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Let's get physical: Biomechanical influences on human pluripotent stem cell differentiation towards vascular engineering

Xin Yi Chan[1], Joon H. Eoh[1] and Sharon Gerecht

Abstract

Regenerative medicine provides a promising avenue of research in which tissue lost from disease, trauma and congenital defects can be replaced from substitutes created in the laboratory. Human pluripotent stem cells (hPSCs) are of great interest in the field of cell therapy due to their ability to provide a patient-specific cell source for the supplementation of tissue engineering constructs. In the field of vascular tissue engineering, blood vessels are composite tissues comprised of various cell types, mainly endothelial cells and smooth muscle cells. Therefore, proper attention must be given to the differentiation process so that the appropriate cell type with the necessary functional properties can be obtained. A larger emphasis needs to be placed on optimizing the functional properties of these cells so that they can withstand physiologically relevant forces in the native environment and integrate into the patients' vasculature. Despite the importance of biomechanical cues in vascular development and engineering, few studies have investigated these critical factors during the differentiation of hPSCs into functional vascular cells and tissue. In this review, we summarize recent findings that elucidate the role of biomechanical influences on the differentiation of hPSCs. Specifically, we focus on their role in the differentiation of hPSCs into endothelial cells and smooth muscle cells. It is now evident that the use of these factors during differentiation can not only better direct cell fate, but can in fact enhance the specification and functionality of the differentiated cells. Finally, future directions and additional considerations for the use of biomechanical cues in the field of vascular bioengineering will be discussed.

Addresses

Department of Chemical and Biomolecular Engineering, Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218, United States

Corresponding author: Gerecht, Sharon (gerecht@jhu.edu)
[1] Authors contributed equally.

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Keywords

Human pluripotent stem cells, Endothelial cells, Smooth muscle cells, Biomechanical cues, Vascular differentiation.

Abbreviations

Con-vSMCs, contractile vSMCs; CXCR4, C-X-C chemokine receptor type 4; ECM, Extracellular matrix; ECs, Endothelial cells; hESCs, Human embryonic stem cells; hiPSCs, Human induced pluripotent stem cells; hiPSC-EC, hiPSC-derived ECs; hPSCs, Human induced pluripotent stem cells; KDR, Kinase insert domain receptor; Mech-mSMLCs, Mechanically stimulated mSMLCs; mSMLCs, Mature SMLCs; PDMS, Polydimethylsiloxane; PEGdma-PLA, Poly(ethylene glycol) dimethacrylate/poly (L-lactide); SMLCs, Smooth muscle like cells; Syn-vSMCs, Synthetic vSMCs; VEcad, Vascular endothelial cadherin; vSMCs, Vascular smooth muscle cells; (vSM-tissue), Vascular smooth muscle tissue; YAP, Yes-associated protein.

Introduction

The field of vascular tissue engineering has greatly advanced throughout the years, improving upon previously employed strategies and slowly moving towards more translational and clinical paths. Human pluripotent stem cells (hPSCs) provide great potential due to the ability to create autologous tissue engineered constructs. While many robust protocols exist for the differentiation of hPSCs into the various vascular cell types, namely endothelial cells (ECs) and vascular smooth muscle cells (vSMCs), these protocols focus on the use of different signaling molecules and growth factors. More attention should be given to methods enhancing the functionality of these differentiated cells so that engineered tissues can better withstand the rigors of the physiological environment and have an improved likelihood of integrating into the patient's body. A potential solution lies in the introduction of biomechanical cues during the differentiation process with the hopes of enhancing the differentiation outcome, and specification and maturation of these vascular cells through improved functionality. In this review, we summarize and provide insight into recent studies that employ biophysical cues during the differentiation and maturation of hPSCs into ECs or vSMCs for use in vascular bioengineering. Current limitations as well as future directions and considerations for this topic of research are discussed.

Endothelial Cells

ECs line the inner blood vessels and play a crucial role in carrying out physiological functions such as nutrient and oxygen exchange. ECs are remarkably heterogeneous in their morphology, molecular profile and function depending on the type of blood vessel they

