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Blood-brain barrier on-a-chip and its application

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Abstract. In the past five years, the organ-on-a-chip technology developed quickly and provided a novel platform for in vitro modelling and experimental testing. Blood-brain barrier (BBB) is an important barrier separating the brain from the rest of the body, thereby protecting the brain from toxins. It is important to study the BBB in terms of its permeability to various molecules, not only identifying potential toxins that might harm the brain, but also to design administration routes for drugs targeting the central nervous system (CNS). This review will summarize various recent designs of the BBB chips and their pros and cons, as well as the future directions for the organ-on-chip technology.

Keywords: Blood-Brain Barrier, Organ-On-A-Chip, In Vitro Modelling, Neurological Diseases, Drug Delivery.

1. Introduction

Blood-brain barrier (BBB) refers to the microvasculature of the central nervous system (CNS). It is created by the endothelial cells that form the walls of the capillaries, with tight junctions between neighbouring cells that allow limited number of molecules to pass through [1]. It provides the shortest route for molecules to enter the brain, as no brain cell is further than about 25 μm from the capillary, once the blood-brain barrier is crossed, solutes and drugs can easily gain access to neurons and glial cells [1]. It has been a popular topic of research to investigate the permeability and selectivity of BBB to certain molecules, not only toxins, but also drugs targeting the brain. While human patients have very limited availability due to issues in terms of ethics and limitations in techniques, the Organ-on-a-chip technology allows mimicry of human physiology by engineered or natural miniature tissues grown inside microfluidic chips [2]. This BBB-on-a-chip approach enables high degrees of freedom for experimental approach and can be used to reveal the unspecified natural mechanisms and how they are affected in various neurological diseases, as well as developing novel drug administration approaches. However, a major problem that is yet to be solved is to ensure the chips capture the actual properties of the physiological environment. This paper will review the development of blood-brain barrier on-a-chip in the recent 5 years and discuss potential applications of in vitro models.

2. Blood-brain barrier is closely associated with neurological diseases

The tight junctions of the blood-brain barrier have high selectivity and protects the brain from toxic drugs and exogenous molecules. BBB disruption has been observed in multiple neurological disorders, including Huntington Disease, Parkinson's Disease, Alzheimer's Disease, etc. While the causes of some disorders are still unclear and it is generally believed that they are the results of genetic and

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environmental factors, there is also evidence showing that the abnormalities are partially caused by inflammation and bacterial infection. For instance, Adams et al. in 2019 carried out a study using fluorescent antibodies to detect the presence of gingipain protease in a population with Parkinson's Disease [3]. They showed that the protease comes from the bacteria P. gingivalis, and that its entry, dissemination and infection, as well as its virulent machinery in a systemic manner may be an etiological and/or driving factor for Parkinson's Disease.

Regarding the disruption of BBB in different disorders, another study by Munji et al. in 2019 profiled the mouse brain endothelial transcriptome in health and disease models [4]. They assessed the regulation of CNS endothelial gene expression in models of stroke, multiple sclerosis, traumatic brain injury and seizure. As a result, it was revealed that these disorders exhibits very similar endothelial gene expression changes during BBB disruption, which was proposed to define a core BBB dysfunction module that has the general effect of shifting the states of brain endothelial cells towards that of peripheral endothelial cells [4]. This 'blurs' the boundary between the CNS and the peripheral environment, which leads to potential invasion of pathogens. Therefore, it seems that the pathologies of different disorders, at least in the aspect of BBB disruption, converge into a shared set of genes. Interfering with the expression of these genes provides a possibility to develop a single type of drug that relieves the symptoms of multiple disorders.

Moreover, it has been a challenge in terms of the pharmacokinetics of different types of CNS drugs in order to cross the BBB into the brain. Different molecules have different chemical properties and therefore differ in their permeabilities across the BBB, which can be referred to as the BBB score [5]. However, it is not practical for drug administration to rely on the passive transport across membrane and diffusion afterwards as this process has low efficiency. Another route for molecules is to be recognized by the membrane protein and enter the brain via an active, dedicated process, for example, receptor-mediated transcytosis (RMT). This ensures the efficiency, but also has very high specificity and requires large numbers of trials for experiments. Therefore, to carry out tests for different drugs, in vitro models are required. Specifically, BBB on-a-chip can be used to mimic the environment using human cells and can thus better capture the characteristics in the human body than animal models. The next section will assess various recent approaches of BBB on-a-chip technology and their application in different fields.

