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# Integrating *in vitro* organ-specific function with the microcirculation

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There is significant interest within the tissue engineering and pharmaceutical industries to create 3D microphysiological systems of human organ function. The interest stems from a growing concern that animal models and simple 2D culture systems cannot replicate essential features of human physiology that are crucial to predict drug response, or simply to develop new therapeutic strategies to repair or replace damaged organs. Central to human organ function is a microcirculation that not only enhances the rate of nutrient and waste transport by convection, but also provides essential additional physiological functions that can be specific to each organ. This review highlights progress in the creation of *in vitro* functional microvessel networks, and emphasizes organ-specific functional and structural characteristics that should be considered in the future mimicry of four organ systems that are of primary interest: lung, brain, liver, and muscle (skeletal and cardiac).

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## Introduction

Recent advancements in both biology and microfluidic technologies have generated unprecedented opportunities to create sophisticated microphysiological systems that mimic human organ function. Within the last decade, 3D systems that recapitulate the human organ microenvironment under highly controlled conditions have emerged and been met with much excitement [1•,2•,3•]. Such systems provide new tools for basic research of both pathological and physiological states, but are also predictive of human physiology and hence attractive for drug efficacy and toxicity screening.

Global challenges to develop *in vitro* organ systems include cell sources, selection of matrix, and the development of a vascular supply. Advances in human induced pluripotent (iPS) stem cell technology offer a promising solution to the cell source issue while continued innovation in synthetic and native biomaterials can potentially address the hurdle of creating realistic cell–matrix interactions; however an often simplified challenge in organ microphysiological system development is the creation of a vascular network. Essentially all human tissue contains a vascular supply, and thus new microphysiological systems must include a vascular supply if they want to truly replicate normal human physiology. Initial work in constructing *in vitro* vessel networks was in the form of either printing or coating rigid channels with cells [4–7]. Although such methods provide precise control of vessel architecture, the channels are not dynamic and thus cannot remodel or respond to changes in the microenvironment. More recently, cylindrical networks in natural extracellular matrices have been endothelialized and have shown the ability to invade into the surrounding matrix [8–10]. Within the past two years several groups have emerged with microfluidic models that allow for vessels to either sprout or self assemble in a hydrogel compartment resulting in perfused human capillaries [11••,12–15]. To date, only our work has shown physiological flow and shear rate [11••]. The ability of endothelial cells to self-assemble into 3D perfusable networks requires cues in the microenvironment. For example, fibrin is often used as a matrix because of it is naturally pro-angiogenic and promotes production of basement membrane such as collagen [16,17]. Another key feature is the presence of stromal cells which can generate freely diffusible growth factors and matrix proteins such as collagen, vascular endothelial growth factor, transforming growth factor β-induced protein, hepatocyte growth factor and fibronectin [18,19].

The next iteration or natural progression of these designs is establishing tissue or organ specificity. Although the most basic role of microvessel networks is to provide the exchange of nutrients, oxygen and waste, the microcirculation is often coupled and integrated into many of the organ system's function in addition to carrying out regulatory functions in response to environmental cues. As a result, there is significant heterogeneity in the structure and function of the microcirculation between different organs. This review will focus on the unique features of the microcirculations of four organs (lung, brain, liver and heart) to emphasize the need to create organ-specific functional and structural characteristics of microvessel

networks in the development of realistic 3D microphysiologic systems.

## Lungs

The lungs are the major organs of the respiratory system, and are primarily responsible for respiratory gas exchange (oxygen and carbon dioxide). During inspiration, air high in oxygen content is delivered first through the branching airway tree where the air is warmed, humidified, and particulate matter is filtered. In the alveolar region, oxygen diffuses from the air into the blood, and carbon dioxide diffuses from the blood into the air. On expiration, the carbon dioxide enriched air reverses the path. Inhalation is a major route for toxic substances to enter the body, but can also be a unique mode of therapeutic drug delivery. Thus, primary functions to mimic in a microphysiological system are the airway filter, and respiratory gas exchange. These physiological functions create unique anatomical features including: (1) an air-tissue interface; (2) an airway epithelium that produces mucus with beating cilia; and (3) a large, dynamic surface area in the alveolar region characterized by a thin (<1 micron) membrane that stretches and contracts during a 2 second inspiration and expiration, respectively to increase by >100%.

