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Engineered In Vitro Systems of the Microcirculation

Monica L. Moya, Christopher C. W. Hughes, and Steven C. George

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7.1 Introduction

Although the microcirculation refers to the smallest blood vessels in the body, its contribution to defining microenvironmental conditions in tissues is anything but small. Serving as the intermediate between the larger vessels of the arterial and venous circulation and the tissues to which it delivers blood and nutrients, the microcirculation is responsible for meeting the metabolic demands of the tissues. Impairments or dysfunction in the microcirculation can lead to and propagate pathologies, including cardiovascular disease, cancer, and diabetes mellitus.

Recapitulating the function of the microcirculation *in vitro* is a pursuit of biologists and engineers alike, who aim to better understand the microcirculation in hopes of being able to manipulate it for therapeutic and regenerative purposes. Many of the *in vitro* platforms created to meet that need seek to imitate specific features of the *in vivo* environment. The challenge that arises in this pursuit, however, is that often there is limited understanding

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of what is being imitated. Only after a fundamental understanding of basic biology is achieved can more effective therapeutics be designed. Therefore, the role and challenge of these models of the microcirculation is to not simply provide models that create potential therapies but also to contribute fundamental understanding of the physiological and pathophysiological processes of the microcirculation.

This chapter will highlight and assess *in vitro* models of the microcirculation that have been developed over the past four decades. This overview will examine their potential as tools to guide the design of new therapies as well as increase our understanding of the microcirculation. While many of these models have been designed to study specific diseased states, or to examine a particular pathophysiological mechanism, this review will focus more on categorizing the basic principles and methods on which these models are built. We have organized this review into two major sections broadly categorizing these models based on their design for studying vascular function. The first part will focus on methods used to understand the microcirculations' transport and flow processes while the second part will focus on models used to investigate growth and remodeling processes of the microcirculation.

7.2 Transport and Flow

The microcirculation performs, or is characterized by, several fundamental physiological processes, which include non-Newtonian fluid mechanical properties (i.e., shear thinning), endothelial shear, platelet aggregation, leukocyte attachment and margination, selective permeability, and transport of the respiratory gases (oxygen and carbon dioxide). Our understanding of these processes has been significantly enriched by a wide range of models of the microcirculation that broadly fall into seven categories: (1) cone and plate rheometry, (2) parallel plate rheometry, (3) porous membranes, (4) glass (or other material) tubes, (5) packed columns, (6) polymeric hydrogels, and (7) microfabricated networks. Each system has advantages and disadvantages and can be successfully employed to understand features of the microcirculation depending on the objective.

7.2.1 Cone and Plate Rheometry

Blood has particulate (cells, platelets) and fluid (plasma, including fibrinogen) phases that can aggregate depending on the chemical and mechanical environments. As such, blood is a non-Newtonian fluid, displaying characteristics consistent with a Casson fluid (nonzero yield stress and shear thinning). In addition, the anatomy of the vascular tree is generally a bifurcating tree structure in which the daughter branches are shorter in length and smaller in diameter compared to the parent. This latter feature, which conforms to the Hess–Murray law, creates a wide range of fluid velocities, although shear rate at the wall is nearly constant.

These unique features of blood and the vascular system were first explored more than four decades ago using a traditional cone plate rheometer (Charm and Kurland 1969, Charm et al. 1969, Parker 1968) (Figure 7.1a). The cone and plate rheometer consists of a cone-shaped top structure that points downward and touches a stationary flat plate. As the cone spins, it creates a constant shear stress in the radial direction. The cone and plate rheometer recreates the shear and fluid velocities present in the microcirculation, and thus

