

# DEVELOPMENT OF AN IMPROVED INJURY DEVICE FOR NEURAL CELL CULTURES

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## ABSTRACT

Traumatic Brain Injury is hypothesized to occur as a function of the strain and strain rate experienced by neural tissues during a traumatic event. *In vitro* studies of TBI at the cellular level have used a variety of methods to subject neural cell cultures to potentially injurious strains and strain rates. A device used in previous investigations of neural cell injury was limited in its ability to control strain and strain rate independently or simulate quick repetitive loading. Here we present the design of an improved cell injury controller based on an experimental setup previously used. The new device has the ability to independently control strain and strain rate and can strain cell cultures grown on a stretchable membrane from 0.1 to 0.60 at rates up to 25 s<sup>-1</sup>.

**Keywords:** Mild Traumatic Brain Injury, Experiment, Injury Tolerance, Neural Tissue

## INTRODUCTION

Traumatic Brain Injury is hypothesized to result as a function of the strain and strain rate experienced by neural tissues during a traumatic event. Using a combination of animal and physical models it has been estimated that diffuse axonal injury, a condition associated with Traumatic Brain Injury (TBI) in humans, occurs at strains between 0.05 and 0.10 [1]. Additional studies that directly measured the strain on a slice of rat brain reported that a strain of 0.20 at 10 s<sup>-1</sup> produced a moderate amount of injury; with higher strain rates causing irreparable damage to the cells resulting in their death [2, 3]. Therefore the current estimated threshold for injury at the cellular level for brain tissue lies around 20% strain at a rate greater than 10 s<sup>-1</sup>.

Previous experiments have utilized a device that delivered a controlled pulse of compressed gas to a culture of cells growing on a flexible medium [4, 5]. The air pulse increases the pressure in the cell culture well, which causes the flexible membrane supporting the cells to bulge out as illustrated in Figure 1. Assuming there is good adhesion between the cells and the flexible membrane, increasing the pressure in the well has the effect of applying a stretching force to the cells in the culture. The amount of cell stretch is affected by the pressure, pressure profile and duration of the pulse.

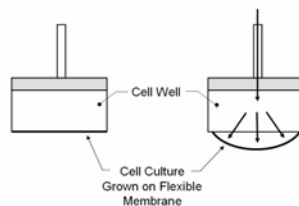


Figure 1. Stretch of Flexible Membrane

The device used in previous experiments was capable of generating a pressure pulse controlled by an internal solenoid valve that is held open by a timer. The duration of the pulse is controlled by adjusting the timer. A pressure regulator integrated into the unit allows the user to adjust the output pressure. A plug with a pressure tube fitting forms a pressure tight seal over the cell culture well allowing the cell culture well to connect to the injury controller with flexible tubing. When the solenoid valve is opened, pressurized air flows from the cell injury controller to the cell culture well. After delivering the pressure pulse, the solenoid valve is closed allowing the pressurized air in the cell culture well to escape.

It is known that both strain and strain rate independently affect cell injury. However the effect of strain cannot be studied independently of strain rate with the current device. Nor can the effect of strain rate be studied independently of strain. Both restrictions are due to the solenoid valve used to deliver the pressure pulse. Thus, a more sophisticated device was needed to perform more detailed experiments while still using the same flexible membranes to mechanically load the neural cell cultures.

## METHODS

A new device was designed which sought to address four areas of improvement: (1) improved control over the membrane deflection speed, (2) better control of the shape of the air pulse, (3) allow the deflection of the membrane to be measured for each test and (4) provide a mount for the culture trays during the injuring event. In this study the strain and strain rate performance of the new device was compared with the original device. A computer controlled proportional valve was used to regulate the flow of compressed air entering the cell culture well. Due to the unique ability of the computer controlled valve to shape the pressure pulse we are able to independently control the maximum strain and the strain rate. Additionally, the new device allows the culture trays to lock into a base unit positioned above a sensor allowing the deflection of the membrane to be measured. The unit is controlled by a PC connected via a USB cable. Figure 2 shows the base of the unit.

