Integrity Disruption of an *In Vitro* Blood-Brain Barrier Model Following Exposure to Blast Overpressure

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Abstract Traumatic brain injury (TBI) is the signature injury of modern military conflicts due to the prevalence of improvised explosive devices (IEDs). However, the pathobiology of blast-induced traumatic brain injury (bTBI) and its effects on the blood-brain barrier (BBB) – a structure essential for maintaining brain homeostasis – remain poorly understood. This work utilized a helium-driven shock tube to generate militarily relevant overpressure/duration histories to injure an *in vitro* BBB model, which exhibited integrity disruption following exposure to 571 ± 15 kPa peak incident overpressure with 1.06 ± .007 ms duration and 186 ± 1.5 kPa·ms impulse in-air. Significant changes to barrier integrity were quantified by trans-endothelial electrical resistance (TEER), hydraulic conductivity and zona occludens-1 (ZO-1) immunofluorescence. The acute post-injury TEER dose-response suggested that a tentative threshold for blast-induced barrier opening exists between 469 kPa and 571 kPa peak overpressure. Significantly increased hydraulic conductivity indicated compromised tight junctions, confirmed by altered ZO-1 morphology and significantly reduced immunofluorescence. TEER in blast-exposed cultures remained significantly depressed compared to age-matched controls up to 2 days after injury, and recovered to control levels at day 3. Elucidating BBB disruption caused by primary blast will guide the development of strategies to mitigate BBB neuropathologies associated with bTBI.

Keywords blast injury, shock tube, blood-brain barrier, endothelial cells

I. INTRODUCTION

Blast-induced traumatic brain injury (bTBI) is one of the most serious wounds sustained by warfighters in modern military conflicts [1,2]. Mounting clinical and experimental evidence, along with reports of military combat casualty care, underscores the potential for short- and long-term neuropathologies caused by blast exposure [2-4]. While the underlying biophysics of bTBI remains poorly understood, it is thought that brain deficits can result from direct interaction with a fast-moving pressure transient associated with blast overpressure [4-6], called primary blast injury. As such, it remains an unresolved controversy as to the mechanism(s) by which primary blast forces directly damage the brain or specialized structures like the blood-brain barrier (BBB), which is essential for maintaining brain homeostasis [6,7]. Given the fine structure of the BBB – only 300 nm thick with a combined surface area of 20 m^2 lining 600 km of brain capillaries [8] – damage to the barrier could be a primary mechanism of bTBI.

The BBB is a selective barrier comprised of specialized brain endothelial cells characterized by complex tight junctions, highly regulated permeability and polarity [9-12]. Tight junction proteins expressed by cerebral vascular endothelial cells form the basis of the BBB and are crucial to determining barrier characteristics [13-15]. Intercellular tight junctions are formed by plasma membrane proteins including occludin, claudins and zonula occludens accessory proteins (ZO-1 and ZO-2), which together help to maintain the function of the BBB [11,15]. Tight regulation of the transport of plasma constituents into the brain is essential for sustaining and protecting its microenvironment, and damage to the BBB may play a critical role in bTBI [5,7,16-19]. However, correlations between blast injury and the loss of BBB integrity must be understood in greater detail and warrant further investigation.

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Brain edema and increased cerebrovascular permeability due to BBB disruption are characteristic features affecting both morbidity and mortality in patients with TBI [16,20]. Severe blast injury in humans often results in delayed death attributed to cerebral edema [2,21]. Brain swelling is thought to be initiated by BBB rupture, allowing the infiltration of plasma fluids into the brain parenchymal extracellular space that may also elicit excitotoxic effects [20,22]. Furthermore, progressive brain edema formation can contribute to ensuing ischemic injuries due to impaired perfusion and oxygenation that critically determine a patient's fate. Together, these phenomena make pathologies caused by cellular injury or BBB breakdown of major clinical importance [20,23].

There is a strong body of evidence showing that rapid contusion and compression associated with the mechanical impact of concussive head injury can result in damage to the BBB. Animal models of experimental TBI and clinical data both suggest a high incidence of BBB breakdown following head trauma that may last from several days to years after the initial injury event [24,25]. Early pathophysiological symptoms of cerebrovascular compromise manifest as blood flow irregularities leading to metabolic imbalance, ischemia, hypoxia and excitotoxicity [26]. Furthermore, a number of mechanisms have been proposed for the emergence of secondary brain damage following BBB disruption, including edema, inflammation and cell death, among other long-term TBI-associated complications such as Alzheimer's disease, cognitive impairments and epilepsy [26,27]. Restoration of BBB permeability and function has generally been shown to occur within days to weeks following concussive injury; however, there is limited quantitative data describing the relationship between BBB damage and the mechanism and severity of TBI [28,29].

A number of bTBI studies in rodents and small animals have demonstrated brain microvasculature and BBB breakdown resulting from blast exposure [1,5,17]. However, the relationship governing BBB damage resulting from primary blast injury, as opposed to a combination of primary and secondary (inertia-driven) blast injury, is still not fully understood. This study utilized an *in vitro* model comprised of a brain endothelial monolayer, mimicking cell-specific phenotype and BBB properties, to elucidate the pathophysiological effects of blast exposure on the BBB. Because the *in vitro* model allowed for isolation of the BBB and generation of reproducible loading conditions, this work is the first to determine a threshold for primary blast-induced barrier opening, report a time-course for spontaneous recovery, and show associated effects on BBB permeability and tight junction morphology. Our results indicate that exposure to blast overpressure directly disrupts the integrity of an endothelial monolayer, as supported by significant changes in trans-endothelial electrical resistance (TEER), hydraulic conductivity and ZO-1 immunostaining of blast-injured monolayers compared to sham controls.

