ORGAN-ON-A-CHIP FOR DRUG TESTING IN BRAIN DISEASES
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ABSTRACT
Engineering the physiological relevance of organs-on-chips is critical important for drug testing, toxicological and therapeutic applications. The development of safe and effective drugs for central nervous disorders is greatly hampered by the presence of highly selective blood-brain barrier (BBB) in vivo and the poor predictive ability of existing animal models. We proposed the microfluidic-based strategies to engineer BBB-on-a-chip and brain disease-on-chip to achieve the greater patho-physiological relevance in vitro. We described the representative efforts to engineer the biomimetic organ-on-a-chip for drug testing in brain diseases.

KEYWORDS: Organ-on-a-chip, Blood-brain barrier, Drug testing

INTRODUCTION
The development of neuropharmaceuticals is becoming one of the largest portions of the global pharmaceutical market owing to the increase in average life expectancy and many neurological disorders. However, the discovery of safe and effective drugs for central nervous disorders are greatly hampered by the presence of highly selective blood-brain barrier (BBB) in vivo and the poor predictive ability of existing preclinical animal models [1]. The BBB dysfunction has been implicated in pathologies such as neurodegenerative disorders, stroke, infectious processes and brain tumor [2]. In spite of major advances, existing in vitro culture systems or BBB models still lack many of the structural and signaling characteristics of native BBB microenvironment, the temporal and spatial structural features and physical regulatory factors.

Micro-scale engineering technologies combining with cell biology have been expected to address these challenges by bridging the gap between human and preclinical models [3]. We described a robust microfluidic-based 3D dynamic BBB system consisting of multiple blood-brain interfaces with endothelial barrier integrity function, physiologically relevant cell-cell interaction, cell migration on three dimensional extracellular matrix (3D ECM), and mechanical fluidic microenvironment. We validate this system by measuring the permeability of clinically relevant drugs for targeting glioma cells and brain metastasis coming from breast, lung and melanoma.

EXPERIMENTAL
The two layer microfluidic device consists of sixteen independently addressable units, in which each unit is composed of four uniform 3D blood-brain interface regions (Fig 1). To demonstrate the formation of 3D blood-brain barrier consisting of brain microvascular endothelial cells (BMEC), astrocytes and ECM, the suspensions of two types of cells were infused from the cells/medium inlets into the main channels (vessel side), separately, and adhered to the surface of the side channels filled with collagen ECM (brain side). Then, the cells proliferated continuously followed by the formation of blood-brain barrier interface. Because blood-brain barrier normally occurs during blood flow in vivo, we examined the key properties of formed BBB barrier under both static and fluidic flow condition. We validated the system by measuring the expression of tight-junction protein (VE-Cadherin et al), transporters, trans-endothelial electrical resistance (TEER), and permeability of small molecules in endothelial cells. To test whether the BBB system responded to multiple anti-cancer drugs as a human organ does, we seeded primary glioma cells in the brain side and infused eight clinically known drugs into the vessel side. We then evaluated the permeability of these clinically used drugs crossing BBB and characterized the induced apoptosis rate in glioma cells after treatment with anti-cancer drugs.
RESULTS AND DISCUSSION

Three-dimensional ECM can provide an enhanced environment for multicellular organization of BBB system consisting of cell-cell and cell-matrix interactions, and fluidic flow induced mechanical stimuli to the surface of the endothelium. Structurally, the addition of astrocytes in closest proximity to the brain capillary endothelial on 3D ECM were shown to significantly facilitate the formation of more stringent inter-endothelial tight junctions and the overall expression of BBB features. As shown in Fig 2, the results indicated a marked increase in the expression of VE-Cadherin, P-glycoprotein (Pgp) and glucose transporter-1(Glut1) under flow condition, indicating the contribution of dynamic mechanical factor and astrocyte component to the barrier function of the 3D BBB system.

The paracellular permeability of the endothelial layer was further monitored by measuring TEER and the influx of sodium fluorescein crossing BBB. TEER value is typically higher in BMEC-astrocyte co-culture on 3D ECM in flow condition than in static condition. The TEER measurement may achieve a value of 1300 $\Omega$/cm$^2$, which is almost 3-fold higher than co-cultures in static conditions, and 2-fold as the value of BMECs alone (data not shown). The significant difference indicates formation of a more stringent and selective vascular bed than monocultures on 3D ECM. The results also demonstrated a low permeability to small molecule of green sodium fluorescein in co-cultures than BMEC alone (Fig 3). The permeability is higher in flow condition than that in static condition, indicating the inclusion of fluidic flow and astrocytes to improve the integrity and barrier function against small molecules.

We further utilized the established in vitro BBB system to evaluate the transmigration behaviors of the types of cancers prone to cross BBB to form brain metastasis. The results are agreement with the clinical findings, in which lung cancer is the most cancer that can metastasize to brain, breast cancer is the second and melanoma is the third (data not shown). In addition, we performed the drug testing of eight clinically used drugs with known properties against brain tumor (Fig 4). We showed that only Temozolomide (TMZ) could induce the apoptosis of glioma cells due to its permeability to BBB with effective effects on glioblastoma, which is consistent with the findings from clinics.

Figure 1. Design of the microfluidic BBB model device for drug testing in brain disease. A) Schematic illustration of brain metastasis. B) Design of the 3D microfluidic blood brain barrier system consisting of endothelial cells, astrocytes, extra cellular matrix (ECM) and flow stimuli.

Figure 2. Fluorescent images of functional proteins expression in endothelial cells under both static and flow condition. The expression of tight junction protein (VE-Cadherin), P-glycoprotein and Glut-1 was strengthen significantly in co-culturing endothelial cells with astrocytes in a perfusion condition.
CONCLUSIONS

We successfully constructed physiologically relevant high throughput 3D dynamic microfluidic system mimicking BBB physiology with improved organ-specific phenotype and function. The established system is applicable for drug testing in brain tumor and brain metastasis, which is also attractive as an in vitro organ model for drug screening in neurodegenerative disease, stroke and inflammatory diseases.

ACKNOWLEDGEMENTS

This research was supported by International Science & Technology Cooperation Program of China (2015DFA00740), and National Nature Science Foundation of China (Nos. 81273483, 81201689).

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