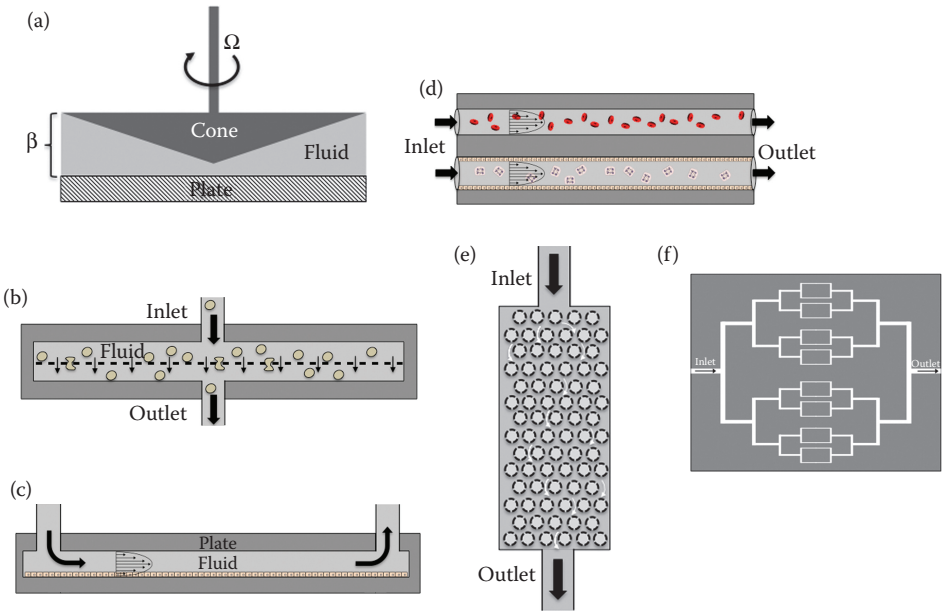

FIGURE 7.1
Schematic depiction of the major model systems used to simulate flow and mass transport features of the microcirculation. (a) The cone and plate rheometer is characterized by a cone-shaped piece that points downward toward a flat plate, and the space between the two surfaces is filled with a fluid. An angle β characterizes the shape of the cone, and ensures that the shear stress within the fluid when the cone rotates at angular speed Ω is constant with respect to radial position. (b) A porous membrane can be used to simulate the permeability of the capillary to molecules and cells. Fluid containing a particulate phase of interest (e.g., red blood cells) can be forced by an external pressure gradient through the membrane. The rate of appearance of the particulate phase on the other side of the membrane provides an index of microcirculation permeability. (c) Two parallel plates can be separated by a fixed distance and lined by endothelial cells. A fluid can then flow between the plates (approximate laminar velocity profile depicted) to effectively mimic the controlled shear stress environment in the microcirculation. (d) Tubes made from transparent materials such as glass can be embedded in a supporting material to mimic the cylindrical shape of small arterioles or capillaries. Fluid-carrying cells such as red blood cells (top tube) or white blood cells (bottom tube) can flow through the tubes (approximate laminar velocity profile depicted), which can also be lined by endothelial cells. (e) Small beads made from glass or dextran can be coated (circular dashed black line) with endothelial cells and packed gently into a long cylinder. Fluid mimicking plasma, lymph, or blood can flow through the pack bed to simulate the resistance to flow in the microcirculation as well as endothelial absorption functions. (f) A finely controlled network of fluidic channels can be microfabricated into a polymer such as PDMS to mimic flow and resistance patterns present in the microcirculation.

constitutes one of the earliest models of the microcirculation. The cone and plate rheometer has been employed successfully to investigate the non-Newtonian features of blood, including values for the yield stress, and range of shear in which the viscosity decreases with increasing shear (shear thinning). In addition, the cone and plate arrangement has been used to generate numerous and sustained observations related to the role of shear and platelet aggregation (Giorgio and Hellums 1988, Levy-Shraga et al. 2006). Finally, endothelial cell response to fluid shear was initially investigated by a cone and plate rheometer (Dewey et al. 1981, Davies et al. 1984). These early seminal studies demonstrated that fluid shear stress leads to cytoskeletal reorganization and alignment with flow, altered cell migration, and enhanced pinocytosis.

