

NUMERICAL SIMULATION OF A CELLULAR-LEVEL EXPERIMENT TO INDUCE TRAUMATIC INJURY TO NEURONS

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Abstract

Previous research has developed a pneumatically driven device for delivering a controlled mechanical insult to cultured neurons. The neuronal cell culture was injured by applying a transient air pulse to a culture well fitted with a highly elastic Silastic culture well bottom. In response to the pressure pulse, the Silastic culture well bottom deformed, stretched the attached cell culture, and resulted in observable cell injuries and death. The goal of this paper was to computationally model the spatial distribution of membrane strain, stress, and strain rate to which these cultures were subjected. The simulation results, using a finite element model of the culture well membrane, compared well with the results from the original experiments. When peak air pressure was varied from 69 kPa to 345 kPa (10 to 50 psig), numerical simulations showed that the corresponding membrane strains varied from 20 to 95% and the stress response varied from 0.5 to 1.2 MPa.

Key Words: finite element model, cell injury, culture wells, mechanical response, traumatic brain injury

Introduction

Ellis et al [1] have developed a pneumatically driven device for delivering a controlled mechanical insult to cultured neurons. This apparatus has been used extensively to study the neural and glial cell cultures of neonatal Sprague-Dawley rat pups after 2-4 weeks of undisturbed growth [2][3][4].

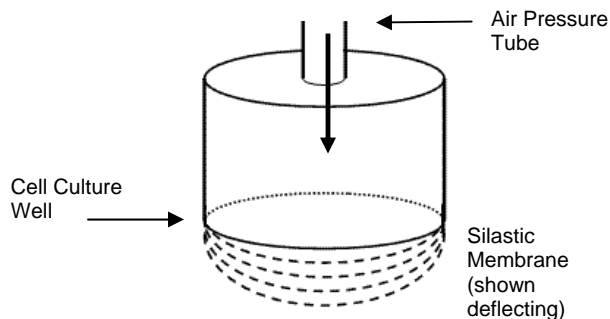


Figure 1: Schematic of culture well with a compliant membrane bottom for inducing cell injury [1]

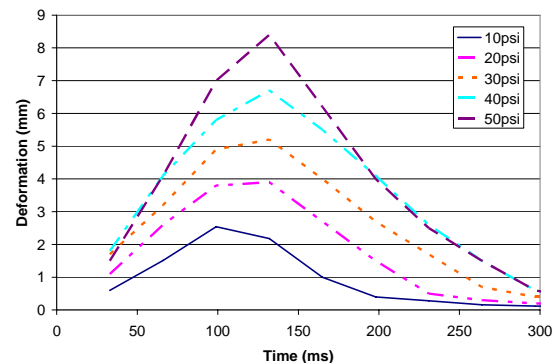


Figure 2: Experimental measurements of membrane deflection vs. time for maximum pressures [1]

The neuronal cell culture response to injury was determined by applying an air pulse to the culture wells under the control of a Commonwealth Biotechnology Model 94A Cell Injury Controller as shown in Figure 1. The cells used in this test were cultured in FlexCell Collagen I circular cell wells. These wells were constructed with a highly elastic bottom manufactured with the silicone elastomer Silastic. Under pressure, the Silastic culture well bottom deformed and stretched the attached cell culture. Figure 2

presents the mechanical responses of the Silastic membrane response to varying pressure pulses as reported by Ellis et al, hereafter referred to as the MCV (Medical College of Virginia) experiments.

The mechanical stimulus was described in global terms such as maximum membrane deflection and the global strain at peak membrane deflection. Damage to cells on the membrane was classified as mild, moderate, or severe, corresponding to maximum vertical membrane deflections of 5.5, 6.5, and 7.5 mm respectively. We hypothesize that finite element modeling may allow us to investigate some measures of mechanical stimulus, e.g. local stress, which could not be measured in the original experiments. Stress and strain will vary spatially, and may lead to regional variation in cell death rates.

Goals

The goal of this study was to quantitatively characterize the mechanical response of the membrane. In particular, the objective was to develop a finite element model that was capable of simulating the original experiments and reconstructing the associated spatial distribution of membrane stress and stain. The long term goal of this study will be correlate this local characterization of membrane mechanical response to physiological measures of cell injury.

Approach

The Silastic membrane under transient pressure loading was modeled using the LS-DYNA non-linear finite element code [7]. One challenge was that many of the mechanical property measurements, which were not relevant in the original MCV experiments, were crucial for the development of the finite element model. Our initial modeling efforts focused on estimating these parameters through numerical simulation. Future efforts will measure these parameters directly through experiments.