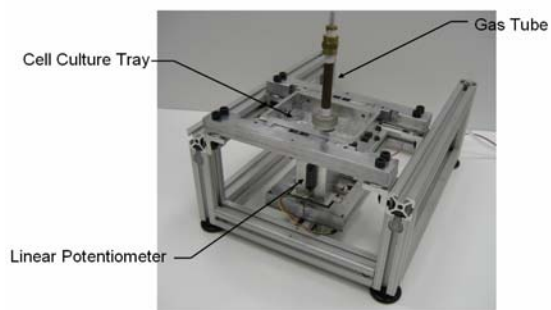


Figure 2. Base of improved Cell Injury Controller

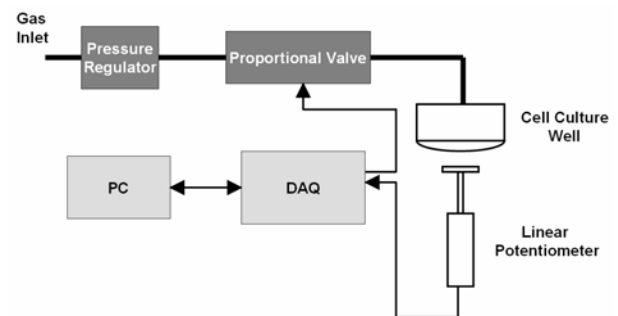


Figure 3. New Device Diagram

The new injury device allows each cell culture well to be positioned above a linear potentiometer. When the membrane bulges out under pressure it engages the potentiometer which generates an electrical signal proportional to distance. The linear potentiometer is lightly spring loaded to keep the plunger in contact with the membrane at all times. The pressure pulse is delivered via the gas tube which seals over the cell culture well.

The proportional control valve is controlled by a signal generated by custom software written in LabView run on a PC. The data acquisition system also measures the signal from the potentiometer and stores deflection versus time data for each test. Figure 3 presents a functional diagram of the system.

The software allows the user to select the shape of the air pulse signal from a preset list. The air pulse signal can have a *square*, *ramp up*, *ramp down* or *ramp up-down* shape which corresponds to how quickly the valve opens and closes. For example, a *square* shaped pulse completely opens the valve at the start and closes the valve at the end of the *square* pulse. By comparison, a *ramp up* shaped pulse gradually opens the valve allowing the flow of compressed gas to increase gradually. The duration and maximum opening of the valve can also be controlled.

Using the equations for strain on a flat plate given in Equations 1 and 2, the membrane strain can be estimated [6]. Expansion of the membrane is modeled assuming a parabolic shape.

$$\varepsilon_r = \frac{2 \omega_0^2}{3 R^2} \quad (1)$$

$$\varepsilon_t = \varepsilon_r \left( 1 - \frac{r^2}{R^2} \right) \quad (2)$$

For Equations 1 and 2,  $\omega_0$  is the maximum membrane deflection at the center,  $R$  is the radius of the cell culture well and  $r$  is the radial distance from the center of the membrane. Based on the geometry of a circular flat plate it is assumed that under uniform load the greatest strain will occur at the center of the plate where  $r = 0$ . This reduces the expression in Equation 2 so that  $\varepsilon_t = \varepsilon_r$ .

## RESULTS

Previous experiments performed by Ellis et al using the original cell injury device identified three distinct injury thresholds: *mild*, *moderate* and *severe* [4]. These thresholds were defined to reflect the extent of cell death after the injury event and were a function of membrane deflection occurring at 5.7, 6.5 and 8.6 mm respectively. For this study, we determined the pressure settings on the original cell injury device that corresponded to the three membrane deflections identified previously. Membrane deflection was measured using high speed video. Table 1 presents the experimentally determined correlations between pressure and membrane deflection for *mild*, *moderate* and *severe* levels of injury severity calibrated using high speed video. The pressure pulses were 50 ms in length. For comparison, the maximum deflection measured using the linear potentiometer on the new injury device is also given.

Table 1. Experimentally Determined Membrane Deflection Calibration

Injury Severity	Pressure (PSIG)	Max Deflection (mm)	
		High Speed Video	Linear Potentiometer
Mild	25	5.7	3.56
Moderate	29	6.5	3.91
Severe	32	8.6	4.26

Table 1 clearly shows that for the same pressure setting a membrane expanding in the presence of the linear potentiometer will have a lower maximum deflection when compared to a membrane allowed to expand freely. Figure 4 shows the calculated maximum strain at the center of the culture well for “mild,” “moderate,” and “severe” injury settings based on the deflection data collected by the linear potentiometer.

