Linking Macro- to Micro-Biomechanics in Closed-Head Traumatic Brain Injury Based on Acute Biophysical Disruptions and Evolving Neurodegeneration

James P. HARRIS, University of Pennsylvania, jamhar@mail.med.upenn.edu
Kevin D. BROWNE, University of Pennsylvania, k browne@mail.med.upenn.edu
John A. WOLF, University of Pennsylvania, wolfj@mail.med.upenn.edu
D. Kacy CULLEN, University of Pennsylvania, dkacy@mail.med.upenn.edu

Traumatic brain injury (TBI) is caused by a physical insult to the head and may result in prolonged or permanent dysfunction for a significant portion of the 3.5 million afflicted annually in the U.S. [1]. TBI is a heterogeneous disorder, with long-term outcomes dependent on the type and severity of the initial physical event and compounded by multi-faceted pathophysiological consequences [2]. Due to chronic and progressive mechanisms of cell dysfunction/death, TBI may be considered both an acute biophysical trauma as well as a longer-term neurodegenerative disease. The vast majority of cases are closed-head TBI, which in general is caused by rapid angular acceleration-deceleration of the head. Such “inertial” loading leads to complex stress-strain patterns within the brain. However, the mechanisms by which neural cells sense, and are pathologically affected by, the transduction of traumatic forces remains poorly understood, although these are believed to be key incentive events in subsequent dysfunction and degeneration. Therefore, it is important to accurately represent cell- and tissue-level biomechanics to fully describe clinical TBI as well as to validate experimental models.

Conventional Versus Blast-Induced TBI. Most recent military TBIs have resulted from blast exposure, which may cause injury due to direct pressure propagation (primary blast: yet to be fully described), impact (secondary blast: focal, often penetrating injuries), or inertial forces (tertiary blast: diffuse acceleration-deceleration injuries). Mild blast TBIs are likely due to primary and/or tertiary effects, which underscore the importance of understanding shockwave and inertial forces independently and collectively. In closed-head TBI, the brain primarily experiences shear strain due to a low shear modulus relative to the bulk modulus [3,4]. Moreover, many biophysical responses are strain rate-dependent owing to the viscoelastic nature of cells where at high strain rates - characteristic of TBI - the elastic component dominates whereas low strain rates engage the viscous component [5]. Notably, different TBI modalities will produce different strain profiles. Conventional TBI (absent shockwave) results in high strain magnitudes (0.10-0.50) with high strain rates (10-50 s^-1), whereas blast shockwave (absent head motion) is predicted to result in lower strain magnitudes (0.01-0.05) with ultra-high strain rates (100-1000 s^-1) [6,7]. We currently have an incomplete understanding of the role of primary, secondary, and tertiary blast to TBI thresholds and pathology, and any synergistic effects are unknown.

Experimental Framework. Our objective is to link macro- to micro-TBI biomechanics based on measures of immediate biophysical disruptions in relation to evolving neuropathological sequelae across various models of TBI. Thus, our framework is to establish the relationships between defined cell- and tissue-level biomechanical inputs, acute plasmalemmal disruptions and cytoskeletal discontinuities, and neurophysiological and neurodegenerative changes. We have applied this approach across in vitro, rodent, and swine models of conventional as well as blast-induced TBI.

Results. As the base step to linking macro to micro, we utilize an in vitro model capable of applying controlled three-dimensional shear strain fields to neural cells within an extracellular matrix. Importantly, this model applies a broad range of strain rates representative of inertial and/or blast TBI. Here, we found strain-rate dependence for immediate plasmalemmal disruptions that correlated with evolving cell death based on the extent of initial damage [8]. Translating to in vivo models, we have mapped patterns of trauma-induced biophysical damage based on type of TBI (e.g., blast vs. inertial), neuroanatomical locale, cell type, and cell morphology. For example, we mapped heterogeneous biophysical disruptions in rodent models of both blast and inertial TBI (Figure 1). This revealed stark differences in the patterns and extent of plasma membrane damage between these injuries (at levels matching the incidence of mortality). We have also assessed acute biophysical damage using swine
models of closed-head inertial and blast TBI. Following inertial TBI, we found plasmalemmal permeability that increased with increasing angular velocity and acceleration in a region-specific manner. Heterogeneous permeability was observed in deep layers of the cerebral cortex predominately at grey/white matter interfaces, regions predicted to develop stress concentrations based on microstructural discontinuities. Moreover, there was significant neural cell permeability in peri-vascular domains in the cortex, subcortical white matter, and thalamus, suggesting vasculature has an important biomechanical role in acute biophysical responses.

**Figure 1. Plasmalemmal vulnerability in blast vs. inertial TBI in rats.** Inertial TBI (BOTTOM) resulted in exacerbated plasma membrane damage compared to blast (TOP). The granular layer and hilus were the most vulnerable regions in blast; however, these regions had more extensive permeability following inertial TBI. Permeabilized neurons from blast maintained normal morphology (arrow heads), whereas neuronal somata/neurites following inertial injury often had a pathological tortuous “corkscrew” morphology (arrows), potentially due to larger strain magnitudes.

**Discussion and Conclusions.** Correlating trauma-induced biophysical alterations with predicted cell/tissue biomechanics is important to identify unique features of TBI pathology. Using a multi-level *in vitro* and *in vivo* approach, we found differential patterns of trauma-induced biophysical damage based on neuroanatomy and cell phenotype that varied based on TBI type (e.g., blast vs. inertial loading in rats or pigs). This underscores the need to consider both the specific injury biomechanics and to categorize consistent mechano-responses. We are currently correlating ultrastructural damage with predicted micro-strain fields as well as hallmark TBI pathologies including neuronal/axonal degeneration and reactive astrocytosis, allowing us to elucidate tolerances to subtle biophysical disruptions and degeneration. Models with sufficient biomechanical fidelity to human TBI are important to replicate mechanisms of primary damage, acute reparative processes, as well as the underlying environment in which secondary damage ensures. Understanding these parameters may inform the links between the physical and physiological consequences of TBI, thus guiding development of targeted therapeutics to address the predominantly afflicted cell populations based on the mechanisms of injury.

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