The vascular system in the lungs is comprised of the smaller systemic bronchial circulation, which nourishes the lung tissue itself, and the larger pulmonary circulation, which is the source of respiratory gas exchange. Of particular interest to the pharmaceutical industry is the fact that the pulmonary endothelium is exposed to the entire cardiac output. Success in simulating lung function in 3D microphysiological systems began with the more simple airway mucosa and included primary airway epithelial cells, a subepithelial stroma, and capillary networks [20–26]. We will focus on the unique structural and functional features of the pulmonary circulation that are of primary interest to mimic in a microphysiological system. The anatomical structure of the pulmonary microcirculation [27] is complex and central to its function (Figure 1). Pulmonary endothelial cells comprise one of three thin layers that comprise the alveolar membrane, and have important metabolic functions related to the processing of vasoactive substances. For example, angiotensin converting enzyme (ACE) is expressed uniquely on the surface of the pulmonary endothelium and not only converts angiotensin I to angiotensin II, it also inactivates bradykinin [28]. In addition, the pulmonary endothelium is actively antithrombogenic, primarily through its ability to secrete prostacyclin and express ADPases [29–32]. Finally, the pulmonary endothelium plays an important role in the immune response through the expression of high levels (>30-fold over other organ systems) of adhesion molecules (e.g. ICAM-1, P-Selectin) specific for circulating leukocytes [33,34].

