Impulsive Pressurization of Neuronal Cells for Studying Traumatic Brain Injury

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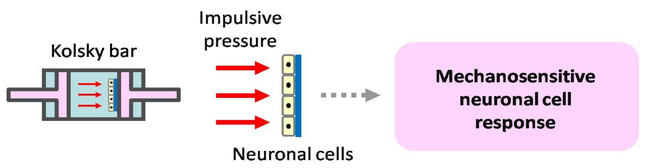
TBI: Brain Cell Response to Impulsive Pressure

Objective



Impulsive pressure effect
- Magnitude of pressure (0.1-3 MPa)
- Duration of pressure (0.1-1 ms)

Cell physiology effect
- Cell interaction (isolated vs. communicating)
- Co-culture effect (neuron-astrocyte)

Biomimetic pressurization
- Lower end pressure (0.2 MPa or less)
- Pressure of cells on ECM layer
- Co-culture effect (neuron-astrocyte)
- Long-term cell response (up to 4 wks)

Mechanism of neuronal loss
- Calcium-calpain-microtubule (spectrin)
- Pressurization magnitude and duration
- Use of primary neuronal cells

Experiments

Cell culture : SH-SY5Y human neuroblastoma cells were maintained in DMEM with 10% FBS and 1% penicillin/streptomycin at 37˚C. The cells were differentiated to neuronal cells with 10 μM retinoic acid (RA).

Pressurization : SH-SY5Y cells cultured on glass cover slip were treated with RA to induce axonal outgrowth. These cells were placed inside the pressure chamber and exposed to 0.5, 1, and 2 MPa level pressurization at a duration of about 0.5 ms. The SH-SY5Y cells exposed to pressure were further incubated for 0 h and 24 h.

Cell viability : For MTT assay, SH-SY5Y cells were incubated for 24 h after pressurization. After incubation, the cells were treated with 0.2 mg/ml MTT for 3 h at 37˚C. After the reaction, the absorbance of each sample was measured at 570 nm.

Fluorescence staining : After pressurization, the SH-SY5Y cells were observed by immunofluorescence after 0 h or 24 h incubation. The SH-SY5Y cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Actin cytoskeletons were stained with rhodamine phalloidin and nuclei were stained with DAPI.

Results

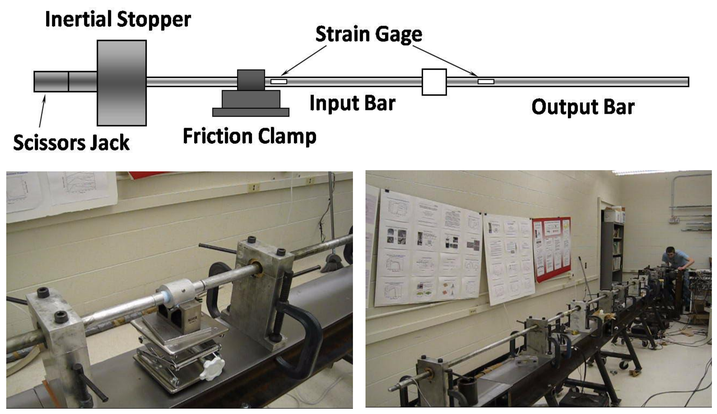


Figure 1. Blast mimicking impulsive pressurization by Kolsky bar. The direct compression Kolsky bar works by storing strain energy in the bar between the engaged friction clamp and the compressing scissors jack. When the friction clamp is released, by fracturing the locking bolt, the energy in the stored section is released as a near square wave of strain. When the wave reaches an impedance change, such as is at the sample, part of the wave will bounce back while the remainder will pass through the material, in this case an in-vitro cell containment chamber. The pressure history of the in-vitro cell containment chamber is proportional to the wave that is recorded by the output bar's strain gauge.

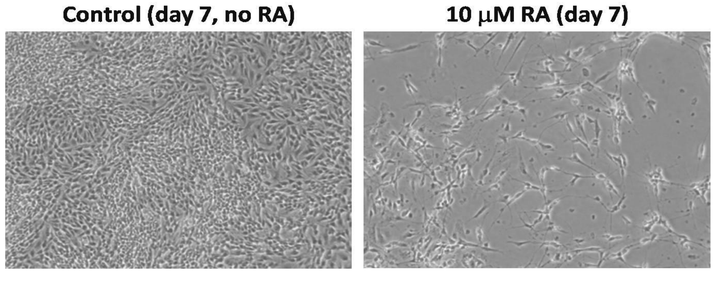


Figure 2. Neuronal differentiation of SH-SY5Y cells. After induction with 10 μM retinoic acid (RA), SH-SY5Y cells are differentiated into neuronal cells with long neurites/axons.

