DISCUSSION

In Vitro Models to Mimic the Endothelial Barrier

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Microfluidic technologies permit the replication in vitro of geometrical features essential for the homeostasis of all vascularised tissues in vivo*, including the contribution of pericytes to the endothelial barrier*

Introduction

A functional microvasculature is critical for the homeostasis of all vascularised tissues. Accordingly, several diseases are associated with alterations in the microvasculature. For example, tumour angiogenesis is a major factor in determining the burden of the disease. Furthermore, the formation of new vessels by angiogenesis and vasculogenesis is critical in the restoration of tissue function in ischaemic diseases. In tissue engineering, sufficient neovascularisation and early vessel anastomosis is thought to be a prerequisite for the integration of the implant. These conditions have been extensively studied in animal models. However, *in vivo* studies have several limitations, including species differences and limited possibilities for imaging and tracking cells in the living animal. They also do not permit high-throughput and multiplexing applications. The development of microfluidic models of microvasculature and the endothelial barrier could help to overcome these problems and, most importantly, would replace a significant amount of animal experimentation. Nevertheless, microfluidic science is still an evolving research field, and many models do not address the endothelial barrier in its full complexity — for example, taking into account the contribution of pericytes.

The contribution of pericytes to the endothelial barrier

Pericytes are vascular mural cells associated with microvessels. The most common definition of pericytes goes back to their localisation, embedded in the endothelial basement membrane.[1] Besides sharing the basement membrane, close interactions between endothelial cells and pericytes have been described, such as peg–socket contacts representing tight-, gap- and adherence-junctions.[2] The relationship between the two cell types, particularly the anatomy of the pericyte coverage, reflects the function of the individual tissues. In organs with high exchange rates of gas and metabolites, the distribution of pericytes is such that diffusion is minimally hindered.[1,3] Pericytes play an important role in vessel stabilisation, and underlying molecular signalling pathways have been described. Here, signalling through angiopoietin and Tie2 is critical; mutation of either angiopoietin 1 or Tie2 leads to mid-gestational death by cardiovascular failure in mice.[2] In angiogenesis, the recruitment of pericytes is required for the stability of newly-formed vessels. Various studies have identified platelet-derived growth factor B (PDGF-B) as a major chemoattractant for pericytes.[4] Capillary and arteriolar pericytes also play an important role in inflammatory processes by guiding extravasating leukocytes toward their target.[5] Pericytes are also involved in several disease states, including airway remodelling in chronic allergic asthma[6] and stroke.[7] The identification of mesenchymal stem cells and progenitor cells at perivascular sites, and the subsequent isolation and characterisation of such cells, suggests that pericytes have a role as multipotent progenitors.[8-10]

Current animal models

The function of pericytes in physiologically and pathologically relevant situations has been studied in transgenic mouse models containing LacZ-expressing or fluorophore-expressing pericytes.[5,6,11] The migration and stimulation of pericytes in different disease situations was subsequently studied by the administration of pro-inflammatory factors, or by backcrossing mice to a transgenic disease mouse model.[5,12] Interactions between labelled leukocytes and pericytes have been studied by lethal irradiation and the subsequent injection of bone-marrow cells from GFP

mice.[5] In addition, specific knockout lines have been used to study specific components of the cell junction and signalling complexes involved in pericyte-endothelial cell crosstalk, e.g. Sparc-deficient and Ccn2-deficient mice.[13,14] Various animal models have also been developed, for studying the basic mechanisms of angiogenesis and vessel sprouting, including the cornea model, the chick chorioallantoic membrane (CAM) model, matrigel plug assays, and the dorsal skin fold chamber.[15]

Current microvascular models

Many factors determine the phenotypes of cells *in vivo*, including cell–cell interactions, interaction with the surrounding extracellular matrix, and the influence of various paracrine factors. Current cell culture vessels (dishes, flasks) cannot recapitulate these aforementioned complex interactions. However, in recent years, microfluidic technologies have shown the potential to more-closely mimic the cellular microenvironment, at both the spatial and temporal levels.[16] Typical microfluidic systems have geometrical features ranging in size from tens to hundreds of microns, and can host single cells or millions of cells arranged in a 2-D or a 3-D fashion. Microfluidic devices can accommodate flow control and therefore induce shear stress, which is known to have significant effects on the endothelial layer.[17] This shear stress is absent in classical culture dishes. Furthermore, microfluidic technologies enable the generation of gradients over long periods of time, and also permit the control of paracrine factors in complex co-culture systems.[18]

In the past, various strategies were pursued to generate perfused microvessels *in vitro*.[19] Different levels of complexity were achieved, ranging from straight or branched channels within a material such as polydimethylsiloxane (PDMS), toward more complex microvascular networks within extracellular matrix hydrogels. In order to study cell–cell and cell–matrix interactions, microfluidic chips were designed that comprised two or more parallel channels, allowing the seeding of different cell types or hydrogels next to each other.[20–22] Chen *et al*. reported on an alternative approach to studying the interactions between endothelial cells and other cell types.[23] Here, each cell type was seeded in a different layer of the microfluidic device, separated by porous membranes; perfusion was applied to the endothelial layer of the chip.[23] The embedding of microvessels in a 3-D matrix, potentially mimicking the perivascular tissue, represents another level of complexity. Several groups have addressed this challenge by generating endothelial cell-aligned microchannels within a collagen hydrogel.[24–28] Such a set-up also allows the incorporation of pericytes in the hydrogel, which might eventually reassemble at the perivascular site of the endothelial cell layer.[24,26,28,29]

Conclusions

Microfluidic technologies permit the replication of geometrical features found *in vivo* (i.e. small channels mimicking capillaries). In addition, due to the small dimensions involved, screenings are much less costly and time-consuming, as compared to similar *in vivo* studies. This is particularly interesting for applications such as testing the delivery of drugs. For the *in vitro* models to be successful, it is critical to consider all parameters of the endothelial barrier, including cell–cell and cell–matrix interactions, and the surrounding perivascular tissue.

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