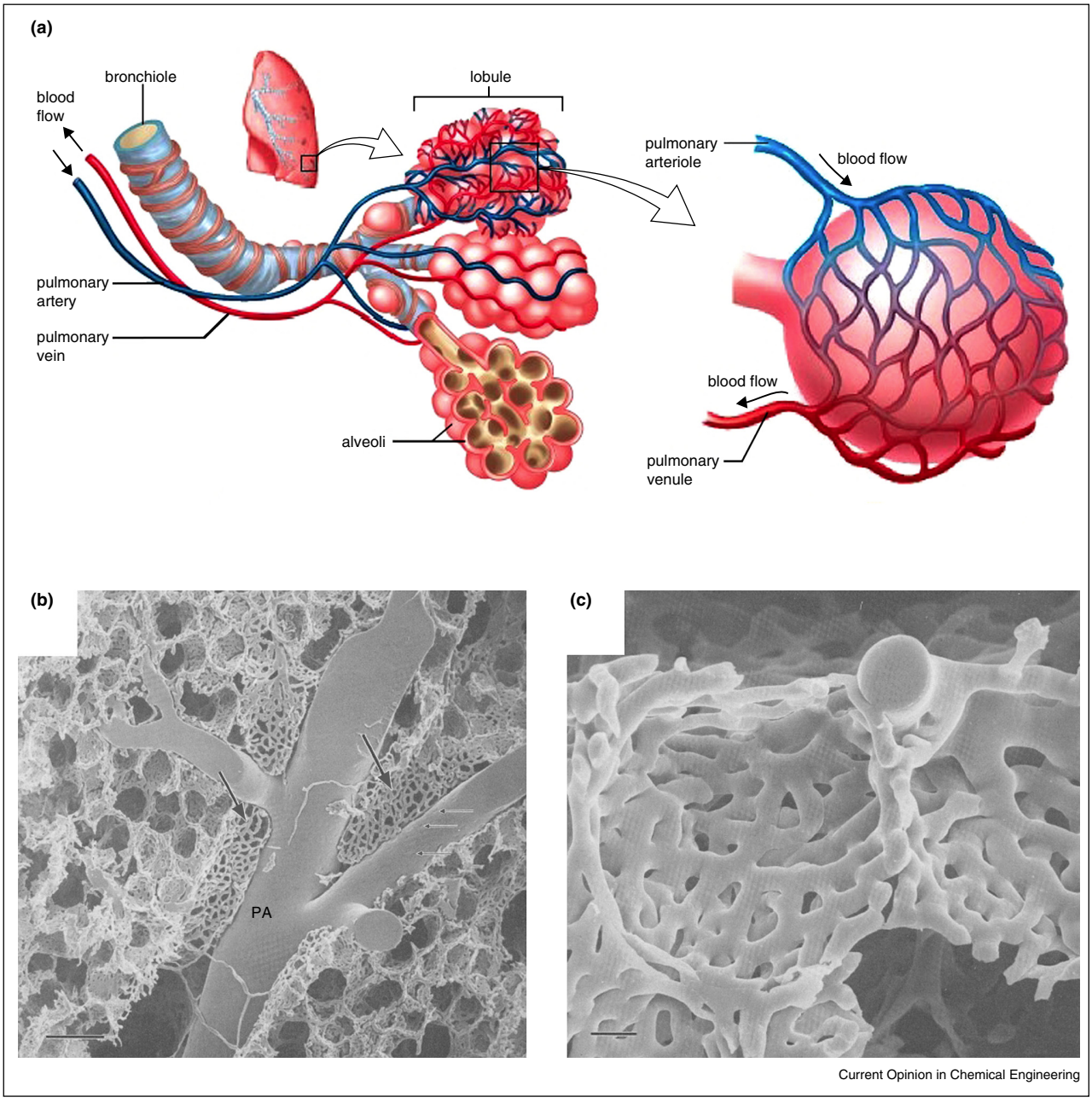
Attempts to mimic the pulmonary circulation *in vitro* have been dominated by monolayer culture of pulmonary endothelial cells. These studies have been able to recapitulate barrier properties [35–37], enzymatic function [38,39], as well as simulate the impact of dynamic stretch that occurs during normal tidal breathing [40–42]. Recently, alveolar epithelial cells and pulmonary endothelial cells have been cleverly combined in a polydimethyl siloxane (PDMS) microdevice to recapitulate alveolar membrane stretch and permeability to fluid and immune cells [2•,43]. Although these studies represent a significant step forward, key features of the vascular system(s) in the lungs that have not been simulated include perfused systemic capillary networks, and the inclusion of blood components such as red blood cells and platelets.

## Brain

The brain or central nervous system (CNS) is clearly complex and regulates essential physiologic functions such as cognition, emotion, motor function, sensation, vision, hearing, and taste. Although the structure of the microcirculation is remarkably similar across different regions of the brain that control these functions, the brain microcirculation has several very distinctive features that are crucial to mimic in any *in vitro* model system. Of particular relevance to the pharmaceutical industry is the fact that most circulating drugs and biologics cannot penetrate the CNS because of the blood–brain barrier (BBB). In addition, brain endothelial cells also uniquely express several proteins to circumvent the BBB and meet the special metabolic demands of the brain. The various techniques that have been used to mimic the BBB *in vitro* has recently been reviewed, with a particular emphasis on the use of these models in neuropharmaceutical discovery [44]. As such, we will review only the salient features of the BBB that are likely to be crucial for *in vitro* mimicry, and the current state of the art for these models.