II. METHODS

BBB Cell Culture Model:

A mouse brain microvascular cell line, bEnd.3 (ATCC, Manassas, VA, USA), was used to generate models of the BBB for its rapid growth, maintenance of barrier characteristics over multiple passages, and ability to form functional barriers [11,30-32]. For TEER and hydraulic conductivity experiments, a total of 60,000 bEnd.3 cells were seeded on 1.12 cm^2, poly-L-lysine coated Transwell inserts in a 12-well plate (Corning Costar, Corning, NY, USA), and were cultured for 8 days at 37^0 C and 5 % CO_2 in accordance with published methods [11,33]. The same culturing procedure was followed for ZO-1 immunostaining experiments, with the exception of using an 80,000 bEnd.3 cell seeding density and culturing period of 4 days. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % newborn calf serum and 4 mM GlutaMAX (Invitrogen). Feeding medium was changed in the upper (0.5 mL) and lower (1.5 mL) chambers every 2-3 days.

Blast Injury of BBB:

Endothelial monolayers were injured by primary blast using a shock tube and *in vitro* receiver as previously described [33]. Briefly, individual Transwell cultures were sealed inside sterile bags (Whirl Pak, Fort Atkinson, WI) filled with serum-containing culture medium pre-equilibrated (37° C and 5 % CO_2/95 % O_2) to prevent contamination, maintain medium pH, and minimize bulk flow in close proximity to the sample. Samples were then placed in a fluid-filled sample receiver test column, and care was taken to prevent entrapment of air bubbles. The culture and bag were submerged in the test column and oriented perpendicular to the direction of wave propagation (Fig. 1).

Blast injuries were generated using a 76 mm-diameter aluminum shock tube with an adjustable-length driver section (50 mm used for current studies) pressurized with helium, and a 1240 mm-long driven section [34]. This shock tube was designed to produce ideal shock overpressure/duration profiles that are similar to those seen in free field blasts. Similar to previously published shock tube characterization [33], a range of blast injury input

parameters for the open-tube configuration were tested including peak incident overpressure, duration and impulse in-air (Table 1). The pressure history of the shock wave in the open-tube was recorded using pressure transducers (8530B, Endevco, San Juan Capistrano, CA, USA) flush-mounted at the tube outlet (Fig. 2) to record incident pressure. Fluid pressure within the sample receiver was measured by a submersible transducer (SPR-524, Millar Instruments, Houston, TX, USA) located beneath the submerged BBB sample (Fig. 3). Sham-exposed controls were sealed into bags and submerged in the receiver for an equivalent period, but the shock tube was not fired. In addition, materials within the sample receiver were carefully chosen to be impedance-matched to the surrounding fluid in order to eliminate cause for any attenuation of the pressure transient through the BBB culture (including culture well, bag and PTFE membrane) [33].

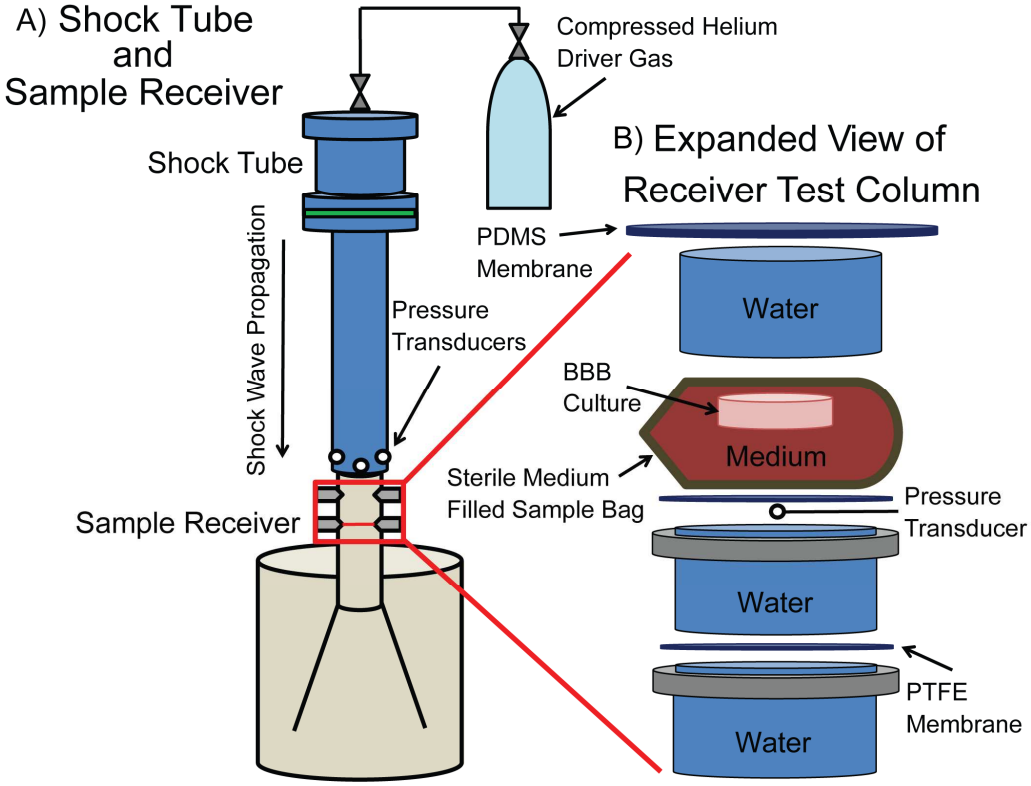


Fig. 1: Schematic of the shock tube with sample receiver as previously described [33]. (A) Compressed helium gas was connected to an adjustable driver section of the shock tube, and in-air transducers were flush-mounted at the tube's exit. (B) The BBB culture in the sample bag rested on top of a PTFE membrane within the fluid-filled sample receiver. A submersible pressure transducer was located directly beneath the culture bag. Reproduced with permission [33].