populate such as arteries or veins, as well as their microenvironment [1]. Morphologically and molecularly, arteries and veins differ significantly [2]. Arteries are made of a single layer of ECs surrounded by thick layers of vSMCs and extracellular matrix (ECM) to provide strength and elasticity to withstand high blood pressure and high flow rate. Conversely, EC layer in veins is surrounded by thinner layers of vSMCs and ECM. Arterial ECs typically express ephrin-B2, CXCR4 and Notch pathway proteins such as Notch1 and Dll4 [3,4], while venous ECs are generally marked with EphB4 receptor and COUP-TFII expression [5,6]. Due to their vital role in maintaining homeostasis in all tissues, the ability to generate large amounts of ECs is deemed essential. Currently, the main impediments to the field of vascular engineering are a lack of protocols to derive specific EC types (arterial or venous) from hPSCs, and minimal control over the size and mechanical properties of the resulting vascular construct [7]. hPSCs—human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have emerged as promising source for generating different cell types including ECs. When given the appropriate signaling molecules and developmental cues, hPSCs can be differentiated into ECs, recapitulating the developmental processes [8–11]. In addition to supplementing small molecules or signaling proteins biomechanical cues have recently been proven to be crucial in directing stem cell fate [12–15]. Compliant hydrogel substrates have been demonstrated to enhance mesoderm differentiation of hESCs. In addition, biomechanical cues are shown to mature hPSC derived ECs into functional arterial ECs that mimic the phenotypes *in vivo*.

In this section, we will discuss in detail two recent advances in vascular differentiation and specification, where biomechanical cues, a less discussed parameter, are employed to guide EC differentiation from hiPSCs on polydimethylsiloxane (PDMS) substrates [16], and maturation of hiPSC-derived ECs into the arterial phenotype using a biomimetic flow bioreactor [17].

Effects of substrate stiffness on hiPSC differentiation into EC fates

Researchers in the field of vascular bioengineering have only just begun to explore the effects of biomechanical cues in differentiating hiPSCs into various cell types [12,15]. For instance, compliant hydrogel substrates have been shown to enhance mesoderm differentiation of hESCs via stabilization of adherens junctions, proto-oncogene tyrosine-protein kinase Src activity and nuclear translocation of β-catenin [18]. Expanding upon the concept of regulating cell fate using biomechanical cues, in a recent study by Smith et al., the role of substrate stiffness in hiPSC differentiation towards EC fate in a two-step differentiation protocol was studied. In the first step, hiPSCs were differentiated towards mesodermal fate, followed by the second step in which mesodermal cells were directed towards endothelial cell fates [19,20]. hiPSCs were first directed towards mesodermal induction on either a physiologically soft substrate (E∼3 kPa) or physiologically stiff substrate (E∼1.7 MPa) fabricated from PDMS, with tissue culture plastic (E∼3 GPa) used as a control. In the second step of the differentiation, the downstream effects of stiffness on these primed mesodermal cells going towards EC fate was assessed on tissue culture plastic. In the first 24 h of the mesodermal differentiation, they examined the contribution of different stiffnesses by observing the localization of yes-associated protein (YAP), a protein known to play a role as a sensor for biomechanical cues at the cell's microenvironment [21], in sparsely seeded hiPSCs. They demonstrated that, on the stiff tissue culture plastic (E∼3 GPa), most of the cells were spread out and activated YAP was observed in the nucleus. From a physiological stiff substrate (E∼1.7 MPa) to a physiologically soft substrate (E∼3 kPa), YAP was gradually deactivated and localized to the cytoplasm devoid of the nucleus.

During the mesoderm induction step, a combination of YAP and Wnt/β-catenin (a transcription factor of the Wnt pathway known to promote differentiation of hESCs) [22] localization was utilized to determine the degree of mesoderm induction from hiPSCs. It was observed that the ratio of nuclear to cytoplasmic YAP localization was significantly higher on PDMS substrates (E∼1.7 MPa and E∼3 kPa) in comparison to E∼3 GPa control throughout mesoderm induction. In addition, they found higher ratios of junctional-to-cytoplasmic β-catenin expression on the PDMS substrates (E∼1.7 MPa and E∼3 kPa) on of the second day of mesoderm induction, opposite to observations of cells differentiated on E∼3 GPa control. Collectively, these observations suggest that the kinetics of both YAP and β-catenin activities are influenced by different substrate stiffnesses and might function in coordinating downstream transcriptional activities of the mesodermal genes during hiPSC differentiation.

Based on the established YAP and Wnt/β-catenin kinetics during mesodermal induction, the authors were prompted to determine if stiffness primed-hiPSCs have enhanced mesodermal induction and/or endothelial commitment. They verified that hiPSCs differentiated on compliant substrates in both serum and serum-free conditions had upregulation of mesodermal genes, T, kinase insert domain receptor (KDR), MESP-1 and SNAIL-1 but, only hiPSCs differentiated on E∼3 kPa has GATA-2 upregulation. Furthermore, they demonstrated that compliant substrates primed mesodermal induction improved the total EC yield during EC differentiation by at least 2-fold as determined by EC markers including vascular endothelial cadherin (VEcad), CD31, von Willebrand factor and endothelial