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Review article

In vitro models and systems for evaluating the dynamics of drug delivery to the healthy and diseased brain

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ABSTRACT

The blood-brain barrier (BBB) plays a crucial role in maintaining brain homeostasis and transport of drugs to the brain. The conventional animal and Transwell BBB models along with emerging microfluidic-based BBB-on-chip systems have provided fundamental functionalities of the BBB and facilitated the testing of drug delivery to the brain tissue. However, developing biomimetic and predictive BBB models capable of reasonably mimicking essential characteristics of the BBB functions is still a challenge. In addition, detailed analysis of the dynamics of drug delivery to the healthy or diseased brain requires not only biomimetic BBB tissue models but also new systems capable of monitoring the BBB microenvironment and dynamics of barrier function and delivery mechanisms. This review provides a comprehensive overview of recent advances in microengineering of BBB models with different functional complexity and mimicking capability of healthy and diseased states. It also discusses new technologies that can make the next generation of biomimetic human BBBs containing integrated biosensors for real-time monitoring the tissue microenvironment and barrier function and correlating it with the dynamics of drug delivery. Such integrated system addresses important brain drug delivery questions related to the treatment of brain diseases. We further discuss how the combination of *in vitro* BBB systems, computational models and nanotechnology supports for characterization of the dynamics of drug delivery to the brain.

1. Introduction

The cross-talk between the brain and the periphery occurs via flow and diffusional processes through different central nervous system (CNS) barriers at the cerebral microvascular endothelium, choroid plexus epithelium, and avascular arachnoid epithelium [1–3]. These barriers contribute to bioavailability of drugs and translocation of endobiotics and immune cells. Among CNS barriers, the cerebral microvascular endothelium forms the largest barrier in the brain and functions as an effective brain barrier, so called “blood-brain barrier” (BBB). The BBB along with blood-cerebrospinal fluid (CSF) barrier control the passage of a variety of molecules and contribute to maintaining the homeostasis of the ions and cells between the blood and neural tissues [1–3]. The BBB stringently regulates the extracellular environment of neural tissues and protects the CNS from the passage of blood-borne toxins, proteins and cells restricting the access of different cells and molecules to the brain tissue [4–6]. External forces like microgravity can also interrupt endothelial cells (ECs) and may lead to the BBB dysfunction [7]. The breakdown of BBB results in detrimental neurological disorders and might lead to neurodegeneration. On the other hand, cerebral microvascular endothelium restricts the drug uptake limiting the appropriate treatments of neurological disorders. The development of physiologically relevant models of BBB supports the recognition of cellular and molecular mechanisms regulating the BBB functions and provides valuable insights for the design of appropriate drugs with efficient delivery to the brain.

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In this review, we discuss the BBB functions and signaling pathways for drug transport, and the performance of *in vivo* animal BBB models and a variety of different *in vitro* BBB models. We further explain modern technologies used to generate biomimetic human BBB models, and finally discuss BBB-on-chip models with integrated monitoring systems for drug discovery application. The complementary role of computational models and nanotechnology is further discussed along with the experimental setups for controlling the transport mechanism of medicines and enhancing the permeability and stability of drugs and nano-formulations. The future predictive BBB models need to consider the advantages of new technologies like microtechnology-based biomimetic human BBB models, integrated biosensors, computational analysis and nanomedicine for systematic analysis of the transport and stability of neuro-pharmaceuticals across the healthy or diseased BBB in order to provide a reliable tool complementing animal and human studies.

2. Blood-brain barrier

The capillary bed of CNS vasculature is mainly formed by endothelial cells (ECs) and maintained by pericytes around the capillaries while astrocytes end-feet surrounding these two layers. The association of neuronal cells with vascular cells and extracellular matrix components (like collagens, laminins and heparin sulfate proteoglycans) is called neurovascular unit. Tight junctions (TJs) hold ECs together constitute the most apical intracellular complex [8]. However, given the complexity of the CNS vasculature, there is a vast heterogeneity in the function and signaling pathways of transport for distinct regions of the BBB in the CNS.

2.1. Heterogeneity and functions of BBB

Several different proteins and cells take participate in BBB formation and functioning. TJs in the BBB are internal membrane proteins and interact with cytoplasmic scaffolding proteins including zonula occludins (ZO), actin cytoskeleton and other associated proteins comprising the protein kinases, small GTPases, and heterotrimeric G-proteins [8,9]. Claudins, as the most important TJs, consist of PMP22/EMP/MP20/claudin family characterized by a W-GLW-C-C domain in their first external loop [10]. More than 20 claudins have been reported in mammals in which different barriers are composed of distinctive claudin family members [11]. Claudin type in TJs is suggested as the key element of the barrier permeability. For instance, claudin 5 (the most abundant claudin in endothelium) deficient mice have a leaky BBB to small molecules and die at the birth [12]. However, the exact role of some claudins such as claudin 3 and claudin 12 in BBB formation and consistency is not clear yet [12,13]. Cytoplasmic TJ proteins like ZO-1, ZO-2, ZO-3, Jacop, MUPP1, MAGI, and cingulin bind to TJs and link them to the cytoskeleton, adherent junctions and other polar complexes [14]. ZO proteins, especially ZO-1 and ZO-2, are reported to be vital for TJ formation via binding to claudin and occludin proteins through their PDZ domain [4]. Additionally, TJs connect to basal adherent junctions, which is made of VE-cadherin and PECAM1, holding ECs together in an integrated manner [4].