TABLE 1
BLAST INJURY PARAMETERS

Diaphragm Thickness	Peak Incident Overpressure	Duration	Impulse
508 µm	377 ± 8 kPa	0.89 ± .007 ms	96 ± 1.5 kPa·ms
762 µm	469 ± 21 kPa	0.99 ± .005 ms	143 ± 1.5 kPa·ms
1016 µm	571 ± 15 kPa	1.06 ± .007 ms	186 ± 1.5 kPa·ms

Parameters of shock wave in-air measured at exit of the open shock tube (mean ± SEM; n=2)

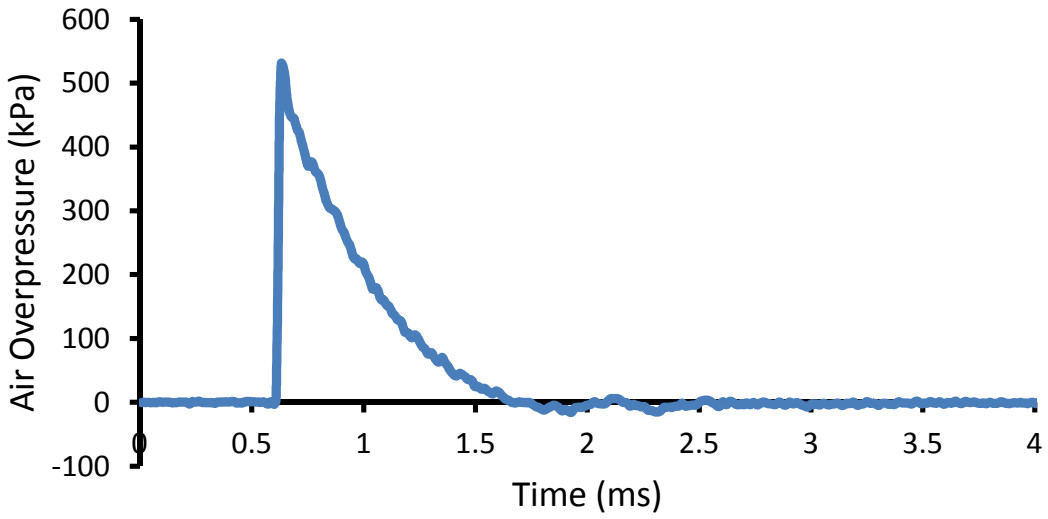


Fig. 2: Pressure history of the shock wave associated with a 1016 µm-thick diaphragm used with the open shock tube configuration described. The pressure trace was detected by transducers at the exit of the shock tube and was used to characterize peak incident overpressure, duration and impulse in-air.

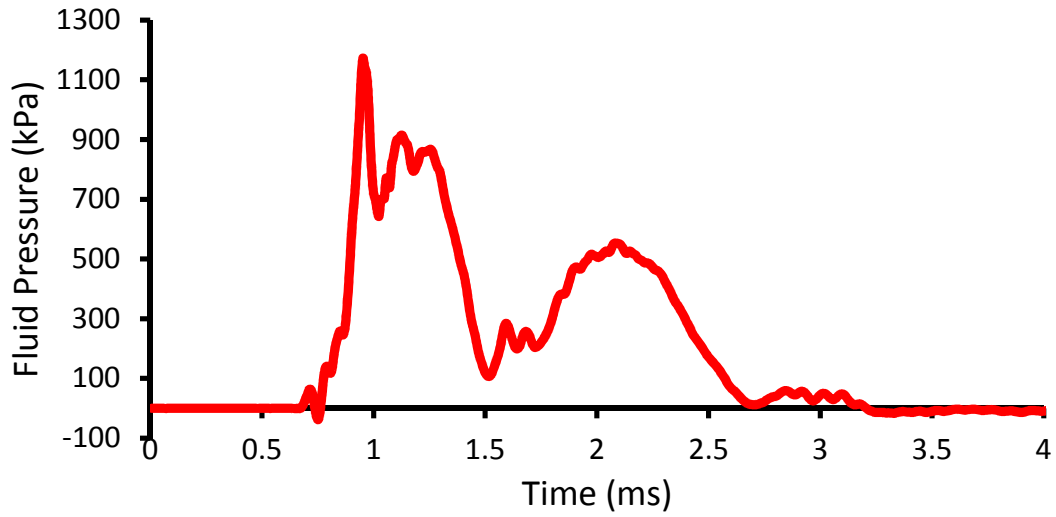


Fig. 3: Fluid pressure history associated with a 1016 µm-thick diaphragm. The pressure trace was detected by a submersible transducer located beneath a submerged BBB culture.

