Transient disruption of a Blood-Brain Barrier on-chip using focused ultrasound and monodisperse microbubbles

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ABSTRACT

Drug delivery to the brain is complicated due to the presence of the blood-brain barrier (BBB) [1]. BBB disruption has been demonstrated *in vivo* using ultrasound-driven microbubbles. However, the underlying mechanisms are not clear thereby limiting clinical translation [2]. Here, we aim to unravel this physical and biological multi-timescale problem by using an optically accessible BBB on-chip model, ultra-high-speed imaging of the bubble dynamics, transendothelial resistance measurements, and monodisperse microbubbles. The results show that our organ-on-chip platform together with monodisperse microbubbles is a powerful tool to correlate microbubble dynamics to the induced bioeffects.

KEYWORDS: blood-brain barrier, organ-on-chip, focused ultrasound, monodisperse microbubbles, sonoporation.

INTRODUCTION

The blood-brain barrier (BBB) is responsible for the strict regulation of the passage of molecules thereby protecting the brain from harmful compounds and pathogens that circulate in the blood [1]. However, it also restricts the passage of most therapeutics due to the presence of tight junctions (TJs) between the endothelial cells in the vasculature of the brain. A successful method to temporarily disrupt the BBB uses focused ultrasound (US) and microbubbles injected into the bloodstream. The US forces the microbubbles to oscillate thereby causing mechanical stress on the cells. As a consequence, the BBB is locally opened allowing the passage of drug molecules. The successful opening of the BBB, without causing necrosis and bleeding, is rather delicate since the mechanical action of bubbles depends on both microbubble and US parameters, such as the microbubble size and concentration, acoustic frequency, and pressure. Understanding the role of these parameters will allow to speed up the clinical translation. Here, we characterize microbubble dynamics at the nanosecond timescale and aim to correlate it to BBB integrity and viability measured at the timescale of hours using fluorescence protein staining and transendothelial electrical resistance (TEER) measurements.

EXPERIMENTAL

A BBB-on-chip was fabricated as previously described by our group [3]. In short, hCMEC/D3 cells were cultured on a porous 2-µm thick polydimethylsiloxane (PDMS) membrane embedded in the chip (Fig. 1A). The cells were seeded on the membrane in the bottom channel and were cultured for 4 days. On the 4th day, BBB disruption was performed as shown in Fig. 1B. Monodisperse 6-µm diameter bubbles formed in a microfluidic flow-focusing device [4] were used for a controlled oscillation amplitude across the full microbubble population. The microbubble oscillation amplitude was characterized at the nanosecond timescale using ultra-high-speed imaging at 10 million frames per second. For this, a concentration of 5×10^6 microbubbles/ml was injected inside the bottom channel of the chip placed in the water bath at 37°C. Focused US at a frequency of 1MHz and at an acoustic pressure amplitude of 1 MPa was then applied with a duration of either 100 or 1000 cycles. To

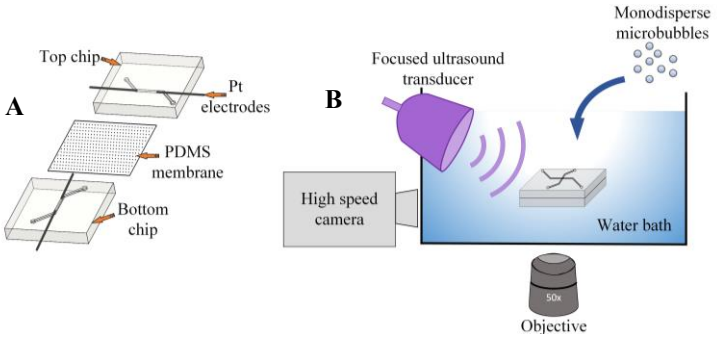

Figure 1: (A) Schematic representation of the chip with a 2-µm thick PDMS membrane between the channels with dimensions of 0.375 mm×0.5 mm×18 mm (h×w×l). Four Pt electrodes were inserted on both sides of the channels. The BBB chip with microbubbles was placed in a water tank at 37°C. (B) Focused US was generated by a transducer placed under a 45° angle with the chip's surface. The chip was imaged from below using a high-speed camera connected to a microscope.

determine the barrier tightness, we measured the impedance at a frequency range from 100 Hz to 1 MHz and then retrieve the TEER values at the selected frequency of 10 kHz. The TEER measurements were performed before applying US, and after 30 min, 2 h, and 16 h. Furthermore, a Live-Dead assay was performed 2 days post-experiment.