The primary function of the BBB is to restrict or control exposure of sensitive neurons to compounds in the circulation, and is therefore broadly described as reducing the permeability of the microcirculation to macromolecules. The BBB is comprised of several structures (Figure 2) all of which contribute to the overall function including intercellular tight junctions (TJs), adherens junctions, the basal membrane, the pericyte, and the astrocyte [44]. In addition, brain endothelial cells uniquely express glucose transporters (e.g. GLUT1) on the basal and apical membranes to meet the high metabolic demand of neurons for glucose [45,46]. Additional metabolic enzymes expressed by brain endothelial cells in a unique pattern that are commonly used to validate models of the BBB include alkaline phosphatase, epoxide hydrolase, monamine oxidases, and cytochrome P450s [47–50]. Together, the structural,

**Figure 1**

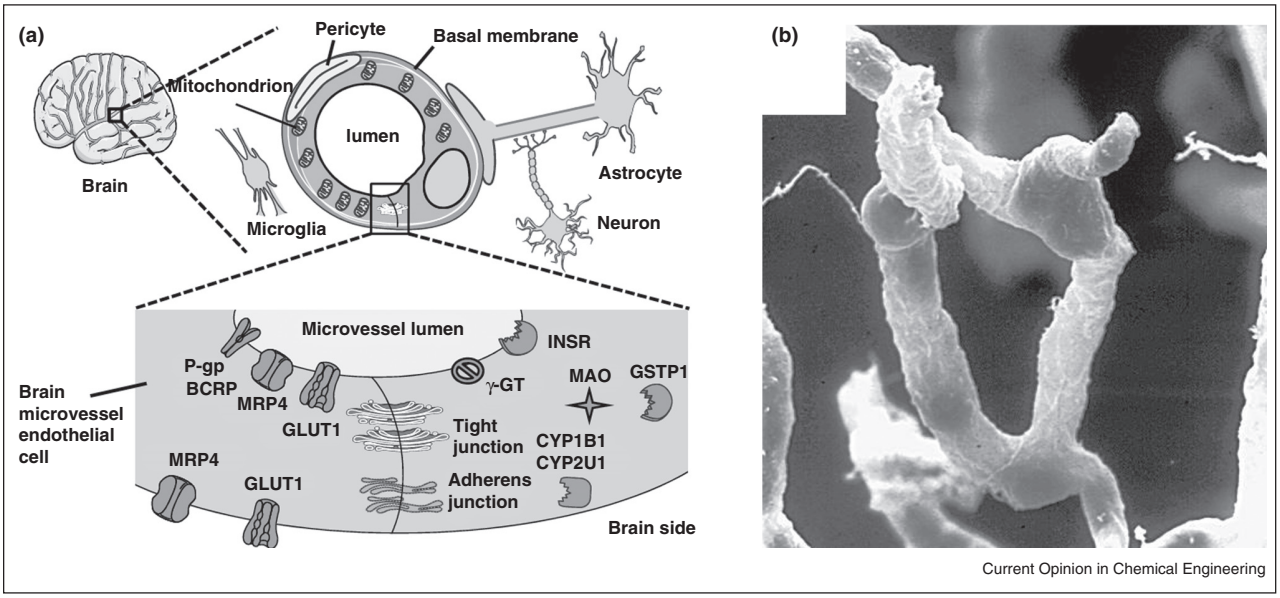



**(a)** Anatomical schematic of the acinar region of the lungs including the alveolar sacs surrounded by a fine mesh of pulmonary capillaries. The spherical geometry is unique to the pulmonary microcirculation (reprinted with permission from http://encyclopedia.lubopitko-bg.com/Respiratory_Tract.html). **(b)** Low and **(c)** high resolution scanning electron microscopy of the microvessel network in the rat pulmonary circulation. The fine mesh of capillaries surrounding the alveolar space is evident. Scale is 100 and 10 microns, respectively in panels B and C (reprinted with permission from [27]).

transport, and metabolic proteins of brain endothelial all contribute to the unique characteristics of the brain microcirculation.