7.2.2 Filters and Membranes

The endothelium that lines arteries and veins, and forms the wall of the capillary in the microcirculation, provides important and selective barrier properties. For example, it has long been known that white blood cells cross the endothelium as they are transported from the blood to the interstitial space. In addition, the endothelium selectively retains larger proteins such as albumin in the blood, but lower-molecular-weight molecules diffuse across relatively freely. The selective permeability functions of the microcirculation were first mimicked using a series of porous membranes or filters (Figure 7.1b). For example, filters with pore sizes ranging from 5 to 8 μm demonstrated the impact of pH, temperature, hydrostatic pressure, osmotic pressure, cell concentration, and cell deformability on the transmembrane migration of peripheral blood leukocytes (Tanner and Scott 1976, Reinhart and Chien 1987, Eppihimer and Lipowsky 1994) and red blood cells (Reinhart and Chien 1987). Similar membranes (polycarbonate, 5 μm pore diameter) have been used to model the microcirculation of the lungs and investigate mechanisms underlying the retarded transit of neutrophils relative to red blood cells (Selby et al. 1991). While porous membranes alone have proven useful to investigate transport properties of white blood cells and red blood cells in the microcirculation, they have also proven to be a helpful mimic of the endothelial basement membrane. As such, they have proven to be a useful substrate for investigating the permeability of cultured endothelial cells to a variety of compounds and cells, including albumin (Smith and Borchardt 1989, Dull et al. 1991), dextran (Albelda et al. 1988), horseradish peroxidase (Behzadian et al. 2003), white blood cells (Giri et al. 2002, McGettrick et al. 2006, Moreland and Bailey 2006), and tumor cells (Lee et al. 2003, Sahni et al. 2009).

7.2.3 Parallel Plates

Following on the success of the cone and plate rheometer and porous membranes, were *in* **Q1** *vitro* devices characterized by laminar flow of cell culture media between two flat parallel plates separated by a fixed distance (Figure 7.1c). Parallel plate devices share the advantages of the cone and plate rheometer and porous membranes in that they can be used to finely control fluid shear at the surface and allow transport across the plates (lined with or without endothelial cells). Thus, they can be used for similar studies of blood viscosity, white blood cell attachment, endothelial cell alignment (Levesque and Nerem 1985), endothelial cell metabolism and transport (Nollert et al. 1991), and platelet aggregation (Muggli et al. 1980, Barstad et al. 1994) within the microcirculation. In addition, the parallel plate design offers much more flexibility in altering the components of the fluid in a temporal fashion, and for visualizing dynamics in real time.

Using the inlet/outlet design, chemical or cellular components can be introduced at precise times, and the duration of exposure can be easily controlled. For example, the duration of exposure time of platelets to endothelial cells in a controlled shear environment demonstrated that platelets can adhere to specific proteins in the extracellular matrix (Sakariassen et al. 1983). Combining relatively simple microscopes and the parallel plate system made of transparent materials such as glass has facilitated real-time visualization of white blood attachment from the top (Forrester and Lackie 1984), as well as from a side view (Lei et al. 1999). The relative simplicity of the parallel plate design also stimulated the first high-throughput platform designs of the microcirculation (Low et al. 1996). More recently, the parallel plate design has been adapted to include a porous membrane and thus allow coculture studies involving endothelial cells with smooth muscle cells

(Chiu et al. 2003). More advanced designs have been able to layer parallel plate flow chambers to create models of ischemia–reperfusion injury (Lee et al. 2009).

7.2.4 Small-Diameter Tubes

A primary limitation of parallel plates is the flat geometry of the flow chamber. To overcome this limitation, while still maintaining the potential for real-time visualization, small-diameter tubes made of glass or other polymers (Figure 7.1d) have been used to mimic features of the microcirculation. Although circular in shape, the major disadvantage of this technique is a lack of wall flexibility, and in many cases, rather impermeable walls. Nonetheless, this methodology has contributed to our understanding of blood rheology, including the Farheus–Lindquist effect (Thompson et al. 1989, Kubota et al. 1996), effect of shear on endothelial cell phenotype (Eskin et al. 1984), margination of white blood cells (Bagge et al. 1983, Goldsmith and Spain 1984, Nobis et al. 1985), and oxygen transport by perfluorocarbons across permeable capillary tubes (Vaslef and Goldstick 1994).

