DISPLACEMENT-CONTROLLED STRETCH INJURY OF NEURAL CELLS

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INTRODUCTION

Severe impact and/or inertial loadings to the head that occur in motor vehicle accidents, falls, and the acts of violence often induce traumatic brain injuries (TBI), leading to neural cell death and other biological responses [1-3]. Among the many types of head injuries, diffuse axonal injury (DAI), cerebral concussion, and subdural hematomas from the diffuse tearing (shearing) of bridging veins are directly related to the degree of bulk shear deformations of the brain tissue [4].

Many *in vitro* injury models have been developed to deform neural cells in a controlled manner to mimic tissue deformations during axonal injury, and to study different aspects of DAI [5-7]. Most of these models employ a device consisting of a circular elastic silicone membrane on which neural cells are cultured, and a pressure-pulse generator to cause *biaxial* deformation of the membrane. However, in this approach the deformation of the neurons under applied pressure is not uniform, and the effect of strain rate on neural stretch injury is difficult to quantify. To better study the biomechanics issues in TBI, we have developed a novel uniaxial stretching device for neural injury, with rather precise displacement control [8]. Using this device, we can apply a wide range of strains and strain rates to cause controlled stretch injury of neural cells, and quantify the resulting neural damage and cell death.

MATERIALS AND METHODS

The Uniaxial Stretching Device

As shown in Figure 1, this device consists of a voice coil actuator, a linear encoder, a proportional integral derivative (PID) controller/amplifier, a power supply, and a computer interface. The voice coil actuator (BEI Kimco, San Marcos, CA) is a linear actuator that is capable of delivering large forces at high speed. The only disadvantage of a voice coil is its limited range of displacement (about one inch). The voice coil is mounted on a linear bearing slide (Edmund Scientific, Barrington, NJ) for support and for achieving accurate linear movement, and is controlled by a PID servo controller/amplifier (BEI Kimco, San Marcos, CA) with a closed loop feedback through a linear displacement encoder (RSF Electronics, Corning, NY) mounted on the linear bearing slide. The power supply, required to power the controller and the voice coil, can provide up to 12 amps at 50 volts DC. A computer is used to program as well as communicate with the controller for any desired linear motion.

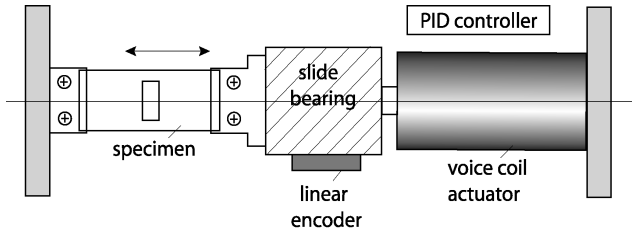

Figure 1. A schematic showing the design and major components of the stretch injury device

Major Features of the New Stretch Injury Model

The major features of the displacement controlled uniaxial stretching device include: (1) achieving accurate motions with at least 70% strain and an overall maximum strain rate of 90 s^{-1} within 2% error; (2) uniform deformation cross the substrate silicone membrane; (3) high reproducibility of motion with different samples; (4) uniaxial deformation of the specimen, with lateral strains (E_{22}) not exceed 5% of the longitudinal strains (E_{11}). Further, the elastic specimens are convenient for cell culture, and the entire device can fit into a biological safety cabinet to maintain sterility.

Cell Culture

The NG108-15 cell line was used for all the cellular assays in this study. Cell cultures were maintained in Dubecco's Modified Eagles containing 4.5g/L glucose, 0.1mM hypoxanthine, 400nM aminopterin,

0.016mM thymidine (Gibco HAT 100X solution, Gaithersburg, MD), 10% FBS (not heat inactivated, Gibco), and 1% penn/strep (Gibco) and incubated at 37°C and 5% CO_2. Cells were passaged at 1:10 twice a week. Subculturing was accomplished by rinsing with DPBS without Ca^{2+} followed by addition of 1mL 0.05% trypsin. Cells were immediately dislodged by striking flask with the palm, suspended with culture media and passed to new flasks.

RESULTS

Cell Adhesion

Although the device can have accurate control over the deformation of the elastic substrate, adequate cell adhesion is necessary to ensure that the neuron and its processes are subjected to the required deformation. Thus, a cell adhesion assay was performed to measure the cell deformation independently. Specitically, After the neural cells were seeded and grown on the silicone substrate, 0.5 micron fluorescent beads (Polysciences, Warrington, NJ) coated with lectin were incubated with the cell culture overnight, allowing them to attach to the membrane of the neurons. Fluorescence microscopy was used to visualize fluorescent beads attached to the top surface of cells cultured on the membrane. Based on two configurational snapshots, i.e., the undeformed configuration and the deformed configuration, a finite deformation analysis was preformed to calculate the actual cell deformation [8].

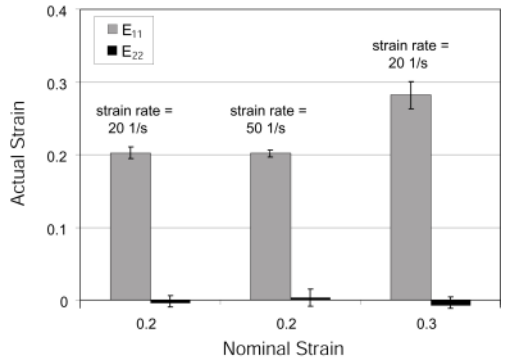

Figure 2. Deformation of NG108-15 cells on the substrate measured using fluorescent beads attached to the cell membrane

Displayed in Figure 2 are the actual strains obtained for different applied strains and strain rates. Note that the transverse strains E_{22} (perpendicular to the stretch direction) were very small indicating uniaxial stretching. Comparing the actual and nominal (applied) strains, it is clear that excellent adhesion of cells to the substrate membrane was achieved.

Stretch Induced Axonal Injury

To further determine the capability of the uniaxial stretching device, differentiated NG108-15 cells were tested at various applied strains and strain rates. Specifically, strains within the range of 20-70% and strain rates within the range of 20-90 s^{-1} were applied to create mild to sever axonal injuries to NG108-15 cells. Injuries to cells were evaluated morphologically by identifying neurite swelling and necrotic-like cell bodies 8 hours and 24 hours following stretch injury. It was found that cells exhibited swellings within 8 hours after injury. However, the major morphological feature of these cells was the appearance of neurite beading. This feature has also been recognized in other *in vivo* and *in vitro* studies of axonal injury. It was also found that the amount of cell death depended critically on applied strain rate.

CONCLUSIONS

In summary, we have developed a unique uniaxial stretching device to study axonal injury and neural cell death resulting from brain tissue deformations common in traumatic head injuries. Using displacement-control rather than force-control, this device is capable of achieving strains > 70% and strain rates up to 90 s^{-1}, well above that currently achievable for studying axonal injury. We have demonstrated that the deformation of the specimen was uniaxial, uniform and highly reproducible; the pre-specified displacement profiles could be realized almost precisely; and adequate cell adhesion could be achieved readily. The entire device can fit into a biological safety cabinet to maintain sterility, and the specimens are convenient for cell culture. This device can be used to investigate a wide range of biomechanical issues involved in diffuse axonal injury.

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