Immunostaining of ZO-1:

Within 30 minutes following exposure of BBB monolayers to blast overpressure, endothelial cultures were fixed, permeabilized, blocked and incubated with anti-ZO-1 rabbit polyclonal antibody (Invitrogen, Carlsbad, CA, USA). Alexa Fluor 488 anti-rabbit secondary antibody (Invitrogen) was used for detection of ZO-1 tight junction proteins. Cell nuclei were counterstained by incubating cultures with 4', 6-diamidino-2-phenylindole (DAPI, Invitrogen). Endothelial cells were imaged using an Olympus IX81 fluorescence microscope and MetaMorph software (Molecular Devices, Downingtown, PA, USA). Five images were acquired for each of the control and injured groups to qualitatively indicate the effect of primary blast injury on tight junction binding.

To quantify ZO-1 immunostaining, five images were randomly selected and analyzed for each of the 5 control and 5 injured cultures (total of 50 images). The same threshold for fluorescence was used to analyze all images of sham and injured samples. ZO-1 immunostaining was quantified as the percent area of a specific region exhibiting fluorescence above the threshold, normalized to the total number of bEnd.3 cells in each region.

Evaluation of Trans-Endothelial Electrical Resistance (TEER):

In vitro models of the BBB must recapitulate *in vivo* characteristics to be useful, and measuring high TEER is one method for verifying the formation of integral monolayers and the presence of tight junctions [14,30]. Changes

in TEER were quantified using an Endohm-12 chamber electrode connected to an EVOMX Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL, USA), accounting for the TEER of cell-free Transwell filters. TEER values were normalized to the membrane surface area. TEER measurements were taken immediately prior to and within 30 minutes following injury. Sham-exposed samples were processed identically to blast-exposed cultures, but the shock tube was not fired.

Evaluation of Hydraulic Conductivity:

The BBB is known to tightly regulate the movement of water and ions, and measuring hydraulic conductivity is one method of assessing endothelial monolayer integrity [11,35]. However, because various pathological conditions including inflammation are associated with the hyper-permeability of vessels [36], it is critical to understand how the hydraulic conductivity of an endothelial monolayer may be modulated by exposure to blast overpressure. A custom-designed permeability device was used to measure hydraulic conductivity in accordance with previous studies [11,31,37]. Transwell inserts were sealed between two polycarbonate pieces to create an enclosed chamber with separate upper and lower compartments. A hydrostatic pressure was applied across each monolayer culture, and fluid flow was measured by tracking the movement of a bubble in a calibrated glass tube. Hydraulic conductivity ($L_{p,}$ cm/s/cmH_2O) was calculated using equation (1) [11,31].

$$L_p = \frac{\frac{\Delta x}{\Delta t} \times F}{S \times \Delta P} \quad (1)$$

Where, $\frac{\Delta x}{\Delta t}$ is the displacement of the bubble over time, F the fluid volume contained in a known length of tubing, S the membrane surface area of the Transwell insert, and ΔP the hydrostatic pressure across the endothelial monolayer.

Statistical Analysis:

Independent samples t-tests were used to analyze the results of injured and sham-exposed BBB cultures in the acute phase, with TEER, hydraulic conductivity or ZO-1 immunostaining as the dependent variables (SPSS v. 19, IBM, Armonk, NY, USA, significance *$p < 0.05$). A repeated-measures analysis was used to determine the overall effect of injury on the time-course of TEER, followed by a one-way ANOVA to identify significant differences between sham and injured cultures for each measurement period (SPSS v. 19, significance *$p < 0.05$).

III. RESULTS

Exposure of endothelial monolayer cultures to controlled blast acutely disrupted monolayer integrity. TEER acutely decreased in a dose-dependent manner as blast severity increased from: 377 ± 8 kPa to 571 ± 15 kPa peak incident overpressure, 0.89 ± .007 ms to 1.06 ± .007 ms duration, and 96 ± 1.5 kPa·ms to 186 ± 1.5 kPa·ms impulse in-air (Fig. 4). Following the 571 kPa peak overpressure blast, TEER significantly ($p < 0.05$) decreased within 30 minutes of injury to 74 ± 2 % compared to 105 ± 5 % in controls (Fig. 4).