RESULTS AND DISCUSSION

The measured oscillation amplitude of the microbubbles is shown in Fig. 2A and it amounts to nearly 0.8 µm at the US driving frequency of 1 MHz (Fig. 2B). The TEER values measured before and after applying the US are plotted in Fig. 3. The data was normalized and is represented in percentage change. Note that the TEER values dropped for both US cases. However, the drop in TEER was much larger for the case with a larger number of US cycles, i.e., leading to a 50% decrease in barrier tightness for 1000 cycles as compared to a 20% decrease for 100 cycles. Additionally, for the 100 cycle case, the cells were not able to fully recover after 16 h as can be observed from the continuous decrease of TEER (blue curve) as compared to 1000 cycles case, although the standard deviation is high (red line). Therefore, 16 h is not enough to fully recover the barrier. Figure 4 shows the Live-Dead assay performed 2 days post-experiment. Interestingly, the cells fully recovered and most of the culture remained viable (Fig. 4A.) Additionally, the cells were stained for the tight-junction marker ZO-1 expressed by the hCMEC/D3 cells (Fig. 5B) however, the location of the marker was more cytoplasmic and only in a few cells peripheral (Fig. 4B). Such non-conclusive localization of ZO-1 in hCMEC/D3 cells was reported before and therefore, another tight junctional marker will be used in future work.

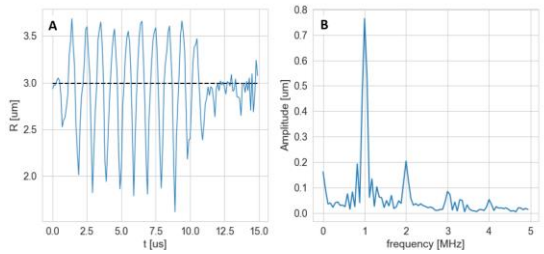

Figure 2: Radius-time curve of a 6-µm diameter bubble driven at 1MHz (A) and its amplitude spectrum (B).

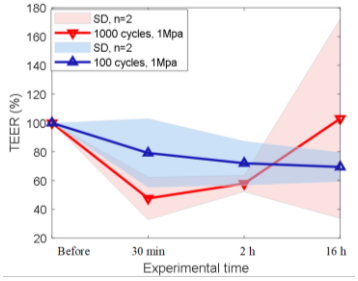

Figure 3: Change in TEER values (%) after exposure to US at different cycles and microbubbles. N=2 chips per experiment.

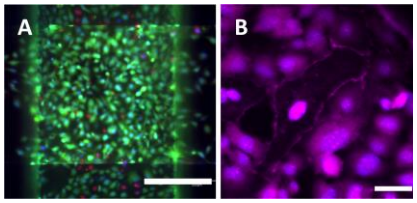

Figure 4: LIVE-DEAD staining of the hCMEC/D3 cells on the 2nd day after the experiment (A). Scale bar 250 µm. ZO-1 staining of the cells after 16h (B). Scale bar 40 µm.

CONCLUSION

In conclusion, we studied the role of the number of US cycles on BBB disruption. We were able to monitor the changes in the barrier tightness by measuring TEER and the results showed that the disruption of the barrier was dependent on the number of cycles applied. The viability of the cells was completely recovered on the second day. Overall, the present platform with a BBB on-chip model and monodisperse microbubbles allows standardization of US and microbubble parameters that will help to provide physical and biological insight into the US-mediated BBB disruption which is envisioned to boost clinical translation.

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