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Development of a blood-brain barrier model in a membrane-based microchip for characterization of drug permeability and cytotoxicity for drug screening

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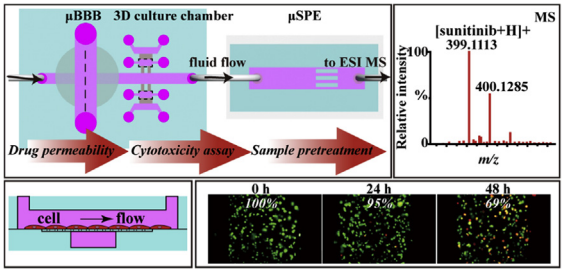
HIGHLIGHTS

- An integrated microfluidic platform is established to recapitulate *in vivo* blood-brain barrier and glioma microenvironment.
- The drug permeability is directly analyzed by ESI MS after μSPE pretreatment.
- It is the first report to simultaneously evaluate drug permeability and cytotoxicity of CNS drug candidates.
- The device enables the rapid analysis of drug candidates, which may accelerate the drug development.

GRAPHICAL ABSTRACT



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ABSTRACT

Since most of the central nervous system (CNS) drug candidates show poor permeability across the blood-brain barrier (BBB), development of a reliable platform for permeability assay will greatly accelerate drug discovery. Herein, we constructed a microfluidic BBB model to mimic drug delivery into the brain to induce cytotoxicity at target cells. To reconstitute the *in vivo* BBB properties, human cerebral microvessel endothelial cells (hCMEC/D3) were dynamically cultured in a membrane-based microchannel. Sunitinib, a model drug, was then delivered into the microchannel and forced to permeate through the BBB model. The permeated amount was directly quantified by an electrospray ionization quadrupole time-of-flight mass spectrometer (ESI-Q-TOF MS) after on-chip SPE (μSPE) pretreatment. Moreover, the permeated drug was incubated with glioma cells (U251) cultured inside agarose gel in the

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CNS drugs
Drug permeability
Drug cytotoxicity

downstream to investigate drug-induced cytotoxicity. The resultant permeability of sunitinib was highly correlated with literature reported value, and it only required 30 min and 5 μL of sample solution for each permeation experiment. Moreover, after 48 h of treatment, the survival rate of U251 cells cultured in 3D scaffolds was nearly 6% higher than that in 2D, which was in accordance with the previously reported results. These results demonstrate that this platform provides a valid tool for drug permeability and cytotoxicity assays which have great value for the research and development of CNS drugs.

1. Introduction

The development of new therapies for brain maladies such as Alzheimer's disease is an urgent medical need for our ageing world. However, few novel CNS drugs have been approved in recent years, and there is a considerable attrition rate during the research and development. This is because that the drug delivery to brain targets is greatly restricted by the BBB and complex brain structures [1]. For one thing, the BBB, a unique structure which is mainly formed by endothelial cells lining the cerebral microvessel lumen with tight junctions, hinders a majority of chemical entities from entering the brain [2,3]. For another, the notorious complexity of brain structures imposes restriction on free diffusion of drugs, so most drugs cannot reach an adequate concentration at target sites [4]. Therefore, it's extremely important to evaluate the CNS drug candidates regarding their permeability across the BBB and cytotoxicity at target cells at the early stage of drug development.

In recent years, many *in vitro* models have been developed to recapitulate organ-level functions for preclinical prediction of drug responses [5,6]. In particular, many BBB platforms have been fabricated to study the permeability of CNS-related drugs [7]. Before permeability assays, these models should be validated to possess *in vivo* BBB properties, which include (1) tight junctions developed between adjacent endothelial cells to directly avert leakage of most compounds [8], (2) adequate fluid flow to promote the endothelial cells to obtain some relevant physiological characteristics [9,10], (3) selective permeability of the constituted structure [11]. However, conventional BBB models are mostly performed in a static culture condition, which generally leads to several physiological defects especially the absence of some tight junctions [12]. Besides, some models misused cell lines from some species like rat or porcine other than human beings, which brought in interspecific differences that cause some distinct properties especially the altered expressions of tight-junction proteins [13]. Therefore, it is urgently needed to develop a reliable human *in vitro* BBB model based on human cerebral microvessel endothelial cells and dynamic culture mode.

Microfluidic technology has attracted an increasing interest in the reconstruction of specific organs and tissues to implement rapid drug evaluation, such as lung-on-a-chip, endothelium-on-a-chip [14,15]. Especially, several microfluidic BBB models have been developed to closely imitate human blood flow, cerebrovascular construction and co-cultured microenvironment [16,17]. However, most of them mainly focused on the BBB model design, but the drug permeability across the BBB and efficacy at brain targets were rarely exploited [18]. Moreover, to better predict the drug action on target cells, 3D cell culture mode has been widely implemented on microfluidic devices [19]. 3D cell culture could help cells retain their native tissue specific functions and recapitulate spatiotemporal chemical gradients and mechanical microenvironments by encapsulating and culturing cells inside matrices such as agarose gel or extracellular matrix [20,21]. In particular, some researches have successfully reconstituted the features of human brain models to study cell behaviour in 3D scaffold [22,23]. However, to our knowledge, there is no report about the study of CNS drug efficacy in 3D cultured brain cells on microfluidic devices.

Recently, many detection methods have been utilized to accomplish the pharmacokinetic studies on the microfluidic platforms, such as fluorescent labelling and spectroscopic detection. In comparison, mass spectrometry (MS) remains the most widely used for its merits of label-free analysis, high sensitivity and semi-quantitative analytical ability [24,25]. However, biological samples usually contain certain matrixes that will lead to severe ion suppression and ion source pollution [26]. Thus, many researches inclined to remove the matrixes by integrating an on-chip pretreatment unit, such as liquid-liquid extraction and solid-phase extraction (SPE), among which on-chip SPE (μSPE) is much easier to manipulate and more compatible with microfluidic platforms [27].

In this report, we assembled a membrane-based microfluidic device for *in vitro* reproduction of the BBB structure and the brain structures, which were then used for evaluation of CNS drug candidates. The BBB model was reconstituted by using human cerebral microvessel endothelial cells under a dynamic culture condition. Moreover, to validate its feasibility for drug screening, sunitinib was used as a model drug to determine the permeability across the BBB and cytotoxicity on brain cells. For permeability assay, the permeated sunitinib was directly determined with an electrospray ionization quadruple time-of-flight mass spectrometer (ESI-Q-TOF MS) after pretreatment with a μSPE column. The detection system showed several advantages including the significantly reduced analysis time and reduced sample consumption. In order to evaluate drug efficacy, U251 cells cultured inside agarose gel were utilized to mimic *in vivo* microenvironment, followed by incubation with the permeated sunitinib. The result showed that the drug-induced cytotoxicity increased with time on the device. In summary, the established system provides a BBB model and a 3D *in vitro* brain tumour model to enable reliable analysis of potential CNS drugs.

2. Experimental section

2.1. Microfluidic device design and fabrication

The microfluidic platform was established by connecting two separate microchips. As shown in Fig. 1A and B, the first microchip was fabricated by assembling two PDMS sheets and a porous.polycarbonate (PC) membrane. The μBBB module and the 3D cell chambers were integrated in this microchip to reconstruct *in vivo* BBB structure and 3D brain microenvironment, respectively. In order to form the μBBB model, human cerebral microvessel endothelial cells (hCMEC/D3) were seeded on the upper side of the membrane to grow into an intact cell monolayer. A dynamic culture condition was then applied to imitate shear stress on the cells. Moreover, cell culture chambers on the bottom PDMS sheet in the downstream were connected to the main channel with shallow