Most of the BBB junction proteins such as claudin 5, claudin 12, ZO-1, ZO-2, VE-cadherin, and PECAM1 are also found in ECs of other tissues which would suggest that the expression of these molecules is not high enough to form the BBB [13]. However, particular mechanisms that form the highly resistant TJs of the CNS are still unclear. In addition, two more proteins are present in the endothelium layer: tricellulin that forms tripartite junctions where three ECs meet, and lipolysis-simulated lipoprotein receptor (LSR) that is responsible for the localization of tricellulin proteins [15]. Recent studies revealed a higher expression of tricellulin and LSR in ECs of the BBB compared to other tissues, proposing the idea that these structures may be crucial for the BBB formation [13].

ECs of the CNS display distinguished luminal and abluminal surfaces due to their highly polarized nature, leading to the expression of distinct transporters in the plasma membrane. These transporters are responsible for both removing potential toxins and supplying specific nutrients, and dictate the movement of various molecules and ions through the BBB [16]. ECs of the BBB express efflux transporters such as MDR1/P-glycoprotein (Pgp) and restrain the xenobiotics trafficking between blood and brain tissues. There is a wide variety of different transporters within brain ECs that sometimes shows an overlapping substrate specificity to provide a better partitioning of small molecules within the BBB [3]. In addition to efflux transporters, ECs represent a series of specific transporters which translocate particular ions, nutrients and proteins from the blood to the brain site using a range of distinct mechanisms. These transporting mechanisms can be categorized as carrier-mediated transporters (glucose and GLUT1/SLc2a1), lactate and amino acids transporters, receptor-mediated transcytosis (like transferrin receptor), and ion pumps (including Na^+, K^+ co-transporters) [17,18]. The recent studies have shown that different metabolic enzymes expressed in ECs of the CNS can supply particular metabolic substances to the CNS [13].

CNS cells are suggested to be the primary regulator of BBB formation and function [19]. Astrocytes reduce the permeability of the BBB to tracers and increase the trans-endothelial electrical resistance (TEER) of the BBB via expressing efflux transporters such as MDR1/Pgp in ECs [20,21]. Astrocytes are also actively involved in the maintenance of the BBB integrity by stabilizing TJs and providing a quiescent immune environment for ECs. However, they are not required for the induction of BBB formation during embryonic development since the BBB is formed prior to astrocyte generation [22]. Furthermore, pericytes play an essential role in BBB development, maintenance, and aging through platelet-derived growth factor B signaling pathways [22]. Transcytosis of ECs and the expression of LAMs are remarkably restricted by pericytes. In addition, *in vivo* studies have shown that the barrier integrity of CNS is improved by transforming growth factor beta or angiopoietin 1 [23].

From the molecular point of view, one of the most crucial signaling pathways involved in regulating the BBB is Wnt/beta-catenin signaling, particularly activated in ECs of the CNS during the development stage [13]. The inhibition of Wnt signaling during embryonic development was shown to be effective in the disruption of angiogenesis in the CNS [13]. Moreover, the expression of claudin 3 and BBB-specific transporters such as GULT1 in ECs of the CNS has been regulated by Wnt signaling pathway [24]. As a result, the BBB gene expression is at least partially regulated by signaling pathways involved in angiogenesis at the CNS. It is a major factor in the pathology of various diseases where leaky vessels are formed due to inappropriate BBB-inducing signals during the new blood vessel formation [4]. The angiogenic invasion of ECs into the CNS is suggested to induce specific gene expression of the BBB. Afterwards, astrocytes and pericytes modulate the properties of BBB during the lifetime. Understanding the molecular mechanisms behind the BBB regulation using a biomimetic BBB model can highly support the treatment of neurological and neurodegenerative disorders [24].

There is also a functional heterogeneity in distinct regions of the BBB in the CNS. Different parts of the brain vasculature have different functions in terms of blood flow and nutrients delivery. While arterial segments show high contractile properties needed for controlling blood flow through different regions, capillaries play an important role in the delivery of nutrients and oxygen, and postcapillary venules provide a primary site for the attachment of immune cells to ECs and their infiltration through the CNS barriers [1]. A wide range of BBB genes are expressed throughout the vascular system of the CNS; however some distinct differences enriched the transport and immune properties in capillaries and venules, respectively [25]. The expression of specific transporters is also different for vessels with different diameters [13]. Furthermore, the circumventricular regions of the brain, including