7.2.5 Microcarrier Beads and Packed Columns

An interesting method to simulate such features of the microcirculation as endothelial cell absorption and blood coagulation is the use of microcarrier beads, either alone or placed in a long cylindrical column (Figure 7.1e). The beads can be made of glass or dextran (Cytodex©), either be coated with endothelial cells or not, and then either placed in a column or a well plate. When placed in a column, cell culture media slowly introduced at the top percolates over the beads. This particular geometry dramatically increases the surface-area-to-volume ratio compared to other methods, and can also effectively mimic the resistance to flow of the entire vascular bed to a single organ such as the lung. However, the technique lacks the control of uniform or homogenous fluid shear and easy visualization offered by parallel plates. Microcarrier beads and packed columns have been used to enhance our understanding of the formation of macromolecular complexes in the blood coagulation (DePaulis et al. 1988), endothelial cell permeability (Killackey et al. 1986, Alexander et al. 1988, Haselton and Alexander 1992, Haselton et al. 1996), and white blood cell entrapment (Haselton et al. 1996).

7.2.6 Microfabricated Networks

The application of microfabrication technology to the study of microcirculation at the turn of the twenty-first century represents a significant advance in our understanding, particularly the impact of a branching network on the microcirculation function. Microfabrication technologies were developed initially by the semiconductor industry, but methods such as plasma etching, photolithography, and polymer micromolding have proved useful for mimicking the complex structure of the microcirculation at submicron resolution (Figure 7.1f).

The earliest examples of microfabricated microvessel networks utilized a silicon wafer as the backbone, and then utilized a mask combined with a photopolymerizable polymer such as SU-8 to create a negative mold on top of the wafer. A soft biocompatible polymer such as polydimethylsiloxane (PDMS) was then placed over the mold, cured, and then peeled off to create a positive mold of the microchannel network (Borenstein et al. 2002). The molded PDMS could then be bonded to another layer of PDMS or glass to create a completely enclosed network of microchannels of diameters as small as 20 μm. This methodology represents an

example of polymer micromolding and is still widely used. The network of channels can be created to precisely mimic the branching network of the microcirculation, including the drop in pressure and the desired shear rates. The channels can also be lined by endothelial cells, which have been shown to easily develop confluency in a matter of days and maintain viability for more than 2 weeks (Shin et al. 2004). Alternate polymers such as poly(glycerol sebacate) (PGS) (Fidkowski et al. 2005) and liquid elastomer RTV 615 A/B (Shevkoplyas et al. 2003) are also amendable to micromolding techniques. More recently, tissue functionality (e.g., hepatocytes) has been added to these networks (Carraro et al. 2008) demonstrating their ability to recreate organ-specific features of the microcirculation.

In general, polymer micromolding has been useful to understand the effect of complex flow patterns and shear in a vessel network on pressure loss (Borenstein et al. 2002), endothelial cell alignment (Song et al. 2005), and cellular transport (Shevkoplyas et al. 2003); however, there remain numerous disadvantages. For example, the natural variability (e.g., asymmetric branching) of the *in vivo* microcirculation has been difficult to simulate. An additional limitation of the early photolithographic and polymicromolding techniques was square channels, which do not completely replicate *in vivo* flow patterns, and endothelial cells do not coat the sharp transitions present in the corners. This has potentially been alleviated by more recent reports that present techniques to create round channels using similar micromolding techniques, and have also included traditional polystyrene in place of PDMS (Wang et al. 2007, Borenstein et al. 2010). Finally, a major limitation of polymer micromolding with synthetic polymers such as PDMS, PGS, or polystyrene is the lack of a natural extracellular matrix to encase the microvessels as well as other functional cells.

More recently, a significant advance has been the creation of microvascular networks within *in vivo* extracellular matrix proteins such as collagen and fibrin. The earliest example utilized collagen cast within a PDMS mold around a removable needle. Once the needle was removed, the resulting channel was completely surrounded by collagen, and following seeding with endothelial cells, created endothelial-lined channels within the collagen gel on the order of arterioles (50–100 μm diameter) (Chrobak et al. 2006). Later advances in this technique employed gelatin as the sacrificial polymer and demonstrated vessels as small as 6 μm diameter or that of a capillary (Golden and Tien 2007). Most recently, this method has been extended to 3D microvessel networks in which a polysaccharide (sugar) is micropatterned and used to create the sacrificial scaffold for the network using 3D printing (Lee et al. 2010, Miller et al. 2012) or melt spinning (Bellan et al. 2009).