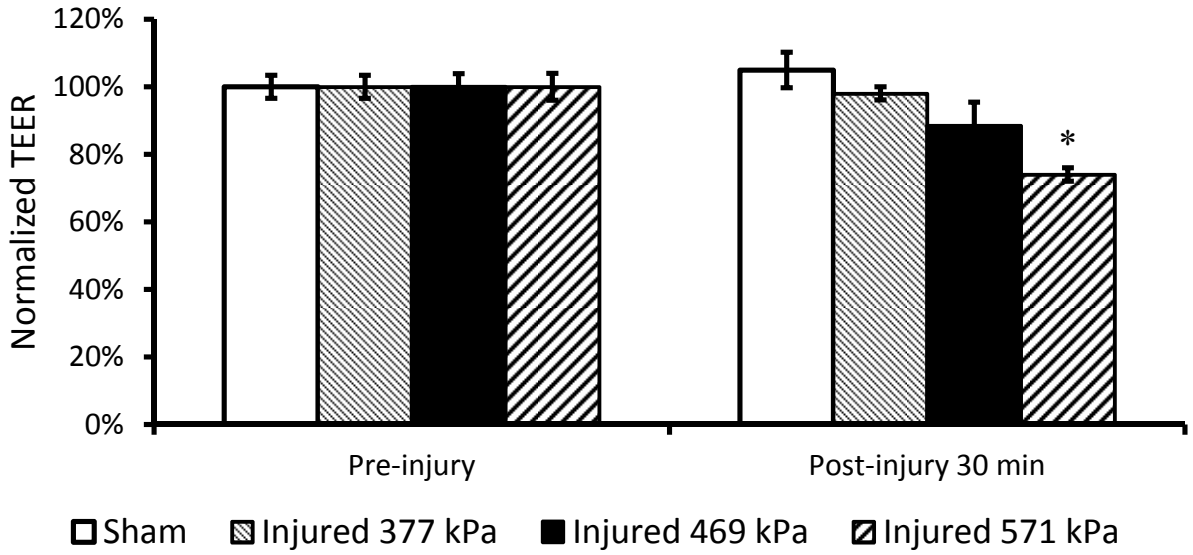


Fig. 4: Dose-dependent response of TEER in endothelial monolayers after blast exposure of increasing severity. Following the 571 kPa peak overpressure blast, TEER significantly and acutely decreased in injured cultures to 74 ± 2 % of pre-exposure levels (*$p < 0.05$; SEM; Sham n=6; Injured n=3).

TEER of cultures exposed to the 571 kPa peak overpressure blast recovered over time, but remained significantly depressed compared to age-matched uninjured shams for up to 2 days after injury (Fig. 5). Injured cultures exhibited full recovery to pre-injury TEER levels at day 3. Sham control cultures maintained consistent TEER levels of approximately 100 % of pre-exposure levels for all time points tested.

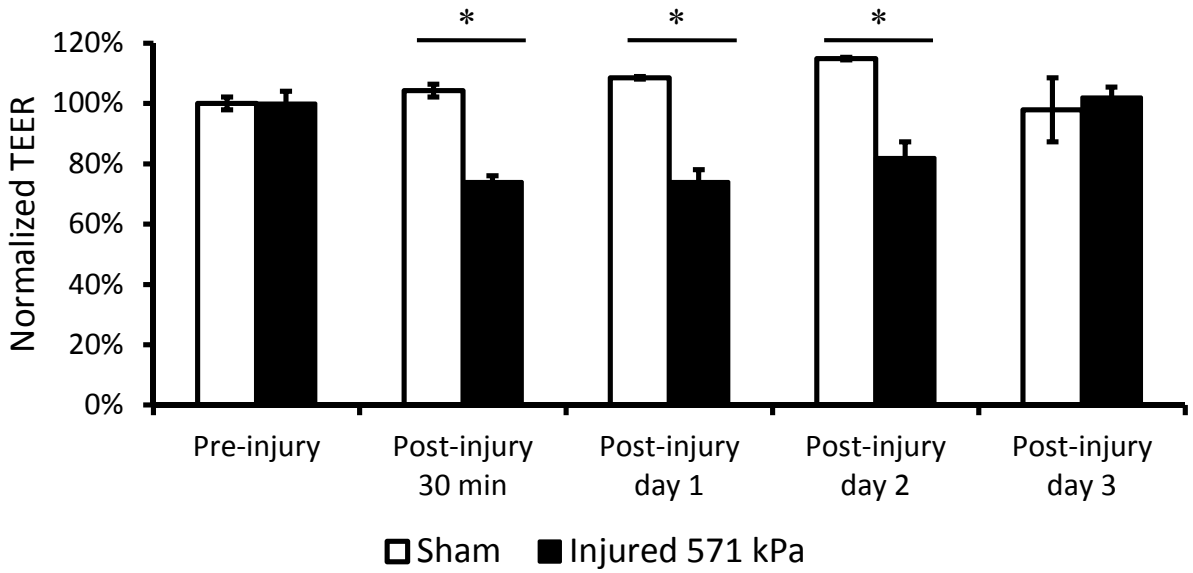


Fig. 5: TEER time-course of blast exposed endothelial monolayers. Depressed TEER of injured cultures fully recovered to pre-injury levels at 3 days post-blast (*$p < 0.05$; SEM; Sham n=3; Injured n=3).

Hydraulic conductivity, L_p, of cultures exposed to the 571 kPa peak overpressure blast exhibited an acute, significant increase to $1.1 \times 10^{-5} \pm 1.5 \times 10^{-6}$ cm/s/cmH_2O compared to $1.8 \times 10^{-6} \pm 3.0 \times 10^{-7}$ cm/s/cmH_2O in sham controls (Fig. 6). Changes were observed within 30 minutes following injury.

