A PARALLEL ARRAY MICROFLUIDIC BLOOD-BRAIN BARRIER MODEL FOR HIGH-THROUGHPUT QUANTITATION OF SHEAR STRESS EFFECTS

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ABSTRACT

In response to demand for innovative *in vitro* models for central nervous system disease and treatment we previously reported the microfluidic blood-brain barrier (µBBB). To further investigate shear stress, a key factor distinguishing the µBBB from traditional models, we substituted the top channel with independently monitored channel arrays to aid in high-throughput analysis. Optimal trans-endothelial electrical resistance (TEER) of 345Ωcm^2 was achieved in channels exposed to shear stress in the range of 0.6-1.2dyn/cm^2, while decreased permeabilities of fluorescent tracers were seen at 1.17dyn/cm^2 compared to 0.078dyn/cm^2, with an average decrease of 2e^{-6}cm/s, indicating optimal flow conditions for subsequent µBBB studies.

KEYWORDS

Microfluidic, blood-brain barrier, shear stress, multi-plexed, parallel array, trans-endothelial electrical resistance, µBBB, endothelial cells, astrocytes, permeability

INTRODUCTION

Due to its prevalent role in drug development and disease progression, innovative approaches to modeling the blood-brain barrier (BBB) *in vitro* are in high demand. The validity of an *in vitro* model is dependent on how closely it mimics properties of the *in vivo* environment, and a key physiological component missing in traditional BBB models is the presence of shear stress [3]. To address this, we previously developed the first microfluidic BBB model, the µBBB, to include dynamic flow across membrane-bound BBB co-cultures [6]. However, model performance has not yet been characterized at specific shear stress rates, and optimal flow conditions for the model have not yet been reported.

Shear stress has a mechanotransductive effect on a myriad of endothelial molecular pathways via activation of membrane-bound receptors including integrins and receptor tyrosine kinases [4]. These pathways promote increased gene and protein expression leading to production of tight junction proteins such as ZO-1 [5], and modulate cytoskeletal structure promoting cell reorientation and restructuring [7], factors known to influence barrier function in the BBB. Physiologically relevant vascular shear stress rates vary significantly depending primarily on vessel size (1-60 dyn/cm^2), so it is necessary to investigate a wide range of applied shear stress rates for development of an effective cerebrovascular model. High-throughput quantitation of shear stress effects on µBBB TEER levels is feasible with employment of parallel arrays **(Fig 1)**.

To quantitate changes in barrier integrity, we developed a modified µBBB system comprising parallel arrays of channels with varying widths using similar fabrication and testing methods as previous [2]. Channel dimensions were designed to promote a significant variance between the fastest and slowest channel velocities to maximize the range of values for each experiment. This range variance was 7x for the single-stage (4-channel), and 60x for the multi-stage (12-channel) device, effectively demonstrating feasibility.