Not surprisingly, attempts to mimic the brain microcirculation have focused on the structural proteins that comprise the BBB, and generally utilize transepithelial electrical resistance (TEER) as a simple index of BBB integrity [51,52]. Primary limitations are species differences (i.e. human BBB has important differences relative to other mammals (e.g. ABC transporters [53]) and poor access or difficulty in harvesting and culturing primary

**Figure 2**




**(a)** Schematic representation of the blood–brain barrier highlighting important structure features such as the tight junction, adherens junction, pericyte, and astrocyte that all contribute to the reduced permeability of the brain microcirculation to macromolecules (reprinted with permission from [44]). **(b)** Scanning electron micrograph of a brain capillary network (reprinted with permission from [97]).

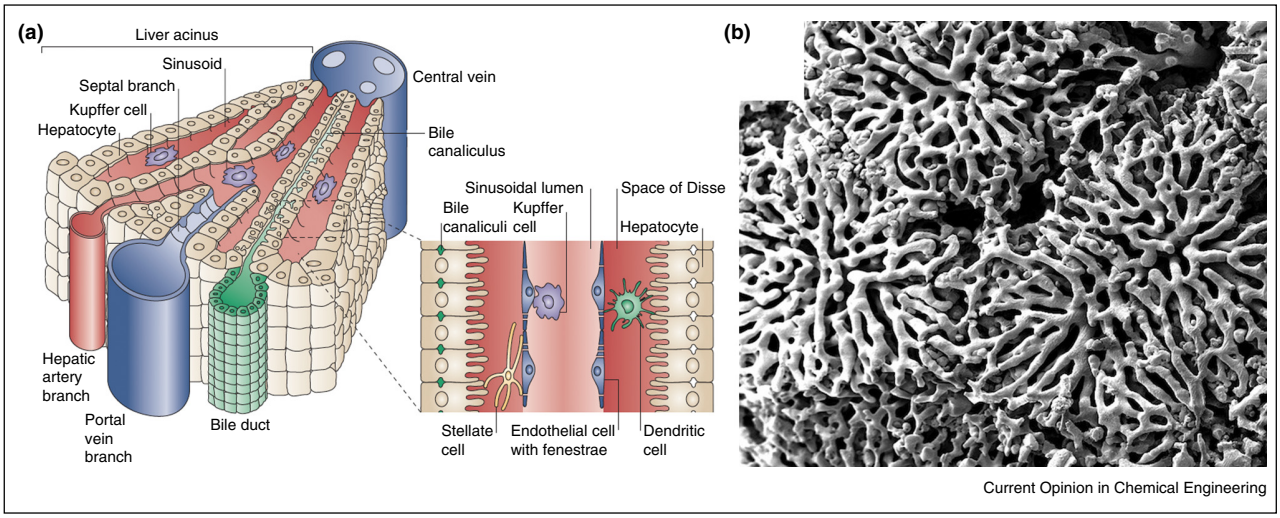
human brain endothelial cells. Although the creation of immortalized brain endothelial cell lines has also been demonstrated [54,55], these lines as well as primary brain endothelial cells both suffer from structural and metabolic features that do not completely mimic the *in vivo* BBB. For example, low passage bovine or murine brain microvascular endothelial cells display a TEER of $<200\ \Omega\ cm^2$, whereas *in vivo* values approach $2000\ \Omega\ cm^2$ [56–59]. Although 2D monolayer culture of brain endothelial cells have dominated the proposed *in vitro* models, some success has been demonstrated using 3D co-culture models of brain endothelial cells and astrocytes [60], and also recognizing the importance of fluidic shear stress on endothelial phenotype [61]. As with microphysiological systems of other organs, the potential of human induced pluripotent stem cells to recapitulate features of the human BBB is promising [62].