7.3 Growth and Remodeling

While early studies of the microcirculation focused on the understanding of fundamental physiological processes, the past three decades have been marked by increased interest in forming or guiding the development of living dynamic vascular networks. An understanding of growth and remodeling processes such as migration, proliferation, vessel sprouting, tubulogenesis, stabilization, and maturation is required to effectively develop strategies for vascularization. This section will focus on the *in vitro* models that have contributed and continue to add to our understanding of these processes. These models will be divided into the following categories: (1) 2D culture (cells grown on various substrates including patterned substrates); (2) 3D culture (static culture of cells within a scaffold); and (3) dynamic 3D culture (3D bioreactor cultures).

7.3.1 Two-Dimensional Cultures

7.3.1.1 Substrates and Coatings

Two-dimensional cultures of cells provided some of the earliest studies of the microcirculation and its growth processes. Among the first of the 2D cultures were cultivation of microvascular endothelial cells on plastic with gelatin and collagen coatings (Figure 7.2a) (Folkman and Haudenschild 1980, Montesano et al. 1983, Ingber and Folkman 1989). These early *in vitro* models provided a simple approach for investigating important factors that stimulate endothelial cell proliferation and rudimentary capillary assembly. This method of growing cells on 2D surfaces continues to be a useful tool for studying singular process such as migration, proliferation, and endothelial cell differentiation. Typically, in these models, a singular vascular cell type (e.g., endothelial cells or mural cells) is investigated. The 2D surfaces that are used to stimulate the interaction of the cells with the extracellular matrix range from coatings of basement proteins such as fibronectin, collagen IV or I, or matrigel to seeding cells on gels of fibrin (Vailhe et al. 1998), collagen (Sieminski et al. 2004, Hong and Stegemann 2008, Francis-Sedlak et al. 2010), or tissue-derived matrix.

7.3.1.2 Patterned Substrates

A more recent variation of this type of 2D *in vitro* model is growing cells on patterned substrates (Figure 7.2b). Adhesive patterns of extracellular matrix proteins such as fibronectin or other biological molecules are patterned on a rigid surface such as silicon or gold (Chen et al. 1998). This approach allows for vascular structures to be patterned in a precise location or in the form of a vascular structure rather than relying on the spontaneous self-organization of cells. Using this technique, growth, apoptosis, migration, and proliferation of vascular cells can be examined with regard to the influence of size and geometry of the patterned substrate.

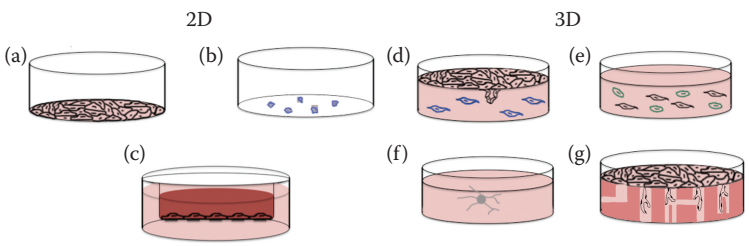

FIGURE 7.2
Schematic depiction of the major model systems used to study growth and remodeling processes of the microcirculation. (a) 2D substrates and coatings can be used to simulate the interaction of cells with extracellular matrix. (b) Cells or vascular structures can be patterned in a precise location or shape using adhesive patterns of extracellular matrix proteins. (c) A Boyden chamber consists of a cell culture insert placed into the well of a cell culture plate. Migration or invasion from cells seeded in the top of the insert can be stimulated by placing chemoattractant in the well below. (d) Assays where 2D cultured cells invade a surrounding 3D matrix allow for visualization of angiogenesis-like processes such as sprouting. (e) Endothelial cells dispersed in a 3D matrix either as a single cell type or mixed with stromal cells self-assemble into tubes. This process resembles the vascularization process of vasculogenesis. (f) A mass of cells embedded in a 3D scaffold, either as an aggregate or a cell-covered bead, can sprout radially and form lumenized capillary networks. Sprouting from a focal point allows for easier quantification of forming capillaries. (g) Patterned 3D scaffolds consist of scaffolds with predefined pathways for cell and vascular growth.

