Exploring Astrocyte Morphological Changes under Shear Stress: A Quantitative Imaging and Laser-Induced Shockwaves Approach

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ABSTRACT

Traumatic Brain Injury (TBI) is the result of external forces impacting the brain. Despite scientific progress, TBI remains a significant cause of impairment and mortality. Recently, laser-induced shockwave (LIS) has emerged as an effective method for TBI simulation. LIS generates shockwaves through pulsed laser-induced plasma formation, allowing for the controlled study of TBI at the cellular level. This study introduces a novel approach to examine cellular morphological changes in response to shear stress, focusing on astrocyte cell type AST-1, by combining LIS with quantitative phase microscopy (QPM). QPM is a label-free technique that allows for real-time cellular dynamics observation through 3D imaging. Integrating LIS and QPM assesses astrocyte responses to shear stress caused by LIS, revealing both immediate and sustained morphological changes. Post-LIS exposure analysis shows significant alterations in astrocyte circularity, volume, surface area, and other features. Statistical tests confirm these observed trends, providing valuable insights into astrocyte responses to mechanical forces. These findings enhance our understanding of how mechanical stimuli affect astrocyte morphology, which may offer the potential for identifying and developing therapeutic strategies in TBI and related neurological disorders.

Keywords: Laser-induced Shockwave, LIS, Quantitative Phase Microscopy, QPM, Astrocytes, Morphology, Traumatic Brain Injury, Cell Segmentation

1. INTRODUCTION

Traumatic Brain Injury (TBI) is a critical public health issue, remaining a leading cause of impairment and death especially among youth.[1] Various models simulate TBI, including blast-induced TBI (bTBI) from open-ended shock tubes and explosive devices. Understanding the bTBI pathologies is critical to developing effective diagnostic and treatment strategies to intercede and prevent post-bTBI neurodegeneration.[2] Laser-induced shockwaves (LIS) offer a controlled method to simulate bTBI, creating shockwaves via pulsed laser-induced plasma formation, which can damage cells mechanically.[3]

Astrocytes, the most numerous CNS cells, maintain ionic balance, homeostasis, blood flow, neurotransmitter recycling, and nutrition.[4] Their injury response, astrogliosis, involves morphological changes depending on insult severity.[5] Astrocyte morphological changes have been observed in neurodegenerative diseases like Parkinson's, Huntington's, and ALS.[6]

This study introduces a system combining LIS and quantitative phase microscopy (QPM) to study astrocyte morphology in response to shear stress. QPM captures 3D images and quantifies optical path lengths, allowing imaging of transparent cell features and dynamics without photo-bleaching.[7-9] The integrated LIS-QPM system monitors shockwave-induced changes in astrocyte membranes and internal structures. Post-LIS, astrocytes exhibited immediate and sustained morphological changes, including increased circularity, altered volume, surface characteristics, and height. These findings enhance understanding of astrocyte responses to mechanical stimuli, aiding the development of targeted therapies for TBI and related neurological disorders.[10-11]

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2. METHODOLOGY

The schematic of the QPM and fluorescence microscope setup can be found in Figure 1. Detailed information on our QPM and LIS setup can be found in our previous publications.[12]

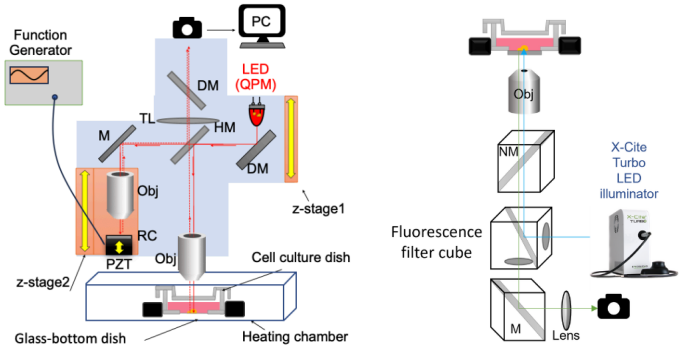

Figure 1. Quantitative phase and fluorescence microscope setup. PD: Photodiode, LED: light-emitting diode, LD: Laser diode, RM: Reference Mirror, HM: Half Mirror, DM: Dichroic Mirror, M: Mirror, TL: Tube lens, Obj: Objective Lens. To avoid confusion, the reflected beams are colored with a strip pattern.

All experiments were performed on the established astrocyte type-I (Ast-1) line (clone CRL-2541) received directly from ATCC. They were separated into two groups: Controlled (non-exposed to shockwaves) and Shockwave-exposed. The control group consisted of 4 dishes and a total of 84 cells. The shockwave-exposed group consisted of 4 dishes and a total of 204 cells. Each round of imaging took two hours at a frame rate of 41 fps, resulting in 1.3 images per second. The shockwave had a pulse power of 40 kW with a beam radius of 1.274 nm and wavelength of 1030nm. All images were captured by a 12-bit CCD camera (acA1920-40um, Basler AG, Ahrensburg, Germany).

3. IMAGE PROCESSING

Wrapped phase images were constructed from interference cell images captured through the QPM through a phase-shifting algorithm.[13] The Goldstein method was used to unwrap the phase and reconstruct the absolute phase distribution.[14-15] Then, the phases were converted to real height images and individually segmented in ImageJ to be processed from grayscale to the final binary mask in MATLAB.[12]

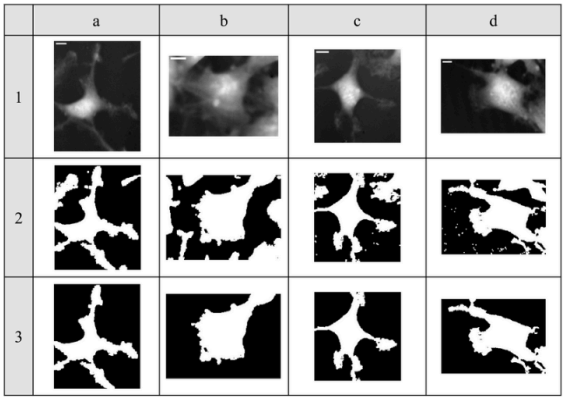

Figure 2: Four example cells (a,b,c,d) and their respective masks. (1) a grayscale image, (2) a mask image created with ImageJ, and (3) a processed image with MATLAB

Features that were considered for this study were dry mass (DM), surface area (SA), surface area to volume ratio (SAV), surface area to dry mass ratio (SDM), sphericity index, height variance (HV), height kurtosis (HK), height skewness (HS), eccentricity (Ecc), circularity (Circ), perimeter (Perim), perimeter to projected area ratio (Perimdivarea) and Complexity score.

4. RESULTS

LIS exposure induces AST-1 to become more circular, with changes in volume, surface characteristics, and height. Increased surface area suggests a response to enhanced chemical interactions, and increased projected area and area-to-volume ratio indicate AST-1 flattening in response to LIS. The changes persisted after 2 hours, suggesting ongoing cellular adjustments. Statistical analysis highlights the astrocytes' dynamic response to mechanical stress.

5. CONCLUSION

In conclusion, this paper's findings on laser-induced shockwaves' impact on astrocyte morphology in combination with quantitative phase microscopy advance knowledge in astrocyte biology and offer a foundation for future research on traumatic brain injuries and cellular responses to mechanical stimuli.

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