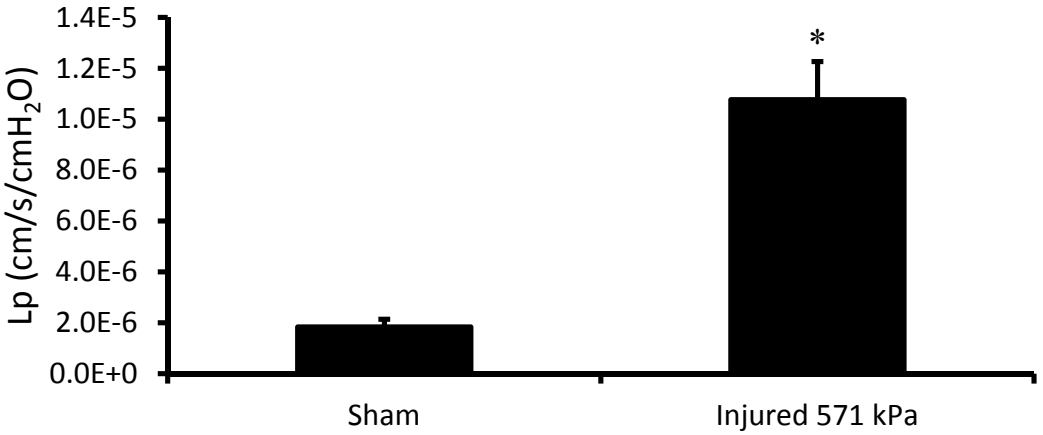
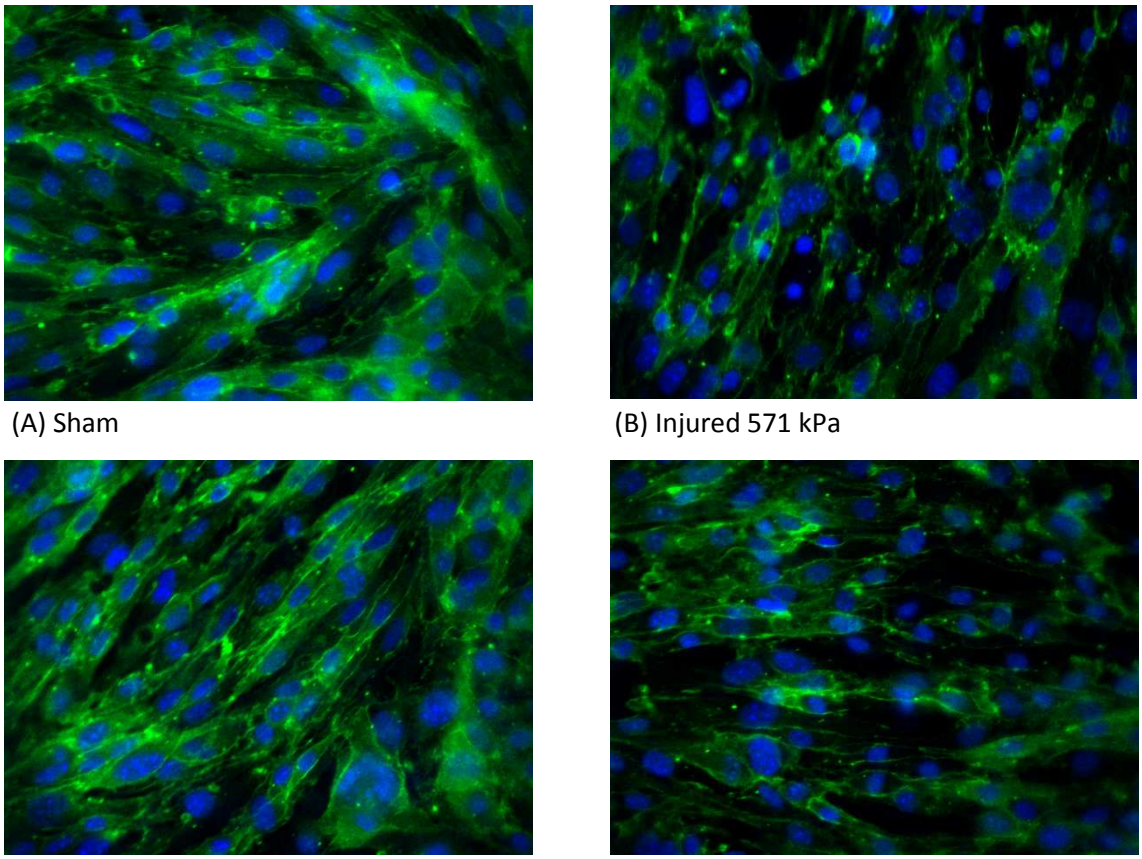


Fig. 6: Hydraulic conductivity of blast exposed endothelial monolayers. Within 30 minutes after injury, exposed cultures exhibited a significant increase in hydraulic conductivity to $1.1 \times 10^{-5} \pm 1.5 \times 10^{-6}$ cm/s/cmH_2O compared to $1.8 \times 10^{-6} \pm 3.0 \times 10^{-7}$ cm/s/cmH_2O in sham controls (*$p < 0.05$; SEM; Sham n=3; Injured n=3).

Compared to sham controls, reduced ZO-1 immunofluorescence was observed in injured cultures within 2 hours of injury, indicating compromised barrier integrity and disrupted tight junctions (Fig. 7). The periphery of individual bEnd.3 cells in sham controls contained prominent ZO-1 localization, confirming the presence of integral tight junctions between adjacent cells. In contrast, however, exposed cultures exhibited compromised ZO-1 staining that revealed an altered morphology of tight junction proteins following injury. The percentage of ZO-1 immunostaining per cell was quantified and showed a significant decrease in injured cultures to 22 ± 2 % compared to 35 ± 2 % in sham controls (Fig. 8).



(A) Sham (B) Injured 571 kPa

(C) Sham (D) Injured 571 kPa

Fig. 7: ZO-1 immunostaining of bEnd.3 cells. Representative images of ZO-1 staining are shown for sham controls (A, C) juxtaposed against blast injured cultures (B, D), confirming that disruption of tight junctions was responsible for TEER and hydraulic conductivity changes after blast (Sham n=5; Injured n=5).