Geometry: Because the membrane is circumferentially symmetrical, computational times were reduced by modeling only one quadrant of the original membrane. The thickness of the membrane was 2 mm. The diameter of each well was 25 mm, with a lip underneath that reduced the effective diameter to 20 mm. The entire membrane was discretized into 96 Belytschko-Lin-Tsay shell elements. Each element was approximately one mm in size. The majority of elements were 4-node quadrilaterals. The use of shell elements assumed that the geometry was much thinner than the length and width. Brown [5] has suggested however that the accuracy of this assumption may be limited for this type of experiment.

Materials: The FlexCell membrane was composed of Silastic, a highly elastic silicone elastomer. Because the MCV experiments resulted in no plastic deformation, our model represented Silastic as a simple, linearly elastic model which can be described by only the density, modulus, and Poisson's ratio. The material was defined by a density of $5.5 \cdot 10^{-4}$ g/mm³, a modulus of $2.5 \cdot 10^6$ Pa, and a Poisson's ratio of 0.46 [6]. These values were considered the default values for this finite element model. The finite element solver was set to account for dynamic changes in thickness during the loading.

Loads and Constraints: Constraints were applied to the model in order to represent the boundary conditions of the membrane. In particular, constraints of symmetry were added to all nodes that lied along the axes of symmetry. In addition, the outer edge of the model was restrained from all translation but was allowed to rotate to simulate the flexure along the lip of the well bottom. The node representing the center of the membrane was restrained from all rotation and allowed to translate only in the vertical direction. A load representative of gravity was also incorporated into the model.

Results

The duration of the air pressure pulse in the MCV experiments was specified as 50 milliseconds. The shape of the pressure pulse however was not described in [1]. The first step in recreating the MCV experiments was to exercise the FE model using several potential waveforms to identify a candidate pulse shape for follow-on simulations and to determine the influence of pressure wave shape on membrane response. Future experiments will measure this wave shape directly. Three candidate waveforms were examined – a sinusoidal wave, a non-ideal square wave, and a non-ideal sawtooth wave. The pressure waves used in the finite element model are shown in Figure 3. The results for each wave form for the default membrane are shown in Figure 4. The sinusoidal wave resulted in a deflection pattern most similar to the experimental observations shown in Figure 2. The remainder of our study was conducted using only sinusoidal pulses. The changes in deflection induced by differing pressure magnitudes of a sinusoidal shaped pulse are illustrated in Figure 5.

The abrupt application of the load pulse to the membrane resulted in some vibration in the membrane, as manifested by the wide variation in the membrane deflection. These vibrations were induced in the beginning of the simulation, when the load was applied to the membrane and again at the end, when the load was removed from the membrane. The vibrations are the cause of the deflections seen after 50 ms. Additional research was conducted to determine the influence of membrane material properties on the membrane deflection. The membrane deflection as a function of the membrane material density was examined, but is not shown. The deflection as a function of the material elastic modulus is shown in Figure 6. Lower moduli led to a higher deflection response. Membrane density primarily affected the vibration of the model. As the density of the model rose, there were increasingly severe vibrations in the membrane model response. The effect of Poisson's ratio was not evaluated.

Discussion

Comparison with Experiment: The results of the finite element model simulation described in the previous section were compared with the experimentally determined membrane responses reported by Ellis et al. Of primary interest was the comparison of the maximum deflections for each of the pressure magnitudes. The pronounced difference in time for the deflection to occur and return to less than one mm from the original position was also investigated. The membrane deflections predicted by the FE model, shown in Figure 5, responded to the changes in pressure much faster than the experimental setup used by Ellis et al, in which the response lasts for 66 to 132 ms. Possible reasons for this difference could be that the tube which connected the cell injury controller to the wells could be altering the duration of the pressure pulse.

The relationship between the maximum observed deflections and the pressure was plotted as well, as the maximum deflection was the most important determinant of the total strain in the membrane. The resulting plot of maximum deflection vs. maximum pressure is shown in Figure 7 for both the experiment and the numerical simulation. There was a good correlation between the maximum deflection ($R^2 = 0.996$). It should be noted however that the MCV deflection measurements provided only a limited check on the accuracy of the FE model. The MCV experiments measured membrane deflection by three methods: (1) averaging the results from a sliding pointer, (2) by static dental acrylic molds, and (3) using frame by frame video analysis [1]. The sliding pointer moved with the membrane as it deformed and remained at the maximum deformation level, allowing for quick measurement. The

resistance of the slider track or the inertia of the moving slider may have influenced the measurements. The dental acrylate tests generated accurate peak deflection measurements. However, the dental acrylate tests were static in nature and may not accurately reflect the membrane displacement experienced in dynamic tests. The video analysis system recorded the motion of the membrane at 30 frames per seconds which provides only a few deflection measurements during the sub-100 ms membrane response. Both the video analysis and sliding pointer measurements were obtained by reading from a fixed ruler with millimeter markings.