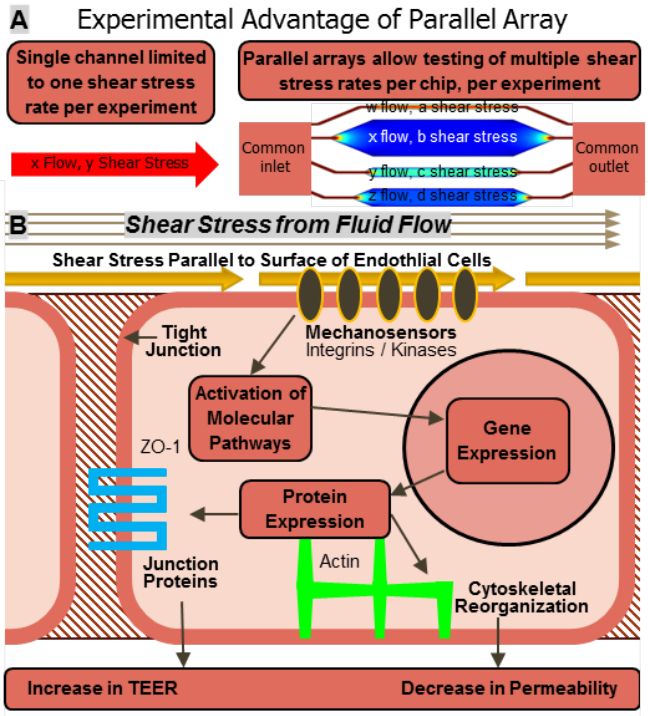

Figure 1: *Quantitating shear stress effects with a parallel array microfluidic device.* ***A.*** *When limited to a single pumping setup, data collection in single-channel µBBB devices [1,2] is limited to a single flow-rate and resultant shear stress per experiment. With a parallel array of varying-width channels, TEER effects of multiple shear stress levels can be quantitated per experiment, allowing more high-throughput data collection.* ***B.*** *Cellular Mechanotransduction. When endothelial cells in the channel are exposed to shear stress, membrane-bound integrins and kinases activate various molecular pathways [4], leading to increases in tight junction expression [5] and cytoskeletal reorganization [7]. These factors are often seen to influence barrier function, leading to increased TEER and decreased permeability.*

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PRINCIPLE AND FABRICATION

Top channels were designed to distribute approximately the same flow-rate through each channel by splitting at equally small widths (200 µm) and widening to varying widths as they cross the bottom channel **(Fig 2)**. Flow distributions and shear stress calculations were determined by Comsol flow simulations and velocity measurements by dye tracking. A glass electrode layer was designed to provide each channel with an independent set of TEER electrodes for measurement of endothelial cell integrity.

To fabricate the device **(Fig 3)**, similar methods were used to the single-channel µBBB [2]. A glass substrate was sputtered with Cr/Au/Ag (20/80/800nm), patterned using liftoff lithography (LOR-10B photoresist), and the silver surface was chlorinated with 30mM $FeCl_3$ for 50s. Channel molds were patterned with SU-8 2075 (100 µm thick) on a silicon substrate, and PDMS was cast with glass electrode layers embedded at the base and cured overnight (60ºC). Polycarbonate sheets (0.4µm pores, 10µm thick) were cut from transwells (Corning) and bonded between channel layers with spin-coated 50:50 PDMS pre-polymer:toluene as described [8]. Marprene or silicone tubing (0.25mm, 0.38mm) was sealed (DC734) to input holes, and tubing was loaded in a 205S cartridge pump (Watson-Marlow).

To further expand the range of shear stresses achievable within a single chip, a multi-stage split-channel design was developed using the same methods just described **(Fig 4)**. In the expanded device, flow is split in two additional stages to three sets of parallel arrays, effectively increasing the applied shear stress range by an order of magnitude compared to the single-stage model, from 7x to 60x.

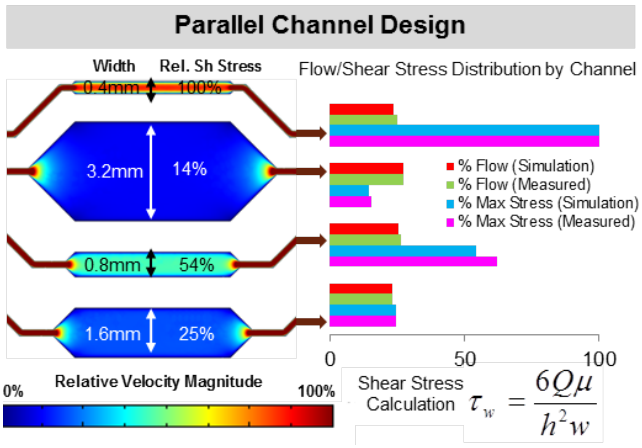

Figure 2: *4-Channel array design. Culturing endothelial cells in the 4-channel µBBB with varying channel widths allows simultaneous collection of 4 data sets. Individual channel widths, relative average velocity magnitudes, percent total flow distributions between channels, and relative shear stress flow distributions are displayed. Flow dynamics were modeled with Comsol simulations and measured visually with dye tracking. Shear stresses were calculated using the displayed equation, where Q is flow, µ is viscosity (0.012dyn·s/cm^2 for complete DMEM/F12), and h & w are channel height (100µm) & width, respectively.*