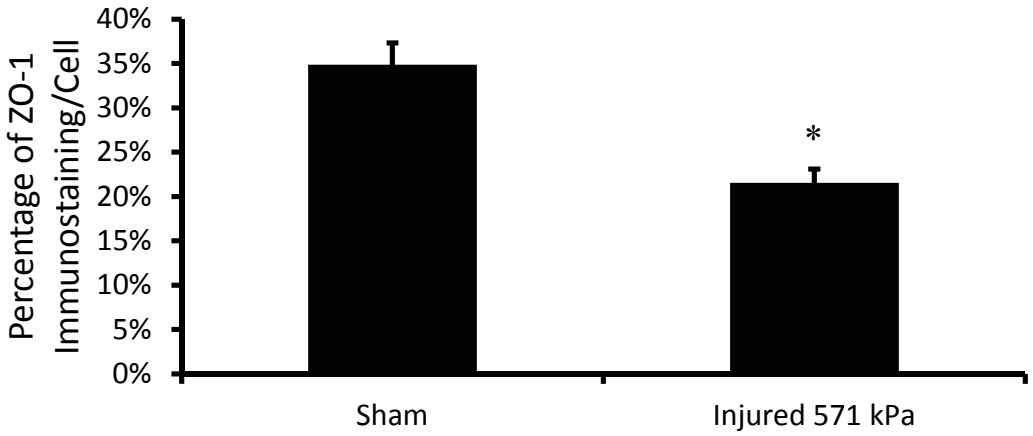


Fig. 8: Quantification of ZO-1 immunostaining of bEnd.3 cells. Following injury, exposed cultures exhibited a significant decrease in percentage of ZO-1 immunostaining per cell to 22 ± 2 % compared to 35 ± 2 % in sham controls (*$p < 0.05$; SEM; Sham n=25; Injured n=25).

IV. DISCUSSION

There is a paucity of published literature on injury tolerance criteria for the BBB associated with primary blast loading. BBB disruption has primarily been reported in several *in vivo* bTBI models by observations of increased permeability to endogenous proteins such as IgG. Readnower et al. [5] demonstrated transient and significant increases in IgG immunohistochemical staining in the cortex following exposure to a 120 kPa peak overpressure blast (unreported duration and impulse) generated by an air-driven shock tube. Garman et al. [17] observed increased IgG immunoreactivity in the contralateral cortex 24 hours after exposure to a 241 kPa blast with approximately a 4 ms duration. In addition, Kuehn et al. [1] detected abnormal IgG immunolabeling in the cerebellum and thalamus following a sub-lethal complex blast (427 to 517 kPa peak overpressure) delivered to the head of rats, signifying microvascular dysfunction. While such evidence provides valuable insight into the vulnerability of the BBB to blast, the challenge remains of harmonizing differences among the injury parameters used by the models. Analysis of the outcomes is further complicated by the inherent complexities of *in vivo* injury models, making it difficult to separate the specific contributions of shearing and stretching forces due to inertial loading from primary interaction of the shock wave with the brain parenchyma and specialized structures like the BBB [33,34,38]. Exposure conditions of Readnower [5], Kuehn [1], and Garman [17] are also quite different from those used in this study. Readnower et al. [5] did not protect the torso of the rats, potentially producing injury cascades in the gut and lungs that affect the BBB and cause transient effects in the brain. The apparatus of Kuehn et al. [1] reflects a high-pressure wave off of the closed cranial surface of the rats, and the integrated impulse of the shock described by Garman et al. [17] is substantially larger than those in this study.

The determination of injury thresholds for mild TBI (mTBI), as related to blast explosions sustained by U.S. warfighters, is an active area of research that has yet to establish a definitive link between blast injury parameters (peak overpressure, duration and impulse) and mTBI. In a recent study by Goldstein et al. [39], exposure to a single sublethal shock wave with 77 kPa incident overpressure using a blast neurotrauma mouse model resulted in persistent hippocampal-dependent learning and memory deficits. Because their model permitted free movement of the head in order to model typical conditions associated with military blast exposure, kinematic analysis revealed blast-induced head oscillation at accelerations sufficient to cause brain injury [40]. Importantly, efforts to immobilize the head during blast exposure prevented said learning and memory deficits, suggesting that inertial forces associated with oscillating acceleration-deceleration cycles imposed on the head, rather than exposure to a pressure transient alone, may be the major contributing mechanism responsible for the observed post-injury sequelae in their model. An advantage of our blast injury model used in the current study, however, is the ability to investigate damage due to a primary blast injury, in the absence of confounding contributions from inertial loading mechanisms. With our ability to separate the shock wave component from the inertial component, the primary blast loading conditions used may be more representative of the thresholds for mTBI associated with a shock wave, whereas mTBI in whole animals or humans at lower overpressures may be attributed to secondary/tertiary blast mechanisms.

