In Vitro Blood–Brain Barrier Models—An Overview of Established Models and New Microfluidic Approaches

ANETTE WOLFF,[1] MARIA ANTFOLK,[1] BIRGER BRODIN,[2] MARIA TENJE[1,3]

[1]Lund University, Department of Biomedical Engineering, Lund, Sweden
[2]University of Copenhagen, Department of Pharmacy, Copenhagen, Denmark
[3]Uppsala University, Department of Engineering Sciences, Uppsala, Sweden

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ABSTRACT: The societal need for new central nervous system (CNS) medicines is substantial, because of the global increase in life expectancy and the accompanying increase in age-related CNS diseases. Low blood–brain barrier (BBB) permeability has been one of the major causes of failure for new CNS drug candidates. There has therefore been a great interest in cell models, which mimic BBB permeation properties. In this review, we present an overview of the performance of monocultured, cocultured, and triple-cultured primary cells and immortalized cell lines, including key parameters such as transendothelial electrical resistance values, permeabilities of paracellular flux markers, and expression of BBB-specific marker proteins. Microfluidic systems are gaining ground as a new automated technical platform for cell culture and systematic analysis. The performance of these systems was compared with current state-of-the-art models and it was noted that, although they show great promise, these systems have not yet reached beyond the proof-of-concept stage. In general, it was found that there were large variations in experimental protocols, BBB phenotype markers, and paracellular flux markers used. It is the author's opinion that the field may benefit greatly from developing standardized methodologies and initiating collaborative efforts on optimizing culture protocols. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2727–2746, 2015

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INTRODUCTION

The pharmaceutical industry is faced with one of the greatest challenges of the modern age—developing medicines that can reach the brain. One of the incentives is the fact that the total cost of the neurovascular health care system in Europe was estimated in 2010 at 800 billion €PPP in direct and indirect costs,[1] with an estimated 85% increase in costs for dementia care by 2030, according to Alzheimers Disease International.[2] These numbers can only be expected to increase with the ageing population. The challenge lies in the existence of the highly effective blood–brain barrier (BBB) that strictly controls what molecules are allowed to pass and reach the brain. Drug screening is very expensive and time-consuming in the current drug development process, where *in vivo* screening is applied at the preclinical test stage. Much research effort is therefore directed toward the development of functional BBB *in vitro* models, which allows the speed by which new drugs are made available to increase.

Already in 1885, Paul Ehrlich found that dyes injected into the circulatory system stained all organs in the mammalian body, except for the brain and the spinal cord.[3] On the basis of this work, in 1900, the term BBB or really *bluthirnshranke*, was coined by Berlin-based Max Lewandowsky.[4] One of Ehrlich's students went on, in 1913, to show that the effect could be seen in the reverse situation as well, by injecting *trypan blue* into the cerebrospinal fluid and observing that it did not spread outside of the central nervous system (CNS).[5] It then became apparent that the brain is protected by a very effective barrier shown later to be the result of tight connections between the cerebral endothelial cells.[6]

The BBB can be characterized as a highly effective and selective barrier in the interface between the blood of the brain microvasculature and the brain tissue and is crucial for achieving a normal function of the CNS. Small lipophilic gases, such as O_2 and CO_2 may diffuse freely across the BBB, but tight junctions (TJs) restrict paracellular fluxes of hydrophilic molecules. Various nutrients and large molecules are actively transported across the cellular membranes through transporter proteins or receptor-mediated endocytosis.

Recent excellent reviews in the field has dealt with only microfluidic systems,[7] had a more biological approach,[8–11] or a focus on drug permeability and applications.[12,13] This review intends to compare the various different models designed to imitate the BBB. Papers have been selected based on influence on the field, as well as model development, and little focus has been given to application-oriented studies. Current state-of-the art BBB *in vitro* research models are presented both with respect to the cell cultures used and the engineering of the cell culture setup and its integration. This is, to our knowledge, the first time an all-encompassing view is taken on the research where equal efforts have been made to analyze the biological as well as the technical aspects of the complete *in vitro* systems.

Abbreviations used: BAEC, bovine aortic endothelial cells; BCEC, brain capillary endothelial cells; BBEC, bovine brain endothelial cells; PBEC, porcine brain endothelial cells; HBEC, human brain endothelial cells; HBMECs, human brain microvascular endothelial cells; HBVEC, human brain vascular endothelial cells; hCMEC, human cardiac microvascular endothelial cells; MBEC, murine brain endothelial cells; NPC, neural progenitor cells; RBCECs, rat brain capillary endothelial cells; RBECs, rat brain endothelial cells; RBMECs, rat brain microvascular endothelial cells; TEER, transendothelial electrical resistance; TJ, tight junction.

Correspondence to: Anette Wolff (Telephone: +46-709948974; Fax: +46-46222 4527; E-mail: anette.wolff@bme.lth.se)

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This work intends to contribute to a stronger interdisciplinary approach to BBB research.

The Neurovascular Unit

The neurovascular unit is the five cell types that constitute the BBB: endothelial cells, astrocytes, pericytes, neurons, and microglia (Fig. 1).

Although the different cell types of the BBB have been identified,[14,15] their interdependence and individual roles are still not fully understood. Here, we list the current level of understanding of the roles of the different cell types: the endothelial cells in the brain are specialized BBB cells that form the microvessels permeating the brain. They differ from regular endothelial cells in four aspects:

1. An absence of fenestrae.
2. A continuous basement membrane that is shared with pericytes, as have been discovered in recent years.[16–18]
3. A high complexity of TJs.
4. Limited pinocytic vesicular transport.