3. Mimicking the environment of BBB

Wevers et al. in 2018 made an approach to model the BBB with various cell types [6]. They firstly used a two-lane OrganoPlate for the fluorescent barrier integrity assay, with human brain microvascular endothelial cells (TY10 cell line) separated with ECM gel. The leakage of dye from the TY10 cells into the adjacent compartment was assessed by acquisition of fluorescent image over time. As a result, for the chip containing leak-tight TY10 microvessel all fluorescent dye was retained within the vessel; while for the cell-free control chip fluorescent dye could freely diffuse into the adjacent gel channel, showing the properties of endothelial cells as barrier.

In this study, another three-lane OrganoPlate was used in which TY10 brain endothelial cells were co-cultured with hAst and hBPCT cells that corresponded to astrocytes and pericytes respectively. It was shown that both compartments on each side of the vessel retained the dye to a great extent, with the minimal passive permeability of molecules similar in size to antibodies. BBB's selectivity for antibodies was also tested by comparing the permeability of the chip between two different types of antibodies, MEM-189 and anti-HEL, among which MEM-189 binds transferrin receptor hTfR expressed by TY10 endothelial cells and undergoes receptor-mediated transcytosis (RMT). It was shown that passage of MEM-189 was remarkably higher than anti-HEL in BBB co-cultures. In comparison, when no barrier was present, both types of antibodies diffused freely between compartments [6]. Therefore, the results of this study revealed that not only the brain endothelial cells but also the astrocytes and pericytes exhibit characteristics of a barrier, and that the in vitro model successfully mimicked the selectivity of human BBB for antibodies.

Nevertheless, it was noted in this study that the cells were isolated from normal brain tissues and immortalised. This process might change the properties of cells and therefore posed questions about the

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reliability of results [6]. By contrast, many other BBB Chip studies, for example Vatine et al. 2019, used induced pluripotent stem cells (iPSCs) instead of extracting cells from patients, which was thought to have better viability for the studies [7].

Furthermore, the layout of different compartments in the chip was also explored by more recent studies. Ahn et al. 2020 developed a model that combined a 2D endothelial monolayer with a 3D brain microenvironment, with vascular space of the brain microvasculature in the upper layer and pericytes underneath the membrane and astrocytes in the lower layer [8]. Normally, the gene expression of reactive gliosis markers (for example, vimentin (VIM) and LCN2) is upregulated in pathological conditions. It was discovered that the 3D model had downregulated astrocytic vimentin and LCN2 than 2D cultures but not the other astrocytic proteins, and that the reactive gliosis marker expression can be further regulated in a dose-dependent manner in response to an inflammatory cytokine treatment [8]. Therefore, the 3D layout was more physiologically relevant than the conventional 2D ones, as it better captured the gene regulation in response to inflammatory conditions by creating a specific 3D layout which resembled the actual in vivo environment.

As an interim summary, researchers are still exploring the design of BBB Chips to fully mimic the actual environment. Factors that need to be considered include the sources of cells, spatial relationship between different compartments, as well as the non-biological conditions, such as temperature and oxygen concentration. Thinking from another angle, these can also be manipulated as variables to study the impact of changing environment to some extent on the filtering properties of BBB, in order to gain knowledge of processes in the body or to develop novel drug delivery methods. This will be discussed in the next section.

4. Applications of BBB Chips

The replication of an environment in vitro has several contributions to scientific research: to help understand natural processes, to help reveal the changes in human body under pathological conditions, and to help develop novel therapeutic strategies for diseases. A linked model separating the influx across BBB, parenchymal compartment and efflux across BBB was used by Maoz et al. in 2018 in order to examine the interactions between each individual compartment [9]. In addition to the blood-brain barrier, the concept of neurovascular unit (NYU) was used. Specifically, metabolic fluxes and conversions over the NVU rely on interactions between brain microvascular endothelium, perivascular pericytes, astrocytes and neurons. It was revealed in the study that the coupling of the three compartments led to significant changes in protein expression in endothelium, perivasculature and neurons, and that paracrine signalling via fluidic coupling of the organ chips changes the phenotype of the cultured cell populations [9]. Also, the levels of GABA synthesis in the coupled brain chip were significantly higher than in the uncoupled chips, so that factors produced by vascular endothelium and/or cells of the perivascular niche can influence neurotransmitter synthesis in the brain neuronal compartment, providing further evidence of interactions between different parts of the brain [9]. This however added concern to the design of devices as well. As there is a bi-directional interaction between the BBB and neurotransmission in the brain, modelling the BBB alone might not be able to take all factors that could affect BBB functioning into account. This can be a tricky problem, as different parts in the body act as an inter-connected network, and it is likely that factors that can affect one organ are distributed around the body. Therefore, a possible direction of development for BBB on-a-chip is to combine with other organ chips and establish an in vitro physiological environment in a larger, whole-body scale.