## Liver

Consuming 20% of the total body energy production, and receiving nearly a quarter of the cardiac output, the liver's impact on human physiology rivals that of the brain. The liver performs many essential functions related to digestion, metabolism, and immunity. Perhaps one of its most important functions is detoxification, where the blood from the gastrointestinal tract passes through the liver via the hepatic portal vein before traveling to the heart allowing the primary cells of the liver, hepatocytes, to monitor and remove (detoxify) many of the toxins from the blood before they reach the rest of the body ('first-pass' metabolism). Detoxification, especially as it relates to drug absorption and metabolism, is a key function to replicate in a microphysiological system. The traditional paradigm for *in vitro* models of culturing primary cells in 2D is especially not feasible for hepatocytes, as they do not replicate nor display a normal phenotype in 2D monolayer culture [63]. Although liver cell lines can be grown in 2D culture, they are not optimal for predicting drug toxicity as they lack some of the key metabolic enzymes [64]. Thus, a microsystem that captures the *in vivo* liver microenvironment is essential to not only predict drug-induced liver toxicity, but also *in vivo* pharmacodynamics and pharmacokinetics.

The liver's unique microcirculation plays a central role in the clearance of toxins from the blood. The liver's microcirculation is unique in that it has a supply of blood from both the hepatic artery and the hepatic portal vein. Blood from both the hepatic arterial and venous circulations of these sources supply blood to the hepatic sinusoids. Changes in portal venous flow can lead to compensatory flow changes in the hepatic artery. The sinusoids are comprised of a fenestrated, discontinuous endothelium [65,66]. These fenestrae can either contract or dilate in response to changes in sinusoidal pressure or flow and act as a selective sieve to control the exchange of material between the blood and liver cells [67]. These sinusoid

**Figure 3**




**(a)** Schematic representation of a section of a hepatic lobule. Hepatocytes radiate in rows from the central vein in between radiating rows of small blood vessels called sinusoids. Many cells make up the sinusoids including Kupffer cells (macrophages), hepatic stellate cells (fibroblast), and fenestrated endothelial cells (reprinted with permission from [98]. **(b)** Scanning electron micrograph of a vascular corrosion cast of the complex network of hepatic sinusoids (reprinted with permission The Journal of Hepatology).

endothelial cells are arranged in a radiating pattern forming hexagonal lobules of endothelial cells and hepatocytes, providing a unique and optimal configuration for interactions between the blood, hepatocytes, and sinusoidal endothelial cells (Figure 3).

Various hepatocyte cell culture methods have been used to capture the complexity of the liver. Although many of these models have focused on capturing cell–cell interactions by co-culturing hepatocytes and non-parenchymal cells either as spheroids [68] or using transwell inserts [69], others have focused on recreating the unique architecture arrangement of the liver lobules [70]. In the absence of perfused *in vitro* human capillary networks, microfluidic devices have been employed to provide perfusion to the cells [3•,71]. Although these models have led to insightful discoveries, without a dynamic capillary network that can respond to environmental cues and cellular cross-talk, the applications of these *in vitro* models are limited in understanding and predicting drug metabolism.