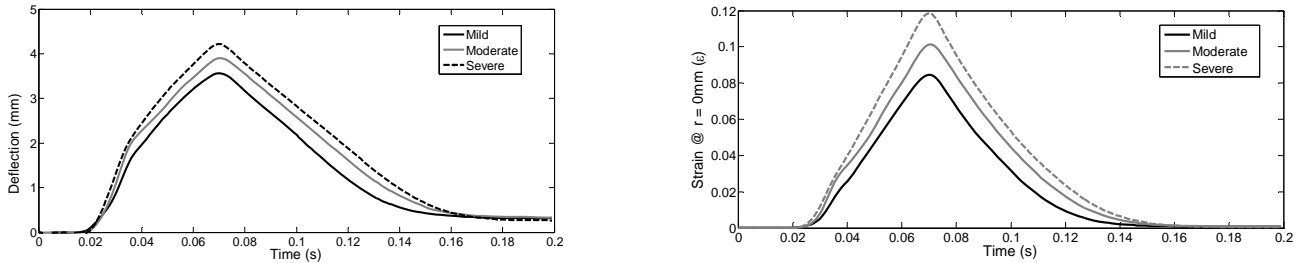


Figure 4. Original Device Strain Curves for Mild, Moderate and Severe Injury Settings

Figure 5 shows the strain vs. time profiles generated using the new device for the *Square*, *Ramp Up* and *Ramp Up/Down* valve control settings, illustrating the device’s ability to shape the pulse using the proportional control valve. These curves were generated at a pressure of 10 PSI. The *Square* setting generates the highest strain at the highest strain rate. The *Ramp Up* and *Ramp Up/Down* settings produce progressively lower strains for the same pressure setting at progressively lower strain rates.

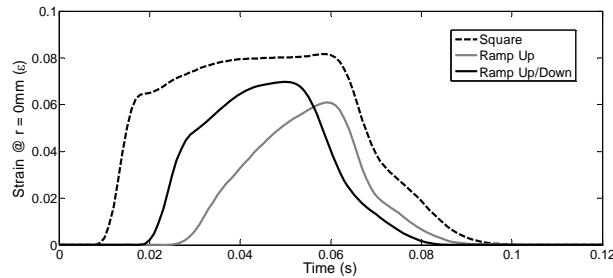


Figure 5. Strain Curve Shapes

Figure 6 shows the deflection and max strain curves for 5, 10, 15, and 18 ms *Square* pulses at a pressure of 30 PSI. At low pressures, the deflection of the membrane approaches equilibrium relatively quickly accounting for the approximately square shaped pulse in Figure 5. However, at high pressures it takes longer for the membrane deflection to reach equilibrium with the pressure in the cell well, explaining the sharp peaks in Figure 6 due to the closure of the valve before equilibrium is reached.

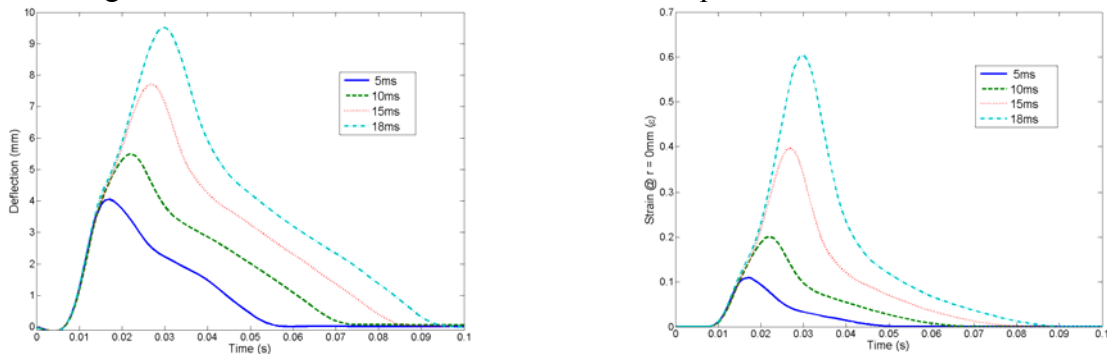


Figure 6. 30 PSI Pulse Deflection and Strain Curves of Varying Duration

A 30 PSI pulse for 18 ms generates a maximum strain of 0.60 at a rate of  $20.1 \text{ s}^{-1}$ . The new device also has the ability to repetitively strain the cell culture which is achieved by applying several quick air pulses in rapid succession. Figure 7 shows the strain curve after four equal air pulses were administered to the culture well. These pulses are separated by 50 ms; however the device can generate pulses separated by as little as 2 ms. The device can also replicate existing patterns. Figure 8 shows the resulting strain curve from an input of three square pulses of decaying magnitude.