Another study by Park et al. 2019 was inspired by the fact that there is a lack of oxygen at early developmental stages of the brain, and exposed BBB Chip to hypoxia environment [10]. They found that the hypoxia model exhibited higher substrate specificity and functionality than other models. The hypoxia environment facilitated cell differentiation, which recapitulated part of the developmental process of the BBB. Although this is not direct evidence of the development of BBB, the chip provides environment for testing and can be used to verify the unknown aspects of current knowledge.

Apart from the natural process, BBB Chips can also be used for disease modelling. Vatine et al. 2019 studied Huntington's Disease by inducing MCT8 mutation using CRISPR/Cas9 technology [7]. MCT8-

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deficient BBB-Chips showed significantly lower permeability for T3, which is a thyroid precursor. The results of this study confirmed the necessity of MCT8 protein for T3 transport across BBB, and given that loss of thyroid hormone-binding proteins increases the vulnerability of striatal neurons in Huntington's Disease [11], the model confirmed the contribution of MCT8 deficiency to the disorder. Similarly, Pediaditakis et al. 2021 showed that abnormal accumulation of alpha-synuclein aggregates led to phosphorylation of αSyn localized in the endothelial cells in the vascular channel and therefore BBB damage in Parkinson's Disease [12]; Shin et al. 2019 showed that in Alzheimer's Disease there is increased BBB permeability induced by reduced expression of tight junction and adherens proteins [13]. These studies were carried out based on the knowledge that the diseases involve changes of BBB properties, and to some extent identified the causes of such changes. Using in vitro models it is possible to derive causal relationships rather than correlations, which is a very significant advantage compared to patient studies. This further step in the understanding of pathology can be used to develop strategies to at compensate for the changes or to reverse the changes, which is related to the development and administration of CNS drugs.

BBB Chips have been used to explore methods of drug administration into the brain. Park et al. 2019 focused on the mechanism of osmotic opening, which can be reversibly induced by intravenous of mannitol solution [10]. They showed that clinically approved therapeutic antibody (cetuximab) can be delivered across the BBB by inducing osmotic opening. Another process that can be targeted is transcytosis. Ribecco-Lutkiewicz et al. 2018 developed a BBB model using endothelial cells generated from iPSC, which can discriminate species-selective antibody-mediated transcytosis mechanisms, shown by the fact that antibodies that were able to cross rat BBB model showed no crossing in the human model [14]. They pointed out that this model could be potentially used to de-risk antibody carriers that are uniquely specific for human antigens [14], emphasizing the benefits of BBB chips over animal models. In terms of specific drug testing, according to Ahn et al. 2020, eHNP-A1 is a potential CNS drug delivery system with their biomimetic ability to cross the BBB via SR-B1 mediated transcytosis. They revealed that blocking SR-B1 activity reduces eHNP-A1 uptake, which confirmed the transport route of the drug [8]. As a result, the model can be potentially used for medicine research to discover novel strategies for drug administration.

Another potential advantage of BBB on-a-chip in medical research is that with improving technology, it may become possible in the future to establish chips for patients by growing endothelial cells with genes from different individuals. As inter-individual difference has always been a factor that affect the effects of drugs on patients, BBB chips provide a way to design treatments for specific individuals. This might lead to a huge step forward in the field of using CNS drugs to treat various neurological diseases.

5. Conclusion

Blood-brain barrier on-a-chip is a very promising topic of research. It can be used to test for knowledge regarding natural processes, such as the changes during developmental stages and the cross-talk between the BBB and the neurotransmitter system inside the brain. It also contributes to disease modelling in terms of how neurological diseases alter the permeability of the endothelial membrane to certain molecules. In addition, BBB chips contribute significantly to drug testing and the exploration of mechanisms that can be used as drug administration route. However, the development of this technology is still in an early stage and there are still issues unsolved. For instance, to mimic the actual physiological environment the cells used in the chips need to have identical properties as in vivo cells; the arrangement of different compartment should also replicate the in vivo model as much as possible; it is also important to consider factors outside the BBB that influence membrane protein expression and therefore permeability. Thus, the development of BBB chips requires further consideration to achieve vital contribution in the field of medical research.

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