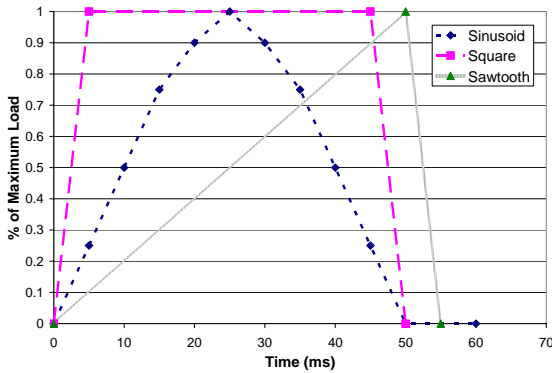


Figure 3: Plot of the three applied wave forms, the square wave, sinusoid wave, and the sawtooth wave.

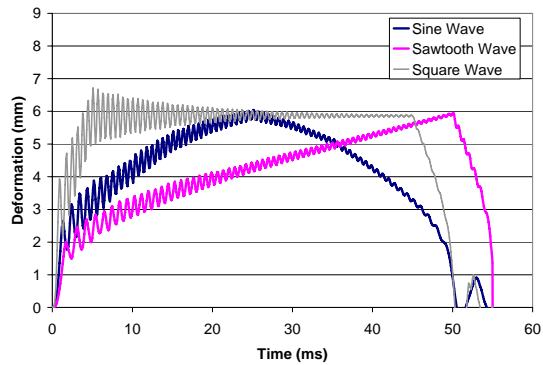


Figure 4: Plot of deflection vs. time. Membrane was subjected to sawtooth, square, and sinusoidal pulses.

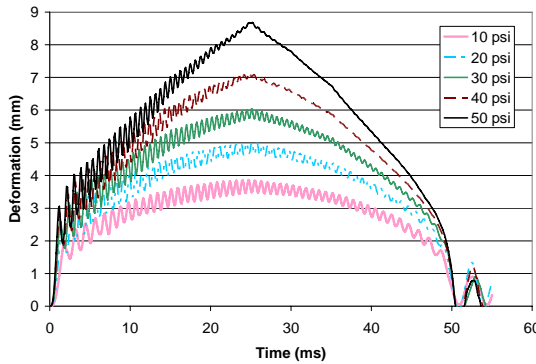


Figure 5: Plot of deflection vs. time as a function of pressure magnitudes.

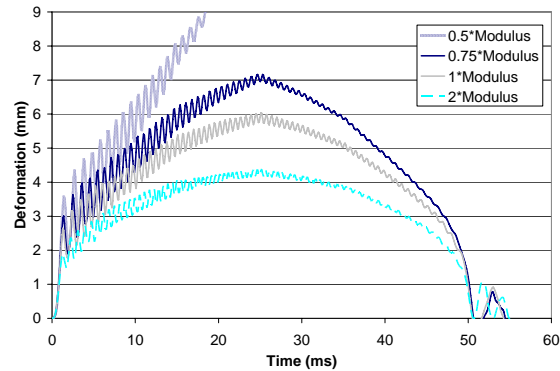


Figure 6: Plot of deflection vs. time for membranes of varying elastic modulus. (30 psi).

Stress and Strain Field: In addition to comparisons using the FE model, the stress and strain patterns predicted by the FE model were examined. Figure 8 shows the Von Mises stress in the membrane as a function of the distance from the center of the membrane at the top, bottom, and midplanes of the membrane thickness. As shown in the plot, the stress in the membrane was dependent on the location at which the stress was being evaluated. As expected, stress was found to also vary through the thickness of the membrane. It should be noted that the model deformed downward, meaning that the upper surface of the membrane represents the interior surface of the well while the lower surface represents the exterior surface of the well. The stresses increased non-linearly with respect to radius as the position moved closer to the center. The maximum stresses for the model occurred in the center of the membrane. The upper surface of the membrane showed some unusual behavior in that the stress at the

center was low and the stress at the edge was high. This may have been caused by the flexure of the membrane near the center and the imposed boundary conditions at the edge, respectively.

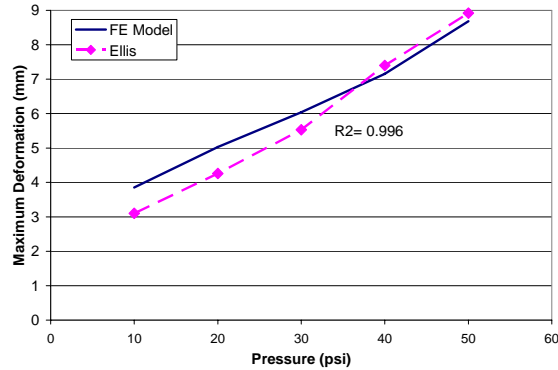


Figure 7: Plot of maximum deflection of finite element model vs. maximum deflection from Ellis et al [1].