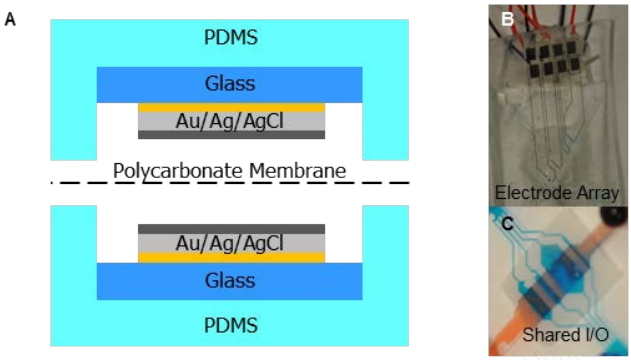

Figure 3: *Device fabrication.* ***A.*** *Channel cross-section. Au/Ag/AgCl TEER electrodes were fabricated on a glass substrate, which was embedded into PDMS layers cured over SU-8 replica molds. Polycarbonate membranes were bonded between top and bottom layers to serve as the co-culture surface.* ***B.*** *Electrode arrays are displayed in the finished device.* ***C.*** *Parallel channels (blue) cross a common 4mm wide channel (red). Electrodes were omitted for imaging purposes.*

EXPERIMENTAL

Devices were sterilized with 70% ethanol, and the membranes were coated with 10 µg/mL poly-D-lysine for 6h on the underside, and 10 µg/mL fibronectin + 10 µg/mL collagen IV for 2h on the topside. Mouse astrocyte cell line C8D1A was seeded in the bottom channel at 6e^4 cells/cm^2, inverted and allowed to attach for 2h. After 2D, brain endothelial cell line b.End3 was seeded in the parallel channels at 6e^4 cells/cm^2 and allowed to attach for 2h. Completed DMEM:F12 growth medium was circulated at near-static flow-rates overnight, subsequently followed by experimental flow-rates. TEER values were measured with an EVOM2 (WPI). In single-channel µBBB chips, fluxes of the following fluorescent tracers were measured across the membrane after D3: FITC-dextrans 4kD, 20kD, & 70kD, propidium iodide, and FITC-conjugated G4 dendrimers [9]. Permeability coefficients were calculated as described [10].

RESULTS AND DISCUSSION

Measured TEER levels (**Fig 5)** for BBB co-cultures where endothelial cells were exposed to shear stress ranging from 0.6-1.2 dyn/cm^2 were significantly higher than those under 0.2 dyn/cm^2, as well as those over 1.2 dyn/cm^2 with a notable drop-off for those over 2 dyn/cm^2. This suggests that physiologically optimal flow conditions for µBBB studies lie within the range of 0.6-1.2 dyn/cm^2. Permeability testing was done in single-channel µBBB chips at the high end of the optimal TEER dataset (1.17 dyn/cm^2) and compared to the lower end shear stress rate (0.078 dyn/cm^2). Permeabilities of fluorescent tracers through single-channel µBBBs were significantly lower at higher stress rates (**Fig 6)**, in agreement with the TEER data. These improvements in barrier function over near-static flow conditions can be explained by mechanotransductive effects on cellular physiology [5,7,11], while the TEER drop-off at higher levels may be due to practical limitations of cell adhesion to the membrane. Though the mechanism of these differences in barrier function need to be investigated further, these results indicate that optimal barrier function in the µBBB is achieved at shear stress of 1.2dyn/cm^2, which falls into the approximate range of *in vivo* shear stresses (1-60 dyn/cm^2) [11].