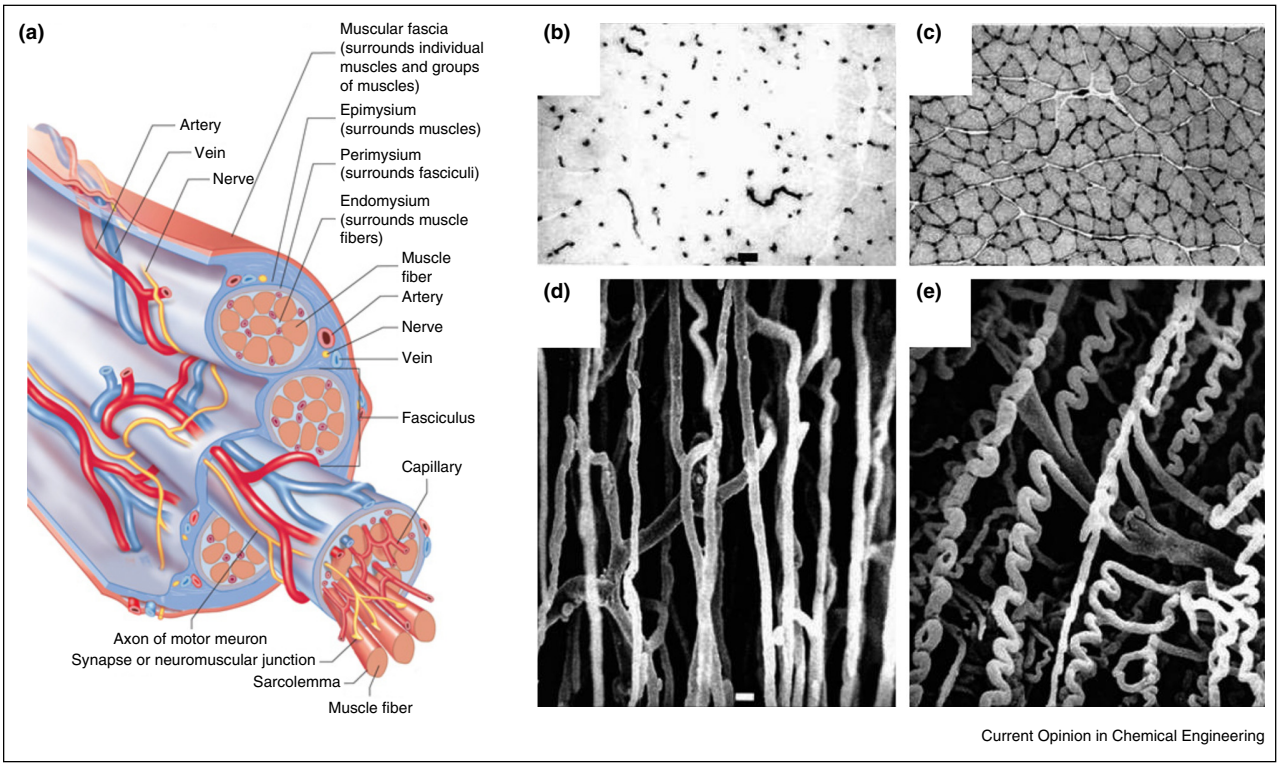
Although primary human hepatocytes are very robust *in vivo* exhibiting impressive regenerative ability, when these cells are cultured *in vitro* they loose phenotypic function and their ability to proliferate [72,73]. The human liver models that are available are generally limited to liver slices that are viable for <24 hours [63,74], liver microsomes, or liver cell lines with abnormal enzyme levels [74]. Successes culturing iPS-derived human hepatocytes *in vitro* have demonstrated functional success *in vivo* and have the potential to be useful in the creation of a liver microphysiological system with a microcirculation [75]. Additionally aside from hepatocytes, in one study murine iPS cells were differentiated into hepatocyte-like cells and some mesodermal cells in the process were found to express genes and proteins of liver endothelial cells [76]. Functionality of liver endothelium was not the primary focus of this work but such results are promising for the potential to derive liver-specific endothelium from iPS cells.

## Cardiac/skeletal muscle

Cardiac and skeletal muscles are comprised of individual muscle fibers in parallel surrounded radially by connective tissue and capillaries running longitudinally and branching from arterioles (Figure 4). The primary function of both skeletal and cardiac muscle is to generate force that results in the movement of either the body (skeletal) or blood (cardiac). The force is created by the contraction of muscle that is either voluntary (skeletal) or involuntary (cardiac). Thus, muscle is metabolically demanding, and the number of capillaries surrounding the muscle fibers depends on the metabolic need of the muscle [77].

A distinguishing feature of the capillary network in skeletal and cardiac muscle is that muscle contraction impacts microvasculature function and vice versa [78]. For example, capillaries in both the endocardium and myocardium modulate cardiac performance, rhythmicity and growth [77]. The impact is not limited to physical changes in vessel network volume and flow caused by extravascular compression, but many of the soluble

**Figure 4**




**(a)** Schematic representation of how the capillary beds surround the muscle fibers (reprinted with permission from The McGraw-Hill Companies). **(b)** and **(c)** Cross sections of muscle show capillaries embedded (black dots) within muscle fibers. **(d)** and **(e)** Scanning electrograph of capillaries running along the muscle (reprinted with permission from [81]).

mediators that promote myofiber transformation can also initiate a remodeling response in the capillary network [79].