7.3.1.3 Boyden Chamber

Another commonly used variation of 2D *in vitro* models is the Boyden chamber assay. This assay aims to recapitulate the migration of endothelial cells from existing blood vessels into surrounding tissue areas during angiogenesis. For this assay, endothelial cells are grown as a monolayer on a cell-permeable membrane (8+ μm pore size) sometimes coated with extracellular matrix, a Transwell insert inside the wells of a multiwell plate (Figure 7.2c). Cell medium is placed above the monolayer while medium with chemoattractants or test agent is placed below the membrane. This assay is commonly used to test the migratory response of endothelial cells to angiogenic inducers or inhibitors. A major advantage of this assay is that it requires low levels of angiogenic stimulus to induce migration of endothelial cells into the second chamber.

7.3.2 Three-Dimensional Cultures

Since the early studies using 2D models, interest has been growing in 3D cultures as most cells in the body are in a 3D environment that is not recapitulated at all in monolayer, 2D cultures. Migration, proliferation, endothelial cell differentiation, tubulogenesis, branching, and stabilization are all crucial steps in the formation of the microvasculature, both embryologically and in the adult during both normal and diseased processes such as would healing and tumorigenesis. 3D models of the microcirculation are now becoming important tools for screening and developing potential therapies associated with the microvasculature.

7.3.2.1 Cells Dispersed in 3D Scaffolds

Many of the early studies with substrate coatings led to a growing awareness of the importance of cell–ECM interactions. Cells not only respond to the proteins and soluble factors associated with the ECM but they also respond to biomechanical cues that may serve to regulate capillary development. *In vitro* models of cells within a scaffold aim to recapitulate this environment. Initial variations to mimic the development of vessel networks in a 3D environment consisted of seeding endothelial cells sandwiched between gels (Montesano et al. 1983), under gels, or on top of malleable gels made from fibrin (Pepper et al. 1990, Vailhe et al. 1998, Bayless et al. 2000), collagen (Kubota et al. 1988, Davis et al. 2000), or Matrigel. Similar to *in vivo* angiogenesis, confluent monolayers of endothelial cells are stimulated to invade into the surrounding 3D scaffold by the addition of growth factors or other angiogenic factors (Montesano and Orci 1985). These 2D/3D assays allow for investigation of several stages in angiogenesis (Figure 7.2d) specifically allowing for the visualization of endothelial cell invasion and morphogenesis into 3D matrices.

The addition of exogenous factors for coaxing single-cell cultures to form tube structures was especially important with assays in which endothelial cells were dispersed within 3D scaffolds (Sieminski et al. 2005). Similar to the 2D cultures, these types of assay relied on the self-assembly of endothelial cells into tubes, a process that resembles the vascularization process of vaculogenesis rather than sprouting angiogenesis, where endothelial cells suspended as single cells form lumens and tubes through the coalescence of vacuoles (Davis and Camarillo 1996). Although these single-cell 3D models required addition of exogenous factors such as TGFβ (Madri et al. 1988), VEGF or bFGF (Yang et al. 1999), or HGF/SF (Lafleur et al. 2002), they allowed for the study of capillary morphogenesis in a controlled manner and leading to successful identification of many signaling components involved in tube formation (Sacharidou et al. 2012). Such systems

are amenable for studying cell proliferation and migration, and also provide the ability to study remodeling processes such as matrix degradation (Vailhe et al. 1998, Sacharidou et al. 2010).

