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Modeling the blood–brain barrier: Beyond the endothelial cells

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Abstract

The blood–brain barrier (BBB) regulates molecular transport to help maintain proper brain function. This restrictive interface formed by brain microvascular endothelial cells (BMECs) excludes the majority of drugs from the brain, and BBB dysfunction is a signature of many neurological diseases. Thus, *in vitro* models of the BBB based on BMECs have been developed for drug permeability screening. However, while BMECs form the main interface, they work in concert with other brain-resident cells such as neural progenitor cells, pericytes, astrocytes, and neurons to form the neurovascular unit (NVU). Importantly, non-endothelial cells of the NVU play key roles in eliciting BBB phenotypes and in regulating the dynamic responses of the BBB to brain activity and disease. As a result, emerging BBB models have incorporated these NVU cell types in addition to BMECs, and have found increasing application in studying complex cellular and molecular mechanisms underlying BBB biology and disease.

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Introduction

The blood–brain barrier (BBB) comprises highly specialized brain microvascular endothelial cells (BMECs) that maintain the delicate balance of ions, nutrients, and other molecules essential for proper brain function, while also excluding toxins from the central nervous system (CNS). Among the specialized properties of BMECs are (i) lack of fenestrae, (ii) tight junctions between adjacent endothelial cells, (iii) presence of solute carriers that regulate ion and small molecule transport, (iv) expression of efflux transporters including P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP), and Multidrug Resistance Proteins (MRPs), (v) low levels of pinocytosis, and (vi) receptor-mediated processes for specific uptake of macromolecules (reviewed in Refs. [1–3]).

Although the microvascular endothelium constitutes this restrictive interface, other cell types present in the neurovascular microenvironment during development and adulthood including neural progenitor cells, pericytes, astrocytes, and neurons contribute significantly to the BBB phenotype. Increasing appreciation of the importance of multiple cell types in regulating dynamic BBB responses to physiological and disease stimuli has led to the concept of an integrated neurovascular unit (NVU), which minimally consists of BMECs, pericytes, astrocytes, and neurons (Fig. 1A), and for some studies can extend to include neural stem cells or microglia.

The development of *in vitro* BBB models has been driven by the desire to understand BBB function in development, health, and disease. Moreover, because the BBB excludes the vast majority of small molecule, protein, and gene therapeutics [4], *in vitro* BBB models also offer a platform for screening drug candidates for BBB permeability. To date, considerable effort has led to the generation of many BMEC-based models of the BBB (reviewed in Refs. [5–7]). Importantly, *in vitro* models that incorporate multiple NVU cell types can have advantages over BMEC-only models. First, the presence other NVU cell types can induce or improve barrier properties, such as the formation of continuous tight junctions to reduce paracellular diffusion or “leakiness”. When used for drug permeability screening, such models may therefore yield results that are more predictive of *in vivo* permeability. Second, multicellular models can provide a tool to interrogate paracrine and juxtacrine signaling that may underlie elements of BBB development and maintenance. Finally, given emerging knowledge about the roles of neurovascular dysfunction in many diseases of the CNS (reviewed in Refs. [3,8]), *in vitro* models of the NVU, including those derived from patient-specific induced pluripotent stem cells (iPSCs), may provide opportunities to better understand molecular and cellular mechanisms of CNS diseases.

We will first briefly discuss the roles of neural progenitor cells, pericytes, astrocytes, and neurons in regulating the development and maintenance of the BBB. We will then review recent advances in BBB modeling resulting from incorporation of NVU cells to form multicellular BBB models, and highlight several examples of the utility of such models in understanding BBB biology and disease.

