disease	RNF213 R4810K	-In vitro tube formation↓ -Proliferation no effect -Integrin β3 expression↓	2016	Hamauchi *et al.*	58
Intravenous immunoglobulin (IVIG) resistant Kawasaki disease	-	-CXCL12 expression↑ -Interleukin-6 pathway↑	2016	Ikeda *et al.*	109
Atrial or ventricular septal defects, pulmonary valve stenosis, cardiomyopathy	GATA4 G29S	-EC related gene expression↑	2016	Ang *et al.*	121
Huntington's Disease (HD)	CAG repeat expansion in Huntingtin (HTT)	-In vitro tube formation↑ -Barrier function↓ - WNT/β-Catenin pathway↑	2017	Lim *et al.*	78
Pulmonary arterial hypertension (PAH)	-	-In vitro tube formation↓ -Adhesison↓ -Migration↓ -Survival↓ -BMPR2 expression↓ -Kisspeptin 1 expression↑	2017	Sa. *et al.*	120
Familial pulmonary arterial hypertension (FPAH)	BMPR2	-In vitro tube formation↓ -Adhesison↓ -Migration↓ -Survival↓	2017	Gu *et al.*	121

Figure 1

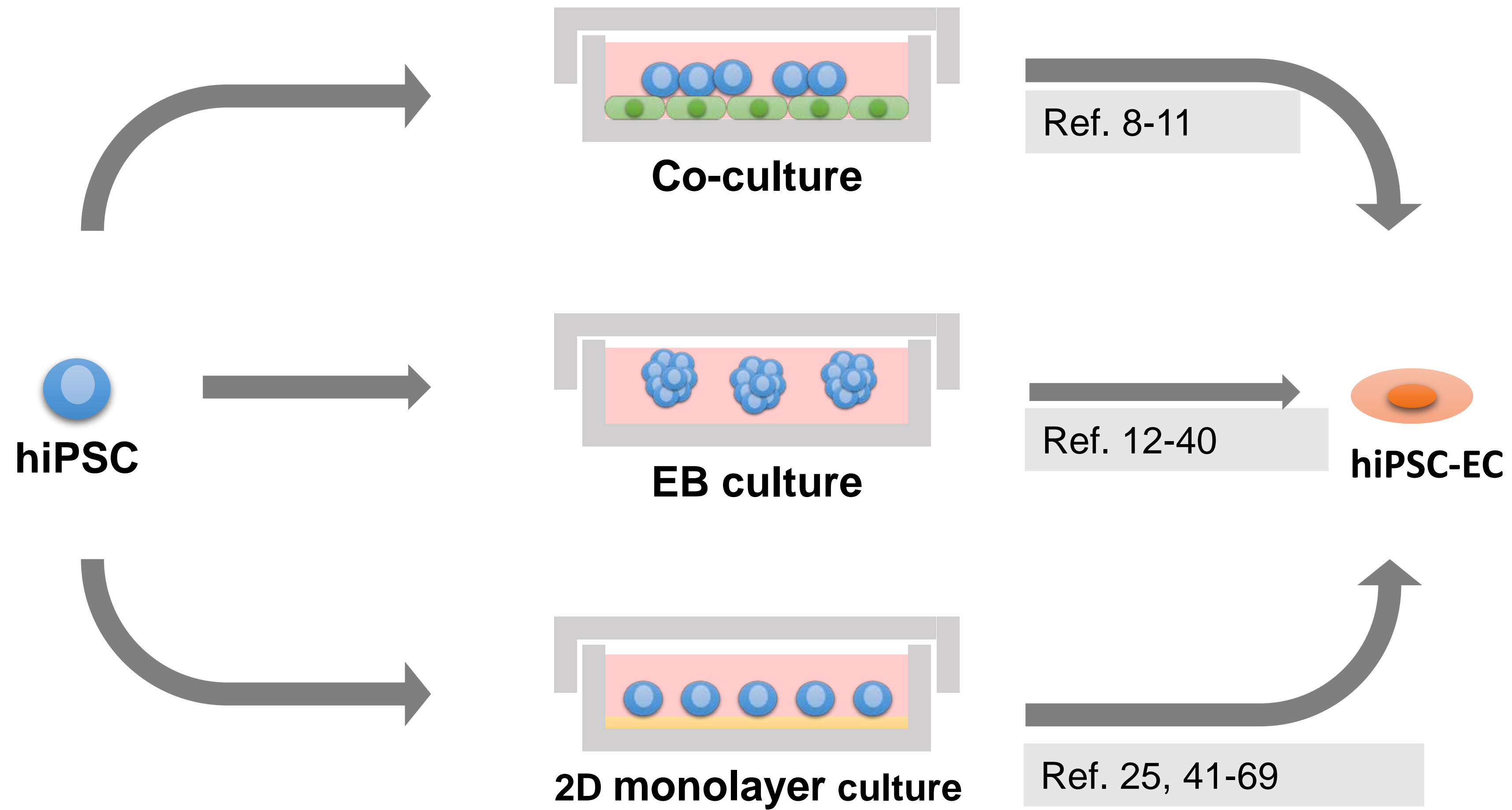


Figure 2

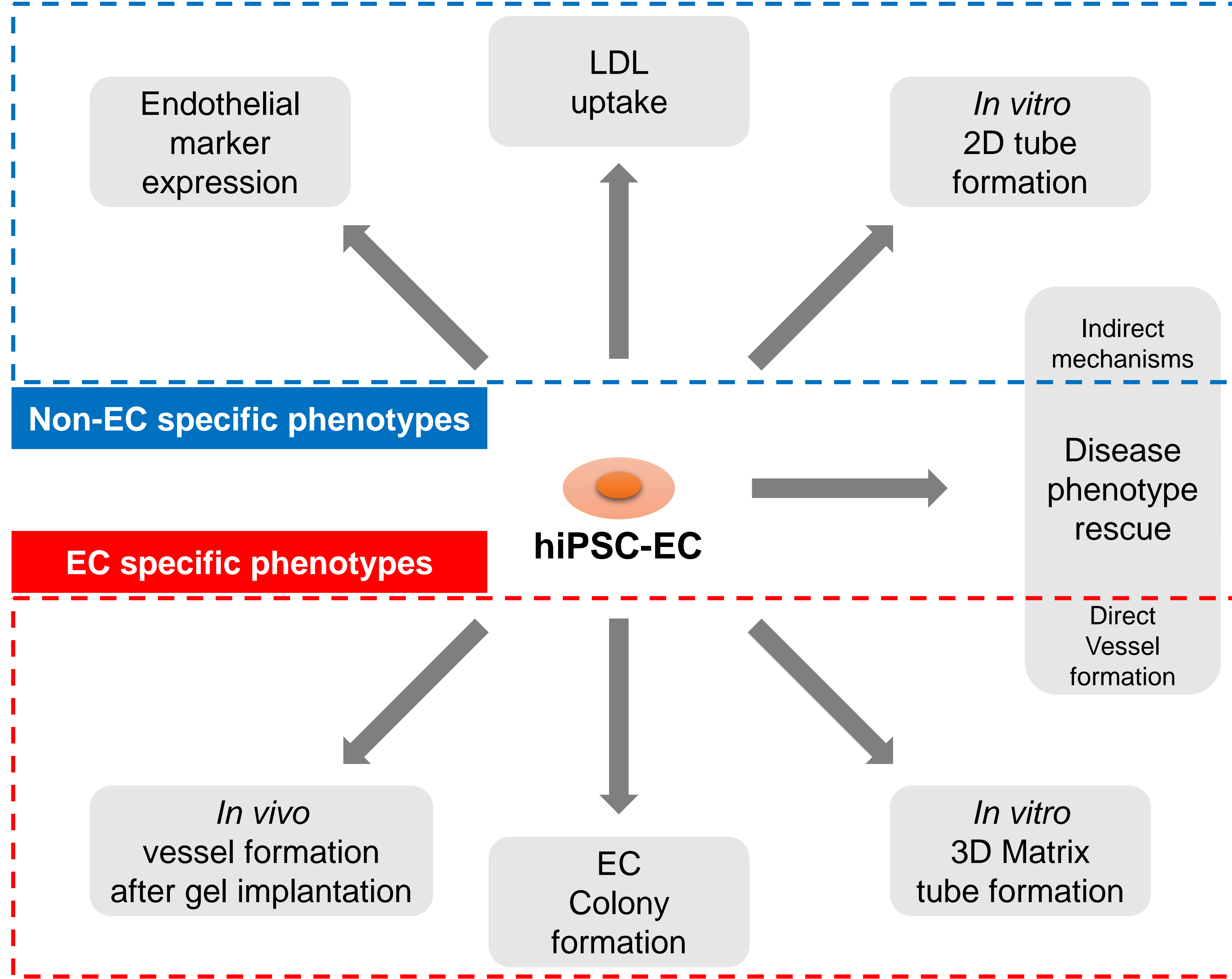


Figure 3