While dispersing cells in a scaffold provides cell–ECM interaction, another feature of the microcirculation microenvironment is cell–cell interaction. A shift toward 3D culture of multicellular systems allowed for this interaction to be studied, and produced systems that do not require exogenous factors. In the 2D/3D models, rather than being cultured on top of acellular gels, endothelial cells are cultured on top of gels with dispersed stromal cells. This not only creates a more *in vivo*-like environment, where the endothelial cells are responding to cues on the basal side rather than medium (and growth/morphogenic factors) placed on their apical side, but also allows for the creation of an environment where the cells are responding to paracrine soluble factors from stromal cells (Montesano et al. 1993) (Kuzuya and Kinsella 1994, Tille and Pepper 2002).

Additional cell types were also eventually added to the model of dispersed cells in 3D scaffold (Figure 7.2e). Endothelial cells can be mixed with fibroblasts or other stromal cells in a 3D scaffold. Like the monoculture dispersed model, capillary networks self-assemble but without addition of exogenous factors. In addition to providing support for growth, stromal cells have been found to wrap around endothelial cells and thus take on a pericyte-like behavior allowing for this type of cell–cell interaction behavior to be observed (Darland and D'Amore 2001, Chen et al. 2010). One benefit of these models is that their functionality can be tested *in vivo* (Sieminski et al. 2002, Koike et al. 2004, Levenberg et al. 2005, Chen et al. 2009). This offers the advantage of being able to examine not only their functionality *in vivo* but also to observe, and thus understand, the process of anastomosis (Chen et al. 2009, White et al. 2012). To further examine the cell–matrix interaction some of these models have used other matrices that better approximate the mechanical and physical properties of tissue *in vivo* (Levenberg et al. 2005).

7.3.2.2 Cell Aggregates or Cells on Beads

One limitation of *in vitro* models with dispersed cells in 3D scaffolds is the difficulty in quantifying the formation of capillary-like structures. Models with cell aggregates or cell-covered beads embedded in scaffolds provide an easier method for observing the radial formation and sprouting of capillary-like structures (Figure 7.2f). Interestingly, while the culturing of single endothelial cells dispersed in a matrix requires the addition of exogenous factors for survival, cells in aggregate form do not (Korff and Augustin 1998). Although these cells survive, they still require the addition of angiogeneic factors to stimulate sprouting. For these models, endothelial cells are coated onto Cytodex beads (Nehls and Drenckhahn 1995) or embedded as a mass of cells (Pepper et al. 1991, Korff and Augustin 1998) and are allowed to sprout, forming lumenized capillary-like structures that grow out from these spheroids into the surrounding scaffold. In some variations of these models, multiple cell-covered beads or aggregates are placed within a scaffold whereas in other variations a single aggregate is placed within a gel (Vernon and Sage 1999). The use of multiple beads or aggregates has the added advantage of allowing for the study of anastomosis between sprouts from neighboring beads or aggregates. These models are well suited for studying different steps of the angiogenesis process in response to various stimuli, including degradation of basement membrane, proliferation, sprouting of endothelial cells, and branching. Endothelial migration or sprouting is induced by various factors, including FGF-1 (Uriel et al. 2006, Moya et al.) and VEGF (Korff and Augustin 1999, Xue and Greisler 2002). Addition of a stromal cell **Q2**

in these models as coembedded fibroblast-coated Cytodex beads, cocultured spheriods (i.e., aggegates made up of both endothelial cells and stromal cells) (Korff et al. 2001) seeded on top of the gel (Nakatsu et al. 2003), or disperesed within the gel (Ghajar et al. 2008) allows for the investigation of not only the cross talk between endothelial cells and the stromal cells but also allows for the study of vessel maturity and stability (Newman et al. 2011, 2013).

7.3.2.3 Patterned 3D Scaffolds

Like its 2D predecessor, patterning in 3D scaffolds allows for the precise control of vascular-like structure assembly (Figure 7.2g). The creation of engineered scaffolds aims to mimic the complexity and microarchitecture of the tissues. Other *in vitro* models of angiogenesis or vasculogenesis allow for cell-guided formation of vessel structures that are often randomly structured and do not recapitulate vessel hierarchy. Although patterning scaffolds allows for the spatial arrangement of endothelial tubulogenesis within 3D ECM, many of these models do not provide the flexibility of allowing the vessel network to adapt or remodel in response to an agonist or to flow.