Using our previously characterized *in vitro* bTBI model [33,34], results indicate that integrity of the BBB decreases in a dose-dependent manner with increasing severity of primary blast injury. A significant decrease in TEER was observed at the highest blast condition tested compared to sham controls, suggesting a tentative threshold for barrier opening between 469 kPa and 571 kPa. However, one limitation with the current findings is that the blast injury parameters tested represent one range of operationally-relevant peak overpressure, duration and impulse. The highest, short-duration loading condition in this study (1.06 ms for 571 kPa overpressure level) is similar to real-world blast threats including 105 mm mortar rounds at close range [41]. Other real-world exposures may have 50 to 1000 kPa peak incident overpressure with 2-10 ms duration, as predicted by the Conventional Weapons Effects Program [34,42] or may have complex overpressure/duration histories. Our sample receiver was designed to replicate *in vivo* pressure histories – whereby a shock wave in-air is converted to a fast-rising intracranial pressure wave [21] – for *in vitro* cell and tissue samples subjected to an overpressure pulse. Receiver performance achieved single overpressure pulses between 0.5 and 2 ms in duration, similar to loading conditions observed in cadaveric and computational blast studies [34,43,44]. Studies are currently underway to assess thresholds for BBB damage resulting from milder overpressure levels with longer durations to further explore the injury parameter space of blast loading conditions, and to support the overarching goal of bTBI research to more accurately replicate clinical outcomes of blast victims using animal or *in vitro* injury models [34,45].

Exposure to blast significantly reduced TEER, which then fully recovered at 3 days following injury, suggesting an intrinsic ability of the monolayer for self-repair. The time-course for spontaneous recovery was consistent with findings from Readnower et al. [5] and Garman et al. [17], both of which report that IgG immunoreactivity (a marker for BBB disruption) returned to control levels at 3 days post-exposure [5,17]. BBB self-repair mechanisms have also been reported using other TBI models. Following fluid percussive injury in rats whereby IgGs were found to be immunolocalized in brain regions due to a compromised BBB, re-establishment of the barrier to circulating proteins ensued within hours after injury, with the most delayed time-course occurring at the site of impact [46-48]. Such findings hold promise in light of limited current understanding as to whether endothelial cell injury can be associated with loss and subsequent recovery of normal function [49]. It is also encouraging to note that BBB integrity of blast injured monolayers, as measured by TEER, exhibited an increasing trend for up to 3 days post-injury, suggesting that acute treatments to potentiate repair of the damaged BBB after blast could provide significant clinical utility.

A normally functioning BBB excludes water due to the presence of tight junctions between endothelial cells; however, various neuropathologies may cause hydraulic conductivity of the capillary wall to increase and favor water flux across the endothelium [50]. Measurement of water flux across the barrier has previously been used to quantify changes in vascular permeability [51,52]. Cerebral capillaries are especially known to have a relatively low hydraulic conductivity compared to most other tissues primarily due to the tight junctions between endothelial cell membranes [53-55]. The measured hydraulic conductivity for sham-treated monolayers was $1.8 \times 10^{-6} \pm 3.0 \times 10^{-7}$ cm/s/cmH_2O, which is approximately one order of magnitude higher than published values for bEnd.3 monolayers cultured under similar conditions. Li et al. [31] and Simon et al. [11] report hydraulic conductivity for bEnd.3 monolayers to be on the order of 10^{-7} cm/s/cmH_2O, indicating that resistance to water flux across cultures used in this study may have been less than ideal. Despite this, a significant six-fold increase was observed in the hydraulic conductivities of cultures injured at the 571 kPa overpressure level, compared to controls.

Disruption of tight junction proteins is a hallmark of many CNS pathologies, including stroke, Alzheimer's disease and multiple sclerosis, among others [7], and may be one underlying mechanism accounting for compromised integrity of the blast-injured BBB. It is thought that post-traumatic BBB dysfunction can be attributed to direct mechanical disruption or redistribution of tight junctions, or other phenomena such as elevated expression of matrix metalloproteinases (MMPs) or inflammatory cytokines [56-58]. While there are a number of proteins that contribute to tight junction formation, ZO-1 is often the marker of choice in pathological studies because proper cellular localization and sealing of tight junctions is thought to depend on its scaffolding properties and cytoskeletal interaction [59-62]. Currently, there is a lack of studies specifically related to blast-induced tight junction disruption; however, the ability of MMPs to degrade tight junction proteins including ZO-1 (a substrate of MMP-9) following experimental TBI has been the subject of a number of investigations [63-66]. In a controlled cortical impact model, densitometric analysis showed a 50 % decrease in ZO-1 levels 24 hours following injury [65]. Adding to such findings of abnormal tight junction structural integrity following brain injury [67], our results revealed altered morphology of tight junction proteins and a significant decrease in ZO-1 immunostaining in cultures exposed to primary blast.

V. CONCLUSIONS

In summary, these data demonstrate that the barrier integrity of an endothelial monolayer (BBB model) was disrupted by primary blast overpressure exposure. A major function of the BBB is to restrict the movement of water and ions between the brain and systemic compartments, making TEER and hydraulic conductivity functional indicators of monolayer integrity. The acute TEER dose response observed post-injury suggested that BBB disruption can occur in bTBI and that a tentative threshold for barrier opening exists between 469 kPa and 571 kPa peak overpressure with duration between 0.99 ms and 1.06 ms. Exposure to blast significantly reduced TEER in the acute phase, which then fully recovered 3 days after injury, suggesting an intrinsic ability of the monolayer for self-repair. Significantly increased hydraulic conductivity of cultures injured at the highest overpressure level indicated compromised tight junctions, which was confirmed by disrupted morphology and significantly reduced ZO-1 immunofluorescence. These results suggest that BBB breakdown caused by direct interaction with a primary blast wave may be a key contributing factor to the neuropathology associated with bTBI. Future studies will test for BBB damage resulting from additional operationally-relevant peak overpressures and durations, identify a threshold for blast-induced increases in hydraulic conductivity, and examine the underlying mechanisms for tight junction disruption.

VI. ACKNOWLEDGMENT

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