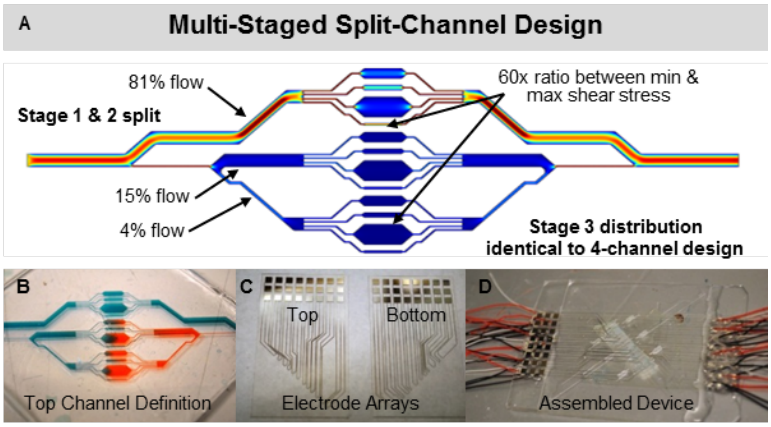

Figure 4: *Expanded multi-staged split-channel design.* ***A.*** *A 60x range variance was achieved by unevenly dividing flow via split-stages to three parallel arrays in combination. Flowrates determined by Comsol and visual dye tracking.* ***B.*** *Top channels displayed with red dye, currently being displaced by blue dye.* ***C.*** *Electrode arrays were embedded in the channel layer in fully assembled device* ***(D)****.*

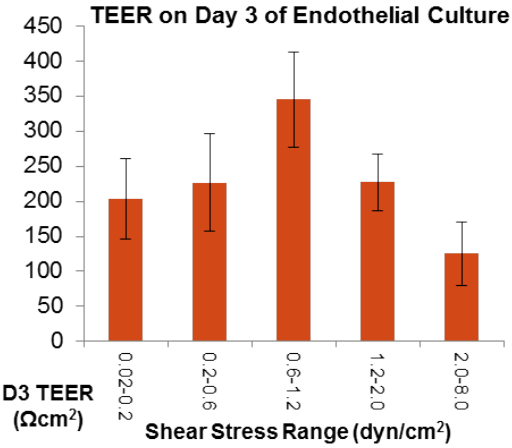

Figure 5: *Day 3 TEER at varying shear stress rates TEER at varying stress rates indicate optimal barrier integrity for µBBB channels in the range of 0.6-1.2 dyn/cm². All conditions. n>3.*

CONCLUSION

This report demonstrates the validity of using a multi-plexed parallel array of channels of varying widths to generate varying shear stress rates within a single device as a high-throughput method of data collection. For the application of optimization of µBBB flow conditions, the device gave results indicating an optimal shear stress range of 0.6-1.2 dyn/cm^2 for optimal barrier function. Though the precise mechanisms of this improvement in barrier function remain unclear and warrant further investigation, future studies using the µBBB to study BBB function and predict drug permeabilities, shear stress of 1.2 dyn/cm^2 will be used as a standard flow condition.

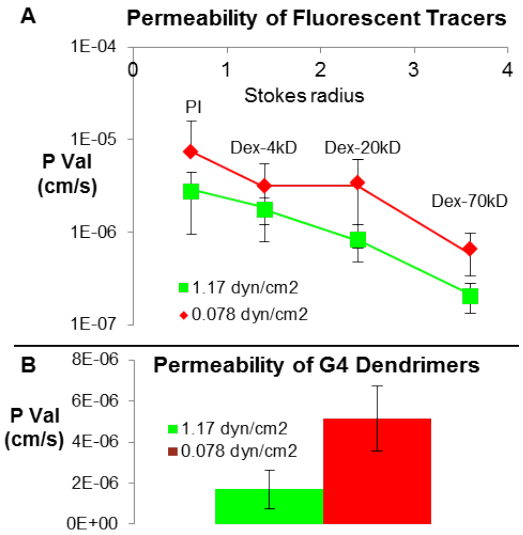

Figure 6: *Permeabilities at distinct shear stress rates.* ***A.*** *Permeabilities of fluoro-tagged tracers were lower at 1.17dyn/cm² (optimal TEER range) than at 0.078dyn/cm² (near-static).* ***B.*** *Permeability of PAMAM Dendrimer drug carriers (G4, FITC-tagged) also show a significant decrease from 1.17dyn/cm² shear stress. All conditions, n≥3.*

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