For these models, various methods are used to create patterned structures of adhesion proteins or growth factors into either natural scaffolds such as collagen (Oh et al. 2011), Matrigel (Nahmias et al. 2005), or synthetic scaffolds. These methods include 3D laser printing, microfrabrication technologies, and photopolymerizable chemistries of polymers such as PEG-based hydrogels (Chiu et al. 2009). These models serve to help understand the interaction between cells and their scaffold (Tan and Desai 2003), tubulogenesis (Raghavan et al.), and cell migration along predefined pathways (Lee et al. 2008). Q2

7.3.3 Dynamic 3D Cultures

Cells are responsive to their microenviroment sensing both mechnical and biochemical cues. While growing cells in static 3D cultures has allowed for the investigation of some mechnical and biochemical cues, these static cultures do not recapitulate the dynamic forces that drive morphogenesis *in vivo*. Bulk fluid flow can generate gradients of chemokines as well as exert forces directly on cells, or indirectly by exerting forces on the scafffolds. Dynamic 3D cultures not only help elucidate transport and flow processes (see the previous section) but are also important for understanding growth and remodeling processes in response to chemokine gradients or intersitial flow.

Early work that introduced bulk perfusion into gels demonstrated significant influence on capillary morphogenesis, cell migration, and remodeling (Ng and Swartz 2003, Helm et al. 2005, 2007). More recently, the use of microfabricated networks (see the previous section) has increased the number of dynamic 3D cultures (Wong et al. 2012). Microfabricated networks add dynamic components to 3D cell culture by typically circulating media through a hydrogel through the use of microfabricated channels. Various designs in the microfluidic network allow for numerous patterns and methods to control mass transport gradients. This percision is advantageous as it allows for fine spatial and temporal control. In some models, the design is derived from static culture but with the added complexity and sophistication of mass transport. For example, one such model uses the endothelial cell-coated Cytodex beads to examine sprouting in a microfluidic device under VEGF gradients (Shamloo et al. 2012). Like the static model, this dynamic model can examine tip sprouting, migration, and branching; however, it has the added advantage that this model can examine sprouting navigation dynamics in response to VEGF gradients.

These dynamic 3D cultures allow us to investigate all the endpoints that are possible with static cultures, but with more complexity and physiological accuracy as they also combine transport and flow processes with growth and remodeling processes. These models can be used to look at angiogenic sprouting into a 3D scaffold, but unlike previous static models, this sprouting process involves perfused native-like vessel structures (i.e., channels lined with endothelial cells) (Song et al. 2012b, Zheng et al. 2012) that can respond to flow. In addition, the sprouting is in response to not just bolus addition of growth factors but to *in vivo*-like gradients of solutes and growth factors. Some of these models have also been designed to specicifically demonstrate the vascular response to other cell types such as tumor cells (Jeon et al. 2013) or even encapsulated cells (Kim et al. 2012). More uniquely, these dynamic cultures allow for examining and understanding the development of vessels into continuous networks in response to dynamic cues such as pressure and interstitial flow (Vickerman and Kamm 2012, Hsu et al. 2013). Finally, the true utility of these models is being realized, as they not only capture the growth of vascular networks but have now been shown to support flow. The further development of perfusable microvessels *in vitro*, capable of supporting the growth of surrounding tissues, will be a major step forward in the now rapidly evolving world of tissue engineering (Yeon et al. 2012, Song et al. 2012a, Moya et al. 2013).

7.4 Conclusions

Despite the numerous *in vitro* models developed over the years, an epitome of the microcirculation is not currently available. A lack of a standardized assay provides a challenge when comparing findings from *in vitro* assays across groups. Variations in cell origins, substrate or matrix material, and growth media all need to be considered when attempting to extrapolate *in vitro* observations to an *in vivo* setting. Research in developing more *in vivo*-inspired *in vitro* models will continue to enhance our understanding of the microcirculation and provide opportunities for new therapies.

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