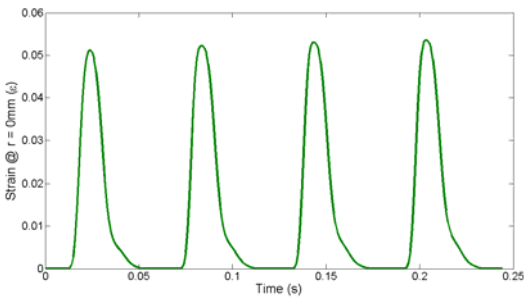


Figure 7. Equal Amplitude Repeated Loading

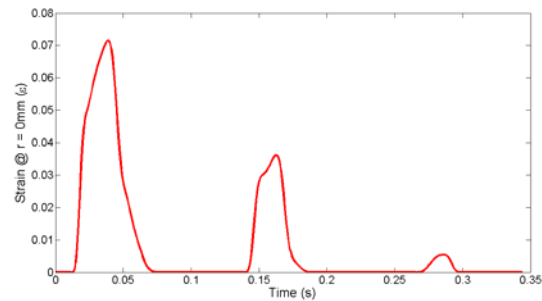


Figure 8. Repeated Decaying Loading

Using the computer controlled valve, any arbitrary curve can be used as an input to control the gas flow into the culture well. This allows values measured in other experiments to be used as a driving signal for the simulation of many different injury scenarios.

## DISCUSSION

Examination of Table 1 shows that the use of the linear potentiometer to measure the deflection of the membrane significantly impedes the membrane's motion. The added mass of a potentiometer and the spring force used to keep the potentiometer plunger in contact with the bottom of the membrane absorb some of the energy of the air pulse. The result is that membrane deflection using the potentiometer was lower than membrane deflection without this instrument in experiments conducted at the same air pressure. This is an undesired effect of using a direct contact instrument such as the potentiometer to measure the deflection. To remedy this issue other non-contact methods for measuring the deflection of the membrane are being investigated.

The new injury device has a lower flow resistance than the original device. This is best illustrated by comparing the deflections given in Figure 4 from the original device to the deflections achieved using the new injury controller given in Figure 6. A comparison of the deflection curves generated using approximately 30 PSI shows that a 5 ms pulse from the new device will result in the same maximum deflection as a 29 PSI pressure pulse generated by the original device lasting 50 ms. Figure 6 also shows that as the duration of the pulses increase using the new injury device, so do the maximum deflections because there is more time for air to flow into the culture well.

Calculation of the strain at the "severe injury" level shown in Figure 4 using the original injury device reveals that a maximum strain of 0.11 and an average strain rate of  $2.32 \text{ s}^{-1}$  is achieved. Compared to other studies, these values border on the low end of the injury envelope established for neural tissue.

These values appear low because the potentiometer limited the travel of the membrane. The unimpeded strain and average strain rate of the membrane is most likely higher.

The original device employed a solenoid valve to generate the pressure pulse, thus strain and strain rate were not independent of each other and the shape of the pressure pulse was a function of inlet pressure and pulse duration only. The use of the proportional valve allows the user to control how quickly the valve opens. Maximum strain is controlled by varying pressure, and the strain rate is controlled by the valve opening rate. Thus, opening the valve a small amount or in a ramping-up shape leads to lower strain rates while quickly opening the valve leads to higher strain rates.

The deflection and strain curves given in Figure 5 demonstrate the ability of the new device to vary the maximum strain and average strain rate independently by varying the pressure pulse shape. The new device can generate pulses from 2 to 1000 ms in duration and strains from 0.1 to 0.60 at rates up to  $25 \text{ s}^{-1}$ . The ability to generate controlled repetitive pulses is a feature not found in the original device. Strain profiles highlighting this feature are given in Figure 7 and Figure 8. This can lead to experiments to examine the effects that vibration, blast loading and multiple impacts have on neural tissue.

## CONCLUSIONS

Previous studies have allowed researchers to make some of the first estimates of the injury tolerance of neural tissue cultures. However, the injury device used in previous investigations of neural cell injury was limited by its inability to control strain and strain rate independently. Additionally, it did not have the capacity to simulate quick repetitive loading and the controller did not provide for automated execution of this function. We have constructed a more sophisticated pneumatic controller for use in cell culture injury research. The controller utilizes a proportional control valve allowing it to generate an infinite number arbitrary pulse shapes. The device can also simulate repetitive loading. Analysis of the full range of capabilities has shown that the new device can generate strains from 0.1 to 0.60 at rates up to  $25 \text{ s}^{-1}$ . These values fully envelope the published injury parameters for neural tissue injury and allow for the exploration of cell responses at higher strains.

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