Roles of non-endothelial NVU cells in BBB formation and function

Stewart and Wiley [9] used quail-chick transplantation studies to show that developing neural tissue was necessary for endothelial BBB development. Subsequent work established the ability of both astrocytes [10,11] and neurons [11,12] to induce BBB phenotypes in endothelial cells. In addition, during early embryogenesis the BBB initially forms in the presence of neural progenitor cells when astrocytes are not yet present. Studies have demonstrated the ability of embryonic neural progenitor cells (NPCs) to induce BBB properties such as decreased endothelial permeability and improved tight junction formation *in vitro* [13], and it was later determined that Wnt/β-catenin signaling driven by NPCs is required for CNS angiogenesis and contributes to barriergenesis during development [14]. In addition, signaling through retinoic acid secreted by radial glial cells [15], Hedgehog secreted by astrocytes [16], and GPR124 [17,18] have also been implicated in aspects of BBB development. Key roles for pericytes in barriergenesis have also been described, as pericytes regulate BBB endothelial tight junction morphology, transcytosis, and expression of leukocyte adhesion molecules [19]. Pericytes are also required for the maintenance of the BBB in adulthood, as demonstrated by pericyte-dependent endothelial gene expression, reduction in endothelial transcytosis, and astrocyte endfoot polarization [20]. Furthermore, given the ability of astrocytes to induce and maintain endothelial BBB properties *in vitro*, the close association of astrocytes with endothelial cells *in vivo*, and correlations between astrocyte pathologies and BBB breakdown (reviewed in Ref. [21]), it is likely that continued astrocyte-endothelial signaling is necessary for BBB maintenance. Neurons similarly have the ability to induce and maintain BBB properties *in vitro* [11,12,22], but currently a detailed picture of neuron-endothelial crosstalk is lacking. Taken together, there is a clear impact of non-BMEC cell types on BBB formation and function motivating the development and use of multicellular NVU-type models to continue to advance our understanding of these complex phenomena in neural health, disease, and therapy.

Advances in multicellular BBB models

Recently developed multicellular BBB models have incorporated neural progenitor cells, pericytes, astrocytes, and neurons. These models have employed both primary and immortalized cells from human, rodent, bovine, and porcine sources. NVU cells derived from pluripotent stem cell or neural stem cell sources have also been used (Table 1). Most models have been constructed using either Transwell culture inserts or microfluidic devices, and models based on cell aggregates are an emerging alternative (Fig. 1B). Below we will summarize each of these configurations as they pertain to the contribution of NVU cells to the BBB model.

Transwell models

Transwell-based BBB models typically consist of endothelial cells cultured on an extracellular matrix-coated permeable membrane of a cell culture insert, which is then suspended within a well of a 12- or 24-well plate (Fig. 1B). Benefits of the Transwell platform include ease of use, moderate scalability, and the ability to rapidly and nondestructively quantify barrier integrity via measurement of transendothelial electrical resistance (TEER). Additionally, for permeability screening, molecules or cells can be added to the culture medium in the top (apical or "blood-side") chamber and their accumulation in the bottom (basolateral or "brain-side") chamber evaluated over time, or vice versa. Drawbacks of the Transwell system include the lack of fluid flow and the relatively large medium volume, which may attenuate the effect of cell–cell signaling through soluble factors. Additionally, the permeable membrane prevents substantial contact between BMECs and other NVU cell types.

The Transwell system can be readily adapted to multicellular BBB models, and offers flexibility in the arrangement of different cell types depending on the intended application of the model. NVU cell types can be cultured on the bottom of the well, allowing the exchange of soluble factors with BMECs cultured on the insert. For example, human pluripotent stem cell (hPSC)-derived BMECs have been co-cultured sequentially with primary human pericytes and human neural progenitor cell-derived neurons and astrocytes in this manner, demonstrating robust increases in TEER up to 5000 $\Omega \times cm^2$ [23]. In addition to allowing sequential co-culture with different cell types, the Transwell platform also allows simultaneous co-culture of three distinct cell types while maintaining spatial separation of each cell type for subsequent molecular analysis. BMECs are typically cultured on the top surface of the membrane, a second cell type is cultured on the bottom surface of the membrane (sometimes referred to as "contact" co-culture, though the membrane prevents *in vivo*-like cell–cell contact), and the third cell type is cultured on the bottom of the well (Fig. 1B). For example, Thomsen et al. developed a Transwell BBB model incorporating primary porcine