Although the contractile response is the obvious function to replicate *in vitro* for cardiac and skeletal muscle, integrating the microvasculature with the muscle would improve the physiological fidelity of the microsystem. In the heart microenvironment, cardiomyoctes do not actually comprise the majority of the cells. They are outnumbered by endothelial cells 3 to 1 as there is a capillary adjacent (<1 μm) to essentially every cardiomyocyte [77]. Some studies have even demonstrated that the survival of the cardiomyocytes *in vitro* is improved when co-cultured with endothelial cells [80]. The endothelium also plays a prominent role in the electrophysiology of muscle. Endothelial cells sense the initial changes in vessel dilation [81] (caused by changes in flow or metabolic changes in muscle) but also play a role in communicating these changes. For example, endothelial cells can detect the local vasoactive response caused by an agonist and can propagate the signal through gap junctions between the endothelial cells to external vessels that supply the muscle [79]. Dysfunction or loss of integrity in the endothelium can exacerbate or initiate cardiovascular diseases such as hypertension; or, in the case of heart failure, lead to perfusion deficiencies and increased muscle fatigue [79,81]. Thus, maintaining the integrity of muscle's microvasculature is important to not only maintain adequate blood flow, but also normal muscle function and homeostasis.

Early attempts to mimic skeletal or cardiac muscle function *in vitro* consisted of simple models employing strips of muscles to examine contractility. For skeletal muscle, in particular, cells from both embryonic and adult tissue have led to *in vitro* models of 3D skeletal muscle tissue that are capable of generating contractile forces in a directed manner [82,83•,84]. Although the source of human cardiomyocytes for cardiac tissue engineering still remains a challenge, engineered cardiac tissues using neonatal rat heart cells have demonstrated impressive strides in mimicking features of adult cardiac tissues, such as spontaneous and rhythmic contraction [85,86]. Human cardiomyocytes from induced pluirpotent stem cells is an emerging technology that presents a novel alternative to animal cells. Although the phenotype of these cells tends to be immature [87], these cells have

been shown in 2D culture to exhibit predictable response to cardioactive drugs [88] and have been shown to respond to mature under electrical stimulation in 3D cell culture [89••].

A more recent trend to improve the performance of *in vitro* microphysiologic models includes the use of bioreactors or microfluidic chambers to enhance environmental control [90–93]. The microfluidic chambers have been used to enhance transport via convection in the absence of a vasculature. Microphysiological systems of cardiac and skeletal muscle have not only shown the ability to create a vascular network, but have demonstrated functional success *in vivo* [94–96].

## Conclusions and future directions

Advances in biotechnology, in particular induced pluripotent stem cells, combined with microfabrication and microfluidic technology provide exciting opportunities to recreate human physiology at the microscale. These microphysiological systems provide new opportunities to alter the paradigm of regenerative medicine and drug discovery. Central to the success is the creation of vascular network to mimic the convective transport process present in essentially all human tissue. A key challenge in this endeavor will be the production of organ-specific endothelial cells. Beyond arterial, venous or lymphatic, endothelial cells undergo further differentiation within each organ. The molecular mechanism underlying endothelial heterogeneity remains largely unknown but is thought to occur in part through the interaction between endothelial cells and their microenvironment (e.g. growth factors, extracellular matrix or mechanical forces). The quest to differentiate induced pluripotent cells into organ-specific endothelium may provide insight into endothelial specification. Although recent work demonstrates the ability to create perfused vascular microvessel networks, the next major advance will be the creation of organ-specific microvessel networks to capture organ-specific physiology.

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- •• of outstanding interest

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