The barrier is tight for polar compounds because of their low lipid permeability, and the presence of extremely tight TJs, leaving only very small polar molecules crossing the barrier.[19] The transport pathways available for larger molecules are the paracellular and transcellular pathways.[20] The paracellular pathways are passive, thus relying solely on the concentration gradient and the permeability, which is very low for macromolecules. The transcellular pathways are energy dependent and substance specific.

Tight junctions are structures assisting the endothelial cells in accomplishing the tightness of the BBB. They comprise at least three different types of transmembrane proteins; claudins, occludins, and junctional adhesion molecules.[21] Claudin and occludin have both been shown to be paramount to the selective paracellular permeability.[22,23] Three specific transmembrane proteins are primarily present in cells with effective barrier functions, such as the BBB endothelial cells. They are: ZO-1, claudin-5, and occludin, where ZO-1 is needed as an anchor point for the claudin and occludin proteins[24] and is necessary for TJ formation.[25] Because of the specificity of these transmembrane proteins to the BBB, they are often used as markers for successful BBB formation.

Astrocytes are characteristically star-shaped glial cells found only in the CNS and they perform different tasks in the brain, one of them being to give biochemical aid to the endothelial cells that form the BBB.[26] It has been found that astrocytes secrete factors are needed for BBB function[27,28] and assist in regulating transport across the capillaries.[29]

Pericytes cover 22%–33% of a capillary,[30] varying between microvessel type,[31] seemingly correlating with the degree of tightness of the endothelial junctions,[32] and pericyte deficiency is known to increase permeability of the BBB.[33] Although it has been suggested that pericytes affect the BBB phenotype,[34] it is believed that they instead inhibit the expression of molecules that increase vascular permeability[35] and induce polarity to the astrocyte end feet that surrounds the microvessels,[31] resulting in a tightening of the barrier. Pericytes also synthesize elements necessary for the differentiation of the BBB[36] and are involved in transport across capillary walls.[31]

Neurons are the main functional constituents of the brain. They need to be protected against the fluctuations inherent in the mammalian system, for example, temperature fluctuations, variations of O_2 or CO_2 levels, or chemical concentration variations, which is the reason for the existence of the BBB. Although the exact mechanism is unknown, Minami[37] has recently shown that the presence of neurons increases the barrier effects, suggesting that neurons, or communication to the barrier, are also a crucial part of the BBB.

The final group of cells that form the neurovascular unit are microglia, which are responsible for clearing debris and handling apoptotic cells in the brain. They are found in the perivascular space but will not be discussed further, as

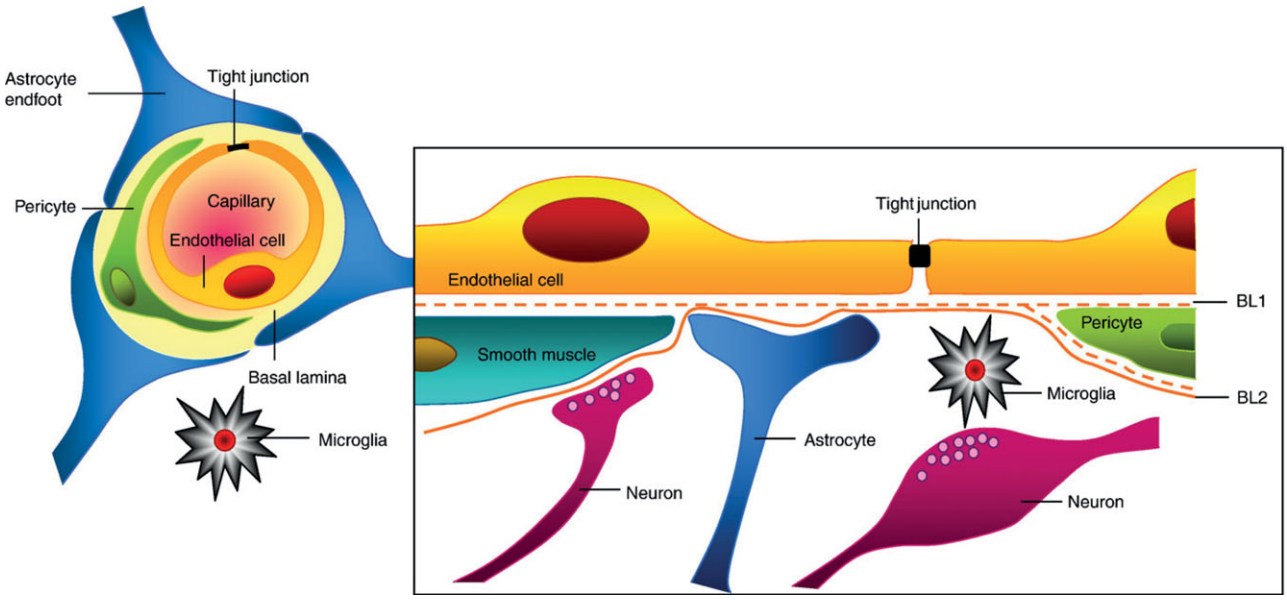

Figure 1. The overview of the neurovascular unit, with the basal lamina denoted as BL. Reproduced from Abbott et al.[14] with permission from Elsevier B.V.

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