The relationship between the maximum midplane stress and the magnitude of pressure was explored (data not shown). The Von Mises stress of the midlevel of the shell was recorded for each pressure value. The resulting curve was also non-linear, and increased more sharply as the pressure reaches higher values. As expected, a higher pressure led to an increase in the overall stress and deflection in the membrane.

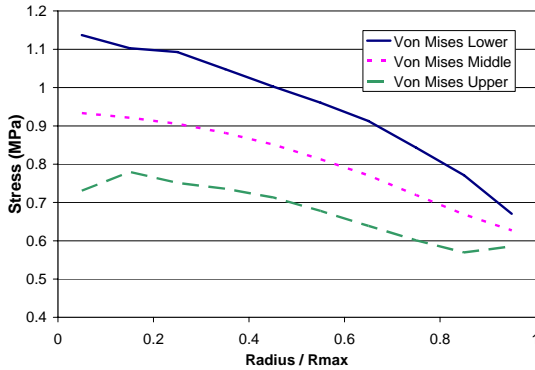


Figure 8: Stress vs. distance for membrane model. Default properties were used. The Von Mises stresses are displayed.

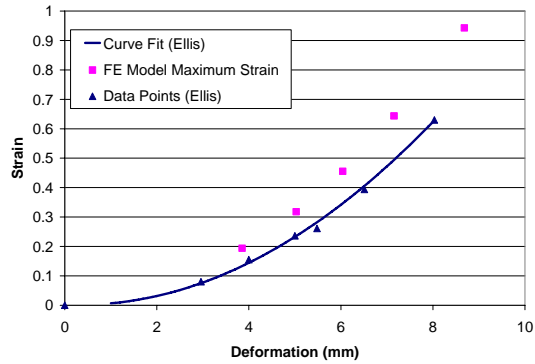


Figure 9: Strain vs. deflection. Data points from model and Ellis are plotted along with a curve fit given in the previous literature.

Strain is believed to be directly related to cell damage. Ellis et al provided a plot of membrane strain vs. deflection. This strain was measured under static testing by measuring the imprint of the membrane into dental acrylic and calculating the entire membrane stretch [1]. Figure 9 shows the comparison between the FE model and the static MCV experiments. The strains reported in the FE model were the highest regional strains observed in a radial strip of elements. The predicted strains of the model were higher than the strains predicted by the static test. The differences may be attributed to the methods by which the deflections were measured. Also, our idealization of the membrane as a perfect membrane of uniform properties and dimensions could have influenced the results.

Conclusions

Through the use of a finite element model, the mechanical response of the FlexCell culturing wells to transient pressure loading was reproduced. The pressure pulses were applied to the model in the form of a sinusoidal wave, which resulted in a response very similar to responses shown in the previous literature. The correlation between the model maximum pressure and the experimental deflection was very good ($R^2 = 0.996$).

The stress and strain response of the model was examined and the strain was compared to the documented strain in the previous experiments. As expected, stress and strain curves varied non-linearly with radial distance from the membrane center. In an analysis of strain vs. deflection, the FE model strain was consistently 25 to 45% higher than the experiments. This disagreement may be due to differences in the way in which strain was computed. Ellis et al computed the entire membrane strain based on measurements from a static test in which the membrane was inflated into dental acrylic. In contrast, the finite element model displayed the maximum strain observed in a strip of radial elements. The loss of dynamic forces or the resistance of the acrylic may have influenced the strain data points. It is also possible that our idealization of the membrane as a perfect geometry with no ambient factors may have an effect.

Limitations. The analysis has shown that the response of the membrane was strongly dependent on the shape and duration of the applied pressure pulse. There is a need to more accurately model the air pressure pulse. For this study, a representative pressure pulse was identified by numerical experimentation. An improved model will be developed by directly measuring the shape of the pressure pulse at the cell culture well. Additional improvements can also be made to the geometric model of the membrane. Our study assumed that the membrane was sufficiently thin to allow the use of shell elements. Although the finite element shells can be described with a finite thickness, it may be beneficial to evaluate the difference in response using solid elements. Additionally, the effects of the control device and the air tubes were not considered in this analysis. Finally, the study assumed that a simple three parameter linear elastic model was sufficient to describe the constitutive properties of Silastic. Although this material model appeared to give good results, a more rigorous experimental evaluation of Silastic constitutive properties should be conducted to verify the adequacy of this assumption.

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