INTRODUCTION

The mechanism of mild traumatic brain injury (mTBI) is directly related to the relationship between the mechanical response of neurons and their biological/chemical functions since the neuron is the main functional component of brain. The hypotheses is that the external mechanical load will firstly cause the mechanical deformation of neurons, and then, when the mechanical deformation of neurons reaches to a critical point (the mechanical deformation threshold), it will initiate the chemical/biological response (e.g. neuronal function loss). Therefore, defining and measuring the mechanical deformation threshold for the neuronal cell injury is an important first step to understand the mechanism of mTBI.

Typically, the mechanical response of neurons is investigated based on the deformation of in vitro model, in which the neurons are cultured on the elastic substrate (e.g. PDMS membranes). The elastic membrane is deformed by the external load, e.g. equibiaxial stretching. The substrate deformation is considered to be the deformation of neurons since the substrate is several orders stiffer than the neurons and the neurons are perfectly bonded with the substrate. The fluorescence method is typically used to test the cell injury, e.g. the cell vitality and the neuron internal ROS level.

EXPERIMENTAL METHODS

Neuroblastoma cells from the SH-SY5Y cell line were used to measure the neuron injury threshold caused by the mechanical injury. The neurons were cultured as monolayers on the collagen-coated six-well FlexPlates (Flex, Hillsborough, NC, USA) in DMEM medium supplemented with 10% foetal calf serum (FCS). Cultures were maintained in humidified incubator (5% CO2, 37°C). Cells were seeded at the density of 5000 cells/cm². In order to make neurons fully differentiated, neurons were treated with 10µM retinoic acid (RA) over 4 days after 24hs cell culturing.

The cells were injured using Flexcell 5000 tension system (Flex, Hillsborough, NC, USA). The bottom of the FlexPlate wells are made by PDMS membrane with the thickness of 0.2mm. The membrane is stretched by a negative pressure pulse. The schematic of the cell stretching is shown in Figure 1. The cell deformation is same as the deformation of the PDMS membrane. In the neuron injury evaluation tests, initially an arbitrarily given deformation e.g. 30% will be applied on the cultured neuron monolayer, which should be strong enough to kill most of the PPDMS membrane. Then, the magnitude of applied strain will gradually be decreased until the injury thresholds are measured. The loading rate is about 0.01/s.

The nonlethal injury of neurons is interested since it might be directly related to the mTBI. The nonlethal
injury can be defined as follows. The mild injury means that the neuron can still survive for a period of time (24h) after unloading. The critical injury strain ($\varepsilon_{cr}$) is defined as the value of the applied external deformation beyond which the neuron will be mildly injured, while below which there will be no detectable injury. Since the neuron injury is determined based on the statistical results in current work, the applied deformation will cause about 80% of the neurons in each test (more than 200 neurons are counted) to have the detectable injury (nonlethal) defined to be the critical injury strain ($\varepsilon_{cr}$). The upper limit of the nonlethal injury of neurons ($\varepsilon_{max}$) is also defined. If the applied external deformation is beyond $\varepsilon_{max}$, the neuron will finally die. $\varepsilon_{max}$ is defined as the value of the applied external deformation, causing about 30% of neurons to die in each test (more than 200 neurons are counted).

Dual fluorescence, live cell time-lapse confocal microscopy is carried out for identifying the neuron injury. Two different fluorescent reagents will be used: 1) a fluorescent ROS indicator, 2,7-dichlorofluorescin (DCF, 1 uM); 2) a HEPES-MEM containing 0.5 uM propidium iodide (PI, viability indicator); The stretched neurons will be loaded with both reagents. An increase in DCF green fluorescence strength (usually in the cell cytoplasm) indicates the ROS reactions occurring as a result of a higher stress level of the cells in response to the external load (mechanical stretch and contraction). When cell viability changes as a result of alteration of membrane permeability in response to the mechanical stretch or contraction, the PI will enter the cells and binding DNAs/RNAs (proteins inside nucleus), resulting in red fluorescent nucleus structure.

RESULTS

Figure 2 shows the fluorescence images of SHSY5 neuronal cells. The cell density variation caused by the stretching is measured using the software Image J (NIH). Figure 3 shows the neuron density variation with $\varepsilon=10\%$, 20\% strain after 24h, and the control unit after 24h is also shown in Figure 3. The results show that the equibiaxial strain of 10\% is the upper limit of the non-lethal injury ($\varepsilon_{max}$), which caused the death of about 30\% neurons. The lower limit of the non-lethal injury ($\varepsilon_{cr}$) is about 5\%, which is measured using the strength of DCF. The results of each well are based on the average values of 15 images.

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