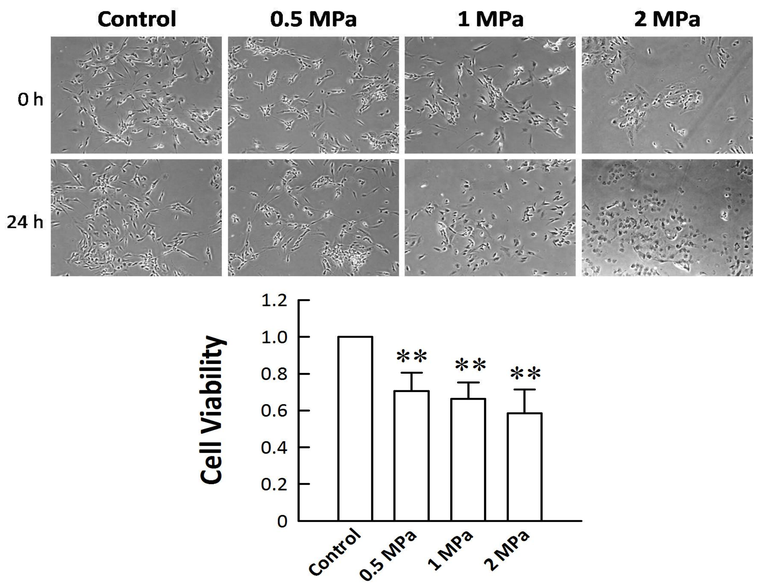


Figure 3. Effect of impulsive pressure level (0.5, 1, and 2 MPa) on the viability and growth rate of SH-SY5Y cells. After pressurization, the number of neurites decreased at 2 MPa right after pressurization and at above 1 MPa for 24 h post-incubation (upper). Cell viability, assessed by MTT assay after 24 h incubation, shows significant decrease at all pressurization level relative to unpressurized control (**: < 0.01) (bottom).

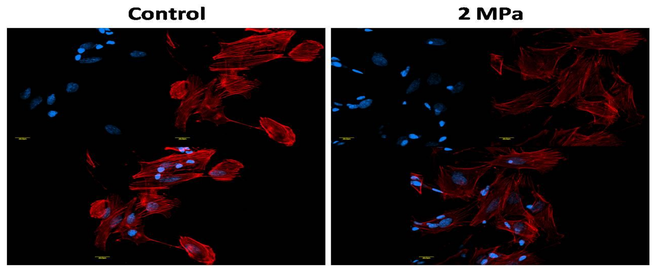


Figure 4. Actin cytoskeleton in neuronal cells was damaged at 2 MPa pressurization (Actin: Red, Nuclei: Blue)

Discussion

Traumatic brain injury (TBI) is one of the leading incidents that lead to morbidity of soldiers during or after the military operations. To date, it is not fully understood what is the cellular and molecular mechanism of blast induced TBI and its secondary progression. Based on the assumption that blast-induced overpressure is the main cause of the TBI, we investigated the effects of impulsive pressurization on neuronal cell functions. A novel impulsive pressurization apparatus capable of controlling pressure level and duration time has been built using the Kolsky bar set-up. SH-SY5Y human neuroblastoma cells were treated with retinoic acid for these cells to make axons. These cells were placed inside the pressure chamber and exposed to 0.5, 1, and 2 MPa pressures at a duration of ca. 0.5 ms, which is relevant to the blast-induced pressure conditions. Preliminary data show that pressurization at 1-2 MPa severely damaged axons and actin cytoskeletons and drastically altered cell morphology. At relatively lower pressurization level of 0.5 MPa (but still known to induce TBI), cell morphology including axons were not very different up to 24 h post-incubation while the MTT assays show significant loss in cell viability. With assays for assessing gene expression specific to apoptosis and neuronal cell markers (e.g., MAP2), we expect to reveal the pressure criteria for TBI and mild-TBI.

This will be the first step towards revealing correlation between pressure level and neuronal cell responses in TBI conditions.

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